

SYNTHESIS, CHARACTERIZATION, AND ACTIVITY ASSESSMENT OF AMOXICILLIN/CuO COMPOUNDS AGAINST PATHOGENIC BACTERIA

Hadi Barru Hakam Fajar Siddiq*, Siti Nur Azizah

Diploma III Farmasi, Akademi Farmasi Jember

*Email: hakamfajar@gmail.com

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Abstract

Infection is a disease caused by bacteria. Some bacteria that can cause infection are *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis*. Infection can be prevented by antibiotic therapy. Excessive use of antibiotics such as misuse of indications, free use in the community, inappropriate doses, and timing of application will cause new problems such as increasing bacterial resistance to antibiotics. The prevalence of drug-resistant bacteria is increasing in many parts of the world. However, this increase is accompanied by a downward trend in the development of new antibiotics. Thus, to overcome this problem new antibiotics have to be developed. This study aimed to synthesize a new antibiotic, namely to test amoxicillin/CuO and its antibacterial activity against *E. coli*, *S. typhi*, *S. aureus*, and *B. subtilis*. Antibacterial activity test using disc diffusion method. The XRD characterization results showed that the diffraction peaks of amoxicillin/CuO in the image above started from 21.13; 29.54; 30.67; 36.82; 39.5; 42.05; and 47.88. The SEM results of the Amoxicillin/CuO compound showed that the particle size of the compound was still in the form of a graph of 196-345 nm. The results of testing the antibacterial activity of amoxicillin/CuO compounds against *E. coli*, *S. typhi*, *B. subtilis*, and *S. aureus* showed that amoxicillin/CuO compounds had activity against all bacterial samples. Thus, amoxicillin/CuO compounds can be used as antibacterial therapy.

Keywords: *synthesis, antibiotic, amoxicillin-CuO, bacteria*

Introduction

Indonesia is one of the countries with high morbidity and mortality rates due to infectious diseases. Pathogenic bacteria are one of the causes of infectious diseases (Darmadi, 2008). Some bacteria that can cause infection are *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Escherichia coli is a gram-negative facultative anaerobic bacterium that can thrive in the oral cavity. These bacteria can enter the human body through hands and eating utensils that are contaminated by dirt or bacteria (Paramitha, et al., 2010).

Salmonella typhi is a group of gram-negative bacilli that can enter the human body through food and drink. The

bacterium *S. typhi* is endemic in developing countries (Faseela, et al., 2010). Infections due to *S. typhi* in humans are seen in two types, namely non-typhoid gastroenteritis and typhoid or paratyphoid fever (Zhang, et al., 2008).

Staphylococcus aureus is a gram-positive bacterium. Acne, boils, and wound infections characterized by tissue damage accompanied by purulent abscesses are some of the infectious diseases caused by *S. aureus*. Antibiotic therapy is used in the treatment of *S. aureus* infection.

Bacillus subtilis is a bacterium that can cause eye infections, meningitis, endocarditis, and others. The bacterium *B. subtilis* is known to cause acidity in canned foods due to the formation of



fermentable sugars contained in these ingredients (Buckle, 1985).

On the other hand, antibiotic therapy is an effort to control infectious diseases. Inappropriate use of antibiotics causes bacterial resistance to antibiotics (Dzen, 2003; Nah et al., 2004). The incidence of bacterial resistance continues to increase in various parts of the world. However, the increase in resistance was not accompanied by the development of new antibiotics (Buntaran, 2007; Finch, et al., 2006). Thus, efforts are needed to develop new antibiotics (Spellberg et al., 2004; Yoneyama, 2006).

The development of antibiotics that has been carried out includes the manufacture of N-benzoylamoxicillin (Soekardjo et al., 1999). The compound obtained had greater antibacterial activity against *Pseudomonas aeruginosa* than amoxicillin. This compound also has activity against other Gram-positive and Gram-negative bacteria (Soekardjo et al., 2000).

The synthesis of other derivative compounds from amoxicillin has also been carried out by Siddiq et al. (2018) by reacting amoxicillin with 4t-butylbenzoyl chloride and has activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* bacteria. In addition, amoxicillin has also reacted with p-aminophenol to produce amoxicillin derivative compounds and has been tested on several pathogenic bacteria (Siddiq and Aziziah, 2019).

On the other hand, copper is an important element in biomedical applications, such as anti-inflammatory, anti-proliferative, and biocidal (Szymanski, et al., 2012). CuO nanoparticles can be used as an antibacterial against *S. aureus*, *E. coli*, and *P. vulgaris* (Manyasree et al., 2017).

Based on the explanation above, in this study modification of the structure of amoxicillin will be carried out by making amoxicillin/CuO using the impregnation synthesis method. Furthermore, the amoxicillin/CuO were characterized and

tested for their antibacterial activity against the pathogenic bacteria *E. coli*, *S. typhi*, *S. aureus*, and *B. subtilis*.

Research Methods

Instruments and materials

Equipment used in this study included an Ohaus CP 214 analytical balance, a PanAnalytical X-ray diffraction instrument Type: Expert Pro, FEI brand SEM (*Scanning Electron Microscopy*) Instrument, Type: Test-S50, Petri dish, Calipers, Autoclave (Westlab), Incubator, Micropipettes, Axis, Bunsen, Laminar Loft Flux (Indiamart stainless steel), colony counter and laboratory glass hand tools. The materials used in this study were amoxicillin trihydrate (pharmaceutical grade), copper sulfate (E. Merck), sodium hydroxide (E. Merck), ethanol p.a. (E. Merck), Methanol p.a. (E. Merck), Acetone (Merck), Aquabiden, nutrient agar, and nutrient broth, *E. coli*, *S. typhi*, *P. aeruginosa*, *S. Aureus*, and *B. subtilis* and paper plates.

Synthesis of amoxicillin/CuO

Amoxicillin / CuO was synthesized by the impregnation method. The steps taken are to adopt the work procedures carried out by Siddiq (2014). Solutions of amoxicillin, copper sulphate, and sodium hydroxide are prepared in a molar ratio of 0.1: 0.1: 0.2 per 100 ml. The mixture was homogenized at 80 °C until a paste formed. The mixture was then cooled to room temperature to give a precipitate. Next, the precipitate was washed several times with distilled water to remove toxins. The next step is to dry the precipitate to give a dry solid at 150 °C.

Identification of amoxicillin/CuO structures using X-Ray diffraction

The steps taken are to adopt the work procedures carried out by Rajabai and Ramachandran (2014). Synthetic amoxicillin/CuO and amoxicillin were tested by X-ray diffraction at a

wavelength of 1.54 at an angle of 2θ 10° – 70° .

Identification of amoxicillin/CuO particle size using Scanning Electron Microscopy (SEM)

The steps taken are to adopt the work procedures carried out by Rajabai and Ramachandran (2014). The Amoxicillin/CuO SEM (*Scanning Electron Microscopy*) photometer was obtained from the Hitachi Model S-3000N test at 20 kV for different magnifications.

Disc diffusion test (Kirby Bawer Test)

In this study, the method of antibacterial activity diffusion was performed with paper plates with a diameter of 6 mm. Various concentrations of the test compound were taken up to 20 l and kneaded on a napkin and filled (Ningsih, 2013). A total of 100 L of bacterial suspension was added to nutrient agar (NA). Then pour into sterile Petri dishes and allow to set. Saturated sandpaper is placed on the surface of the agar. In this study, the positive control was amoxicillin, while the negative control was 70% ethanol. The incubation

was done at 37°C for 24 hours (Saraswati, 2015).

The results of the antibacterial activity test are considered positive when a clear inhibition zone is formed around the plate. The diameter of the blocking zone was measured by reducing the diameter of the paper plate (6 mm) with the diameter of the barrier (Hermawan, 2007). When the diameter of the inhibition zone is 0-3 mm, the resistance is weak. Inhibition zone diameters between 3 and 6 mm are classified as moderate inhibitory reactions and inhibition zone diameters greater than 6 mm have strong inhibition reactions (Pan et al., 2009).

Results and Discussion

XRD test results

Amoxicillin/CuO and synthetic CuO compounds were characterized by their X-ray diffraction (XRD) crystal structures between 20° – 80° . The refractive peak of the amoxicillin/CuO compounds is 29° , starting at 21.13° ; 29.54° ; 30.67° ; 36.82° ; 39.5° ; 42.05° ; and 47.88° . The results of the diffraction images are shown in Figure 1.

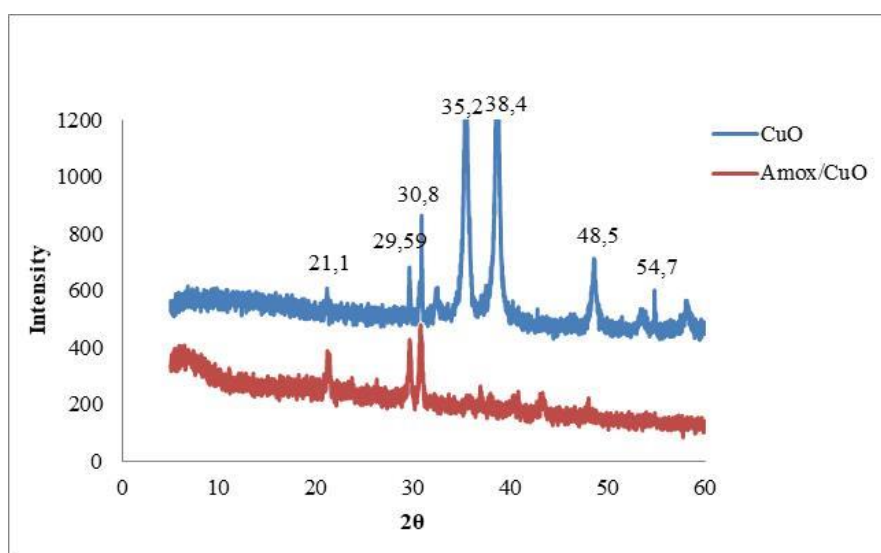


Figure 1. Amoxicillin/CuO XRD diffractogram

Figure 1 shows reduced signal peaks for CuO compounds and some missing for amoxicillin/CuO compounds. This shows that CuO is adsorbed into the pores of amoxicillin. In other words, the amoxicillin compound is loaded with CuO. According to the results of research conducted by Razzak et al. (2008), amoxicillin shows the three strongest intensity peaks that occur at an angle of 2θ by 15.144° ; 18.045° ; and 19.350° which has an orthorhombic crystal system with a primitive unit cell (P212121) and lattice parameters $a = 15.75$; $b = 18.80$; and $c = 6.684$. On the other hand, according to the results of research conducted by Padil and Cernik (2013) CuO solids showed XRD results that there were peaks at 32.47° , 35.49° , 38.68° , 48.65° , 53.36° , 58.25° and

61.45° were assigned to the reflection lines of monoclinic CuO nanoparticles.

SEM test results

SEM tests on amoxicillin/CuO nanoparticles were operated with a Hitachi Model S-3000N at 20 kV at 20,000x magnification. The SEM results for amoxicillin/CuO are shown in Figure 2. The SEM results for amoxicillin/CuO show that the particle size of the compound is still between 196–345 nm. This site is still relatively large on a nanoparticle scale. In addition, the particle size distribution of the amoxicillin/CuO compound was not seen. This may be due to a lack of heating temperature of 150°C .

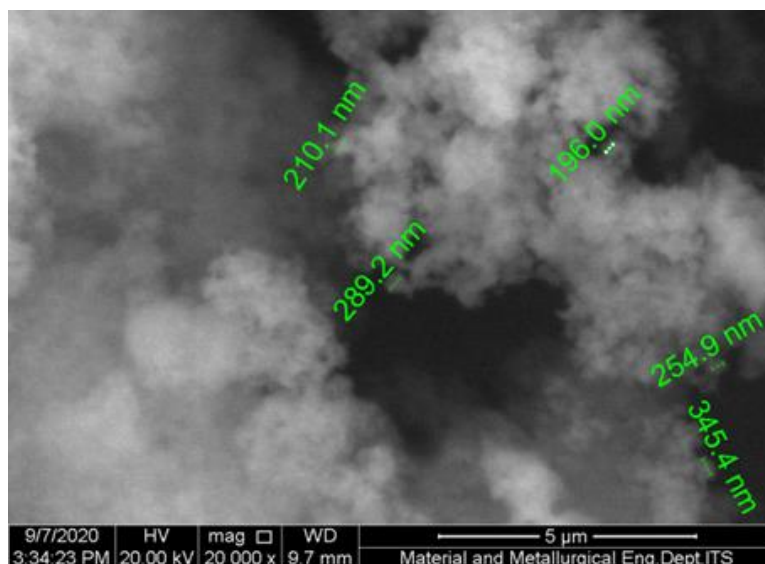


Figure 2. Amoxicillin/CuO XRD particle size

According to the results of research that has been carried out, CuO nanoparticles have a particle size of 50–70 nm (Eslami et al., 2017), while the particle size of

amoxicillin is 450 nm (Tenorio et al., 2010), as shown in Figure 3.

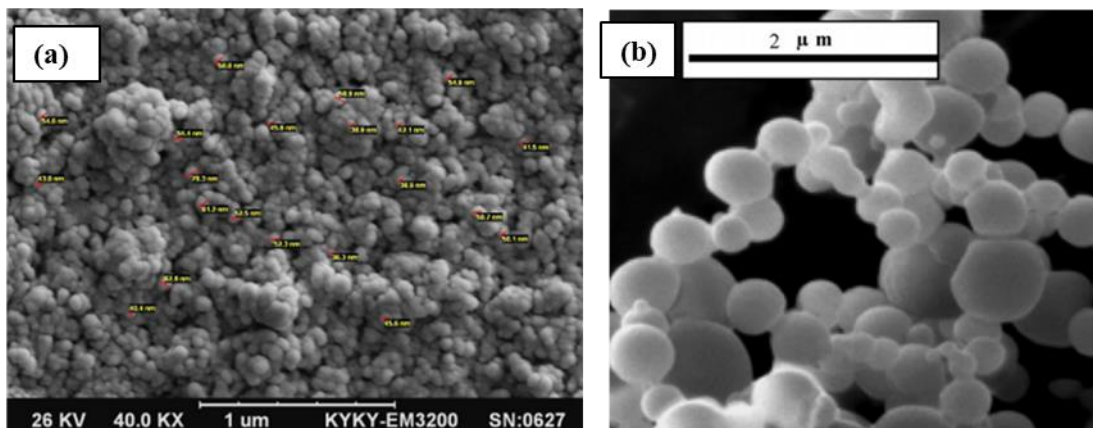


Figure 3. SEM image of (a) copper oxide nanoparticles (Eslami et al., 2017), (b) amoxicillin (Tenorio et al., 2010)

Antibacterial activity test results

The first step to test the antibacterial activity of amoxicillin/CuO is to observe the morphology of the bacteria. Observations were made by gram staining to determine cell shape and gram bacterial species. According to microscopic morphological observations, *S. aureus* is a salt of round cells of positive bacteria (Figure 4a). According to Taylor and Unakal (2017), *S. aureus* is a gram-

positive bacterium with clustered cell types such as grapes and violet. *Bacillus subtilis* belongs to the group of gram-positive bacteria and has a purple color, as shown in Figure 4b. *E. coli* was included in the group of gram-negative bacteria with stem cell conformation (Figure 4c), which was also consistent with the study by Rafika et al. (2018). *Salmonella typhi* is a very small rod-shaped red blood cell that shows gram-negative (Figure 4d).

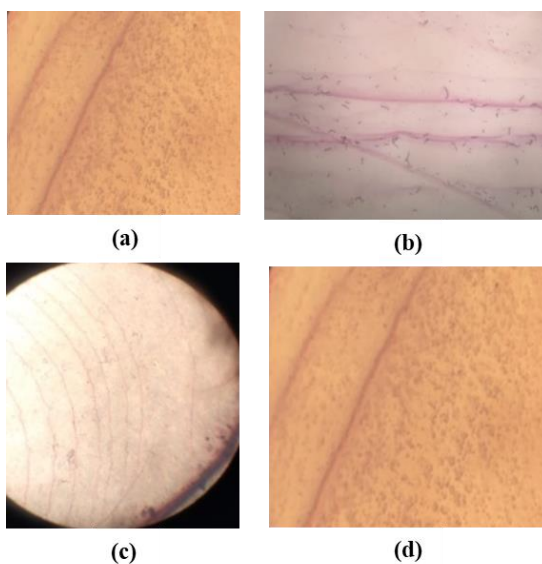


Figure 4. The results of microscopic observation of bacterial morphology cell shape (a) *S. aureus*, (b) *B. subtilis*, (c) *E. coli*, (d) *S. typhi*

The antibiotic activity test was performed using a disc diffusion method containing an antibiotic. The creation of the field indicates the presence of antibiotic activity. The amoxicillin/CuO

variants used were 1.56%, 3.12%, 6.25%, and 12.5% (w/v). The results of the antibiotic activity test are shown in Figure 5.

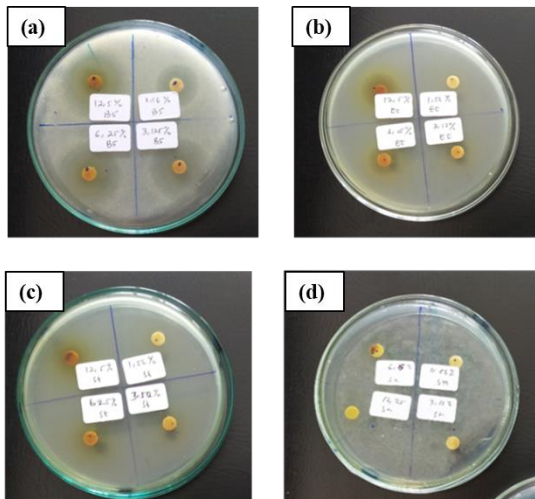


Figure 5. The results of the inhibition zone against (a) *B. subtilis*, (b) *E. coli*, (c) *S. typhi*, (d) *S. aureus*

The test results show that any concentration of amoxicillin/CuO had antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *S. typhi* by forming a transparent zone. The amoxicillin/CuO compound had a strong inhibitory response to *B. subtilis* because it had an inhibition zone diameter greater than 6 mm at all concentrations (Figure 5). A strong inhibitory response was also

demonstrated in other bacteria, namely *E. coli* and *S. typhi*, but only at a concentration of 12.5%. *S. aureus* bacteria show a strong inhibitory response at concentrations of 6.25% and 12.5%. However, this study was not considered a positive control for amoxicillin. Therefore, the efficacy of amoxicillin derivative compounds cannot be compared with amoxicillin.

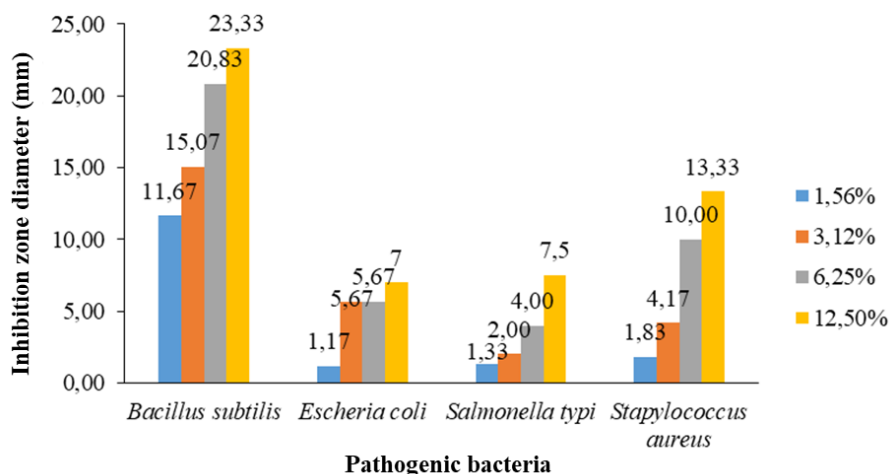


Figure 6. Amoxicillin/CuO antibacterial activity test results against various concentrations of pathogenic bacteria

Conclusions

Amoxicillin/CuO nanoparticle compounds synthesized by the impregnation method have specific chemical properties. The use of synthesis temperature can affect the properties and characteristics of the Amoxicillin/CuO compound. Amoxicillin/CuO compounds have antibacterial activity on *S. aureus*, *B. subtilis*, *E. coli*, and *S. typhi* bacteria. Thus, the compound Amoxicillin/CuO can be used as an antibacterial.

Suggestion

Further characterization of amoxicillin-CuO nanoparticles is required, for example by NMR spectrophotometer. It is necessary to test the activity against other bacteria and to test the toxicity of the synthesized amoxicillin/CuO on other preclinical animals.

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