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Green synthesis of copper oxide (CuO) nanoparticles using *Anredera cordifolia* leaf extract and their antioxidant activity

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Abstract

Our present study describes the synthesis of copper oxide nanoparticles (CuONPs) using a leaf extract from *Anredera cordifolia* (AC) by the green synthesis method. The presence of flavonoids, saponins, and tannins in the plant extract was confirmed by phytochemical analysis, and these chemicals can be used as reducing, stabilizing, and capping agents. UV-Vis Spectrophotometry, XRD, FT-IR, SEM-EDS, and PSA were used to characterize the CuONPs. The UV-Vis spectroscopy of the biosynthesized CuONPs revealed an absorption peak at 325 nm. The XRD graph also revealed that the Debye-Scherrer formula observed an average crystalline size of around 43.47 nm. EDS analysis confirmed the composition and purity of CuONPs. The presence of functional groups -OH, C=O, and C-O triggered the synthesis of CuONPs, according to FT-IR analysis. Furthermore, the CuONPs antioxidant property inhibited free radicals as antioxidants with an IC_{50} value of 34.20 g/mL.

Keywords: Green synthesis; *anredera cordifolia*; copper oxide nanoparticles; antioxidant

1. Introduction

Green synthesis of nanoparticles has many potential applications in the environmental and biomedical fields with an aim to reduce the use of toxic chemicals, for instance, by using biological materials such as plants that are usually safer. Plants also contain the reducing and stabilizing agents that can be used in the nanoparticle synthesis process [1].

Nanoparticles have a large surface area and small particle size that exhibit better material characteristics compared to other materials and are widely applicable in various applications in recent years [2]. Currently, quite a number of physical and chemical procedures are presented to fabricate various types of nanoparticle scales with different shapes and sizes. Despite having better characteristics than other materials, nanoparticles are expensive and often contain toxic materials (e.g. solvents, reducing agents, acid and base reagents) that have the potential to damage the environment and serious health hazards. As a consequence, it then, limits their applications. Whereas nanoparticle formation procedures using biological materials are less toxic, economical, and most importantly, environmentally friendly [3]. In response, recent research has attempted to use natural material-based sources such as plant extracts, fungi, bacteria, and marine organisms as a sustainable green synthesis method [2].

Although plants contain highly active bio-organic molecules, eco-friendly plant-based methodologies using plant parts such as roots, flowers, seeds, leaves, bark, and fruits are emerging as highly promising biological sources for the formation of a wide array of nanoparticles [4]. The active biomolecules present in plants are likely to consist of bio-functional groups such as amino, hydroxyl, and carboxyl that will act as the metal reductants and as protective agents to form stabilization layers in nanoparticle biosynthesis [5].

Copper nanoparticles and its derivatives as one type of metal nanoparticles that are well stable, economical and more widely available compared to other expensive precious metals such as Au, Pt, and Ag are widely used in many applications, catalysts for chemical reactions, solar cells, chemical sensors, absorbents, drug delivery systems, anticancer activity, antimicrobial agents, and efficient antioxidant agents [6-8].

Secondary metabolites in plants used as bioreductor in nanoparticle synthesis act as antioxidant compounds, protecting cells from free radicals. They also play an important role in protecting the body from various diseases, such as cardiovascular and neurodegenerative conditions in which free radicals play a major role [9].

Plant with potential as an antioxidant is binahong. Research conducted by Souhoka, et al. (2021) [10] showed that *Anredera cordifolia* leaves contain chemical compounds that can be used as antioxidants. Antioxidant activity tests

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were carried out using the DPPH method with the results showing a strong antioxidant activity of ethanol extract of binahong leaves, as evidenced by the IC_{50} value of 87.423 g/mL.

Ghosh et al. (2015) [7] reported the results of their research related to the synthesis of copper nanoparticles. Nanoparticles synthesized using *Dioscorea bulbifera* water extract as a bioreductor showed inhibitory activity against DPPH, nitric oxide, and superoxide radicals. Ghosh et al. (2020) [11] also successfully synthesized copper nanoparticles using green synthesis method using *Jatropha curcas* leaf extract as a bioreductor.

The potential of *Anredera cordifolia*, which has secondary metabolites as antioxidants can be formed as nanoparticles so that they have a smaller size, which is expected to have better antioxidant activity. Recently, it has been reported the synthesis of silver nanoparticles with good biological applications using *Anredera cordifolia* such as research conducted by Wattimena and Patty (2017) [12] who successfully synthesized Ag nanoparticles using Binahong (*Anredera cordifolia*) stem extract with quite good antimicrobial activity.

Given the above background, it is imperative to investigate the synthesis of copper nanoparticles (CuNPs) by *Anredera cordifolia* and examine their antioxidant properties. Previous studies have shown chemical, biochemical and catalytic applications of CuNPs regarding electronic and optical properties. Systematic studies on CuNPs against radical compounds towards the exploration of antioxidant potential followed by evaluation of reported inhibition are few to date. In this study, we have studied the inhibitory potential of CuNPs against DPPH radical compounds to reveal the inhibitory action of CuNPs as antioxidants.

2. Materials and Methods

In this study, several tools were used to support the results of the research used for the inhibition test of the DPPH as an antioxidant. Materials and methods can be seen in the section below.

2.1. Chemicals and Instrumentation

The materials used included sample of binahong (*Anredera cordifolia*) leaves, Copper (II) nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) acquired from Sigma-Aldrich and utilized without further purification. Deionized water was used in all experiment steps for making molar solutions, washing, and dilution. It also used Whatmann filter paper grade 42, $FeCl_3$ 1 M (Merck), $Pb(CH_3COO)_2$ 0.5 M (Merck), H_2SO_4 5 M (Merck), acetic anhydride 1 M (Merck), $CHCl_3$ 1 M (Merck), HCl 1% (Merck), Dragendorff reagent, Mayer reagent, 2,2-diphenyl-1-picrylhydrazyl 0.5 mM, and etahnol absolute 70% (Merck). Meanwhile, the instruments used included pyrex glassware, Velp Scientifica multistirrer, magnetic stirrer, Gallenhamp angle head centrifuge, Shimadzu UV-2600 UV-Vis spectrophotometer, Shimadzu Prestige-21 FTIR spectroscopy, Shimadzu 7000 X-Ray Diffraction Instrument, and JEOL Scanning Electron Microscope-Energy Dispersive Spectroscopy.

2.2. Preparation of the plant extract

The sample used was the binahong leaves (*Anredera cordifolia*). Binahong extract was produced by boiling 10 grams of fresh binahong leaves that had been mashed and added with 200 mL of deionized water in a glass beaker and stirred continuously for 30 minutes. The sample was then boiled for 45 minutes to speed up the extraction process. The solution was then left to cool at room temperature and filtered with Whatmann grade 42 filter paper to obtain binahong leaf water extract.

2.3. Phytochemical analysis

2.3.1. Tannin analysis

2 mL of binahong leaf water extract was mixed with 2 mL of deionized water and added with few drops of $FeCl_3$. The formation of a green precipitate indicated the presence of tannin [13].

2.3.2. Flavonoids analysis

The aqueous extract of binahong leaves was put into two different test tubes. The first test tube was added with few drops of lead acetate ($Pb(CH_3COO)_2$). The formation of a yellow precipitate indicated the presence of flavonoids. Meanwhile, the aqueous extract of binahong leaves in the second test tube was added with few drops of sulfuric acid (H_2SO_4). The formation of an orange precipitate indicated the presence of flavonoids [14].

2.3.3. Saponins analysis

The method used was the Frothing test. A total of 2.5 mL of binahong leaf water extract was mixed with few drops of deionized water before being vigorously shaken. The formation of foam with a large amount indicated the presence of saponins [15].

2.3.4. Steroids analysis

Acetic anhydride as much as 2 mL was added into 5 mL of water extract of binahong leaves and then 2 mL of sulfuric acid was slowly added. The occurrence of color changes from purple to blue or green indicated the presence of steroids [14].

2.3.5. Terpenoids analysis

The method used was the Salkowski test. A total of 2 mL of chloroform ($CHCl_3$) was added into 5 mL of water extract of binahong leaves and then 3 mL of concentrated sulfuric acid (H_2SO_4) was added slowly until forming a layer. The red formation at the interface layer indicated the presence of terpenoids [16].

2.3.6. Alkaloids analysis

3 mL of 1% HCl was added into 3 mL of aqueous extract of binahong leaves and stirred on a water bath. The mixture

was put into 2 different test tubes of 1 mL each. The water extract of binahong leaves in the first test tube was added with few drops of Dragendorff reagent. The formation of an orange-colored precipitate indicated the presence of alkaloids. Meanwhile, the extract in the second tube was added with few drops of Mayer reagent. The formation of a yellowish-beige precipitate indicated the presence of alkaloids [13].

2.4. CuO nanoparticles synthesis procedure

Binahong leaf aqueous extract and 0.01 M $Cu(NO_3)_2.3H_2O$ solution were mixed at the ratio of (%V/V) 1:1, 1:2, 1:3 and 1:4 [17]. Furthermore, the mixture of binahong leaf water extract and $Cu(NO_3)_2.3H_2O$ solution was stirred for 2 hours with a magnetic stirrer at 3000 rpm. The mixture was then allowed to stand for 24 hours, then a color change occurred and CuO nanoparticles were formed. Next, the mixture was centrifuged at 10000 rpm for 30 minutes. The CuO nanoparticle precipitate was de-watered by drying with a freeze dryer.

2.5. Characterization of synthesized CuO nanoparticles

CuONP was characterized by UV-Vis Spectrophotometry, FT-IR, XRD, SEM, EDS, and PSA. UV-Vis spectrophotometry was used to confirm the formation of nanoparticles and stabilization of nanoparticles as well as to predict the size and shape and number of nanoparticles based on the absorbance value and maximum wavelength of the sample. FT-IR, meanwhile, was used to determine the functional groups playing a role in the metal reduction process in nanoparticle synthesis. From the analysis of functional groups, the group of secondary metabolite compounds that acted as reducing agents in the synthesis of nanoparticles was identified. XRD was used for phase identification and characterization of nanoparticle crystal structure and SEM was used to observe the morphological structure of the nanoparticle surface in high magnification using a high-energy electron beam. Lastly, EDS and PSA were used to observe the purity and element/compound content of nanoparticles, and to determine the particle size distribution of nanoparticles respectively.

2.6. Antioxidant analysis of CuO nanoparticles

The antioxidant activity (AA%) of each substance was determined using the DPPH scavenging of free radicals assay. Various concentrations of CuONps were added to every vials excluding blank. Then, 3 mL of ethanol was got to add, along with 0.3 mL of 0.5 mM DPPH drastic approach in ethanol. The control solution was made by combining 3.5 mL of ethanol with DPPH radical solution (0.3 mL). After 30 minutes of reaction, absorbance was measured at its maximum wavelength. The total antioxidant activity portion (AA%) was calculated with the following equation (1):

$$\% \text{ AA} = \frac{(\text{abs. at blank} - \text{abs. at test})}{\text{abs. at blank}} \times 100\% \qquad (1)$$

3. Results and Discussion

The formation of nanoparticles can be determined by several factors such as temperature, pH, sample concentration and concentration of metal solution. In this study, the comparison of binahong extract and metal solution was carried out to determine the optimum ratio (%V/V) in the synthesis of nanoparticles.

3.1. Phytochemical test of anredera cordifolia leaf extract

Phytochemical tests on the water extract of *Anredera cordifolia* leaves showed positive results for flavonoids, saponins, and tannins. The purpose of this test was to determine the content of metabolite compounds contained in *Anredera cordifolia* extract, which is expected to act as a reducing agent and stabilizing agent in the process of forming copper oxide nanoparticles. Table 1 presents the results of the phytochemical test of *Anredera cordifolia* leaf water extract.

Table 1. Phytochemical analysis

Phytochemical test	Result
Saponins	+
Flavonoids	+
Terpenoids	-
Steroids	-
Alkaloids	-
Tannins	+

3.2. Composition optimization of cu solution

1 mL of *Anredera cordifolia* leaf extract was pipetted and put each into four 100 mL erlenmeyers, and then $Cu(NO_3)_2.3H_2O$ 0.01 M solution of 1, 2, 3, and 4 mL was added to each erlenmeyer. The mixture was stirred with a magnetic stirrer for 2 hours at 3000 rpm, and the wavelength and absorbance were analyzed by UV-Vis spectrophotometer. Table 2 presents the results of the optimization analysis of the composition of $Cu(NO_3)_2.3H_2O$ solution.

Table 2. Determination of optimum ratio

Concentration ratio	Time (day)	wavelength (λ_{maks})	Absorbance
1:1	1	310	2,649
	2	310	3,054
	3	305	3,152
	4	315	3,156
	5	320	2,622
	6	330	2,592
	7	315	2,582
1:2	1	320	2,507
	2	320	2,696
	3	325	2,749
	4	325	2,834
	5	320	2,748
	6	325	2,611
	7	325	2,509
1:3	1	325	2,188
	2	325	2,274
	3	325	2,307
	4	325	2,407
	5	325	2,514
	6	330	2,745
	7	325	2,387
	8	325	2,344
1:4	1	325	1,827
	2	325	1,833
	3	325	1,915
	4	325	2,057
	5	325	2,344
	6	325	2,260
	7	325	2,138

In the 1:1 composition variation, nanoparticles formed after stirring and experienced a wavelength shift on day 3 of analysis. An increase in absorbance occurred on day 4 of analysis but began to decrease on day 5. This indicated the possibility of agglomeration of the synthesized copper nanoparticles.

In the 1:2 composition variation, copper nanoparticles were formed after stirring and did not experience a significant wavelength shift. The absorbance peak in this variation also decreased on day 5, indicating the possibility of agglomeration on the same day as the 1:1 composition variation.

In the 1:3 ratio, the colloidal solution had a fairly stable maximum wavelength around 325 nm for 5 days of analysis and slightly increased on day six with a wavelength of 330 nm. The increase in absorbance occurred on day 1 with an absorbance of 2.188 until day six with an absorbance value of 2.745 indicating that in this comparison nanoparticles tended to be stable for 6 days and then decreased on day 7.

In the ratio of 1:4, the maximum wavelength from the first day to the seventh day of analysis was quite stable, i.e. around 325 nm. This comparison showed an increase in absorbance from day 1 of 1.827 to day 5 of 2.344 and then decreased on day 6 and the following day.

The concentration of nanoparticle solution with a stable wavelength and a stable increase in absorbance is able to reduce Cu^{2+} ions to Cu^{o} more, so that the redox reactions that occur tend to last continuously and prevent interactions between metal particles to form a larger collection of particles. In contrast to nanoparticle solutions that provide less stable wavelength data and increase in absorbance in just a few days, redox reactions tend to be completed more quickly and metal particles interact with each other to form larger aggregates. The large number of Cu nanoparticles formed can be seen from the absorbance value [18]. This shows that copper nanoparticles tend to be stable at a ratio of 1:3 when viewed from the maximum wavelength data which is quite stable and has a fairly stable increase in absorbance when compared to other composition variations. Therefore, the 1:3 ratio was used for the synthesis process of copper oxide nanoparticles.

The synthesized results after 1 minute and 3 hours for the ratio of 1: 1:2; 1:3 and 1:4 are shown in Figure 1.

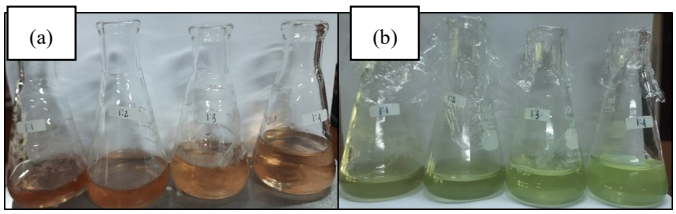

Fig. 1. The synthesized results (a) after 1 minute and (b) after 3 hours

Figure 1 shows that there was a significant color difference between the four nanoparticle samples prepared with different ratios of binahong extract and $Cu(NO_3)_2.3H_2O$ after 3 hours. Nanoparticles synthesized after 1 minute had a light brown color and after 3 hours it changed in to yellowish green. This indicated that copper oxide nanoparticles have been formed in accordance with the research of Mali et al., [19] who synthesized copper oxide nanoparticles by giving the same color. The formation with the highest intensity (1:3) was chosen after composition optimization for the synthesis of substantial quantities of CuONPs.

3.3. Synthesis analysis results of CuO nanoparticles

CuONPs were synthesized by gradually adding *Anredera cordifolia* leaf extract to the $Cu(NO_3)_2.3H_2O$ metal solution, and then were stirred for 1 hour with a magnetic stirrer to homogenize the solution mixing. As seen in Figure 2, the mixing results showed a change in color from brown to light green and gradually formed colloids in the first 1 hour and after 3 hours the color turned into pale green.

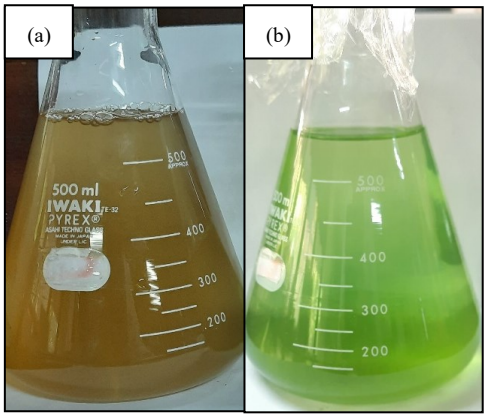

Fig. 2. The synthesized results (a) after 1 hours and (b) after 3 hours

Figure 3 shows UV-visible spectra of CuONPs formation via the supernatant of the Anredera cordifolia extract and Anredera cordifolia extract. CuONPs has illustrated a relatively broad absorption peak at 325 nm most likely attributed to surface plasmon resonance (SPR) of Cu excitation [20].

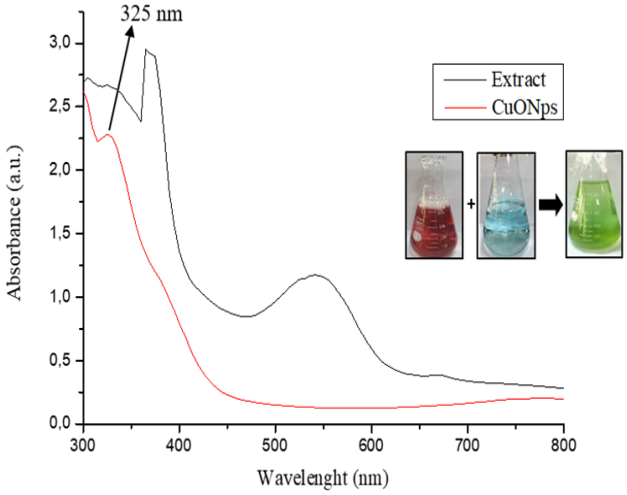

Fig. 3. UV-Vis spectra CuONPs and Extract *Anredera cordifolia*

The results of UV-Vis spectrophotometer analysis of *Anredera cordifolia* leaf extract and copper oxide nanoparticles showed a number of differences in wavelength absorption peaks indicating a peak shift during the formation

of nanoparticles. The wide absorption peak in *Anredera cordifolia* extract indicated the presence of secondary metabolite compounds containing chromophore groups that were able to absorb UV-Vis electromagnetic radiation and transitions at certain wavelengths [21].

3.4. Fourier transform infrared spectroscopy (FTIR) analysis

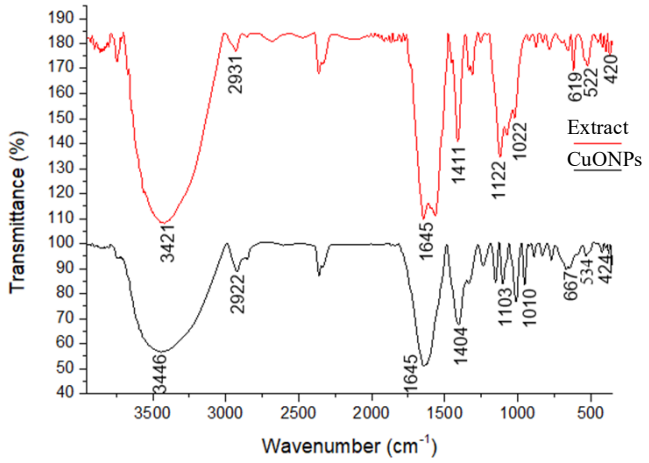

Fig. 4. Spectra of AC extract and CuONPs

Figure 4 shows a decrease in the absorption intensity of *Anredera cordifolia* extract and copper oxide nanoparticles in the O-H, C=O, and C-O groups, indicating that these groups played a role in the copper metal reduction reaction. The similarity in the shape of the spectrum of *Anredera cordifolia* extract with copper oxide nanoparticles indicated that copper oxide nanoparticles functioned as a drug carrier that had a bond with compounds derived from *Anredera cordifolia* extract.

The FTIR spectrum of *Anredera cordifolia* extract showed a sharp absorption with strong intensity at a wave number of 1645 cm^{-1} indicating the vibration of the C=O flavone bond conjugated with C=C at the same wave number. The broad absorption of the C-H bond at wave number 3055 cm^{-1} with moderate intensity was not seen in the FTIR spectra of either binahong extract or copper oxide nanoparticles for having the same absorption area as the O-H functional group. The absorption at 735-770 cm^{-1} indicated ortho-substituted aromatics. The presence of O-H functional groups with a broadened peak character between 3200 and 3600 cm^{-1} with strong intensity was reinforced by C-O bond vibrations (phenol) in the 1022 cm^{-1} region. The absorption at wave number 1411 cm^{-1} was the vibration of O-H bending bond. Furthermore, absorption at wave numbers 534 cm^{-1} and 1010 cm^{-1} indicated the different bending vibration modes of the Cu-O bond. The appearance of the peak at 1645 cm^{-1} indicated the stretching vibration of Cu-O bond of copper oxide nanoparticles.

3.5. X-ray diffraction (XRD) analysis

The nanoparticle solution was then centrifuged at 10000 rpm for 30 minutes in which the centrifugation results were then dried by freeze drying. The final product of copper oxide nanoparticles as shown Figure 5 was in the form of greenish brown powder.

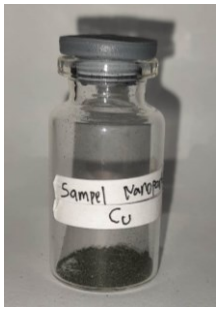

Fig. 5. Product of copper oxide nanoparticles

Nanoparticle product characterization was performed using XRD analysis. Based on the 2θ peak obtained, the crystal structure of the nanoparticles was identified. Figure 6 shows the XRD results of copper oxide nanoparticles showing 3 sharp peaks in the diffractogram.

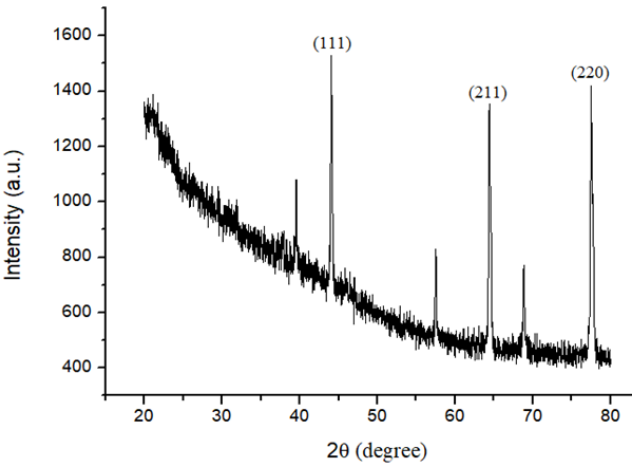

Fig. 6. Diffractogram CuONPs

X-ray diffraction (XRD) patterns in 2θ in the range of 20° to 80° of the synthesized copper oxide showed the presence of various characteristic peaks with 3 sharp peaks that could be indexed for having simple cubic crystal structure. Reference copper oxide nanoparticle crystals [17] can be seen in Table 3.

Table 3. Reference CuO nanoparticle crystals

Crystal structure	$h^2 + k^2 + l^2$
Simple Cubic (SC)	1,2,3,4,5,6,7,8,9,10,...
Body Centered Cubic (BCC)	2,4,6,8,10,12,14,16,...
Face Centered Cubic (FCC)	3,4,8,11,12,16,19,20,...

The results of XRD analysis obtained the reflection fields of (111), (211) and (220). In determining the crystal structure, this number is often called Miller Indices, from the data obtained from the results of numbers 3, 6, and 8.

Figure 6 shows the XRD results of copper oxide nanoparticles showing 3 sharp peaks in the diffractogram. These peaks at 2θ values were 44.08°; 64.42°, and 77.52°, respectively. Based on the 2θ peaks from XRD analysis, the average crystal size was 43.47 nm. Table 4 depicts the crystal size calculations.

Table 4. Crystal size calculations of CuONPs

2θ	FWHM	Cristalite Size D (nm)	D (nm) average
44.0452	0.1880	45.58276	43.4761
64.4016	0.2218	42.3280	
77.5108	0.2396	42.5178	

3.6. Scanning electron microscope (SEM) analysis

Scanning electron microscope (SEM) is one of the most widely used techniques used in the characterization of nanomaterials and nanostructures. The signals that derive from electron-sample interactions reveal information about the sample including surface morphology (texture), and chemical composition of the sample [11].

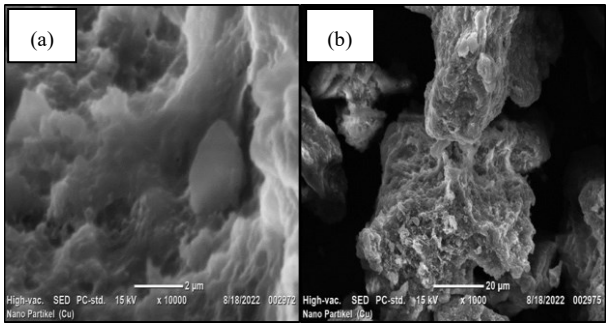

Fig. 7. SEM CuONPs result of (a) 10000 times and (b) 1000 times magnification

Figure 7 illustrates SEM images of copper oxide nanparticles synthesized using *Anredera cordifolia* leaf extract. The surface morphology of the nano-copper by this eco-friendly method showed the nearly monodispersed distribution of particles sizes. It showed that mostly spherical nano copper, as well as the number of aggregates, synthesized nanoparticles and some of them, showed the undefined shape nanoparticles.

3.7. Energy dispersive spectroscopy (EDS) analysis

The EDS spectrum as shown in Figure 8 confirmed the copper elements with carbon, oxygen, and nitrogen. This aggregation may be due to the presence of secondary metabolites in the leaf extract of *Anredera cordifolia*.

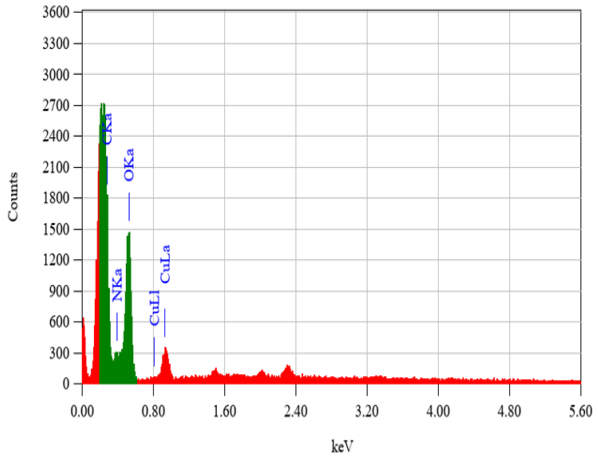

Fig. 8. EDS spectrum CuONPs results

Table 5 presents the percentages of Cu and other biomolecules showing that the highest percentage was found in atomic oxygen, which indicated that it came from secondary metabolite compounds of *Anredera cordifolia*. It is also shown that the type of nanoparticles formed was copper oxide. This was also supported by the results of FTIR analysis, which showed the Cu-O bond.

Table 5. Element percentage of CuONPs

Element	Mass (%)	Atom (%)	Compound
C K	30.27	44.30	C
N K	5.78	7.25	N
O K	37.43	41.12	-
Cu K	26.52	7.33	CuO
TOTAL	100	100	

3.8. Particle size analyzer analysis

The next analysis was conducted by using the Particle Size Analyzer (PSA) instrument used to measure the size of nanoparticles distributed in nanoparticles dispersed in a solution. The results of PSA analysis can be seen in Figure 9.

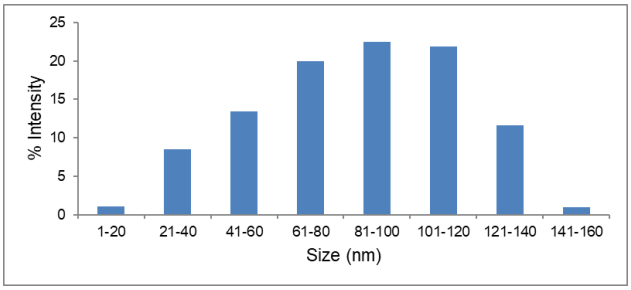

Fig. 9. Intensity Size Distribution of CuO nanoparticles

Figure 9 shows the intensity of the size distribution of copper oxide nanoparticles and it was identified that particles with the nanoparticle category had a dominant intensity percentage with a range of 65.5%. Particle size in the range of 81-100 nm had the highest intensity with a percentage of 22.5%. This showed the potential of that binahong extract to be used to synthesize copper oxide nanoparticles.

3.9. Antioxidant activity by DPPH assay

At room temperature, DPPH is a stabilized free radical that is frequently used to assess the antioxidant activity of various natural compounds. The antioxidant-induced decrease of absorbance at 514 nm was used to determine the reduction ability of DPPH radicals. The antioxidants in CuONps can neutralize the free radical in DPPH by transferring their electron or hydrogen atoms to DPPH, and changing the color from purple to yellow [22]. Figures 10 and 11 show the color change occurred in the DPPH scavenging activity with the standard Vitamin C and the sample (Extract AC, CuONps, and their combination). Table 6 shows the antioxidant activity and IC_{50} of standard Vitamin C and the sample (Extract AC, CuONps, and their combination).

Fig. 10. DPPH Assay of the standard Vit. C

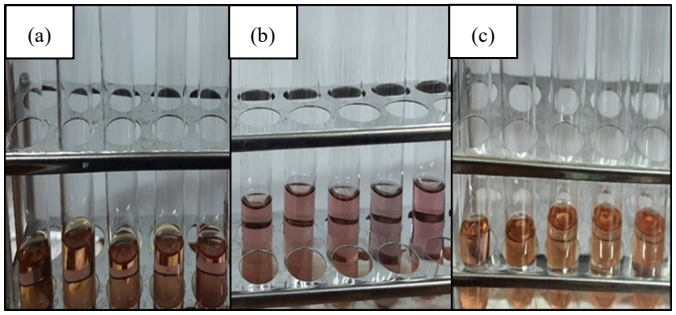

Fig. 11. DPPH Assay of the synthesized (a) Extract AC, (b) CuONPs, and (c) combination extract AC + CuONPs

Table 6. Antioxidant activity of vitamin C and the sample

Sample	Antioxidant Activity (%)	IC_{50} (µg/mL)
Vitamin C 0,25 ppm	39.20	
Vitamin C 0,50 ppm	39.77	
Vitamin C 1 ppm	41.10	4.52
Vitamin C 2 ppm	44.13	
Vitamin C 4 ppm	48.48	
Extract AC 1 ppm	20.27	
Extract AC 2 ppm	21.02	
Extract AC 4 ppm	23.3	31.03
Extract AC 8 ppm	27.27	
Extract AC 16 ppm	35.04	
CuONPs 1 ppm	18.75	
CuONPs 2 ppm	19.70	
CuONPs 4 ppm	21.78	34.20
CuONPs 8 ppm	26.52	
CuONPs 16 ppm	32.58	
Extract AC + CuONPs 1 ppm	29.92	
Extract AC + CuONPs 2 ppm	31.06	
Extract AC + CuONPs 4 ppm	33.33	17.42
Extract AC + CuONPs 8 ppm	37.88	
Extract AC + CuONPs 16 ppm	48.48	

The highest antioxidant activity of the three samples tested was the combination of *Anredera cordifolia* extract + copper oxide nanoparticles by showing the highest antioxidant activity value and the least IC_{50} value of 17.42µg/mL followed by extract AC and copper oxide nanoparticles of 31.03µg/mL and 34.20µg/mL respectively. These results indicated that the inhibition category with IC_{50} values below 50µg/mL [23] from the three samples was strong.

4. Conclusion

In this study, CuONP was synthesized by green chemistry method using *Anredera cordifolia* leaf extract. Copper oxide nanoparticles were obtained at an optimum condition at composition (1:3). The average crystal size of copper oxide nanoparticles was 43.47 nm. Scanning electron microscopy (SEM) analysis showed that most of the copper oxide nanoparticles were spherical and some of them showed the undefined nanoparticle shapes. The flavonoid and other compounds present in Anredera cordifolia leaf extract reduced Cu^{2+} ions to CuONPs, which was confirmed by FT-IR and EDS studies and showed that the type of nanoparticles formed were copper oxide (CuO) nanoparticles. Copper oxide nanoparticles were evaluated for their antioxidant activity by DPPH free radical scavenging assay and it was found that CuO nanoparticles acted as a strong antioxidant agent.

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