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Research Paper

CuO Nanoparticles : Biosynthesis, Characterization and In Vitro Study

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Abstract
The biosynthesis of CuO nanoparticles was studied. The aims of this research were biosynthesis CuO nanoparticles from Cu^{2+}ion solution and leaves aquoeus extract of sweet star fruit and its in vitro study as antibacterial agent of *Escherichia coli*. CuO nanoparticles characterized using FTIR spectroscopy and X-Ray diffractometer. CuO nanoparticles were synthesized by reaction between Cu^{2+} ion solution and leaves aquoeus extract of sweet star fruit. The paper disk method was used as the application of the suspension of CuO nanoparticles. The result showed that Cu-O groups were detected at a wavenumber of 503, 619, 767, and 821 cm^{-1}. Diffractogram of CuO nanoparticles had sharp and narrow diffraction peaks. The crystallite size of CuO nanoparticles was 4.25 nm. The average inhibition zone of CuO nanoparticles at concentration 10.000, 7.500, 5.000 and 2.500 ppm was 17.43 ± 1.81 ; 15.73 ± 0.40 ; 14.50 ± 2.96 and 9.67 ± 0.28 mm respectively.

Keywords
CuO nanoparticles, characterization, in vitro.

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1. INTRODUCTION

Metal nanoparticles are nanotechnology and developed by material scientists until now. Unique is one of the properties of nanometal and it can be applied in various fields. The synthesis, characterization, and application of metal oxide nanoparticles are very essential because the properties of nanomaterials can be used as optical, electronic, catalytic, and magnetic. The properties of nanomaterials are affected by the shape, size, and chemical surroundings (Prabhu et al., 2017)

Nanomaterials such as copper nanoparticles have good properties. There are many applications of copper nanoparticles such as super-strong materials, antibacterial, sensors, and catalysts (Prabhu et al., 2017). CuO nanoparticles can be synthesized by chemical and non-method. Chemical methods such as sol-gel Dadi et al. (2019) and precipitation (Phiwdang et al. (2013); Mewara et al. (2017)). The non-chemical method to biosynthesis CuO nanoparticles is through a green synthesis method. The advantage of this green synthesis is effective, save for the environment, and a few of the dangerous chemicals can be minimized and this method can be done at low temperatures and pressures (Prabhu et al., 2017).

Some plant components, such as roots, leaves, stems, seeds, and fruits have been used for green synthesis (biosynthesis) of CuO nanoparticles (Kumar et al., 2017). These plant components can act as a bioreduction to produce CuO nanoparticles (Prabhu et al. (2017); Kumar et al. (2017); Altikatoglu et al. (2017)). The size and shape of nanoparticles are determined by the presence of phytochemicals in plants (Yugandhar et al., 2017). Das et al. (2017) reported that the process of biosynthesis of metal nanoparticles such as reaction time, temperature, and the concentration of plant extract and metal ion has an effect on the size and shape of nanoparticles.

Plant of *Averrhoa carambola L* is classified to the Oxalidaceae family (Soncini et al., 2011). This plant is cultivated in various other Asian countries, included in Indonesia. Plant of *Averrhoa carambola L* has a fruit-like star and is called sweet star fruit. Fruit and leaf can be used as herbal medicine. Leaves aquoeus extract and methanol extract of sweet star fruit are rich in secondary metabolites (Hitesh and Tejpal (2016); Wahab et al. (2014)). It has various biological activities Hitesh and Tejpal (2016) such as pharmacological activity : hypotensive Shah et al. (2011), antioxidant Chen et al. (2017), and anthelmintic (Soncini et al., 2011). The biosynthesis of metal nanoparticles such as CuO nanoparticles using leaves aquoeus extract of sweet star fruit is not widely explored. This biosynthesis of metal nanoparticles is based on secondary metabolites contained at leaves extract of another plant can be used as bioreduction of divalent metal

ion to nano-sized metal oxide (Prabhu et al. (2017); Kumar et al. (2017); Altikatoglu et al. (2017)).

In this paper, we biosynthesized CuO nanoparticles through a green synthesis method. Leaves aquoeus extract of sweet star fruit was used as a bioreduction of Cu^{2+} ion. Characterization of CuO nanoparticles was analyzed by FTIR spectroscopy and XRD respectively. CuO nanoparticles were used as antibacterial of *Escherichia coli* based on mechanisms of reaction between cell wall bacterial and positive charge of CuO nanoparticles (Prabhu et al. (2017); Altikatoglu et al. (2017)). Abboud et al. (2013) reported a large surface area has an effect on microorganisms. DNA molecules or microbial cells of microorganisms can bind with Cu^{2+} ions and the disorder of the synthesis of protein (Siddiqi and Husen, 2020).

2. EXPERIMENTAL SECTION

2.1 Materials

Copper (II) sulfate pentahydrate ($CuSO_4 \cdot {}_5H_2O$, Merck) and nutrient agar (Merck). Aquadest and *Escherichia coli* from Microbiology laboratory of Bhakti Pertiwi High School of Pharmacy Science. Leaves of sweet star fruit (*Averrhoa carambola L.*) from Palembang, South Sumatera, Indonesia.

2.2 Methods

2.2.1 Preparation of leaves aquoeus extract of sweet star fruit

About 50 g of leaves of sweet star fruit were washed several times with aquadest. Cleaned leaves of of sweet star fruit were incised into small pieces and added to 100 ml aquadest in a 250 ml Erlenmeyer flask. The mixture was boiled at 80-90°C for 15 minutes. After 15 minutes, the mixture was allowed to cool at room temperature. After cooling, the mixture was separated by decantation and filtrate (leaves aquoeus extract of sweet star fruit) was stored in the refrigerator for further experiment (Daphedar and Taranath, 2018).

2.2.2 Biosynthesis of CuO nanoparticles

About 100 ml of leaves aquoeus extract of of sweet star fruit was added to 50 ml of 0.1 M $CuSO_{45}H_2O$ solution in a 250 ml Erlenmeyer flask and boiled at 80°C until the color of mixture changed from slightly green to deep dark. The reaction mixture was allowed to cool at room temperature for one night to form precipitation. The residue was separated from filtrate and residue was washed several times with aquadest. The residue was dried in an electrical oven at 50oC until dry (Nasrollahzadeh et al., 2017).

2.2.3 Characterization

The functional group of CuO nanoparticles and copper (II) sulfat pentahydrate 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}. X-ray diffraction (Shimadzu 6000) used for calculating the crystalline size of CuO nanoparticles and evaluating the crystalline level of chitosan-CuO nanoparticles, CuO nanoparticles, and copper (II) sulfate pentahydrate. 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°-60°

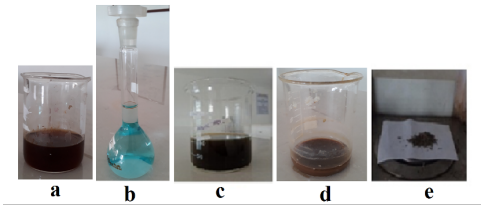

Figure 1. The documentation of biosynthesis CuO nanoparticles

2.2.4 Samples and in *vitro* study of CuO nanoparticles to *Escherichia coli*

The samples in this study were CuO nanoparticles (10^4, 75.10^2, 5.10^3, and 25.10^2 ppm), Cu^{2+} ion solution (10^3 ppm), tween 80 (1% v/v), and amoxicillin solution (25.10^2 ppm). 15 mL of sterilized nutrient agar was poured into Petri dishes and then they were solidified. The fresh inoculum suspension of *Escherichia coli* (10 ml, approximately 1.0×10^8 CFU/mL) was spread on the surface of a solidified agar plate. The steril of paper disks (6 mm diameter) was dropped by 10 μL the suspension of CuO nanoparticles, tween (1% v/v), Cu^{2+} ion solution, and amoxicillin solution respectively. The paper disk contained the sample was placed aseptically on the surface of fresh inoculum suspension of *Escherichia coli.* The Petri dishes were incubated at 37 °C for 24 h and the inhibition zones of bacterial growth were measured after 24 h. *In vitro* study prepared with triplicate.

3. RESULTS AND DISCUSSION

3.1 Biosynthesis of CuO nanoparticles

The photograph of leaves aquoeus extract of sweet star fruit (Fig. 1a), the Cu^{2+} solution (Fig. 1b), the mixture of leaves aquoeus extract of sweet star fruit and Cu^{2+} solution after boiled at 80°C (Fig. 1c) and CuO nanoparticles (wet, 1d and dry, 1e) can be seen in Fig. 1.

Color change visually in Fig. 1a-d (slightly green to deep dark) showed that when copper salt was added to leaves aquoeus extract of sweet star fruit, the formation of CuO nanoparticles occurs and change in the color can be observed. This fact showed Cu^{2+} ions were converted to CuO nanoparticles by leaves aquoeus extract of sweet star fruit.

The main chemical reactions involved in the biosynthesis of CuO nanoparticles using the leaves aquoeus extract of sweet star fruit are either reduction or an oxidation mechanism. The biological materials in leaves aquoeus extract of sweet star fruit were take part in the conversion of metal copper compound to specific nanoparticles. Alkaloids, carbohydrates, glycosides, phytosterols, resins, phenol, tannin, diterpenes, flavonoids, protein and amino acid, quinones, and phlobatannins are the secondary metabolites in leaves aquoeus extract of sweet star fruit (Mewara

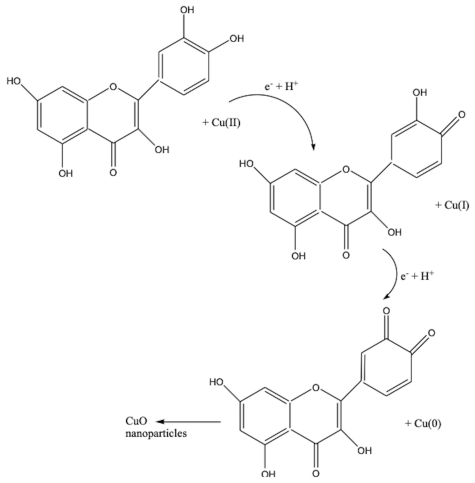

Figure 2. Illustration for biosynthesis of CuO nanoparticles (Nasrollahzadeh et al., 2017)

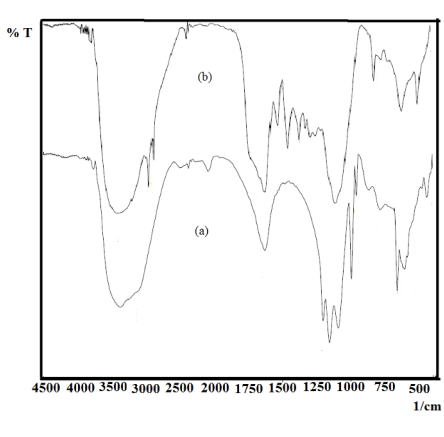

Figure 3. FTIR spectra of: copper (II) sulfate pentahydrate (a) and CuO nanoparticles (b)

et al., 2017). These metabolites are used for the reducing or oxidizing agent of metal ions to nano-sized metal oxide (Vijayakumar et al., 2018). Khandel et al. (2018) reported that metal ions can be reduced by some biomolecules to metal nanoparticles or nano-sized materials. Some biomolecules are flavonoids, terpenoids, alkaloids, and phenolic compounds. Nasrollahzadeh et al. (2017) reported that some biomolecules or secondary metabolites such as polyphenolics can act as a reducing and stabilizing agent. The illustration of Cu(II) ion was converted to CuO nanoparticles as seen in Fig 2.

3.2 FTIR spectra and its analysis of functional group

FTIR spectra of copper (II) sulfate pentahydrate and CuO nanoparticles can be seen ini Fig. 3.

The main bands in copper (II) sulfate pentahydrate (Fig. 3a) are: stretching vibration of O-H groups detected at 3134-3385 cm^{-1}. Stretching vibration asymmetric of H_2O appeared at 1625 cm^{-1}. Bending vibration of O-H groups at wavenumber 1091 cm^{-1}. SO_4^{2-} non-degenerate mode detected at 997 cm^{-1} and SO_4^{2-} degenerate mode appeared at 661 cm^{-1} (Manimekalai and Raja, 2013).

The molecular interactions between the CuO nanoparticles and leaves aquoeus extract of sweet star fruit (as the media) were recorded as FTIR spectra of CuO nanoparticles and as seen in Fig. 3b. The broad peak at 3369 cm^{-1} is stretching vibration of O-H groups from alcohols and phenols but Taran et al. (2017) reported that the band at 3369 cm^{-1} is N-H stretching vibration. The secondary metabolite such as flavonoids has O-H groups. Moresco et al. (2012) reported that *Averrhoa carambola* leaves contained secondary metabolite of glucopyranoside class and this class has N-H groups.

Stretching vibration of C-H groups appeared at 2848-2918 cm^{-1} (Altikatoglu et al., 2017). The peak at 1317 cm^{-1} is a vibration of the C-O group (Nasrollahzadeh et al., 2017). The vibration band at 1099 cm^{-1} is the stretching vibration of the C=O group. C=C stretching detected at 1612 cm^{-1}. 1444 cm^{-1} is a band of O-H bending and 1249 cm^{-1} is C-O stretching (Taran et al., 2017). The stretching vibration of the Cu-O group was detected in wave number 503, 619, 767, and 821 cm^{-1}. All these wavenumbers is confirming the formation of CuO nanoparticles (Hemalatha and Makeswari (2017) ; Berra et al. (2018) ; Altikatoglu et al. (2017)).

3.3 XRD and its interpretation

Diffractogram of copper (II) sulfate pentahydrate and CuO nanoparticles can be seen in Fig.4. The highest peak at 2θ ~ 18° showed the narrow peaks of copper (II) sulfate pentahydrate (Fig. 4a) and this compound has a good quality crystalline nature (Bakr et al., 2017). The peaks in the XRD powder patterns of CuO nanoparticles (Fig. 4b) has sharp and narrow diffraction peaks. Diffraction angles of CuO nanoparticles are 15.99, 18.33, 21.37, 22.61, 23.87, 27.13, 29.19, 31.26, 32.65, 36.97, 51.51 and 56.59 and indicating that the synthesized CuO nanoparticles are crystalline in nature (Taran et al., 2017). Crystalline in nature is defined by sharp peaks (Kurian and Kunjachan, 2014). The diffraction angles at 31, 32, 51, and 56° are the diffraction angles of CuO nanoparticles (Muthukrishnan (2015) ; Kumar et al. (2017) ; Nasrollahzadeh et al. (2017)). The Debye Scherrer's formula can be used for calculating the crystallite size of the biosynthesized CuO nanoparticles. Debye Scherrer's formula can be written as follows

$$D = (0.9.\lambda/\beta.cos\theta)$$

Where D is the crystallite size, λ is the wavelength of X-ray used (1.5406 Å), β is the full width at half maximum (FWHM) and θ is the Bragg's angle (Vijayakumar et al., 2018). From this formula, the average particle size of the synthesized CuO nanoparticles was 4.25 nm. This size is classed in the range 1-100 nm as defined by (Boverhof et al., 2015)

3.4 *In vitro* study

The antibacterial activity of amoxicillin solution (A: 25.102 ppm, positive control), Cu^{2+} ion solution (B, 10^3 ppm), tween 80 1

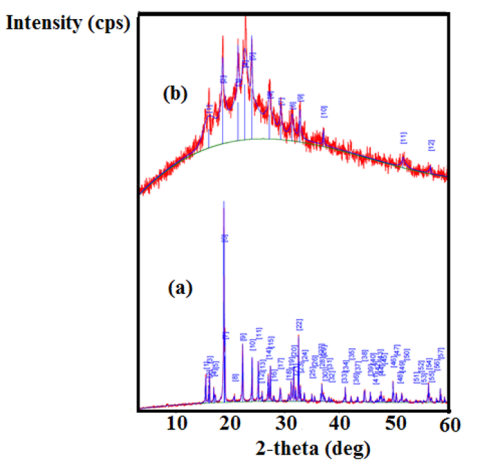

Figure 4. XRD powder patterns of copper (II) sulfate pentahydrate (a) and CuO nanoparticles (b).

% (v/v) solution (C, negative control), and suspension of CuO nanoparticles (D1: 10^4, D2: 75.10^2, D3: 5.10^3 and D4: 25.10^2 ppm respectively) were investigated as in Fig.5 and tabulated at table 1. The average inhibition zone of suspension of CuO nanoparticles (from higher to lower concentration) was 17.43 ± 1.81; 15.73 ± 0.40 ; 14.50 ± 2.96 and 9.67 ± 0.28 mm respectively. The average inhibition zone of Cu^{2+} ion solution was 14.07 ± 1.40 mm. The average inhibition zone of positive and negative control were 30.95 ± 4.34 and 13.41 ± 1.55 mm respectively.

It was seen that the inhibition zone of CuO nanoparticles, increases with increasing the concentrations of CuO nanoparticles but the Cu^{2+} ion solution, positive and negative control had the inhibition zone. The antibacterial mechanism of all compounds is described following: the cell membranes of bacteria are made up of peptidoglycan, a polymer containing sugars and amino acids, which is easy for CuO nanoparticles enter to cell membranes and destroying enzymes of cell membranes. The small size, spherical shape, and a high surface of the CuO nanoparticles affected to destroy the cell membranes of bacteria (Yugandhar et al., 2017). Tween 80 is a surfactant that may affect nutrient availability at cell membranes of bacteria through reducing process the size of nutrient particles, which indicates dispersal of peptide aggregates and a higher surface to volume ratio, acting as a nutrient source itself and increasing the permeability of membranes Nielsen et al. (2016) and Cu^{2+} ion solution as antibacterial was linked by the ability of copper to give and accept electrons, as a consequence produced a hydroxyl radical. Hydroxyl radical can disturb an oxidation process of protein and lipid in the cell wall of bacteria (Gabriela et al., 2016).

4. CONCLUSIONS

Cu^{2+} ion can be changed by leaves aquoeus extract of sweet star fruit to form CuO nanoparticle. The particle size of CuO nanoparticles is 4.25 nm. Cu-O group appeared at wavenumber 503, 619, 767, and 821 cm-1. The inhibition zone of CuO nanoparticles is affected by the concentration of CuO nanoparticles. The inhibition zone of CuO nanoparticles were 17.43 ± 1.81 ; 15.73 ± 0.40 ; 14.50 ± 2.96 and 9.67 ± 0.28 mm respectively (from the higher to lower concentration).

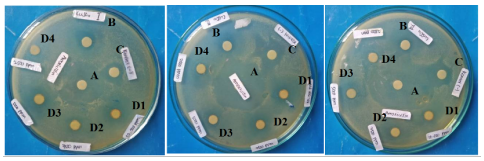

Figure 5. Photograph of the inhibition zone : amoxicillin (A), Cu^{2+} ion solution (B), tween 80 1% v/v (C) and CuO nanoparticles (D1,D2,D3 and D4) to *E. coli* respectively.

5. ACKNOWLEDGEMENT

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