

Green Synthesis of Copper Nanoparticles Using *Anredera cordifolia* Stem Extract and Evaluation of Their Antidiabetic Activity

Nurharis Munandar*, Henry F. Aritonang, Ridho Bonaventura

Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Sam Ratulangi, Manado 95115, Indonesia

Received 30 November 2025; Received in revised form 10 March 2026

Accepted 23 April 2026; Available online 30 June 2026

ABSTRACT

Copper nanoparticles (CuNPs) were successfully synthesized through a green synthesis approach using extract of *Anredera cordifolia* as a natural reducing and stabilizing agent. The formation of CuNPs was confirmed using several characterization techniques including UV–Visible spectroscopy, Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Particle Size Analyzer (PSA). UV–Vis analysis showed an absorption peak at approximately 325 nm, indicating the formation of copper nanoparticles. FTIR spectra revealed the presence of functional groups associated with plant metabolites that contribute to nanoparticle reduction and stabilization. PSA analysis showed particle size distribution in the range of 10–150 nm with a dominant peak around 30 nm and a median particle size (D50) of approximately 36 nm. The α -amylase inhibition assay demonstrated that the combination of *A. cordifolia* extract and CuNPs exhibited stronger inhibitory activity compared to the extract or CuNPs alone, suggesting potential application as a natural antidiabetic agent.

Keywords: α -amylase; Antidiabetic; *A. cordifolia*; Copper nanoparticles; Green synthesis

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from the inability of the pancreas to produce insulin or the body's resistance to insulin, causing elevated blood glucose levels [1]. In 2019, approximately 463 million people aged 20–79 years had diabetes, and this number is predicted to increase to 700 million by 2045 [2]. DM is becoming a global

problem due to the increasing number of patients and complications that damage blood vessels, increasing morbidity and mortality [3, 4].

Oxidative stress results from an increase in free radicals or a decrease in antioxidant activity. Unstable free radicals can damage lipids, proteins and nucleic acids, contributing to various diseases. In DM, redox imbalance

increases the production of superoxide (O_2^-) which reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), exacerbating oxidative stress and endothelial dysfunction [5].

Antioxidant administration inhibits free radicals and prevents vascular complications. Supplements that increase NO can improve endothelial and mitochondrial function and decrease the activity of the enzyme NAD(P)H oxidase. Natural antioxidants such as superoxide dismutase and glutathione, as well as synthetic ones, can control blood glucose levels and prevent complications [6]. However, synthetic drugs often cause side effects, such as drug resistance and gastrointestinal disorders, so safer alternative agents are needed.

Metal ions such as vanadium, zinc, manganese, copper, and chromium have shown antidiabetic activity [7]. Inhibition of α -glucosidase and α -amylase enzymes can suppress postprandial hyperglycemia, but studies regarding metal nanoparticles as antidiabetic agents are still limited [8]. Roseline and Priya (2023) [9] reported that copper nanoparticles synthesized with biological method more effectively inhibited α -glucosidase and α -amylase than acarbose. Ghosh et al. (2015) [10] reported that copper nanoparticles (CuNPs) synthesized with *Dioscorea bulbifera* extract more effectively inhibited α -glucosidase and α -amylase than acarbose.

As a transition metal, copper plays an important role in biochemical pathways such as oxygen transfer and catalytic activity. CuNPs are more biocompatible than other metal nanoparticles, so they have wider therapeutic potential [11]. CuNPs also have high stability, are more economical, and are widely used in catalysts, sensors, antimicrobial agents, and antioxidants [12]. Plant-based CuNP synthesis is an efficient strategy to control hyperglycemia because copper is known to inhibit α -amylase. Binahong (*Anredera cordifolia*) was chosen because it has antidiabetic, antimicrobial,

antihyperlipidemia, antitumor, and anti-inflammatory properties [13]. Bioactive compounds such as flavonoids and tannins act as antioxidants and antidiabetics [10]. Research conducted by Munandar, et al. (2022) [14] showed that copper nanoparticles and Binahong leaf extract used as a bioreductor had antioxidant activity of 34.20 μ g/mL and 31.03 μ g/mL, demonstrating their potential as antidiabetics; and also showed the phytochemical test results of Binahong leaf extract and the content of secondary metabolite compounds, namely flavonoids, saponins, and tannins.

Although previous work reported the synthesis of copper nanoparticles using *Anredera cordifolia* extract and evaluated their antioxidant activity [14], the antidiabetic potential of these nanoparticles, particularly their α -amylase inhibitory activity, has not been explored. Therefore, this study focuses on the green synthesis of CuNPs using *A. cordifolia* extract and investigates their potential as α -amylase inhibitors for antidiabetic applications, together with detailed physicochemical characterization.

2. Materials and Methods

2.1 Chemicals and instrumentation

The materials used included Binahong (*Anredera cordifolia*) stem samples, and Copper (II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$), obtained from Sigma-Aldrich and used without further purification. Deionized water was used in all experimental steps for making molar solutions, washing, and dilution. In addition, $FeCl_3$ 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, Whatmann No. 42 filter paper, Alpha-amylase enzyme (EC3.2.1.1) (Sigma-Aldrich, A8220), maltose monohydrate dissolved in distilled water, phosphate buffer (pH 7) 50 mM, 3,5-dinitro salicylic acid (DNS) reagent 1% (W/V), dimethyl sulfoxide (DMSO) 0.5% (V/V),

acarbose (glucobay) (Sigma-Aldrich, A8980) and ice cubes.

Meanwhile, the instruments and tools used included pyrex glassware, Velp Scientifica stirrer, DraonLab micropipette, Gallenhamp angle head centrifuge, Ohaus AP-110 analytical balance, pH meter (Hanna Instruments), Thermo Scientific Vortex Mixer, DLAB Incubator DHP Series, Memmert UN30 oven, BM100 water bath, Velp Scientifica multistirrer, Shimadzu UV-2600 UV-Vis spectrophotometer, Shimadzu Prestige-21 FTIR spectroscopy, Shimadzu 7000 X-ray Diffraction Instrument, Beckman Coulter Delsa Nano C Particle Size Analysis, and SEM (Scanning Electron Microscopy) JEOL JSM-IT500.

2.2 Preparation of the plant extract

The sample used was Binahong (*Anredera cordifolia*). The sample was dried and ground until smooth, then weighed as much as 5 grams. Next, the sample was boiled using 100 mL of aquabides, allowed to cool, and filtered with Whatmann No. 42 filter paper.

2.3 Phytochemical analysis

Phytochemical tests of secondary metabolite compounds of Binahong stem water extract are Tannin Content Test using FeCl_3 reagent, Flavonoid Test using lead acetate and sulfuric acid, Saponin Test with frothing test, Steroid Test using acetic anhydride and sulfuric acid, Terpenoid Test using chloroform and concentrated sulfuric acid, and Alkaloids test using Dragendorff reagent and Mayer reagent.

2.4. Cu nanoparticles synthesis procedure

Binahong extract solution and 0.01 M $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ solution were mixed in the ratio of (%V/V) 1:1, 1:2, 1:3 and 1:4. Furthermore, the mixture of Binahong extract and copper solution was stirred for 2 hours with a magnetic stirrer at 3000 rpm. The mixture was then allowed to stand for 24 hours, where there was a change in color and

copper nanoparticle precipitates had begun to form. Next, the mixture was centrifuged at 10000 rpm for 30 minutes. The copper nanoparticle precipitate was de-watered by drying with a freeze dryer.

2.5 Characterization of synthesized Cu nanoparticles

Characterization of copper nanoparticles was carried out using several instrumental techniques, namely UV-Vis Spectrophotometry, Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Particle Size Analyzer (PSA).

2.6 Alpha amylase enzyme inhibition analysis

2.6.1. Maltose standard determination

a. Preparation of 1000 mg/L Master Solution

Maltose monohydrate 0.01 g was dissolved with distilled water, then put into a 10 mL volumetric flask and diluted to the limit mark, then homogenized.

b. Preparation of Standard Solution

Preparation of standard solution was through a dilution process of 1000 mg/L master solution. The preparation of standard solution can be seen in Table 1.

Table 1. Preparation of maltose standard solution.

Standard Solution (mg/L)	Volume of Master Solution (mL)	Volume of distilled water (mL)	Total Volume (mL)
80	0.12	1.38	1.50
150	0.225	1.275	1.50
200	0.30	1.20	1.50
300	0.45	1.05	1.50
400	0.60	0.90	1.50
600	0.90	0.60	1.50

c. Maltose Standard Measurement

Test tubes containing blank solutions, and standard solutions with concentrations of 80; 150; 200; 300; 400; and 600 mg/L with a volume of 1.5 mL were prepared and labeled. Then 1.5 mL of DNS reagent was added into

the test tube and homogenized using a vortex for 10 seconds. Each test tube was then put into a water bath and boiled for 10 minutes. Next, each test tube was put into cold water. Then the absorbance was measured at its maximum wavelength.

2.6.2. Activity test

a. Blank

A mixture of 0.5 mL phosphate buffer pH 7.0, 0.5 mL distilled water, and 0.5 mL 2% starch substrate was put into a test tube, then incubated at 37°C for 60 minutes. Next, 1.5 mL of DNS reagent was added to the mixture and shaken with a vortex for 10 seconds. The mixture then was heated in boiling water for 10 minutes, before being cooled in ice water. The absorbance of the reaction was measured at the maximum absorbance wavelength (λ_{max}).

b. Acarbose (Comparator)

A mixture of 0.5 mL of acarbose solution, 0.5 mL of α -amylase enzyme solution, and 0.5 mL of 2% starch substrate was put into a test tube and incubated at 37°C for 60 minutes. Next, 1.5 mL of DNS reagent was added to the mixture, which was then shaken with a vortex for 10 seconds. The mixture was heated in boiling water for 10 minutes, then cooled in ice water. The absorbance of the reaction was measured at the maximum absorbance wavelength (λ_{max}).

c. α -Amylase Enzyme Activity (Negative Control)

A mixture of 0.5 mL of phosphate buffer (pH 7), 0.5 mL of α -amylase enzyme solution, and 0.5 mL of 2% starch substrate was put into a test tube, then incubated at 37°C for 60 minutes. Next, 1.5 mL of DNS reagent was added to the mixture, which was shaken with a vortex for 10 seconds. Then the mixture was heated in boiling water for 10 minutes and cooled in ice water. The absorbance of the reaction was measured at the maximum absorbance wavelength (λ_{max}).

The maltose content obtained was then used to determine the enzyme activity using the α -amylase enzyme activity calculation formula in Eq. (2.1) as follows:

$$EA\left(\frac{U}{\text{mL}}\right) = \frac{[\text{Maltose}] \times DF}{MW \times V \times t} \quad (2.1)$$

The Enzyme Activity (EA) is calculated based on the [Maltose] content produced during the reaction, expressed in ppm. This value is then multiplied by the Dilution Factor (DF) to correct for the dilution effect of the sample. The Molecular Weight (MW) of maltose is used in the calculations to convert the concentration into number of moles. The Volume of enzyme (V) used, expressed in milliliters, as well as the incubation Time (t) in minutes, are also taken into account in determining the rate of enzyme activity quantitatively.

d. Samples (Binahong stem extract, CuNPs, and Combination of Binahong stem extract + CuNPs)

A mixture of 0.5 mL of samples (with concentrations of 1; 2; 4; 8; and 16 mg/L), each added 0.5 mL of α -amylase enzyme solution, and 0.5 mL of 2% starch substrate was put into a test tube, then incubated at 37°C for 60 minutes. Then, 1.5 mL of DNS reagent was added to each mixture, then shaken with a vortex for 10 seconds. Then the mixture was heated in boiling water for 10 minutes, then cooled in ice water. The absorbance of the reaction was measured at the maximum absorbance wavelength (λ_{max}) and the Relative Activity (RA) percent was calculated using Eq. (2.2) and the Percentage inhibition (%) using Eq. (2.3).

$$RA = \frac{\text{Inhibitory Activity}}{\text{Without inhibiting substance}} \times 100\% \quad (2.2)$$

$$\% \text{ Inhibition} = 100 - \% \text{ Relative Activity} \quad (2.3)$$

The IC_{50} value is defined as the concentration of inhibitor that can inhibit 50% of α -amylase enzyme activity. The IC_{50} value is calculated using a linear regression equation with sample concentration as the x-axis and percentage inhibition (%) as the y-axis. From the equation $y = ax + b$, the IC_{50} value can be calculated using Eq. (2.4):

$$IC_{50} = \frac{50-b}{a}. \quad (2.4)$$

In a linear regression equation, the parameter a represents the slope value, which shows the rate of change of the dependent variable with respect to the independent variable. Meanwhile, b is the intercept, which is the value at which the regression line intersects the y-axis when the value of the independent variable is zero.

3. Results and Discussion

The process of nanoparticle formation is strongly influenced by reaction conditions, including temperature, acidity (pH), and the concentration of reactants used. One of the important factors studied in this research is the ratio between Binahong stem extract and copper precursor solution.

3.1 Phytochemical test

Phytochemical analysis of Binahong stem extract used in this study showed the presence of flavonoid and saponin compounds. This finding differs from the results of Munandar et al. (2022) [14], who reported the presence of flavonoids, saponins, and tannins in similar extracts. The difference in results is most likely due to the geographical variation of the plant source, given that the samples in this study were obtained from the Manado region of North Sulawesi, Indonesia.

This phytochemical test was conducted with the aim of identifying secondary metabolite compounds that have the potential

to act as reducing agents in the biosynthesis of copper nanoparticles. The complete data of the phytochemical test results are shown in Table 2.

Table 2. Phytochemical analysis.

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

3.2 Optimization of Cu solution composition

A total of 5 mL of binahong stem extract was added to each of the four 100 mL erlenmeyers. Next, 5, 10, 15, and 20 mL of 0.01 M $Cu(NO_3)_2 \cdot 3H_2O$ solution were added into each erlenmeyer, respectively. The ratios of 1:1, 1:2, 1:3, and 1:4 refer to the volume ratio between *A. cordifolia* extract and $Cu(NO_3)_2$ solution, where one part of plant extract was mixed with different volumes of copper precursor solution. The mixture was then homogenized using a magnetic stirrer at 3000 rpm for 2 hours to ensure optimal interaction between the bioactive compounds in the extract and copper ions. After the mixing process, the samples were analyzed using a UV-Vis spectrophotometer to observe the maximum wavelength and absorbance value as an indicator of nanoparticle formation. The results of the composition optimization analysis of the $Cu(NO_3)_2 \cdot 3H_2O$ solution are presented in Fig. 1.

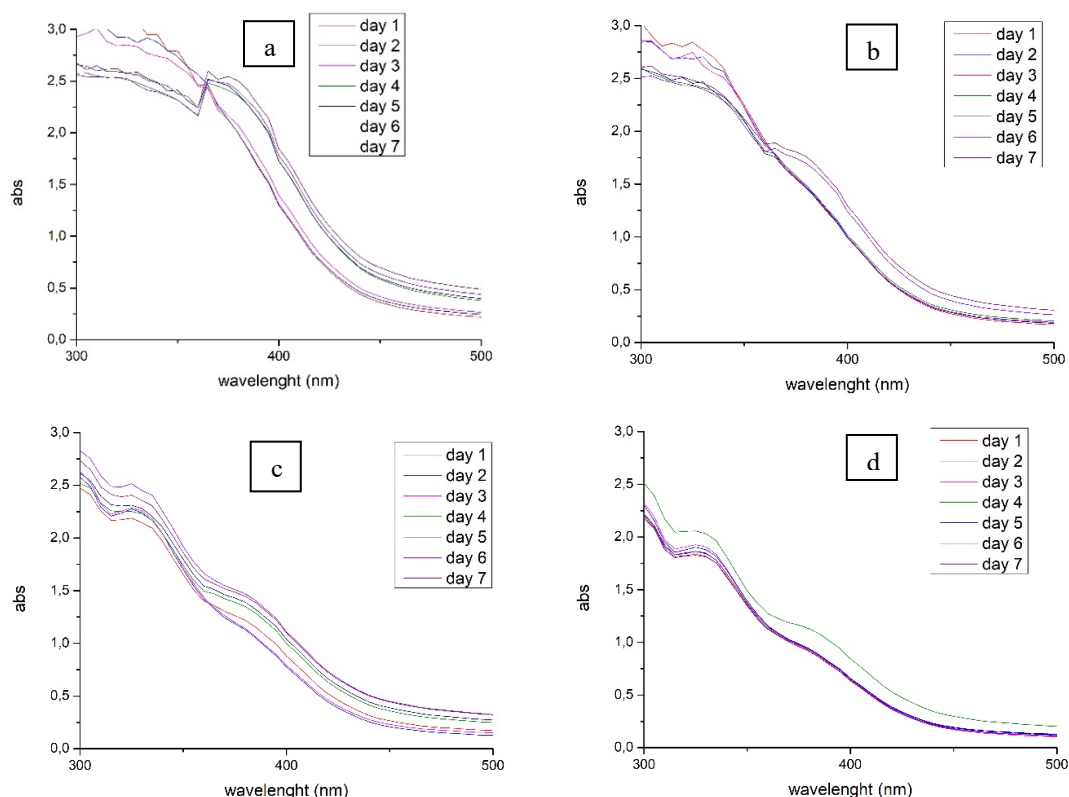


Fig. 1. UV-Vis spectra of Cu nanoparticles in the ratio of (a) 1:1, (b) 1:2, (c) 1:3, and (d) 1:4.

Fig. 1 shows that the UV-Vis spectra of the four ratio variations exhibit relatively similar spectral patterns, but with differences in absorbance intensity during the 7-day incubation period. UV-Vis analysis was conducted to monitor the formation and stability of copper nanoparticles based on changes in absorbance values and maximum wavelength. The absorbance value is influenced by the metal reduction time, the concentration of metal ions, and the concentration of plant extract, which acts as both a reducing and stabilizing agent [15, 16].

The variation in the extract-to-precursor ratio affects the reduction rate of Cu^{2+} ions as well as the stabilization of the formed nanoparticles. At lower extract ratios (1:1 and 1:2), the number of phytochemical compounds acting as reducing and stabilizing agents may be insufficient, resulting in less stable nanoparticle formation. This is reflected by the decrease in the absorbance peak intensity at

around 325 nm from day 1 to day 7. Such a decrease indicates instability of the formed copper nanoparticles, which may result from particle agglomeration or oxidation of Cu^0 into CuO or Cu_2O , leading to the weakening of the characteristic surface plasmon resonance (SPR) peak of CuNPs [17,18]. The absence of a sharp SPR peak within the 320–340 nm range further suggests that nanoparticle formation at these ratios was not optimal [14, 19].

In contrast, the 1:3 ratio provides a more balanced condition, where the amount of reducing agents is sufficient to convert Cu^{2+} ions into Cu nanoparticles while also providing adequate stabilization on the particle surface. This is indicated by the relatively consistent increase in absorbance from day 1 to day 6, suggesting a more stable nanoparticle formation process. This condition is likely associated with the presence of phenolic and flavonoid compounds in the extract that

function as reducing and stabilizing agents for the nanoparticles [20]. However, a slight decrease in absorbance was observed on day 7, which is likely due to partial oxidation of Cu^0 to CuO or Cu_2O , causing the weakening of the SPR peak [21].

At the 1:4 ratio, the increase in absorbance occurred more rapidly and reached its peak by day 4, but subsequently decreased until day 7. This suggests that although a higher extract concentration can accelerate the initial reduction process, an excess of organic compounds may promote particle aggregation and reduce system stability. Based on these results, the 1:3 ratio is considered the optimal condition because it shows the most stable increase in absorbance during the incubation period. This finding is consistent with previous reports stating that the balance between metal precursor concentration and plant extract plays an important role in determining the efficiency of nanoparticle synthesis and stability [20, 22].

3.3 Synthesis of Cu nanoparticles

Nanoparticle synthesis on a large scale was carried out to obtain nanoparticle powder in large quantities. Nanoparticle synthesis was performed at a ratio of 1:3. The ratio of 1:3 refers to the volume ratio between *A. cordifolia* extract and $\text{Cu}(\text{NO}_3)_2$ solution, where one part of extract was mixed with three parts of precursor solution. The results of nanoparticle synthesis are shown in Fig. 2.

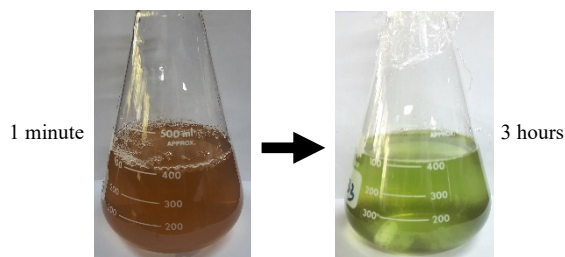


Fig. 2. Synthesis result of Cu nanoparticles.

The color change of the synthesized copper nanoparticles is shown in Fig. 2, where the solution turns from brown to green in a span of about three hours, indicating the formation and oxidation of some CuNPs to

copper oxide. This color change phenomenon is in line with the report of Zambare et al. (2023) [23] on the synthesis of CuNPs using *Ocimum sanctum* extract, who noted a color shift from blue to green within 30-60 minutes as an early indicator of nanoparticle formation through the reduction of Cu^{2+} by phytochemical compounds such as polyphenols and terpenoids. A similar mechanism is explained by recent studies, that the color change from brown to green indicates the conversion of Cu^0 to CuO oxide, which also affects the optical properties, especially the SPR peak in the UV-Vis spectrum [24, 25]. Therefore, the color change in Fig. 2 is not only a marker of the formation of CuNPs, but also an early signal of oxidation of the nanoparticle surface, which should be further controlled to maintain the stability and integrity of the particles.

3.4 UV-Vis spectroscopy analysis results

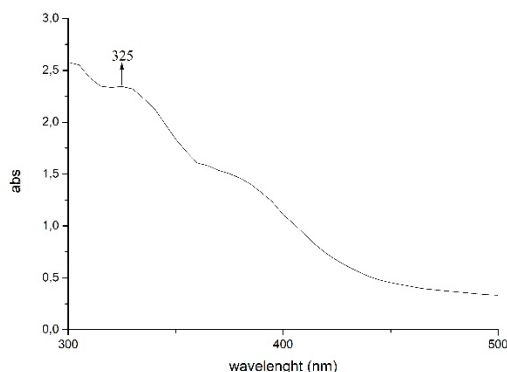


Fig. 3. UV-Vis spectra Cu nanoparticle.

The results of UV-Vis spectroscopic analysis shown in Fig. 3 indicate that the formed copper nanoparticles have an absorbance peak at a wavelength of about 325 nm, which indicates the presence of a local surface plasmon resonance (SPR) phenomenon typical of CuNPs. This peak indicates that the particles formed are nanometer-sized and optically stable in the initial phase of synthesis. This finding is in line with the research of Munandar et al. (2022) [14], who reported that CuNPs synthesized using *Anredera cordifolia* extract showed a

similar peak at 325 nm, with a crystallite size of about 43.5 nm, suggesting that the phenolic and flavonoid groups of the extract act as reducing and stabilizing agents. Another study by Mathanmohun et al. (2024) [26] also supported that green-synthesized CuO NPs showed an absorbance peak in the range of 320-330 nm, which depends on the chemical composition and stability of the natural reductant used. Meanwhile, Ghosh et al. (2023) [27] found that increasing the concentration of the extract can increase the SPR intensity, but if it is excessive, it triggers particle agglomeration characterized by broadening or shifting of the UV-Vis peak. Thus, the absorbance peak at 325 nm not only indicates the formation of copper nanoparticles, but also reflects the balance between the reduction process of Cu^{2+} ions and the stabilization of particles by active compounds in plant extracts.

3.5 XRD pattern of CuNPs synthesized by

3.5.1 *Cordifolia*

The X-ray Diffraction (XRD) analysis of copper nanoparticles synthesized using aqueous extract of Binahong leaves showed one diffraction peak at a 2θ angle of 20.62° , without the presence of sharp peaks that usually appear at positions characteristic of crystalline copper metal, such as at 2θ around 43° , 50° , and 74° for the (111), (200), and (220) crystal planes based on JCPDS No. 04-0836 data. Such diffraction patterns indicate that the nanoparticle structure formed is semi-crystalline or even dominantly amorphous. This is strongly suspected to be caused by the presence of bioactive compounds in Binahong extract such as flavonoids and proteins that act as a stabilizing agent (capping agent) and cover the surface of nanoparticles, thus inhibiting the growth and preparation of an orderly crystal lattice.

These results are in line with research by Chandraker et al. (2020) [28], who reported that the synthesis of CuNPs using Piper retrofractum stem extract produced a broad

XRD peak in the 20° - 22° range that reflects the amorphous character due to the interaction of organic compounds with the particle surface. In addition, a study by Wahyuningsih et al. (2022) [29] also showed that the presence of phenolic compounds from plant extracts can widen and dampen the intensity of CuNPs crystal peaks in XRD patterns, resulting in thermodynamically stable semi-crystalline structures. The results of XRD analysis are shown in Fig. 4.

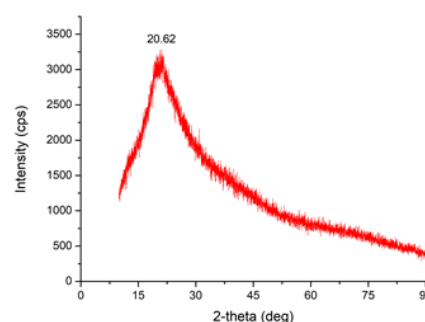


Fig. 4. Diffractogram of Copper nanoparticles.

Although the UV-Vis spectra showed an absorption peak at around 325 nm, which is commonly associated with the surface plasmon resonance (SPR) of copper-based nanoparticles, the XRD pattern did not display sharp diffraction peaks corresponding to crystalline CuO or Cu_2O phases. This indicates that the synthesized material cannot be conclusively assigned as purely CuO nanoparticles. Instead, the results suggest the formation of copper-based nanoparticles with limited crystallinity. In green synthesis systems using plant extracts, bioactive compounds often act as both reducing and capping agents, which can restrict crystal growth and result in nanoparticles with amorphous or poorly crystalline structures.

Furthermore, Dadhwal et al. (2023) [30] reported that in plant-mediated synthesis of copper nanoparticles, a core-shell structure is frequently formed, where the particle core consists mainly of Cu^0 while the surface undergoes partial oxidation to CuO or Cu_2O . Such partially oxidized structures may still produce an optical absorption response in UV-

Vis spectra but may not generate well-defined diffraction peaks in XRD due to their low crystallinity or very small particle size.

Therefore, the nanoparticles synthesized in this study are more appropriately described as copper-based nanoparticles (CuNPs) with possible surface oxidation, rather than purely CuO nanoparticles. This interpretation highlights that UV–Vis spectroscopy is sensitive to optical and surface-related properties, whereas XRD primarily reflects the long-range crystallinity of the material. The combination of these analyses provides complementary information for understanding the structural characteristics of the synthesized nanoparticles.

3.6 Functional group identification by FTIR analysis

The Fourier Transform Infrared (FTIR) spectrum of the copper nanoparticle sample shows strong characteristics indicating that the synthesis was carried out via a green synthesis (biosynthesis) route, in line with the latest research trends. This spectrum is dominated by two main absorption groups: those originating from organic biomolecules and those from metal-oxide bonds.

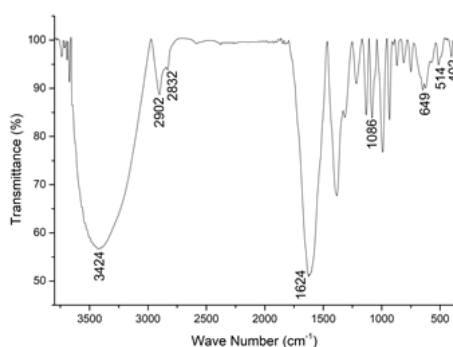


Fig. 5. FTIR Spectrum of CuNPs synthesized by 3.5.1 *cordifolia*.

The broad and strong absorption in the range of 3400–3200 cm^{-1} is caused by the stretching vibration of the hydroxyl group (O-H), which originates from adsorbed water and, more importantly, from stabilizing compounds such as polyphenols, proteins, or carbohydrates present in natural extracts.

Moderate peaks around 2920 and 2850 cm^{-1} confirm the presence of aliphatic groups (C-H). In addition, a significant absorption peak at 1630 cm^{-1} likely originates from the stretching vibration of carbonyl (C=O) or Amide bonds of proteins/amino acids. These organic groups play an important role as reducing agents and capping agents, which reduce copper ions to nanoparticles and stabilize their surface, as found in the literature on green synthesis of copper nanoparticles using *Tithonia diversifolia* or *Capparis spinosa* extracts [31, 32].

Confirmation of the successful formation of Copper Oxide Nanoparticles (CuO NPs) is clearly seen from the sharp peaks in the low wave number region ($<650 \text{ cm}^{-1}$), which are characteristic stretching vibrations of Cu-O bonds. The presence of these Cu-O vibrations is consistent with findings in the synthesis of CuO NPs, as reported by Waris et al. (2021) [33], who observed a characteristic Cu-O absorption band when using herbal extracts, and Letchumanan et al. (2021) [34], who confirmed the successful biosynthesis of CuO NPs through FTIR spectra showing sharp Cu-O peaks.

Other peaks in the range of 1000–1250 cm^{-1} also indicate C-O stretching vibrations, further supporting the presence of biomolecules (e.g., polysaccharides) as stabilizers, a common mechanism in biogenic synthesis, including the use of *Pseudomonas putida* [35]. Overall, this FTIR spectrum provides double evidence: first, the identification of the CuO phase, and second, the validation of the green synthesis method through the detection of functional groups that coat and stabilize the nanoparticles.

3.7 SEM of CuNPs synthesized by 3.5.1 *cordifolia*

The results of morphological analysis using Scanning Electron Microscopy (SEM) on copper (Cu) nanoparticle samples at $\times 440$ magnification show that the particles have an irregular shape with a rough surface and tend to form large aggregates. Automatic

measurement of the SEM image showed an average equivalent diameter of 25.27 μm , with a median of 10.97 μm , and a particle size range of 0.38–117.83 μm .

Based on this range, the particles observed were on the micrometer scale, not

nanometers, indicating agglomeration or sintering between particles. This morphological feature generally appears when nanoparticles undergo excessive growth or rapid drying, causing small nanoparticles to combine to form larger aggregates [36].

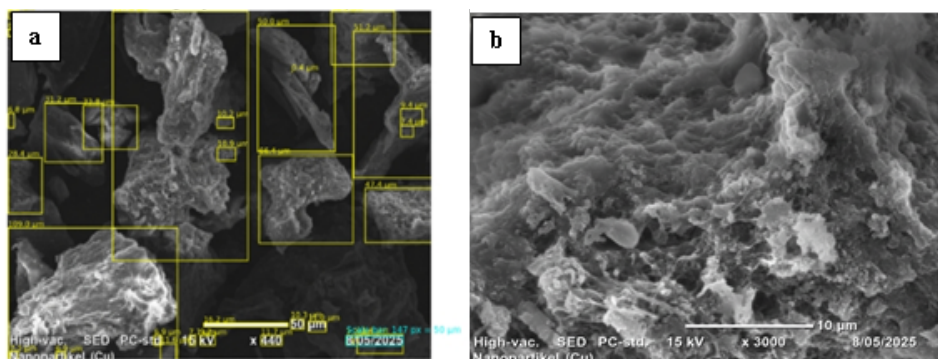


Fig. 6. (a) Distribution of detected particle equivalent diameters; (b) Scanning electron micrograph of CuNPs.

3.8 Distribution of Cu nanoparticle particle sizes using PSA

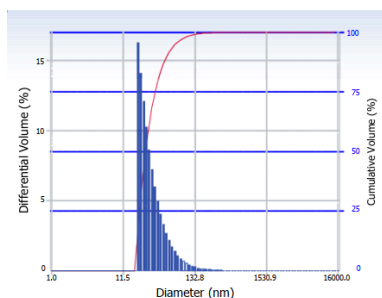


Fig. 7. CuNps intensity distribution.

The particle size distribution analysis shows that the synthesized copper-based nanoparticles are in the nanometer scale, with particle sizes ranging from approximately 10–150 nm. The differential distribution curve exhibits a main peak at around 30 nm, indicating that most particles are concentrated at this size. Based on the cumulative distribution curve, the D50 value (median diameter) is approximately 36 nm, meaning that 50% of the total particles have sizes smaller than this value. Therefore, the average particle size of the nanoparticles in this system can be considered to be around 36 nm. Although most particles are within the tens of nanometers range, a small fraction of larger

particles up to approximately 150 nm is still present. This indicates that the particle size distribution is not completely uniform, and thus the nanoparticle system can be classified as exhibiting moderate polydispersity rather than true monodispersity.

3.9 α -Amylase enzyme inhibition test

α -Amylase inhibition testing was conducted to determine the decrease in α -amylase enzyme activity in breaking down starch. The more maltose produced from starch, the more starch is hydrolyzed into maltose and glucose [37]. The percentage inhibition (%) and IC_{50} values for each sample can be seen in Table 3.

Table 3. Percentage Inhibition Value and IC_{50} of samples.

Sample	Concentration ($\mu\text{g/mL}$)	Inhibition (%)	IC_{50} ($\mu\text{g/mL}$)
Acarbose	1	24,15	5,23
	2	32,58	
	4	48,31	
	8	69,65	
	16	98,87	
<i>A. cordifolia</i> extract	1	7,30	9,06
	2	16,85	
	4	28,08	
	8	50,55	
	16	79,77	

CuNPs	1	1,68	12,67
	2	7,86	
	4	19,09	
	8	38,19	
	16	59,54	
A. cordifolia extract + CuNPs	1	12,91	7,25
	2	22,46	
	4	36,51	
	8	57,29	
	16	93,25	

The α -amylase inhibition results presented in the table show that all samples exhibited increased inhibitory activity with increasing concentration. Acarbose, used as the positive control, showed the strongest inhibition with an IC_{50} value of 5.23 $\mu\text{g/mL}$, while the *A. cordifolia* extract exhibited an IC_{50} value of 9.06 $\mu\text{g/mL}$. The synthesized copper nanoparticles (CuNPs) displayed relatively lower inhibitory activity with an IC_{50} value of 12.67 $\mu\text{g/mL}$. However, when the *A. cordifolia* extract was combined with CuNPs, the inhibitory activity increased significantly, resulting in an IC_{50} value of 7.25 $\mu\text{g/mL}$, which is stronger than that of the extract or CuNPs alone. This result indicates a synergistic effect between the bioactive compounds present in the plant extract and the copper nanoparticles.

The enhanced inhibitory activity can be associated with the physicochemical properties of the synthesized nanoparticles, particularly particle size, surface chemistry, and the presence of phytochemicals acting as stabilizing agents. Particle size distribution analysis showed that the synthesized CuNPs have an average particle size of approximately 36 nm, providing a high specific surface area. The small nanoparticle size facilitates more effective interactions between the particle surface and the active site of the α -amylase enzyme, thereby increasing the likelihood of enzyme inhibition.

Furthermore, the biosynthesis process using *A. cordifolia* extract results in the nanoparticle surface being coated with phytochemical compounds such as flavonoids and phenolics, which function as both reducing and stabilizing agents. These compounds are known to interact with amino acid residues at the active site of α -amylase through hydrogen

bonding or hydrophobic interactions, thereby contributing to enzyme inhibition. When these phytochemicals are bound to the surface of CuNPs, a nano-bioactive system is formed, which can enhance the effectiveness of interactions with the enzyme compared to the free extract.

In addition to the effects of particle size and surface chemistry, partial oxidation on the surface of CuNPs, forming a thin layer of $\text{CuO/Cu}_2\text{O}$, may also contribute to the observed biological activity. This oxide layer can increase the surface reactivity of the nanoparticles and enhance biomolecule adsorption, thereby increasing the probability of interaction with the target enzyme. Therefore, the enhanced α -amylase inhibitory activity observed in the combination of *A. cordifolia* extract and CuNPs is likely the result of a synergistic interplay between nanoparticle size effects, phytochemical capping on the nanoparticle surface, and reactive surface chemistry, which collectively improve interactions with the α -amylase enzyme.

4. Conclusion

Copper nanoparticles (CuNPs) were successfully synthesized using a green synthesis approach with stem extract of *Anredera cordifolia* as a natural reducing and stabilizing agent. The formation of CuNPs was confirmed through UV-Visible spectroscopy, FTIR, XRD, SEM, and PSA analyses. The UV-Vis spectrum showed an absorption peak around 325 nm indicating the formation of copper nanoparticles, while FTIR analysis revealed functional groups from plant metabolites that play a role in the reduction and stabilization processes. PSA results showed a particle size distribution ranging from 10 to 150 nm with a dominant peak around 30 nm and a median particle size (D_{50}) of approximately 36 nm, confirming the nanoscale formation of the synthesized particles. In addition, the α -amylase inhibition assay demonstrated that the combination of *A.*

cordifolia extract and CuNPs exhibited stronger inhibitory activity compared to the extract or CuNPs alone. These findings suggest that green-synthesized CuNPs using *A. cordifolia* extract have promising potential as an environmentally friendly material with prospective application in the development of natural antidiabetic agents.

Acknowledgements

The authors would like to express their sincere gratitude to the Research Program of Universitas Sam Ratulangi for the financial support provided through the 2025 research grant (Grant Number: SP DIPA–139.03.2.693382/2025), which made this research possible.

References

- [1] Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*. 2018; 138: 271–81.
- [2] Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, Ogle GD. Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas, 9th edition, *Diabetes Res Clin Pract*. 2019; 157:107842.
- [3] Prawitasari, DS. Diabetes Melitus dan Antioksidan, *Jurnal kesehatan dan kedokteran*. 2019; 1(1):47-51.
- [4] Pieme CA, Tatangmo JA, Simo G, Biapa PC, Moor VJ, Moukette MB, Sobngwi E. Relationship Between Hyperglycemia, Antioxidant Capacity and Some Enzymatic and Non-enzymatic Antioxidants in African Patients with Type 2 Diabetes, *BMC Research Notes*. 2017; 10(1):141.
- [5] Oyenihni AB, Ayeleso AO, Mukwevho E, Masola B. Antioxidant strategies in the management of diabetic neuropathy, *BioMed Research International*. 2015; 2015:515042.
- [6] Erlidawati E, Safrida S, Mukhlis M. Potensi Antioksidan Sebagai Antidiabetes, Syiah Kuala University Press. 2018; 1–11.
- [7] Kitture R, Chordiya K, Gaware S, Ghosh S, More PA, Kulkarni P, Chopade BA, Kale SN. ZnO Nanoparticles-Red Sandalwood Conjugate: A Promising Anti-Diabetic Agent, *J Nanosci Nanotechnol*. 2015; 15(6): 4046-51.
- [8] Patil AB, Ghosh S, Phadatare SD, Pathak P, Sharma GK, Chopadece BA, Shinde VS. Evaluation of malonic acid diamide analogues as radical scavenging agents, *New J Chem*. 2015; 39: 1267-73.
- [9] Roseline VP, Priya V. Antidiabetic Potential of Copper Oxide Nanoparticles Using Biological and Polymer Functionalized Method Mediated by *Sarcostemma acidum* Stem Extract, *Oriental Journal Of Chemistry*. 2023; 39(2): 387–92.
- [10] Ghosh S, More P, Nitnavare R, Jagtap S, Chippalkatti R, Derle A, et al. Antidiabetic and Antioxidant Properties of Copper Nanoparticles Synthesized by Medicinal Plant *Dioscorea bulbifera*, *J. Nanomed. Nanotechnol*. 2015; S6: 1-9.
- [11] Singh R, Nawale LU, Arkile M, Shedbalkar UU, Wadhwani SA. Chemical and biological metal nanoparticles as antimycobacterial agents: A comparative study, *Int. J. Antimicrob Agents*. 2015; 46: 183-8.
- [12] Buazar F, Sweidi S, Badri M, Kroushawi F. Biofabrication of highly pure copper oxide nanoparticles using wheat extract and their catalytic activity: a mechanistic approach, *Green process synth*. 2019; 8: 691-702.
- [13] Wattimena SC, Patty PJ. Antibacterial properties of silver nanoparticles synthesized using leaf extract of *Anredera cordifolia* as a reducing agent, *WJPPS*. 2017; 6: 1673-83.

- [14] Munandar N, Kasim S, Arfah R, Basir DN, Hala Y, Zakir M, Natsir H. Green synthesis of copper oxide (CuO) nanoparticles using *Anredera cordifolia* leaf extract and their antioxidant activity, *Commun. Sci. Technol.* 2022; 7(2): 127-34.
- [15] Antonio-Pérez A, Durán-Armenta LF, Pérez-Loredo MG, Torres-Huerta AL. Biosynthesis of Copper Nanoparticles with Medicinal Plants Extracts: From Extraction Methods to Applications, *Micromachines.* 2023; 14(10): 1882.
- [16] Pal PK, Sarifujjaman MD, Saha P, Md SMM, Islam E, Ahmmad B, Karim KMDR, Mahiuddin Md. Green Synthesis of Zinc Oxide Nanoparticles Using *Dillenia Indica* and *Mikania Micrantha* Stem Extracts: Applications in Photocatalysis and Antibacterial Activity, *Chemistry Open.* 2024; 13(12): e202400102.
- [17] Sharma S, Sharma N, Kaushal N. Utilization of novel bacteriocin synthesized silver nanoparticles (AgNPs) for their application in antimicrobial packaging for preservation of tomato fruit, *Frontiers in Sustainable Food Systems.* 2023; 7: 1072738.
- [18] Tyagi S, Singh P, Khasa V. Green fabrication of silver nanoparticles with *Syzygium aromaticum* leaf extract: Characterization and applications as a biocontrol agent for multidrug resistant pathogens, *Chemical Physics Letters.* 2024; 855: 141560.
- [19] Weerasinghe W, Weerakoon SR, Sandanayake CLT, Aruggoda B. Optimizing the Bio Synthesis of Silver and Ferrous Oxide Nanoparticles Using *Marsilea quadrifolia* (L.) Stem Extract, *European Journal of Theoretical and Applied Sciences.* 2023; 1: 1533-40.
- [20] Ghosh MK, Sahu S, Gupta I, Ghorai TK. Green synthesis of copper nanoparticles from an extract of *Jatropha curcas* leaves: characterization, optical properties, CT-DNA binding and photocatalytic activity, *Royal Society of Chemistry.* 2020; 10: 22027-35.
- [21] Kumar B, Smita K, Debut A, Cumbal L. Rapid synthesis and antioxidant activity of copper nanoparticles using rambutan peel extract with ultrasound assistance, *Emergent Materials.* 2023; 7.
- [22] Nguyen TD, Hoang YH, Thai NTT, Trinh G. Synthesis of copper nanoparticles by a sonication mediated method using *Malpighia glabra* fruit extract and their applications, *RSC Adv.* 2024; 14: 34119-34.
- [23] Zambare P, Survase A, Kanase S. Green Synthesis of Copper Nanoparticles Using Stem Extract of *Ocimum sanctum* and its Antimicrobial Activity, *Int. J. Pharm. Investigation.* 2023; 13: 106-12.
- [24] Nazir A, Aslam S, Akhter P, Osama A, Mohammed, Ahmed SA, et al. Effect of Iron Doping on Titania Nanoparticles Derived from *Dalbergia sissoo* for Removal of Tetracycline Hydrochloride. *Semiconductors.* 2025; 59: 291-9.
- [25] Ahmed B, Tahir MB, Sagir M, Hassan M. Bio-inspired sustainable synthesis of silver nanoparticles as next generation of nanoproduct in antimicrobial and catalytic applications, *Materials Science and Engineering: B.* 2024; 301: 117165.
- [26] Mathanmohun M, Sagadevan S, Rahman MZ, Lett JA, Fatimah I, Moharana, et al. Unveiling sustainable, greener synthesis strategies and multifaceted applications of copper oxide nanoparticles, *Journal of Molecular Structure.* 2024; 1305: 137788.
- [27] Ghosh A, De SK, Mondal S, Halder S, Barai M, Guchhait KC, et al. Green synthesis of silver nanoparticles and its applications as sensor, catalyst, and antibacterial agent, *Materials Today: Proceedings,* 2023.
- [28] Chandraker SK, Lal M, Ghosh MK, Tiwari V, Horai TK, Shukla R. Green synthesis of copper nanoparticles using leaf extract of *Ageratum houstonianum* Mill. and study of their photocatalytic and antibacterial activities, *Nano Express.* 2020; 1: 010033.

- [29] Wahyuningsih K, Yuliani S, Iriani ES. Nano-encapsulation of Eucalyptus citriodora Oil: Preparation and Characterization, IOP Conf. Ser.: Earth Environ. Sci. 2022; 1024: 012016.
- [30] Dadhwal P, Dhingra HK, Dwivedi V, Alarifi S, Kalasariya H, Yadav VK, et al. Hippophae rhamnoides L. (sea buckthorn) mediated green synthesis of copper nanoparticles and their application in anticancer activity, Front. Mol. Biosci. 2023; 10: 1246728.
- [31] Millavithanachchi SS, Gunasena MDKM, Galpaya GDCP, Priyadarshana HVV, Indupama SVAA, Induranga DKA, et al. Green Synthesis, Optimization, and Characterization of CuO Nanoparticles Using Tithonia diversifolia Stem Extract, Nanomaterials. 2025; 15(15): 1203.
- [32] Khatami M, Ebrahimi K, Galehdar N, Moradi MN, Moayyedkazemi A. Green Synthesis and Characterization of Copper Nanoparticles and Their Effects on Liver Function and Hematological Parameters in Mice, Turk J Pharm Sci. 2020; 17: 412-6.
- [33] Waris A, Din M, Ali A, Ali M, Afridi S, Baset A, et al. A comprehensive review of green synthesis of copper oxide nanoparticles and their diverse biomedical applications, Inorganic Chemistry Communications. 2021; 123: 108369.
- [34] Letchumanan D, Sok SPM, Ibrahim S, Nagoor NH, Arshad NM. Plant-Based Biosynthesis of Copper/Copper Oxide Nanoparticles: An Update on Their Applications in Biomedicine, Mechanisms, and Toxicity, Biomolecules. 2021; 11: 564.
- [35] El-Sayed O, Abd-Elhalim BT, Mosa MA, Hassan EA. Characterization and optimization of biogenic copper nanoparticles synthesized by Pseudomonas putida with cytocompatibility investigation, Sci. Rep. 2025; 15: 32504.
- [36] Manjunatha KB, Bhat RS, Hashidhara A, Kumar HSA, Nagashree S. Antimicrobial and Nonlinear Optical Studies of Copper Oxide Nanoparticles, Journal of Electronic Materials. 2021; 50: 3415-21.
- [37] Samudra AG, Nugroho AE, Husni A. Aktivitas Inhibisict-amilase Ekstrak Karagenan dan Senyawa Polifenol dari Eucheuma denticulatum, Med Farm. 2025;1.