***JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA)**, Vol. 6, No. 2, 2021* *pp. 216-227*
Chemistry Education Study Program, Universitas Sebelas Maret *ISSN 2503-4146*
https://jurnal.uns.ac.id/jkpk *ISSN 2503-4154 (online)*

ZnO NANOPARTICLES AND ITS INTERACTION WITH CHITOSAN: PROFILE SPECTRA AND THEIR ACTIVITY AGAINST BACTERIAL

Ahmad Fatoni[1]*, M. Arif Afrizal[1], Ade Arinia Rasyad[1], and Nurlisa Hidayati[2]

[1]Bhakti Pertiwi High School of Pharmacy Science
Jln. Ariodillah III No. 22, Palembang, South Sumatera, 30128, Indonesia

[2]Department of Chemistry, Faculty of Mathematic and Natural Sciences, Sriwijaya University,
Jln. Palembang-Prabumulih Km. 32, Indralaya, Ogan Ilir, South Sumatera, Indonesia

* Correspondance, Telp. (0711) 315579, Fax. (0711) 358930 e-mail : ahfatoni@yahoo.com

Received: January 23. 2021 Accepted: August 23, 2021 Online Published: August 29, 2021
DOI : 10.20961/jkpk.v6i2.48000

ABSTRACT

The biosynthesis of ZnO nanoparticles and synthesis of chitosan- ZnO nanoparticles were studied. The aims of this research were biosynthesis of ZnO nanoparticles, synthesis of chitosan-ZnO nanoparticles, its characterization and, used as an antibacterial agent of *Escherichia coli***.** ZnO nanoparticles were biosynthesized by reacting with ethanolic extract of guava seeds leaves (*Psidium guajava* L.) and zinc acetate dihydrate solution. Chitosan-ZnO nanoparticles were synthesized by the heating method. ZnO nanoparticles and chitosan-ZnO nanoparticles were characterized by FTIR spectroscopy and X-ray diffraction, respectively. The suspension of chitosan-ZnO nanoparticles was used as an antibacterial agent with a paper disk method. The result showed that the Zn-O group at ZnO nanoparticles was detected at a wavenumber 615.29 and 673.16 cm^{-1} The crystallite size of ZnO nanoparticles was 1.43 nm. The wavenumber of 617-655 cm^{-1} is the Zn-O group at the structure of the chitosan-ZnO nanoparticle. The average of diameter inhibition zone of chitosan-ZnO nanoparticles (1:2) at concentration 0.25 and 0.5 % (w/v) to *Escherichia coli* was 15.7 ± 1.0 and 18.3 ± 0.4 mm respectively.

***Keywords**: ZnO, chitosan-ZnO nanoparticles, Escherichia coli*

INTRODUCTION

ZnO nanoparticles are transition metal oxide nanoparticles, and it was used in wide application. Weldegebrieal [1] reported that the utility of ZnO nanoparticles in any field such as chemistry field (catalysis, sensors, etc.), medicine, for supporting human life (paints, batteries, solar cells, memory devices, and electronic) and in agriculture (bio-fertilizer). There are four methods to synthesis ZnO nanoparticles such as physical, chemical, biological, and hybrid methods [1]. First, chemical methods such as chemical vapour [2], precipitation [3], using diblock copolymers as templates [4], and sol-gel [5]. Physical methods such as super-critical hydrothermal [6], gas condensation [7], laser ablation [8], and microwave [9]. Biological methods include using plant extract [10, 11, 12] and hybrid methods such as electrochemical and chemical Synthesis [13].

Nowadays, the biological method, particularly using plant extract to synthesis metal nanoparticles, is an interesting study of material scientists. The material scientists reported that the synthesis of metal nanoparticles using plant extract is a green synthesis method [14]. The plant extracts are rich in phytochemicals and act as reducing and stabilizing agents in this synthesis [15]. The advantage of this green synthesis is effective, save the environment, and a few of the dangerous chemicals can be minimized, and this method can be done at low temperatures and pressures [11]. The green synthesis of ZnO nanoparticles using plants has been carried out using an aqueous extract of *Trifolium pratense* [10], *Costus pictus* D. Don [15], *Calotropis gigantea* [16], and *atalantia monophylla* [17].

The size and shape of metal nanoparticles are affected by the concentration of plant extract and metal ions [18]. The concentration of plant extract is affected by solvent. The solvent will extract the secondary metabolites of the plant become an aqueous extract. Aqueous extract of the plant is containing secondary metabolites and is obtained by the extraction process. The majority of the extraction process is using aqua dest or ethanol. Aquadest or ethanol can be used as a solvent in the extraction process based on the polarity index. The polarity index between aqua dest and pure ethanol is 9 and 5.2 [19]. Based on this polarity index, aquadest is more polar than pure ethanol. Therefore, Aquadest is more favourable than ethanol on the extraction process above.

In this study, we studied the ZnO nanoparticles combined with chitosan to become chitosan-ZnO nanoparticles. An organic polymer such as chitosan can be used as a modifier agent of ZnO nanoparticles to become chitosan-ZnO nanoparticles. Chitosan is non-toxic, safe for further use [20], bio-compatibility [21], and chitosan has properties as antibacterial [22]. Chitosan-ZnO nano-particles can be used as anti-bacterial [23,24], medicine [25] and antifungal [26].

There is a difference between this study and our previous study [23,24]. In our previous study, the biosynthesis of ZnO nanoparticles was done at an alkaline solution. A previous study reported that biomolecules as biological nanoparticles synthesis were inactive [27]. On the other hand, the biosynthesis of ZnO nanoparticles can be done without adding the alkaline solution. As reported by [28], biomolecules such as alkaloids, terpenoids, steroids etc. can act as bioreduction of Zn^{2+} ion. Biosynthesis ZnO nanoparticles in this study use Zn^{2+} ion and ethanolic extract of leaves of guava seeds (*Psidium guajava* L) without an alkaline solution. Biosynthesis ZnO nanoparticles use Zn^{2+} ion and ethanolic extract of leaves of guava seeds (*Psidium guajava* L). The solvent of ethanol 70%(v/v) is used in this extraction, and this solvent is not widely explored in the extraction process of leaves of guava seeds. The presence of small amounts of water in this solvent will support the extraction process of secondary metabolites in leaves of guava seeds based on the difference of index polarity. Chitosan-ZnO nanoparticles will be as the organic-

inorganic hybrid materials which have synergistic effects as antibacterial of *E. coli.*

METHODS

The material: chitosan (DD 87%) obtained from CV. Ocean Fresh Bandung, West Java, Indonesia. Acetic acid glacial (Merck), zinc acetate dihydrate (Merck), absolute ethanol (Merck) and nutrient agar (Merck). Aquadest and *E. coli* from Microbiology laboratory of Bhakti Pertiwi High School of Pharmacy Science. Guava seeds leaf (*Psidium guajava* L) from Palembang, South Sumatera, Indonesia.

1. Preparation of ethanolic extract of guava seeds leaves

The preparation of ethanolic extract was adopted from Fatimah [29] with slight modification. Ethanolic extract was prepared by maceration process of fresh leaves guava seeds (100 g) in ethanol 70 % (v/v) as solvent (250 mL). The maceration process was done overnight. Then, the mixture was filtered, and the filtrate was stored in the refrigerator for further experiment.

2. Biosynthesis ZnO nanoparticles.

About 50 ml of ethanolic extract of guava seeds leaves was added to 50 ml of 0.1 M zinc acetate dihydrate solution in a 250 ml Erlenmeyer flask and boiled at 80°C for 4 hours until the color of the mixture changed from slightly green to deep green. The mixture was allowed to cool at room temperature for one night to form precipitation. The residue was separated from filtrate, and the residue was washed several times with aquadest. The residue was dried in an electric oven at 50ºC until dry [30].

3. Synthesis chitosan-ZnO nanoparticles

The synthesis of chitosan-ZnO nanoparticles was adopted from Jayasuriya [31] with slight modification. Chitosan-ZnO nano-particles with ration (1: 1, w/w) between chitosan and ZnO nanoparticles were synthesized as follows: chitosan (0.1 g) was dissolved in acetic acid 1 % (v/v, 10 mL) at 250 mL of beaker glass. The mixture was stirred until homogenous. Next, ZnO nanoparticles (0.1 g) was added to the chitosan solution. The mixture was stirred by continuous stirring (60 minutes). After 60 minutes, the mixture was cast in a petri dish until dry at room temperature. The same procedure was used to synthesis chitosan-ZnO nanoparticles (1: 2, 0.1 g of chitosan and 0.2 g of ZnO nanoparticles) and chitosan-ZnO nano-particles (2: 1, 0.2 g of chitosan, and 0.1 g of ZnO nanoparticles).

4. Characterization

The functional group of ZnO nano-particles, chitosan and, chitosan-ZnO nanoparticles were analyzed by FTIR Spectrophotometer (Shimadzu Prestige-21) with the help of KBr pellets and spectra were recorded at a range of 4500–500 cm^{-1}. In addition, x-ray diffraction (Shimadzu XRD 6000) was used to calculate the crystallite size of ZnO nanoparticles and evaluate the crystalline level of ZnO nanoparticles, chitosan, and chitosan-ZnO nanoparticles. The operational condition of X-ray diffraction is Cu Kα X-ray tube at 1.5406 Å, 30 kV and 10 mA with scan speed/duration time 10.000 deg. min^{-1} and the 2θ range of 0°– 80°.

5. *In vitro* study of chitosan-ZnO nanoparticles as antibacterial against *E. coli*.

The samples in this study were the concentration of chitosan-ZnO nanoparticles (0.25 and 0.5 % (w/v)) and acetic acid solution 1 % (v/v). The antibacterial of chitosan-ZnO nanoparticles is determined by the agar disk diffusion method. The *E. coli* was cultured on nutrient agar medium slants in glass tubes and incubated for 24 h at 37°C. The inoculum suspension of *E. coli* was prepared by adding sterile NaCl solution (0.9% w/v) to the fresh culture. UV-Vis measured the optical density of the suspension at 580 nm and until 25% transmittance [32]. The nutrient agar was sterilized in an autoclave, and approximately 15 mL of this nutrient agar was poured into Petri dishes before they were solidified (the first layer). The second layer is the inoculum suspension of *E. coli* (0.1 mL of bacteria suspension in 10 mL of nutrient agar) and spread on the surface of a solidified nutrient agar. The steril of paper disks (6 mm diameter) was dipped (15 seconds) on the suspension of chitosan-ZnO nanoparticles (samples) and acetic acid solution 1 % (v/v), respectively, and placed aseptically on the surface of inoculum suspension of *E. coli*. The Petri dishes were incubated at 37 °C for 24 h. The diameter of the zones inhibition of bacterial growth was measured after 24 h. *In vitro* study was prepared in triplicate.

RESULTS AND DISCUSSION

1. Biosynthesis of ZnO nanoparticles and synthesis of chitosan-ZnO nanoparticles.

Ethanolic extract of leaves guava seeds and zinc acetate dihydrate solution are used on the biosynthesis of ZnO nanoparticles. The product of ZnO nanoparticles is seen in Figure 1. The mechanism of biosynthesis of ZnO nanoparticles is a reduction or an oxidation mechanism [30]. Ethanolic extract of leaves guava seeds contains biological materials or metabolites (primary or secondary) such as carbohydrates, flavonoids, alkaloids, glycosides, tannin, vitamins, and steroids [33]. The biological materials have a key position in the conversion process of metal ions to specific metal nanoparticles and act as reducing or oxidizing agents [17]. The size of metal oxide nanoparticles is controlled by metal salt, reducing, stabilizing, or capping agents [34]. Based on the expression above, the atomic level of zinc can be made to a large nanostructure with biological reaction and called the bottom-up approach [35].

Figure 1. ZnO nanoparticles

The conversion process of zinc ion to ZnO nanoparticles as in the reaction below [36]

Zn^{2+} + ethanolic extract ⟶ [Zn / ethanolic extract]$^{2+}$ (1)

[Zn / ethanolic extract]$^{2+}$ $\xrightarrow{\text{heat}}$ [$Zn(OH)_2$ / ethanolic extract] (2)

[$Zn(OH)_2$ / ethanolic extract] $\xrightarrow{\text{incubation}}$ ZnO nanoparticles (3)

The product chitosan-ZnO nanoparticles as seen in Figure 2. The reaction between chitosan and ZnO nanoparticles become chitosan-ZnO nanoparticles as reported by [25] and [26]: the presence of primary amine and hydroxyl groups in the chitosan framework can form the coordination bonding with metals (such as ZnO) to be chitosan-coated ZnO nanoparticles

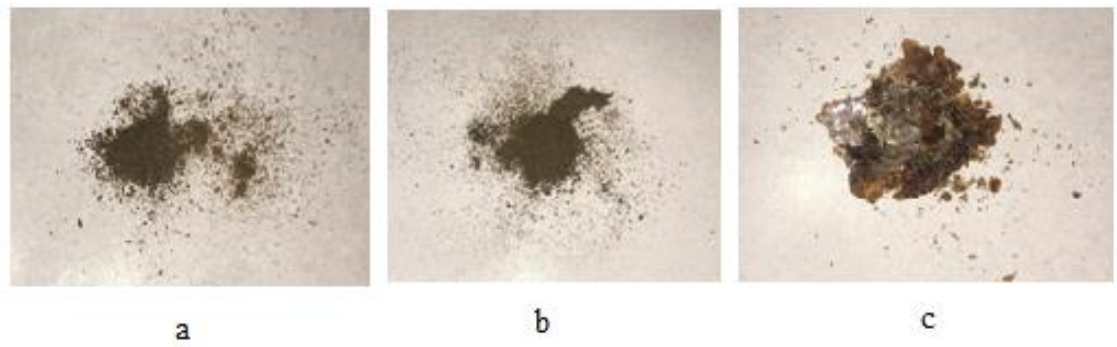

Figure 2. a. Chitosan-ZnO nanoparticles (1:1), b. Chitosan-ZnO nanoparticles (1:2) and c. Chitosan-ZnO nanoparticles (2:1)

2. Analysis of the physical structure

The XRD pattern of chitosan, ZnO nanoparticles, and chitosan-ZnO nano-particles can be seen in Fig.3. The physical structure of chitosan is crystalline form because it has two strong diffractions at around 2θ = 10° and 20° [37]. Chitosan structure has intra and intermolecular hydrogen bonds in the polymer chain. The effect of a hydrogen bond on the chemical structure of chitosan, so the chitosan is stable in the crystalline form (Figure 3a).The diffractogram of ZnO nanoparticles showed a broad peak at around 2θ = 25° and the absence of a sharp peak (Figure 3b). This indicates ZnO nanoparticles in these diffractograms is in the amorphous state, [38] reported that the type of this diffractogram is ZnO with the hexagonal wurtzite structure. Debye Scherrer's formula can be used for calculating the crystallite size of the biosynthesized ZnO nanoparticles. Debye Scherrer's formula can be written as follows [17].

$$D = (0.9.\lambda / \beta.\cos\theta) \text{.....(4)}$$

Where D is the crystallite size, λ is the wavelength of X-ray used, β is the full width at half maximum (FWHM), and θ is the Bragg's angle. The XRD pattern of ZnO nanoparticles showed, the crystallite size of the biosynthesized ZnO nanoparticles was estimated at 1.43 nm. This crystallite size is classed in the range 1-100 nm as defined by [39].

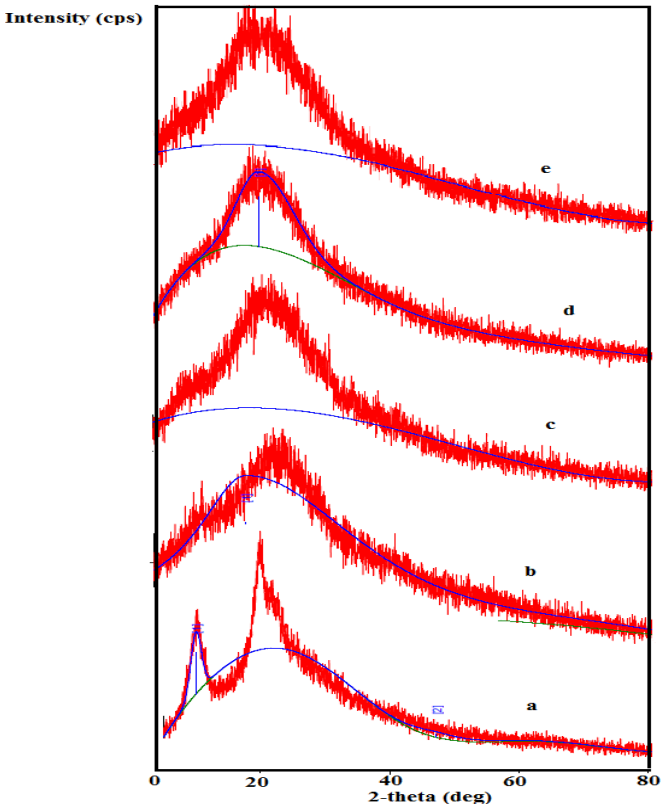

Figure 3.The diffractogram of chitosan (a), ZnO nanoparticles (b), chitosan- ZnO nanoparticles (1 : 1, c), chitosan- ZnO nanoparticles (1 : 2, d) and chitosan- ZnO nanoparticles (2 : 1, e)

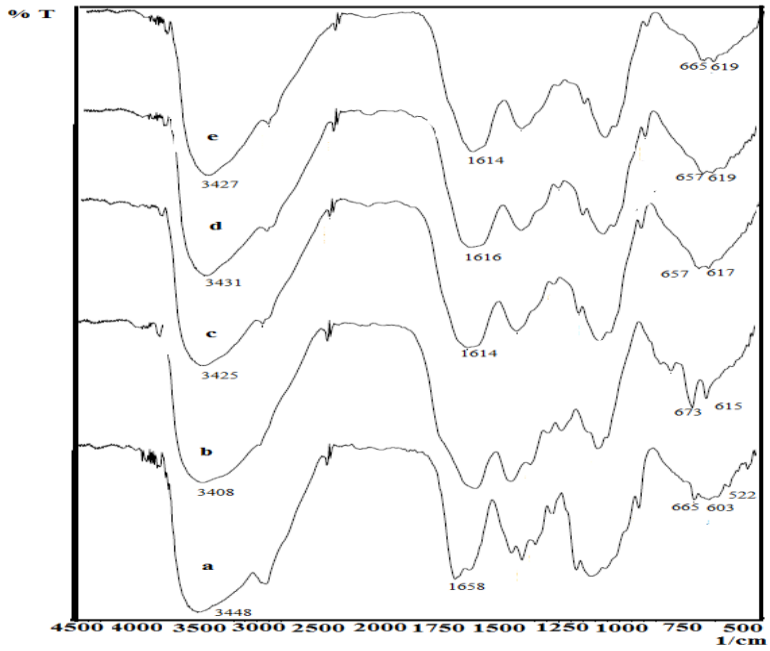

Figure 4. The spectra FTIR of chitosan (a), ZnO nanoparticles (b), chitosan- ZnO nanoparticles (1 : 1, c), chitosan- ZnO nanoparticles (1 : 2, d) and chitosan- ZnO nanoparticles (2 : 1,e)

The X-RD diffractogram of chitosan-ZnO nanoparticles (Figure.3c-e) and they have a similar peak. However, there is no peak obtained and classed in amorphous

nature. The amorphous nature may be related to the effect of ZnO nanoparticles on primary amine (-NH_2) at the chemical structure of chitosan. It will decrease hydrogen bonds and destroy the regularity of the chitosan chain [40].

3. Analysis of Functional Group

FTIR spectra of chitosan showed in fig. 4a. Stretching vibration of –N-H and –O-H groups appeared at 3448.72 cm^{-1}. They were stretching vibration group of –CH_2- detected at 2881.65 cm^{-1}. The band of 1597.06 and 1381.03 cm^{-1} stretch vibrations of –C=O and -C-N- groups, respectively. The band 1033.85 cm^{-1} is stretching vibration of –C-O-C- linkages. Bending vibration of N-H group detected at 1658 cm^{-1}. All these groups were reported by [37] and [12].

FTIR spectra of ZnO result from the interaction between ethanolic extract of jambu biji leaves and zinc acetate dihydrate (Fig. 4b). The result shows that the peak at 3408.22 cm^{-1} is stretching vibrations of the –O-H group. Stretching vibration of C=C, C=O and C-N was detected at the range between 1570.06 and 1427.32 cm^{-1}. C-O group detected at 1066.64 cm^{-1}. The peak at 615.29 and 673.16 cm^{-1} is the hexagonal phase of ZnO [11,41]. [42] reported that the functional group's secondary metabolites can donate electrons that could reduce divalent zinc ion (Zn^{2+}) and finally to zinc nanoparticles (Zn^0)

The spectra FTIR of chitosan-ZnO nanoparticles (Fig. 4c-e) showed that the band in the range 3425-3431 cm^{-1} is stretching vibration of –N-H and –O-H groups. This band is lower than the band in chitosan (3448.72 cm^{-1}). This band's sift indicated ZnO nanoparticles bonded at –N-H and –O-H groups of chitosan framework through coordination bonds [12,43]. The band of the Zn-O group in these spectra appeared between 617-655 cm^{-1} [11,41] This indicates that the existence of ZnO nanoparticles in the chitosan framework

4. The *in vitro* study of chitosan-ZnO nanoparticles

The antibacterial activity of acetic acid solution 1 % (v/v,) and suspension of chitosan-ZnO nanoparticles (0.5 and 0.25 % (w/v)) were investigated as shown in Figure 5 and tabulated in Table 1. The average diameter inhibition zone of acetic acid 1 % (v/v) against *E Coli* bacteria was 12.9 ± 0.1 mm. The average of the diameter inhibition zone of chitosan-ZnO nanoparticles (included of the 6 mm diameter of paper disks) at concentration 0.25 and 0.5 % were 13.5 ± 0.7 and 13.9 ± 0,4 mm for chitosan-ZnO nanoparticles (1:1), 15.7 ± 1.0 and 18.3 ± 0.4 mm for chitosan-ZnO nanoparticles (1:2) and 13.8 ± 1.3 and 15.4 ± 1.7 mm for chitosan-ZnO nanoparticles (2:1) respectively. The diameter inhibition zone of chitosan-ZnO nanoparticles (1:2) is higher than chitosan-ZnO nanoparticles (1:1) and chitosan-ZnO nanoparticles (2:1) but the diameter inhibition zone of chitosan-ZnO nanoparticles (2:1) is higher than chitosan-ZnO nanoparticles (1:1).

This fact showed that the antimicrobial activity was affected by the amount of ZnO nanoparticles and chitosan modified. ZnO nanoparticles have synergistic effects as antimicrobial activity [12]. The mechanism of chitosan-ZnO nanoparticles as the antimicrobial activity reported by [12] is the following: The reactive oxygen species (ROS) is released by ZnO nanoparticles, and ROS and

Zn^{2+} ions attack the negatively charged cell wall of bacterial and will disturb of the synthesis protein. Electrostatic attraction between the positive surface charge of chitosan-ZnO nanoparticles and negatively charged bacterial cell walls will obstruct the growth of bacteria [26]. The synergistic effect of chitosan-ZnO nano-particles developed by [44] was used as an antibacterial finish for textile. The ZnO nanoparticles were obtained by the sol-gel method and coated with chitosan. Finally, the chitosan-ZnO nanoparticles can reduce approximately 92% of bacteria.

Chitosan can act as an antibacterial through two of three methods. The first is the penetration of chitosan into the micro-organisms' nuclei. The second is the interaction between positively charged chitosan compound (NH_3^+ groups) and negatively charged microbial cell membranes through the electrostatic forces [45].

Acetic acid is a weak acid, and the ability to cross bacterial membranes is more readily than strong acids. The ionized and non-ionized form of acetic acid is easy to diffuse cross hydrophobic membranes of bacterial and a disturb of synthesis protein, a consequence of its, a growth of bacterial will be blocked [46].

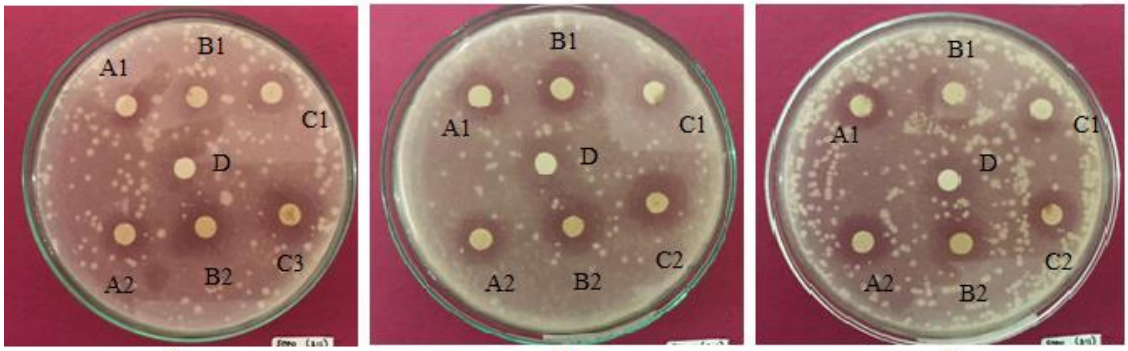

Figure 5. Antibacterial activity of chitosan-ZnO nanoparticles with agar disk diffusion method

Table 1. The calculation of the diameter zone of inhibition acetic acid and chitosan-ZnO nanoparticles to *E. coli*

No	The Petri dishes	The diameter of the inhibition zone (mm)						
		acetic acid (1 %, v/v) (D)	concentration of chitosan-ZnO nanoparticles (%, w/v)					
			(1:1)		(1:2)		(2:1)	
			0.25 (A1)	0.5 (A2)	0.25 (B1)	0.5 (B2)	0.25 (C1)	0.5 (C2)
1	I	13.1	13.9	14.1	16.9	17.5	15.1	13.8
2	II	12.9	14.1	14.3	15.6	18.8	12.4	17.3
3	II	12.9	12.7	13.5	14.8	18.7	14.8	15.2
Average		12.9 ± 0,1	13.5± 0.7	13.9 ± 0.4	15.7 ± 1.0	18.3 ± 0.4	13.8 ± 1.3	15.4 ± 1.7

CONCLUSION

ZnO nanoparticles can be formed by interaction between zinc acetate dihydrate and ethanolic extract of leaves guava seeds. ZnO nanoparticles have an amorphous state. The average crystallite size of the ZnO nanoparticle was 1.34 nm. The existence of

the functional group of Zn-O in the chitosan framework. The in vitro study showed that the antibacterial activity of chitosan-ZnO nanoparticles (1:2) is the best than chitosan-ZnO nanoparticles (1:1) and chitosan-ZnO nanoparticles (2:1).

ACKNOWLEDGEMENT

We are thankful to Bhakti Pertiwi High School of Pharmacy Science (STIFI Bhakti Pertiwi) for funding this research.

REFERENCES

[1] G. K. Weldegebrieal. "Synthesis method, antibacterial and photocatalytic activity of ZnO nanoparticles for azo dyes in wastewater treatment: A review". *Inorg. Chem. Commun*. vol. 120 , no. 108140., 2020. doi: 10.1016/j.inoche.2020.108140

[2] S. Polarz, A. Roy, and M. Merz. 'Chemical vapour synthesis of sizeselected zinc oxide nanoparticles', *Small.* vol, 5, pp. 540–552, 2005, doi: 10.1002/smll.200400085

[3] Z. Hu, G. Oskam, and P. C. Searson., "Influence of solvent on the growth of ZnO nanoparticles", *J.Colloid. Interface. Sci.*, vol. 263, pp.454–460, 2003. doi: 10.1016/S0021-9797(03)00205-4

[4] J. C. Hulteen, and C. R. Martin, "A general template based method for the preparation of nanomaterials", *J. Mater. Chem*., vol. 7, no. 7, pp. 1075–1087, 1997. doi: 10.1039/a700027h

[5] Siswanto, N. T. Rochman and P. R. Akwalia. "Fabrication and characterization of Zinc Oxide (ZnO) nanoparticle by sol-gel method". *J. Phys.: Conf. Ser.* vol. 853, no. 1. 012041, 2017. doi: 10.1088/1742-6596/8531/012041

[6] A. B. Moghaddam, T. Nazari, J. Badraghi, and M. Kazemzad, "Synthesis of ZnO nanoparticles and electrodeposition of polypyrrole/ZnO nanocomposite film", *Int. J. Electrochem. Sci*., vol. 4, pp. 247–257, 2009.

[7] H. Chang, and M. H. Tsai, "Synthesis and characterization of ZnO nanoparticles having prism shape by a novel gas condensation process", *Rev. Adv. Mater. Sci*., vol. 18, pp. 734–743, 2008.

[8] S. C. Singh, and R. Gopal, "Zinc nanoparticles in solution by laser ablation technique', *Bull. Mater. Sci*., vol. 30, pp. 291–293, 2007. doi: 10.1007/s12034-007-0048-z

[9] P. B. Khoza, M. J. Moloto, and L. M. Sikhwivhilu, "The Effect of Solvents, Acetone,Water, and Ethanol, on the Morphological and Optical Properties of ZnO Nanoparticles Prepared by Microwave", *J. Nanotechnol.* vol. 2012, Article ID 195106, 6 pages, 2012. doi:10.1155/2012/195106

[10] R. Dobrucka and J. Dugaszewska. "Biosynthesis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract". *Saudi J. Biol.Sci*., vol. 23, pp. 517–523, 2016. doi:10.1016/j.sjbs.2015.05.016

[11] Y. T. Prabhu, K.V. Rao, S. Sai and T. Pavani." A facile biosynthesis of copper nanoparticles: A micro-structural and antibacterial activity investigation". *J.Saudi Chem. Soc*. vol. 21**,** pp.180–185, 2017. doi:10.1016/j.jscs.2015.04.002

[12] P. M. Rahman, V. M. Abdul Mujeeb, K. Muraleedharan, and S. K. Thomas, " Chitosan/nano ZnO composite films: Enhanced mechanical, antimicrobial and dielectric properties." *Arab. J.Chem*., vol.11, pp. 120–127, 2018. doi: 10.1016/j.arabjc.2016.09.008

[13] R. M. Nyffenegger, B. Craft, M. Shaaban, S. Gorer, G. Erley, and R.M. Penner., "A Hybrid Electro-chemical/Chemical Synthesis of Zinc Oxide Nanoparticles and Optically Intrinsic Thin Films", *Chem. Mater*. vol.

10, no. 4, pp. 1120–1129, 1998. doi: 10.1021/cm970718m

[14] J. Singh, T. Dutta, K.H. Kim, M. Rawat, P. Samddar, and P. Kumar, P. "Green" synthesis of metals and their oxide nanoparticles: applications for environmental remediation". *J. Nanobiotechnology*. vol. 16, no. 84, pp. 1-24, 2018, doi:10.1186/s12951-018-0408-4

[15] J. Suresh, G. Pradheesh, V. Alexramani, M. Sundrarajan and S. I. Hong. "Green synthesis and characterization of zinc oxide nanoparticle using insulin plant (Costus pictus D. Don) and investigation of its antimicrobial as well as anticancer activities, *Adv. Nat.Sci.: Nanosci. Nanotechnol.* vol. 9, no. 1, pp 015008, 2018. doi: 10.1088/2043-6254/aaa6f1

[16] S. K. Chaudhuri and L. Malodia. "Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: characterization and its evaluation on tree seedling growth in nursery stage". *Appl. Nanosc*. vol. 7, pp. 501–512, 2017 doi: 10.1007/s13204-017-0586-7

[17] S. Vijayakumar, S. Mahadevan, P. Arulmozhi, S. Sriram, and P. K. Praseetha, "Green synthesis of zinc oxide nanoparticles using Atalantia monophylla leaf extracts: Characterization and antimicrobial analysis", *Mater. Sci.Semicond. Process*. vol. 82, pp. 39–45, 2018, doi: 10.1016/j.mssp.2018.03.017

[18] R. K. Das, V. L. Pachapur, L. Lonappan, M. Naghdi, R. Pulicharla, S. Maiti and S.K. Brar. "Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. Nanotechnology for Environmental Engineering", vol. 2, no. 18, pp. 1-21, 2017. doi:10.1007/s41204-017-0029

[19] N. Muhamad, N. A. Muhmed, M. M. Yusoff, and J. Gimbun, J. 2014. Influence of Solvent Polarity and Conditions on Extraction of Antioxidant, Flavonoids and Phenolic Content from *Averrhoa bilimb.* J.Food *Sci.Eng*. vol. 4, pp. 255-260. doi: 10.17265/2159-5828/2014.05.006

[20] S.K. Kim and N. Rajapakse. "Enzymatic production and biological activities of chitosan oligosaccharides (COS): a review". *Carbohydr. Polym*. Vol. 62, no. 4, pp. 357–368. 2005 . doi: 10.1016/j.carbpol.2005.08.012

[21] N. A. A. Yusof, N.M. Zain and N. Pauzi. "Synthesis of chitosan / zinc oxide nanoparticles stabilized by chitosan via microwave heating". *Bull. Chem. React. Eng. Catal*. Vol. 14, no. 2, pp. 450-458, 2019, doi: 10.9767/bcrec.14.2.3319.450-458

[22] M.S. Benhabiles, H. Lounici, N. Drouiche, M. F. A. Goosen and N. Mameri. "Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste". *Food Hydrocoll.* Vol. 29, no. 1, pp. 48-56, 2012. doi: 10.1016/j.foodhyd.2012.02.013

[23] A. Fatoni, E. Munarsih, K. Asmadi and N. Hidayati. "Synthesis and characterization chitosan-ZnO nanoparticle and its application as antibacterial agent of Staphylococus aureus". *Sci.Technol. Indones*. vol. 5, no. 1, pp.1-5, 2020, doi: 10.26554/sti.2020.5.1.1-5

[24] A. Fatoni, H. Hilma, A. A. Rasyad, S. Novriyanti, and N. Hidayati, "Biosintesis ZnO nanopartikel dari ekstrak air daun jambu biji (*Psidium guajava* L) dan ion Zn^{2+} serta interaksinya dengan kitosan sebagai antibakteri Escherichia coli". *J. Sains Farm. Klin*. vol. 7, no. 2, pp. 151-157, 2020, doi: 10.25077/jsfk.7.2.151-157.2020

[25] H. Wu and J. Zhang. "Chitosan-based zinc oxide nanoparticle for enhanced anticancer effect in cervical cancer: A physicochemical and biological perspective". *Saudi Pharm. J*. vol. 26, pp. 205–210, 2018. doi: 10.1016/j.jsps.2017.12.010

[26] S. H. S. Dananjaya, R. S. Kumar, M. Yang, C. Nikapitiya, J. Lee, and M.De Zoysa. "Synthesis, characterization of ZnO-chitosan nanocomposites and

evaluation of its antifungal activity against pathogenic Candida albicans". *Int. J. Biol. Macromol.* vol. 108, pp. 1281–1288, 2018, doi:10.1016/j.ijbiomac.2017.11.046

[27] H. M. M. Ibrahim. "Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative micro-organisms". *J. Radiat. Res. Appl. Sci.* vol. 8, pp. 265-275, 2015. doi:10.1016/j.jrras.2015.01.007

[28] M. Nilavukkarasi , S. Vijayakumar , and S. Prathipkumar. "Capparis zeylanica mediated bio-synthesized ZnO nanoparticles as antimicrobial, photo-catalytic and anti-cancer applications". *Mater. Sci. Energy Technol*. vol 3, pp. 335–343, 2020. doi: 10.1016/j.mset.2019.12.004

[29] I. Fatimah, R. Y. Pradita, and A. Nurfalinda. "Plant Extract Mediated of ZnO Nanoparticles by Using Ethanol Extract of Mimosa Pudica Leaves and Coffee Powder." *Procedia Eng*. vol. 148, pp. 43-48, 2016. doi: 10.1016/j.proeng.2016.06.483

[30] M. Nasrollahzadeh, S.S. Momeni and S. M. Sajadi, Green synthesis of copper nanoparticles using *Plantago asiatica* leaf extract and their application for the cyanation of aldehydes using $K_4Fe(CN)_6$. *Journal of Colloid and Interface Science,* 506, (2017),471–477. doi: 10.1016/j.jcis.2017.07.072

[31] A.C. Jayasuriya, A. Aryaei, and A. H. Jayatissa. "ZnO nanoparticles induced effects on nanomechanical behavior and cell viability of chitosan films", *Mater. Sci. Eng. C Mater. Biol. Appl*. vol. 33, no. 7, pp. 3688-96. 2013 doi: 10.1016/j.msec.2013.04.057

[32] Isnaeni, E. Hendradi, and N. Z. Zettira, "Inhibitory effect of roselle aqueous extracts-HPMC 6000 gel on the growth of *Staphylococcus aureus* ATCC 25923". *Turk. J. Pharm. Sci., vol.* 17, no. 2, pp. 190-196, 2020. doi: 10.4274/tjps.galenos.2019.88709

[33] L. Joseph, M. George, G. Singh, and P. Mathews. "Phytochemical investigation on various parts of Psidium guajava". *Ann. Plant. Scie.* Vol. 5, no. 2, pp. 1265-1268, 2016 doi: 10.21746/aps.2016.02.001

[34] M. Sorbiun, S. M. Ebrahim, A. Ramazani and A. M. Malekzadeh. "Biosynthesis of metallic nanoparticles using plant extracts and evaluation of their antibacterial properties." *Nanochem. Res.* vol. 3, no.1, pp; 1-16, 2018 doi:10.22036/ncr.2018.01.001

[35] D. Zhang, X. Ma, Y. Gu, H. Huang, and G. Zhang. "Green synthesis of metallic nanoparticles and their potential applications to treat cancer". *Fron. Chem*. Vol. 8, pp. 799. 2020. doi:10.22036/ncr.2018.01.001

[36] S. A. Akintelu, A. S. Folorunso, F. A. Folorunso, and A. K. Oyebamiji. "Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation". *Heliyon* vol.6, pp.e04508, 2020. doi: 10.1016/j.heliyon.2020.e04508

[37] S. Kumari, P. Rath, A. S. H. Kumar, and T. N. Tiwari, "Extraction and characterization of chitin and chitosan from fishery waste by chemical method". *Environ. Technol. & Innov*., vol. 3, pp.77–85, 2015. doi: 10.1016/j.eti.2015.01.002

[38] S. W. Balogun, O.O. James, Y. K. Sanusi, Y. K and · O. H. Olayinka. "Green synthesis and characterization of zinc oxide nanoparticles using bashful (*Mimosa pudica*) leaf extract: a precursor for organic electronics applications". *S. N. Appl. Sci.* vol. 3, no. 2, pp. 504, 2020. doi: 10.1007/s42452-020-2127-3

[39] D. R. Boverhof, C. M. Bramante, J.H. Butala , S. F. Clancy , M. Lafranconi, J. West and S. C. Gordon. "Comparative assessment of nanomaterial definitions and safety evaluation considerations". *Regul. Toxicol. Pharmacol.* vol. 73, pp. 137-150, 2015 doi: 10.1016/j.yrtph.2015.06.001

[40] N. A. Mohamed and M. M. Fahmy, "Synthesis and antimicrobial activity of some novel cross-linked chitosan hydrogels", *Int.. J. Mol. Sci.,* vol. 13, pp. 11194-11209, 2012. doi: 10.3390/ijms130911194

[41] J. Santhoshkumar, S. V. Kumar, and S. Rajeshkumar, "Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen". *Resource-Efficient Technologies.,* vol. 3, pp. 459–465, 2017. doi: 10.1016/j.reffit.2017.05.001

[42] S. Alamdari, M. S. Ghamsari, C. Lee, W. Han, H. H. Park, M. J. Tafreshi, H. Afarideh, and M. H. M. Ara, M. H. M. "Preparation and Characterization of Zinc Oxide Nanoparticles Using Leaf Extract of *Sambucus ebulus*", *Appl. Sci.,* vol.10, pp. 3620, 2020. doi: 10.3390/app10103620

[43] M. M. AbdElhady, "Preparation and Characterization of Chitosan/Zinc Oxide Nanoparticles for Imparting Antimicrobial and UV Protection to Cotton Fabric", *Int. J. Carbohydr. Chem.,* vol. 2012, Article ID 840591, 6 pages, 2012. doi: 10.1155/2012/840591

[44] A. Farouk, S. Moussa, M. Ulbricht, and T. Textor." ZnO Nanoparticles-Chitosan Composite as Antibacterial Finish for Textiles". *Int. J. Carbohydr. Chem*. Vol. 2012, doi: 10.1155/2012/693629

[45] R. C. Goy, D. de Britto, and O.B. G. Assis. "A Review of the Antimicrobial Activity of Chitosan". *Polímeros: Ciência e Tecnologia*, vol. 19, no. 3, pp. 241-247, 2009, doi:10.1590/S0104-14282009000300013

[46] F. D. Halstead, M. Rauf, N. S. Moiemen, A. Bamford, C.M. Wearn, A.P. Fraise, et al. "The Antibacterial Activity of Acetic Acid against Biofilm-Producing Pathogens of Relevance to Burns Patients". *PLoS ONE*. vol. 10, no. 9, pp. e0136190, 2015, doi: 10.1371/journal.pone.0136190