

## Synthesis and Characterization Chitosan-ZnO Nanoparticles and Its Application as Antibacterial Agent of *Staphylococcus aureus*

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### Abstract

The synthesis of modified chitosan has been studied. The aims of this research were modification of chitosan with ZnO nanoparticle to form chitosan-ZnO nanoparticles and its application as antibacterial agent of *Staphylococcus aureus*. Characterization of chitosan-ZnO nanoparticles was conducted using FTIR spectroscopy and X-Ray diffractometer. ZnO nanoparticle was synthesized by reaction between leaf extract of Sirih hijau (*piper betle L*) and zinc acetate dihydrate. Modified chitosan was synthesized by chitosan and ZnO nanoparticles. Chitosan-ZnO nanoparticles solution can act as antibacterial agent with paper disk method. The result showed that chitosan can be modified with ZnO nanoparticle and detected at wave number of 3427  $\text{cm}^{-1}$ . The crystalline size of ZnO nanoparticle is 16.47 nm. The average inhibition zone of chitosan-ZnO nanoparticles at concentration 10.000, 5.000 and 2.500 ppm are  $28.87 \pm 0.4$ ;  $24.93 \pm 0.15$  and  $19.35 \pm 0.3$  mm respectively.

### Keywords

chitosan-ZnO nanoparticle, antibacterial agent

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### 1. INTRODUCTION

Chitosan is an organic material and natural polymer. It is composed of a linear polysaccharide of  $\alpha$  (1-4)-linked 2-amino 2-deoxy  $\beta$ -D glucopyranose. Chitosan was synthesized by deacetylation of chitin (Mohanasrinivasan et al., 2014). The characteristic of chitosan is nontoxic and safe for further used (Agnihotri et al., 2004; Kim and Rajapakse, 2005) but solubility of chitosan only in dilute acidic aqueous solutions and it is a problem of chitosan. Modification of chitosan will improve properties of chitosan. There are two methods in modification process of  $-\text{NH}_2$  group of chitosan, i.e. physical and chemical method (Guibal, 2004). Physical method is to increase widely of surface area, active site of adsorption, swelling polymer chain, decrease crystallinity and to increase the swell of it. Chemical method can be done by grafting, impregnating and cross linking (Zhang et al., 2016; Kyzas and Bikiaris, 2015). The aim from modification of chitosan is change of the characteristic solubility of chitosan (Kandile and Nasr, 2011; Dai et al., 2012).

Some plant components, such as roots, leaves, stems, seeds, and fruits have been used for synthesis of zinc oxide (ZnO) nanoparticles because plant extracts are rich in phytochemicals and act as reducing and stabilization agents (Suresh et al., 2018).

ZnO nanoparticle can be synthesized in many forms: rods, wires, whiskers, belts, bipods, tetrapods, tubes, flowers, propellers, bridges, and cages. It have been found to exhibit interesting properties such as large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency and strong adsorption ability (Dhillon et al., 2014). ZnO nanoparticle is currently being investigated as an antibacterial agent in both microscale and nanoscale formulations. ZnO nanoparticle exhibits significant antimicrobial activities when particle size is changed to the nanometer range. The result showed that nano-sized ZnO can interact with bacterial surface and/or with the bacterial core where it enters inside the cell and to be as bactericidal mechanisms. The interactions between ZnO nanoparticle and bacteria cell are very interesting and have been developed for antimicrobial agents (Sirelkhatim et al., 2015).

Now, chitosan-ZnO nanoparticle was an efficient approach to produce antibacterial agents with improved functional properties (Perelshtein et al., 2013). In this paper, we synthesized modified chitosan from reaction between chitosan and ZnO nanoparticle to form chitosan-ZnO nanoparticle. Characterization of chitosan-ZnO nanoparticle was analyzed by FTIR spectroscopy and XRD. Application of chitosan-ZnO nanoparticle as

antibacterial *Staphylococcus aureus*.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

Chitosan (DD 87%) obtained from CV. Ocean Fresh Bandung, West Java, Indonesia. Acetic acid glacial, sodium hydroxide, zinc acetate dihydrate, sulfuric acid 97% (v/v) and nutrient agar. All in analytical grade were used without further purification and purchased from Merck. *Staphylococcus aureus* from Microbiology laboratory of Bhakti Pertiwi School of Pharmacy Science. Sirih hijau leaf (*piper betle L*) from Palembang, South Sumatera, Indonesia.

### 2.2 Methods

#### 2.2.1 Preparation of leaf extract

Leaf extract of Sirih hijau (*piper betle L*) was synthesized according to Daphedar and Taranath (2018) procedure with slight modification. The first, about 50 g of *piper betle L* leaves were washed several times with aquadest to remove dust and other impurities. The second, cleaned leaves of *piper betle L* were incised into small pieces and added to 100 ml aquadest in a 250 ml Erlenmeyer flask and boiled at 90 °C for 15 minutes. The reaction mixture was allowed to cool at room temperature. After cooling, the suspension was filtered through Whatman No. 1 filter paper and filtrate was stored in the refrigerator for further experimental use.

#### 2.2.2 Synthesis of zinc nanoparticles

About 7.5 ml of leaf extract of Sirih hijau (*piper betle L*) was added to 92.5 ml of zinc acetate dihydrate solution 1 M in a 250 ml Erlenmeyer flask and incubated at 60 °C for 10 min. The reaction mixture was allowed to cool at room temperature, and pH was adjusted to 10 with added NaOH solution 0.1 M. During the reaction period, the color of the solution changed from slightly yellow to dark yellowish. The change of color, indicates the formation zinc nanoparticle and reduction of zinc ions (Daphedar and Taranath, 2018).

#### 2.2.3 Synthesis of chitosan-ZnO nanoparticles

ZnO nanoparticle (0.1 g) was dissolved in 100 mL of acetic acid 10 % (v/v). Chitosan (0.1 g) was added to this solution. The mixture was stirred continuously for 30 min, after which 1 M of NaOH solution was added dropwise to the solution until the pH was 10. The solution was then heated in a hot plate at 60 °C for about 1 h. Finally, this solution was filtered and the residue was washed several times with aquadest until the residue has neutral pH, and then dried at 50 °C for 1 h in an electrical oven (AbdElhady, 2012).

#### 2.2.4 Characterization

FTIR Spectrophotometer (Shimadzu Prestige-21) used for identifying the presence of functional groups of chitosan and chitosan-ZnO nanoparticle with the help of KBr pellets and spectra were recorded at a range of 4500–500  $\text{cm}^{-1}$ . X-Ray diffractometer (Shimadzu XRD-6000) used for evaluating the crystalline level and the crystalline size.

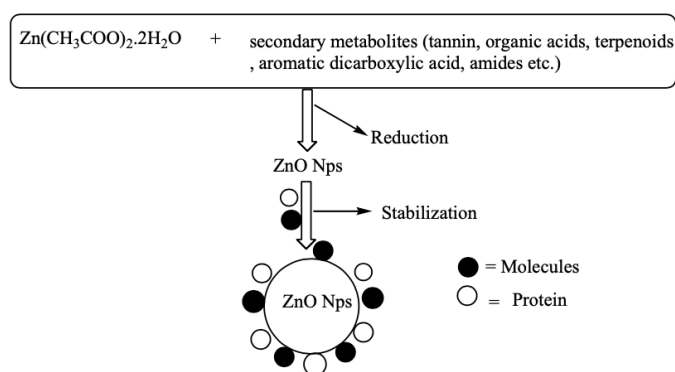
### 2.2.5 Antibacterial Study

The antibacterial activity was studied against *Staphylococcus aureus* (Gram-positive bacteria) according to in vitro. The concentration of chitosan-ZnO nanoparticle were prepared ini 10.000, 5.000 and 2.500 ppm respectively. Paper disks were sterilized in an autoclave and saturated with solution of chitosan-ZnO nanoparticle, acetic acid (10 % v/v, as a solution of negative control), tetracycline solution (104 ppm, as a solution of positive control) and ZnO nanoparticle (104 ppm) respectively. All samples were placed aseptically in the petridishes containing nutrient agar media inoculated with the above mentioned bacteria separately. The petridishes were incubated at 37 °C and the inhibition (clear) zones were recorded after 24 h of incubation period.

## 3. RESULTS AND DISCUSSION

### 3.1 Synthesis of ZnO nanoparticles using the leaf extract of sirih hijau (*piper betle L*)

The main chemical reactions involved in the synthesis of ZnO nanoparticles using the leaf extract of sirih hijau are either reduction or an oxidation mechanism as seen in Fig. 1. The biological materials possess were take part in the conversion of metal zinc compound to specific nanoparticles. The leaf extract of sirih hijau has variety of metabolites such as tannin, organic acids, terpenoids and aromatic dicarboxylic acid, amides etc. This metabolites are responsible for the antioxidant or reducing property that aids in the immediate reduction of zinc ions in to nano-sized ZnO (Vijayakumar et al., 2018). Daphedar and Taranath (2018) reported that the size particle of ZnO nanoparticles was affected by NaOH solution. The photo of product ZnO nanoparticles as seen in Fig 2.



**Figure 1.** Illustration for biosynthesis of ZnO nanoparticles using leaf extract of sirih hijau (Vijayakumar et al., 2018)

### 3.2 Formation of chitosan/ZnO Nanoparticles

When chitosan and zinc oxide are dissolved in acetic acid solution 10 % (v/v), the solution became acid and  $\text{Zn}^{2+}$  metal ions was formed. At pH 6 to 9, the unstable compound of  $\text{Zn}[(\text{OH})_2]$  was formed. Since the  $\text{NH}_2$  and  $\text{OH}$  groups chitosan can form coordination bond with metal ions, by increasing pH of the solution

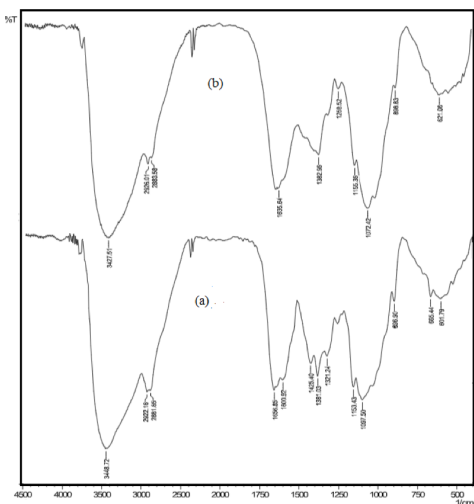


**Figure 2.** The product of ZnO nanoparticles

to pH = 10 with dropwise addition of NaOH 1 M, the stable complex of chitosan-ZnO nanoparticles is formed (Abdelhady, 2012).

**3.3 Analysis of functional group with FTIR**

FTIR spectra of chitosan was compared by FTIR spectra of chitosan-ZnO nanoparticle and as seen in Fig. 3



**Figure 3.** FTIR spectra of: (a) chitosan and (b) chitosan-ZnO nanoparticles

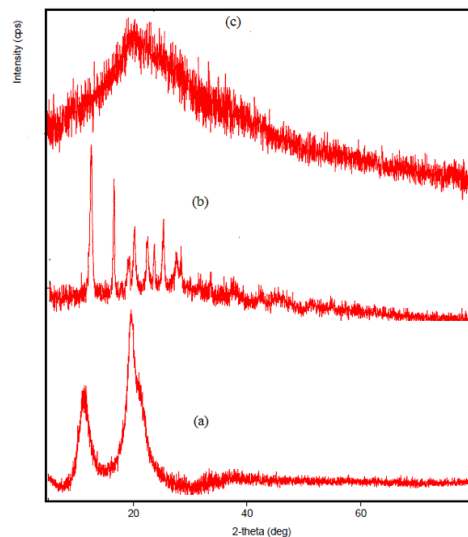
The main bands and their assignments in chitosan (Fig. 3a) are as follows: stretching vibrations of O-H,  $\nu(\text{O-H})$  and overlap with  $\nu(\text{N-H})$  at  $3448 \text{ cm}^{-1}$ . Stretching vibration of C-H,  $\nu(\text{C-H})$  at  $2922 \text{ cm}^{-1}$  (Kumari et al., 2015). Bending vibration of N-H,  $\delta(\text{N-H})$  at  $1656 \text{ cm}^{-1}$  (Mohammed et al., 2013; Kumari et al., 2015) and deformation vibration in primary amine can be found at  $1425 \text{ cm}^{-1}$  (Li and Bai, 2005). Bending vibration of C-H,  $\delta(\text{C-H})$  at  $1381 \text{ cm}^{-1}$  (Huang et al., 2013). Stretching vibration of C-O group,  $\nu(\text{C-O})$  at  $1097 \text{ cm}^{-1}$  (Kandile et al., 2014).

The main band and its interpretation data in chitosan-ZnO nanoparticles (Fig.3b) are as follows: compared with FTIR spectra of chitosan, the stronger peak is stretching vibration -OH group,  $\nu(\text{OH})$  and overlap with  $\nu(\text{N-H})$ . The wave number was shifted to lower wave number at  $3427 \text{ cm}^{-1}$  which indicated the strong interaction between these groups and ZnO (Abdelhady, 2012). The bending vibration of -NH group,  $\delta(\text{N-H})$  at  $1635 \text{ cm}^{-1}$ .

Stretching vibrations of -CH,  $\nu(\text{C-H})$  at  $2956 \text{ cm}^{-1}$ . Stretching vibrations of C-O-C group,  $\nu(\text{C-O-C})$  at  $1156 \text{ cm}^{-1}$  (Coates, 2006). A broad absorption peak for the vibration of the O-Zn-O group was in  $621 \text{ cm}^{-1}$  (Yusof et al., 2019; Abdelhady, 2012).

**3.4 Analysis of physical structure with XRD**

Diffractogram of chitosan, ZnO nanoparticle and chitosan-ZnO nanoparticles can be seen in Fig.4.



**Figure 4.** XRD powder patterns of chitosan (a), ZnO nanoparticles (b) and chitosan-ZnO nanoparticles (c).

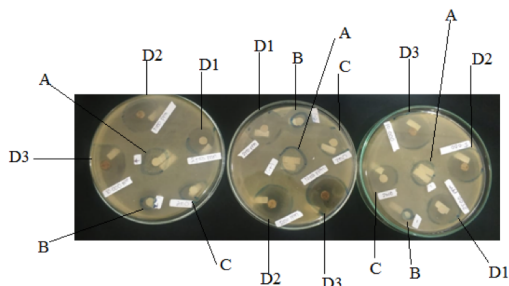
The physical structure of chitosan is crystalline form because it has two strong diffractions at  $2\Theta = 9.90^\circ$  and  $20^\circ$  (Kumari et al., 2015). The crystalline form chitosan is an indication that chitosan structure has intra and intermolecular hydrogen bond included all polymer chain (Fig.4a). The peaks in the XRD spectrum of ZnO nanoparticle (Fig. 4b) have sharp and narrow diffraction peaks, indicating that the synthesized nanoparticles are pure and crystalline in nature. The Debye Scherrer's formula can be used for calculating the crystalline size of the synthesized ZnO nanoparticles. The Debye scherrer's formula can be written as follows:

$$D = (0.9.\lambda/\beta.\cos\Theta) \tag{1}$$

Where D is the crystallite size,  $\lambda$  is the wavelength of X-ray used ( $1.5406 \text{ \AA}$ ),  $\beta$  is the full width at half maximum (FWHM) and  $\Theta$  is the Bragg's angle (Vijayakumar et al., 2018). From this formula, the average particle size of the synthesized ZnO nanoparticles is  $16,47 \text{ nm}$ . Diffractogram of chitosan-ZnO nanoparticles (Fig. 4c) have changed the crystallinity of chitosan form. This fact showed that strong hydrogen bond in chitosan framework can be deformed by insertion of the functional group of chitosan (Ding et al., 2007) because substituted of primary amine of chitosan framework (Pereira et al., 2017).

### 3.5 Antibacterial Study

The efficient antibacterial of tetracycline solution (A : 104 ppm , positive control), acetic acid solution (B : 10% v/v, negative control), ZnO nanoparticle solution (C : 104 ppm) and chitosan-ZnO nanoparticles solution (D1 : 2.500, D2 : 5.000 and D3 : 10.000 ppm respectively) were investigated in Fig.5. The average inhibition zone of A, B and C are  $20.13 \pm 0.8$ ,  $8.45 \pm 0.2$  and  $16.2 \pm 0.3$  mm respectively. The average inhibition zone of D1, D2 and D3 are  $19.35 \pm 0.3$ ,  $24.93 \pm 0.15$  and  $28.87 \pm 0.4$  mm respectively.



**Figure 5.** Antibacterial study of acetic acid 10 % v/v (B, negative control), ZnO nanoparticle (C) and chitosan-ZnO nanoparticles (D) to *S. aureus* respectively. Tetracycline solution (A, positive control).

It was seen that the inhibition zone increases with increasing the concentrations of chitosan-ZnO nanoparticles. The antimicrobial activities of ZnO nanoparticles in contact with bacteria of *S. aureus* is influenced by particle sizes, morphologies and specific surface areas of ZnO nanoparticles (Suresh et al., 2018). In other hand, reactive oxygen species (ROS) including hydrogen peroxide ( $H_2O_2$ ),  $OH^-$  (hydroxyl radicals), and  $O_2^{-2}$  (peroxide) is resulted by ZnO nanoparticle (Sirelkhathim et al., 2015). The increase of chitosan-ZnO nanoparticles propertise as antibacterial agent because there is an interaction between positive charge of  $NH_3^+$  group chitosan and negative charge of surface cell bacteria (Benhabiles et al., 2012).

Acetic acid can act as antibacterial agent because penetration of acetic acid in the cell cytoplasm. The increasing  $H^+$  ion in the cytoplasm and leads to decrease of the local pH of the cell and the cell becomes more acidic (Sedira et al., 2014).

### 4. CONCLUSIONS

Zinc asetat dihydrate can be changed by leaf extract of Sirih hijau (*piper betle L*) to form ZnO nanoparticle. Particle size of ZnO nanoparticle is 16,47 nm. Zn-O group appeared at  $621\text{ cm}^{-1}$ . Chitosan can be modified by ZnO nanoparticle to form chitosan-ZnO nanoparticle and it has wave number at  $3427\text{ cm}^{-1}$ . Application of chitosan-ZnO nanoparticle as antibacterial agent. The inhibition zone from chitosan-ZnO nanoparticles is more high than ZnO nanoparticle. The inhibition zone is increases with increasing the concentrations of chitosan-ZnO nanoparticles.

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