5 (1) (2025) 16-24

Science, Technology and Management Journal

http://journal.unkartur.ac.id/index.php/stmj

GREEN SYTHESIS OF CUO USING *Citrus x microcarpa* BUNGE PEEL EXTRACT AND ANTIBACTERIAL ACTIVITY EVALUATION

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Info Artikel

Sejarah Artikel:

Disubmit: 28-12-2024

Direvisi : 07-01-2025

Disetujui : 10-01-2025

Keywords:
Antibacterial; CuO; green-synthesis; nanoparticles.

Abstrak

Artikel ini bertujuan untuk mensintesis nanopartikel CuO menggunakan metode sintesis hijau dengan ekstrak kulit buah Citrus x microcarpa Bunge, menganalisis karakteristiknya, dan mengevaluasi aktivitas antibakterinya terhadap pathogen S. aureus. Kami juga menyelidiki peran agen akselerator NaOH dalam sintesis hijau nanopartikel CuO. Kehadiran nanopartikel CuO hijau hasil sintesis dikonfirmasi oleh spektrofotometer UV-Vis dan XRD. Penggunaan NaOH dalam sintesis hijau nanopartikel mempercepat munculnya puncak absorbansi di sekitar panjang gelombang 400 nm, di mana puncak tersebut terkait dengan frekuensi plasmon permukaan CuO. Perhitungan ukuran kristalit berdasarkan difraktogram XRD menunjukkan bahwa NaOH juga dapat bertindak sebagai agen pereduksi. Ukuran nanopartikel CuO yang lebih kecil akan meningkatkan stabilitasnya saat dipanaskan. Selain itu, nanopartikel CuO hijau hasil sintesis menunjukkan aktivitas antimikroba yang tinggi terhadap patogen S. aureus. Hasil ini menunjukkan bahwa nanopartikel CuO dengan aktivitas antibakteri dapat disintesis menggunakan metode yang ramah lingkungan dan sederhana.

Abstract

This article aims to synthesize CuO nanoparticles using the green synthesis method using Citrus x microcarpa Bunge peel extract, analyze their characteristics, and evaluate their antibacterial activity against the S. aureus. We investigated the role of the accelerator agent NaOH in the green synthesis of CuO nanoparticles. The presence of green synthesized CuO nanoparticles was confirmed by UV-Vis spectrophotometer and XRD. The use of NaOH in the green synthesis of nanoparticles accelerates the emergence of absorbance peaks around 400 nm, related to CuO's surface plasmon frequency. Calculation of crystallite size based on XRD diffractogram shows that NaOH can also act as a reducing agent. The smaller size of CuO nanoparticles will increase their stability when heated. In addition, green synthesized CuO nanoparticles showed high antimicrobial activity against S. aureus pathogens. These results indicate that CuO nanoparticles with antibacterial activity can be synthesized using an environmentally friendly and simple method.

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p-ISSN 2746-0207
e-ISSN 2807-7865

1. Introduction

The development of nanomaterials is a hot topic that continues to be studied in nanotechnology and nanoscience in the last few decades (Agarwal & Shanmugam, 2020; Jacob, et al., 2021). Nanomaterials have unique properties such as very high surface-to-volume ratios (Guerrini, Alvarez-Puebla,, & Pazos-Perez, 2018) and excellent electrical (Chan, et al., 2019), magnetic (Li, et al., 2017), and optical properties (Zhang & Wang, 2017), making them widely used for catalysts (Xie, Niu, Kim, Li, & Yang, 2019), semiconductor materials (Jiang, et al., 2017), and photonic materials (Gogurla, Kundu, & Ray, 2017). One of the interesting new properties of nanomaterials is related to their antibacterial activity (Yin, et al., 2020; Das, et al., 2020).

In addition to silver nanoparticles, CuO nanoparticles are nanoparticles known to have antibacterial (Zaman, Poolla, Singh, & Gudipati, 2020; Pagar, Ghotekar, Pansambal, Pagar, & Oza, 2020) and antifungal effects (Shammout & Awwad, 2021; Imani, et al., 2020). The price is relatively lower than silver nanoparticles, and its ability to be applied as a coating material on various types of surfaces (Bengalli, et al., 2021) makes CuO nanoparticles be seriously studied as antibacterial agents (Akintelu, Folorunso, Folorunso, & Oyebamiji, 2020; Aziz, Abid, & Hussein, 2020).

One of the problems in producing nanometer-scale materials is that most of the synthesis methods used, such as conventional physical and chemical methods, tend to be expensive and not environmentally friendly (Rana, Yadav, & Jagadevan, 2020; Jadoun, Arif, Jangid, & Meena, 2021). The physical nanoparticle synthesis method requires high energy, while the conventional chemical method is feared to use toxic compounds that threaten the environment (Abegunde, Idowu, & Sulaimon, 2020; Castillo-Henríquez, et al., 2020). Especially for medical purposes and the health industry, the risk of residual toxic compounds is a risk that absolutely must be avoided.

To overcome these problems, we propose a method of green synthesis of CuO nanoparticles using *Citrus x microcarpa* Bunge (*C. x microcarpa* Bunge) peel extract in this study. Instead of using toxic compounds, the green synthesis nanoparticle method synthesizes nanoparticles using various biological materials such as plant extracts (Mustari, J, Noor, Rafsanjani, & Tiandho, 2019; Chandra, Kumari, Bontempi, & Yadav, 2020) and bacteria as reducing agents (Huq, 2020). This method is claimed to be cheaper, safer, and environmentally friendly, so that it has received much attention in recent years (Agarwal, Kumar, & Rajeshkumar, 2017). We used the peel extract of *C. x microcarpa* Bunge because the peel waste is abundant in the Bangka Belitung Islands. By local people, C. x microcarpa Bunge, commonly referred to as a jeruk kunci, is used as an acid enhancer in foods and processed into syrup (Roanisca & Mahardika, 2020). Apart from focusing on the synthesis and characterization of CuO nanoparticles, we also investigated the effect of using the accelerator agent NaOH and evaluated the antibacterial activity of CuO nanoparticles against *Staphylococcus aureus* (*S. aureus*).

2. Material and methods

2.1. Preparation of Peel Extract

The peels of the *C. x microcarpa* Bunge used in this study were collected from Bangka Regency, Bangka Belitung Islands Province, Indonesia. In order to obtain extracts, the peels were washed and dried in the sun. The peels were then grounded using a blender, and the resulted powder is shown in Figure 1. Afterward, 4 gr of peel powder was added to 100 mL of distilled water and stirred for 20 minutes at a temperature of 100°C. The maceration extract was filtered and stored in the refrigerator at 4°C for further use.

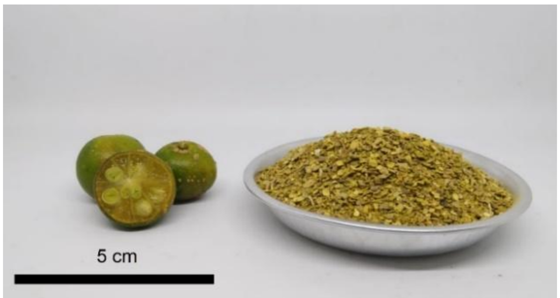

Figure 1. *C. x microcarpa* Bunge fruit and the peel powder

2.2. Synthesis of CuO

60 mL of peel extract to be used to synthesize CuO nanoparticles was heated at 80°C for 15 minutes. CuO nanoparticles were synthesized by mixing 2 gr of $Cu(NO_3)_2.3H_2O$ into the extract and stirred until homogeneous. As an accelerator, we were added NaOH 2 M and stirred at 100°C for 1 hour. To investigate the accelerator agent's effects, we were used different concentrations of NaOH (5 and 10 mL) by keeping the source of copper and extract at a constant level. Finally, the synthesized powder was filtered and dried at a temperature of 100°C using an oven and

heated at 400 C for 2 hours using a furnace. Schematically, the synthesis process of CuO nanoparticles is shown in Figure 2.

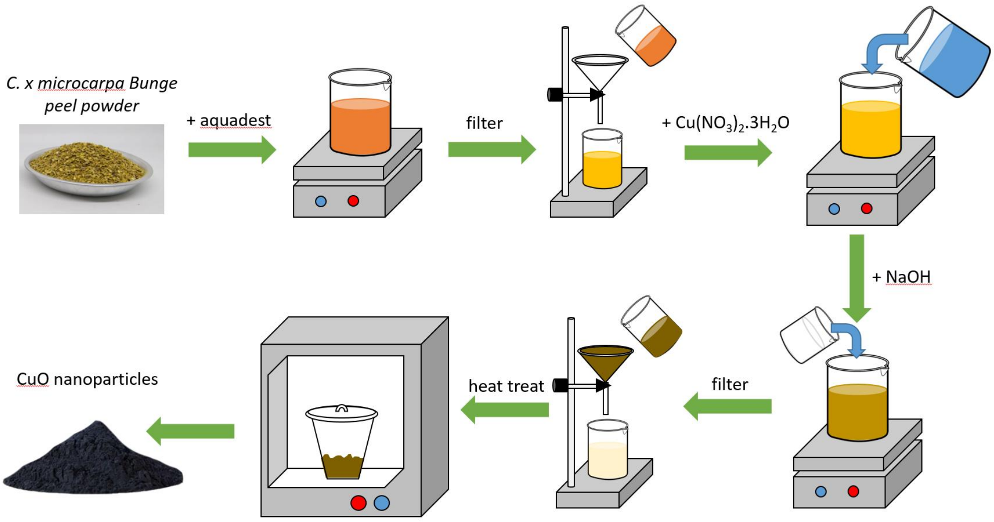

Figure 2. Schematic diagram of green syntesis of CuO nanoparticles.

2.3. Characterization of CuO

To investigate the extract's phytochemical compounds, we analyzed the functional groups of the fruit peel of *C. x microcarpa* Bunge using FTIR at wavenumbers from 4000 – 600 cm-1. The presence of colloidal CuO nanoparticles is indicated by the wavelength absorption pattern of the UV-Vis spectrophotometer in the range of 200 – 900 nm. The absorption peak can be related to the surface plasmon frequency of CuO nanoparticles. X-ray diffraction studies of CuO nanoparticle powder were carried out to characterize the crystalline properties of the nanoparticles. In addition to the refinement process using the Le Bail method, we also calculated the crystallite size of CuO nanoparticles using the Scherrer equation (He, Chen, Wang, Wei, & Chen, 2018):

$$D = \frac{}{\beta}$$

where D is the average size of the crystallite, K is the dimensionless shape factor (in this calculation, we use K=0.9), λ is the X-ray wavelength (in this study, we used the Cu-Kα wavelength = 1.5406 Å), β is the FWHM of the peak diffractogram and θ is the Bragg angle

2.4. Antibacterial activities

We used nutrient agar (NA) media as a medium for microbial growth. *S. aureus* microbial inoculation was carried out by streaking the bacterial suspension on NA media. The antibacterial activity of CuO nanoparticles was observed using disc paper that had been dipped in colloidal CuO nanoparticles (0.1 gr CuO nanoparticles/1 mL distilled water). The disc paper was placed on NA media and incubated for 24 hours at 37°C. The experiment was repeated three times, and the antibacterial effect of CuO nanoparticles against *S. aureus* was indicated by the average diameter of the inhibition zone

3. Results and Discussions

Figure 3 shows the FTIR spectrum of the peel of *C. x microcarpa* Bunge. Through this characterization, the functional groups of the peel can be identified, which have the potential to act as bioreducing and stabilizing agents in the synthesis process of CuO nanoparticles. A broad absorption band occurs between 3000 and 3694 cm-1 due to the O-H stretching frequency. The band at 1613 cm^{-1} was a peak related to the C=O stretching of the carbonyl group and the aromatic C=C stretching vibration. A band at 2922 cm^{-1} was indicated as C-H stretching due to the presence of a CH3 (methyl) group. An intense band at 1031 cm^{-1} corresponds to a C-O-H or C-O-R group (alcohol or ester) and a band around 1448 cm^{-1} was a band associated with aliphatic chains namely -CH2- and -CH3-. The combination of these functional groups was a basic structure of this lignocellulosic material. This analysis also showed that the peel of *C. x microcarpa* Bunge contained aromatic compounds, especially in the form of phenolic compounds or polyphenols (Zapata, Balmaseda, Fregoso-Israel, & Torres-Garcia, 2009; Roanisca & Mahardika, 2020). Phenolic compounds or polyphenols are phytochemical compounds that can reduce and stabilize Cu^{2+} ions during the nucleation process.

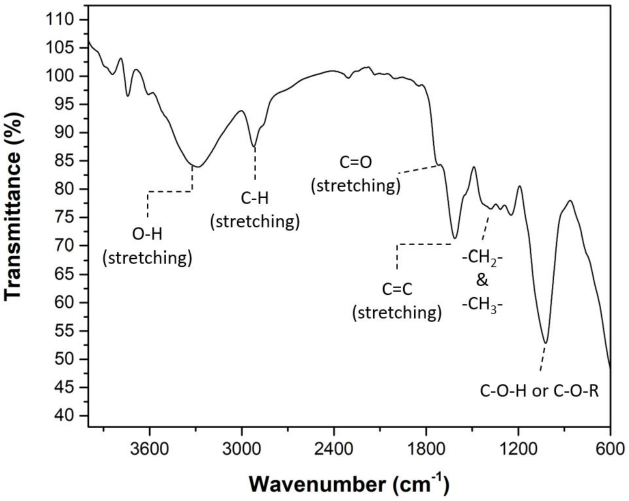

Figure 3. FTIR spectrum of *C. x microcarpa* Bunge peel.

Based on Figure 4, it can be seen that the UV-Vis spectroscopic absorbance pattern of the extract added with $Cu(NO_3)_2.3H_2O$ with and without NaOH is different. It appears that without the addition of NaOH, two sharp peaks occur at 267 nm and 317 nm. The absorbance peaks at around 267 nm and 317 nm were associated with the formation of copper oxide nanoparticles, especially cuprous oxide nanoparticles (Cu_2O) (Abboud, et al., 2014). The addition of NaOH will change the absorbance peak to about 400 nm. The absorbance peak is related to the surface plasmon absorbance characteristics of the CuO nanoparticle formation. The absorbance peak in the sample with the addition of 10 mL NaOH was higher and occurred at a slightly larger wavelength than the sample with the addition of 5 mL NaOH. It is related to the increasing number of CuO nanoparticles formed. Also, the shift in the absorbance peak at larger wavelengths is related to the effect of smaller particle size (Gebremedhn, Kahsay, & Aklilu, 2019). CuO nanoparticles that have different particle sizes will have different surface plasmon resonances. The transformation process can also be seen by changing the color of the mixture, as shown in Figure 5. The addition of NaOH to the mixture of *C. x microcarpa* Bunge and $Cu(NO_3)_2.3H_2O$ will make the color darker brown to indicate the formation of CuO nanoparticles.

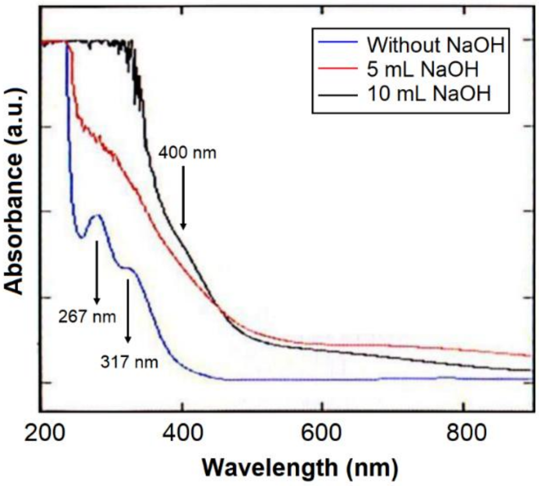

Figure 4. UV-Visible spectra of CuO nanoparticles synthesized via green synthesis method using different concentration of NaOH.

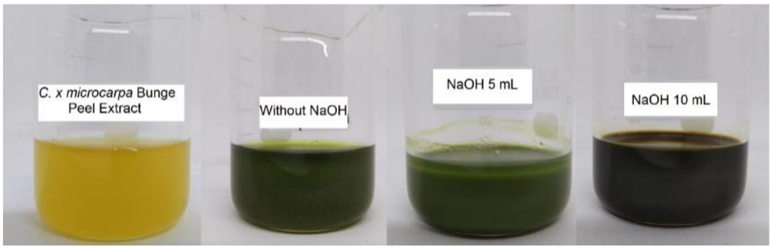

Figure 5. Visible detection of the green-synthesis of CuO nanoparticles using C. x microcarpa Bunge peel extract.

Figure 6 shows the XRD pattern of the nanoparticles synthesized in this study. Both XRD patterns have shown that the synthesis in this study has produced CuO (JCPDS: 00-045-0937). However, it appears that other additions of NaOH can produce slightly different XRD spectral patterns. The addition of 5 mL NaOH into the CuO nanoparticle synthesis process also generates peaks related to Cu_2O (JCPDS: 03-065-3288) sharply. The appearance of Cu_2O in the synthesized nanoparticles due to CuO reduced to Cu_2O when heated. It is known that CuO nanoparticles with a larger particle size will be easier to reduce to Cu_2O (Pike, Chan, Zhang, Wang, & Hanson, 2006). Therefore, this indicates that the size of CuO nanoparticles synthesized with the addition of 10 mL NaOH is smaller than that synthesized with the addition of 5 mL.

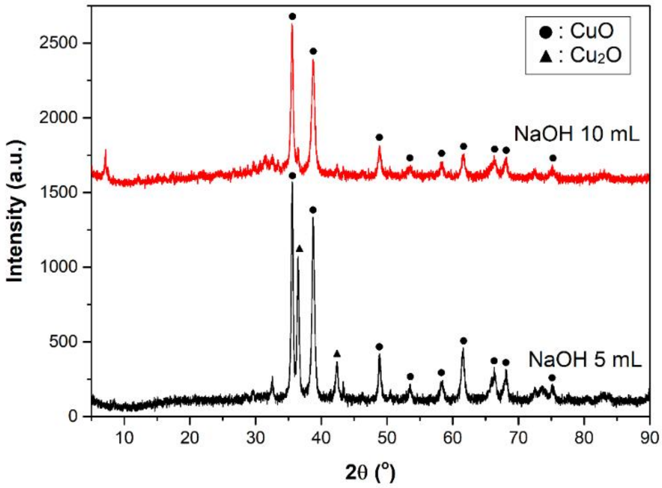

Figure 6. XRD pattern of CuO nanoparticles synthesized via green synthesis method using different concentration of NaOH.

After we refine the XRD pattern by the Le Bail method, we can know the lattice parameters of the CuO crystals, as shown in Table 1. CuO nanoparticles have a monoclinic structure with a C2/C group space. By adding data on the positions of Cu and O atoms [6], the crystal structure of CuO can be described as shown in Figure 7. It appears that each Cu atom will bind to 4 O atoms.

Table 1. The lattice parameter of synthesized CuO nanoparticles with different NaOH concentration.

NaOH concentration (mL)	Lattice Parameter						
	a (Å)	*b* (Å)	*c* (Å)	*α* (°)	*β* (°)	*γ* (°)	*V* (Å^3)
5	4.6824	3.4282	5.1332	90.000	99.525	90.000	81.2633
10	4.6933	3.4311	5.1248	90.000	99.328	90.000	81.4343

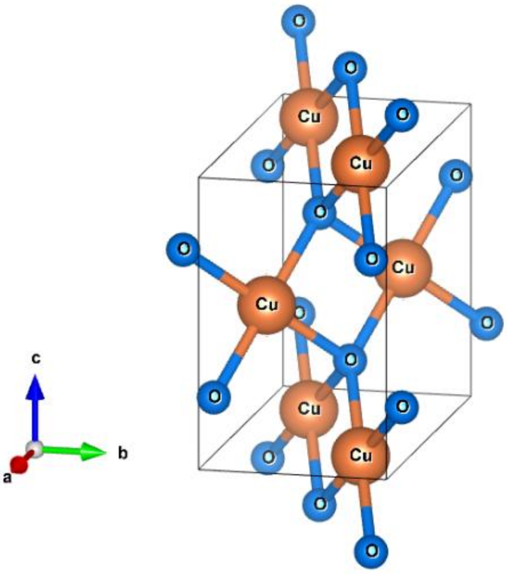

Figure 7. Crystal structure of CuO.

Based on calculations using the Scherrer equation on the XRD pattern, we obtained the average crystallite sizes of the synthesized CuO nanoparticles were 23.9 nm and 18.6 nm, for the addition of 5 mL and 10 mL NaOH, respectively. These results follow the analysis results for UV-Vis characterization related to the shift of the absorbance peak associated with the surface plasmon frequency of CuO nanoparticles. In addition, these calculations also support the mechanism for reducing CuO to Cu_2O, which occurs due to differences in particle size. This size reduction can be understood because NaOH can act as a reducing agent and an accelerating agent that strengthens the role of phytochemical compounds in the extract. When $Cu(NO_3)_2.3H_2O$ is dissolved in distilled water, Cu^{2+} ions are formed, which interact with phenolic compounds in the extract. Various phenolic compounds that have hydroxyl aromatic ring groups in the extract act as ligands. This interaction will convert Cu^{2+} ions into Cu(0) nanoparticles and continuously act as an effective capping agent to avoid agglomeration. The addition of NaOH, which can form OH- ions, will accelerate Cu^{2+} into Cu nanoparticles through the oxidation process. In the end, the procedure will produce Cu(0) nanoparticles that get aerially oxidized to CuO NPs by heating. However, excessive heating will make CuO nanoparticles reduced to Cu_2O. Schematically, the mechanism for forming CuO nanoparticles in the green-synthesis method using *C. x microcarpa* Bunge peel extract carried out in this study is presented as shown in Figure 8.

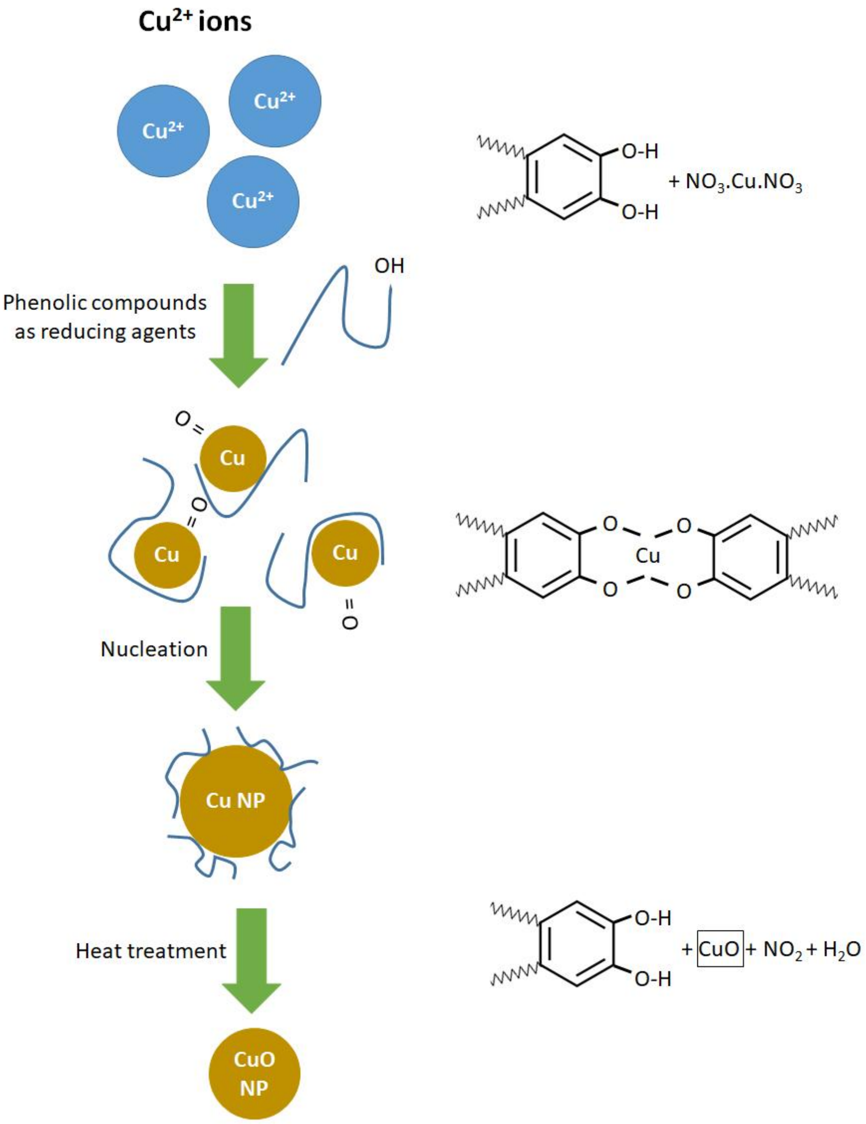

Figure 8. Schematic diagram of the mechanism for the formation of CuO nanoparticles using the green synthesis method using *C. x microcarpa* Bunge peel extracts (Thi, et al., 2020; Makarov, et al., 2014).

The antibacterial activity of CuO nanoparticles in this study was carried out against S. aureus. Figure 9 shows the CuO nanoparticles have an antibacterial effect indicated by the formation of an inhibition zone against the bacterial strain with a diameter of 8.08±1.88 mm. These results indicate that the green synthesis method proposed in this study can be developed for the health industry and agroindustry purposes.

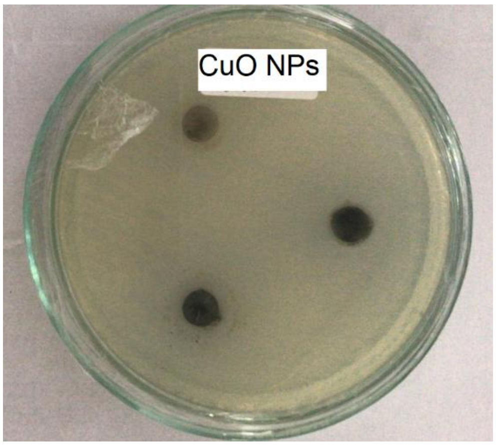

Figure 9. Inhibition zone of CuO nanoparticles against *S. aureus*.

4. Conclusions

This study successfully synthesized CuO using *C. x microcarpa* Bunge peel extract through the green synthesis method. The presence of polyphenolic compounds in the *C. x microcarpa* Bunge peels can act as a ligand to protect Cu metal from agglomeration during the particle nucleation process. In addition, the addition of NaOH as a reaction acceleration agent has a significant impact on the crystallite size of CuO. The use of higher amounts of NaOH will make the peak UV-Vis absorbance associated with the surface plasmon frequency of CuO experience a redshift. It is related to the smaller size of the CuO crystallites. The smaller crystallite size also impacts the stability of CuO compounds when given a heating treatment. CuO with a larger size will more easily transform into Cu_2O. Investigations on the antibacterial activity of green synthesized CuO nanoparticles also showed that the had an antibacterial effect against *S. aureus*. Thus, this research can provide a simple and environmentally friendly method of synthesizing CuO. The abundance of *C. x microcarpa* Bunge waste in Indonesia, especially in the Bangka Belitung Islands Province, makes this method very potential to reduce toxic chemicals and production costs of CuO.

5. Acknowledgements

This research was funded by the Institute for Research and Community Services - Universitas Bangka Belitung (LPPM UBB) through Hibah Penelitian Unggulan scheme in 2021 (No.: 17.29/UN50/PP/III/2021).

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