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Green Synthesis of Copper Nanoparticles (CuNPs) using Chaya (*Cnidoscolus aconitifolius*) Leaves Extract as a Bioreductant

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Abstract
Copper nanoparticles (CuNPs) are metallic particles sized 1-100 nm. This study was conducted to develop an ecofriendly and sustainable approach for CuNP synthesis using *Cnidoscolus aconitifolius* (chaya) leaves extract as a natural bioreductant. The research aimed to determine the optimal synthesis conditions, and characterize the resulting CuNPs. This experimental laboratory study employed a green synthesis method by reducing $CuSO_4{\cdot}5H_2O$ solution with chaya leaves extract. CuNPs were synthesized at various volume ratios (1:1, 1:2, 1:3, 1:4) and pH values (6-12). Data were collected using instrumental analyses (UV–Vis, FTIR, PSA, and XRD). The obtained data were analyzed descriptively based on spectral, structural, and particle size characteristics. The optimal synthesis condition was achieved at a 1:1 ratio and pH 10, producing CuNPs with a maximum absorption peak at 325 nm, an average particle size of 35.17 nm, and a polydispersity index of 0.2656. FTIR spectra indicated Cu vibrations, while XRD analysis shows the typical 2θ peaks for copper (32.57°, 35.60°, 38.72°, 48.91°, 53.32°, 61.67°, 68.02°, and 72.51°). These findings demonstrate the potential of chaya (*Cnidoscolus aconitifolius*) leaves extract as a sustainable bioreductant for green synthesis of copper nanoparticles, supporting the development of ecofriendly nanomaterials.

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INTRODUCTION

Nanotechnology is an emerging field of science and technology that focuses on materials within the nanoscale range of 1–100 nm (Kushwaha, 2021; Sharma et al., 2021). In recent years, nanotechnology has advanced rapidly, driven by its wide ranging applications in diverse fields such as biomedicine and physicochemistry, as well as its ability to integrate with other technological innovations (Afolalu et al., 2023; Sutoyo et al., 2025). The synthesis of nanoparticles with controlled morphology, size, shape, and structure has become a central focus in nanotechnology research, as their crystallinity and geometry critically influence stability, reactivity, and functional performance in optical, mechanical, and chemical applications (Joudeh & Linke, 2022; Munandar et al., 2022).

Conventional methods for synthesizing nanoparticles often rely on toxic chemicals and energy intensive procedures, which raise environmental and safety concerns (Ibrahim et al., 2023; Krishna et al., 2024). This creates an urgent need for greener and safer approaches to nanoparticle synthesis. Green synthesis using plant extracts of nanoparticles has gained attention as an eco-friendly alternative to conventional chemical methods, aligning with green chemistry principles that aim to reduce the use of synthetic chemicals (Verma et al., 2021; Villagrán et al., 2024). Plants offer a natural platform for nanoparticle synthesis, utilizing bioactive compounds such as flavonoids and phenolics that act as reducing and capping agents (Maulana et al., 2022; Mardiyanto et al., 2023) In this study, copper nanoparticles (CuNPs) were selected for synthesis due to their cost effectiveness, strong biological activity, and abundant availability compared to gold or silver. Copper is also more amenable to processing and modification for biomedical applications, making it a practical and sustainable option (Mahmood et al., 2022; Putri & Sutoyo, 2025).

Chaya (*Cnidoscolus aconitifolius*) is a medicinal plant belonging to the genus *Cnidoscolus* and the family *Euphorbiaceae* (Adindu et al., 2024). Chaya is traditionally used in herbal medicine and is increasingly cultivated (Simamora et al., 2022). This species contains a wide range of bioactive compounds such as phenolic, alkaloids, saponins, flavonoids, and terpenoids. Some of these compounds especially alkaloids, flavonoids, and phenolics are known to function as natural reducing agents (Nurjanah et al., 2023; Riskianto et al., 2022). Chaya also exhibits various pharmacological properties, including antidiabetic, hepatoprotective, and notably antibacterial effects (Adindu et al., 2024; Panghal et al., 2021).

Several studies have reported the synthesis of copper nanoparticles using various plant extracts. Atri et al. (2023) synthesized copper nanoparticles using alenda (*Ephedra alata*) plant extract, while Amer & Awwad (2021) used lemon (*Citrus limon*) fruit extract. Similarly, Haruna et al. (2022) utilized dragon fruit (*Hylocereus polyrhizus*) extract, and Prabhu et al. (2022) used butterfly pea (*Clitoria ternatea*) flower extract.

However, the synthesis of copper nanoparticles using chaya (*C. aconitifolius*) leaves extract has not yet been reported. This indicates a clear research gap, as the potential of *C. aconitifolius* leaves extract as a sustainable bioreductant for the synthesis, optimization, and characterization of copper nanoparticles remains unexplored. Therefore, there is a need to evaluate the potential of *C. aconitifolius* leaves extract as a sustainable bioreductant for the green synthesis of CuNPs, to optimize the synthesis conditions, and to characterize the resulting nanoparticles in accordance with green chemistry principles. The successful green synthesis of CuNPs using *C. aconitifolius* may contribute to the development of cost-effective and environmentally friendly nanomaterials for future catalytic or antimicrobial applications.

METHOD

Materials

Chaya (*C. aconitifolius*) leaves were picked from Pucang Village, Gempol District, Pasuruan, East Java, Indonesia. $CuSO_4.5H_2O$ (Merck), double-distilled water (DDW) (Otsuka), and Whatman filter paper No. 42.

Preparation of *Cnidoscolus aconitifolius* (chaya) Leaves Extract

Chaya (*C. aconitifolius*) leaves were washed with DDW to remove the dust particles on them, dried at room temperature for a week and crushed to a fine powder. After that, chaya leaves powder is sieved again using a 100 mesh sieve, so the powder has a smaller particle size (±150 μm) (Wang et al., 2022). Around 10 g of leaves powder was added with 100 mL of DDW and heated at 60°C for 90 min. The leaves extract was allowed to cool to ambient temperature and further filtered by No.42 Whatman filter paper. The collected leaves extract solution was kept at 4°C for further use (Murthy et al., 2020).

Preparation of 0,1 M $CuSO_4.5H_2O$

A total of 2.4969 g of $CuSO_4.5H_2O$ crystals was accurately weighed to prepare a 0.1 M solution. The weighed salt was added to a 250 mL beaker and dissolved in approximately 100 mL of DDW. The solution was stirred with a magnetic stirrer until it became clear and uniformly blue. After achieving a homogeneous solution, it was transferred to a 100 mL volumetric flask and the volume was adjusted to the mark with DDW. The final solution was then stored in a glass bottle for subsequent use (Aliyu et al., 2023).

Synthesis of Copper Nanoparticles (CuNPs)

Copper nanoparticles (CuNPs) were synthesized by reacting chaya (*C.* aconitifolius) leaves extract with a 0.1 M $CuSO_4.5H_2O$ solution at varying volume ratios of 1:1 (E1C1), 1:2 (E2C1), 1:3 (E3C1), and 1:4 (E4C1) with variations volume 30 mL:30 mL, 20 mL:40 mL, 15 mL:45 mL, and 12 mL:48 mL, and each mixture maintaining a final volume of 60 mL. The mixtures were stirred at 60°C and 1500 rpm for 30 minutes using a magnetic stirrer. The solutions colour changed from brownish to greenish, suggesting that CuNPs had formed. After stirring, the solutions were left undisturbed for 24 hours. The resulting colloids were characterized by UV–Vis spectrophotometry in the 200–800 nm range to determine the maximum absorbance wavelength and to identify the optimum bioreductant-to-precursor ratio for nanoparticle synthesis (Putri & Sutoyo, 2025; Sadia et al., 2021). UV–Vis spectra were conducted as single measurements under controlled laboratory conditions.

The obtained volume bioreductant-to-precursor ratio was subsequently optimized for pH at values of 6, 7, 8, 9, 10, 11, and 12. The pH adjustment was carried out by adding 0.1 M NaOH while stirring the solution with a magnetic stirrer. The resulting mixtures were subsequently analyzed using UV–Vis spectrophotometry to determine the maximum absorbance wavelength and identify the most favorable pH condition for CuNPs formation (Putri & Sutoyo, 2025; Sadia et al., 2021).

The colloidal nanoparticles were also characterized using particle size analysis (PSA), which requires the sample in liquid form. For other analyses that require solid samples, such as FTIR, and XRD, the nanoparticles were freeze-dried to obtain powders suitable for solid-state characterization. The steps of synthesis copper nanoparticles (CuNPs) are presented in Figure 1.

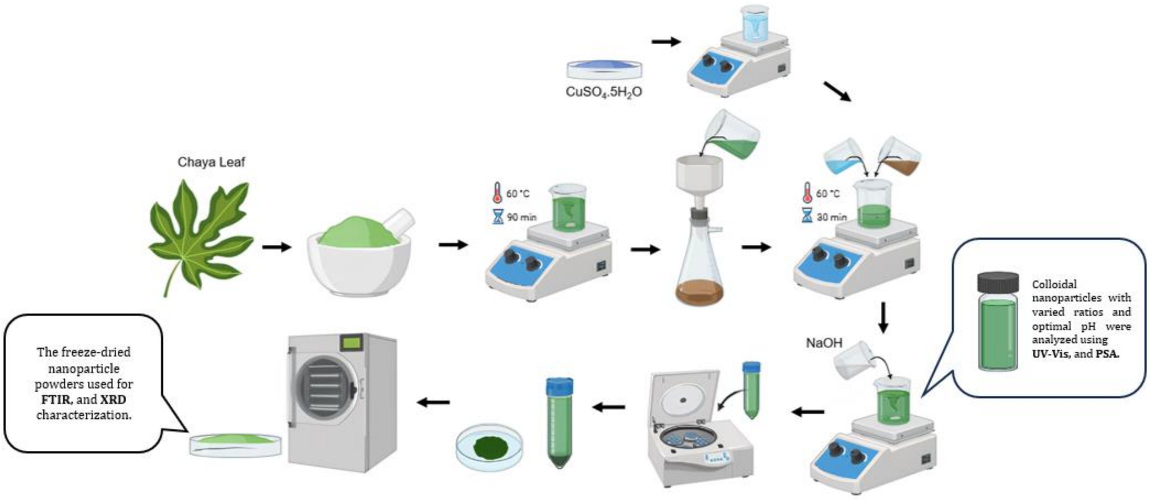

Figure 1. Preparation of Copper Nanoparticles (CuNPs) using Chaya Leaves Extract

Characterization methods

The synthesized CuNPs were characterized using several analytical instruments. UV-Vis spectrophotometry (Shimadzu UV-Vis 1800) was used to determine the maximum absorption wavelength, FTIR spectroscopy (Nicolet iS10) to identify functional groups, and a Particle Size Analyzer (PSA) (Biobase BK-802N DLS Nano) to determine particle size distribution, average particle size, and polydispersity index. X-ray diffraction (Rigaku MiniFlex 600) was employed to confirm the presence of copper in the CuNPs. All characterization in this study were conducted as single measurements under controlled laboratory conditions.

RESULTS AND DISCUSSION

Result

Optimization of CuNPs Using Chaya Leaves Extract

The concentration of bioreductants plays an important role in determining the efficiency of nanoparticle synthesis. The optimal volume of chaya leaves extract increases the reduction of Cu^{2+} ions into elemental copper (Cu^0).

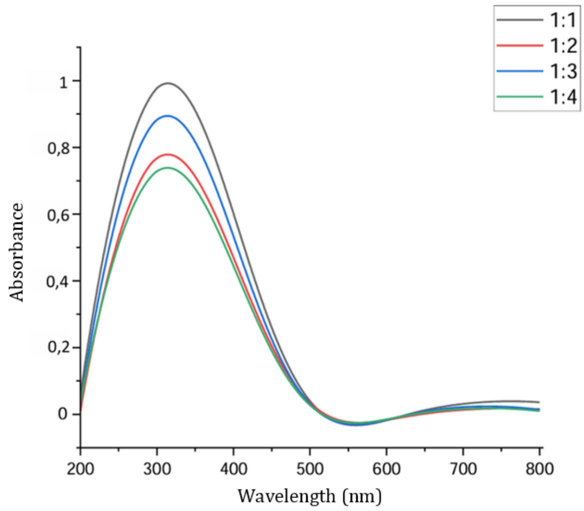

Figure 2. Optimization bioreductant-to-precursor volume ratio of CuNPs using chaya leaves extract with UV-Vis spectrophotometer

As seen in Figure 2., the highest absorbance is obtained at a ratio of 1:1 (E1C1) with an absorption of 0.9919 at a wavelength of 314 nm. The results of the measurement of the maximum wavelength of the UV-Vis spectrophotometer with bioreductant-to-precursor volume ratio variations are presented in Table 1.

Table 1. Results of UV-Vis Spectrophotometer Optimization of CuNPs Using Chaya Leaves Extract with Bioreductor-to-Precursor Volume Ratio Variations

Volume Comparison	Wavelength (nm)	Absorbance
1:1 (E1C1)	314	0.9919
1:2 (E2C1)	314	0.7786
1:3 (E3C1)	314	0.8944
1:4 (E4C1)	314	0.7385

Note: E = extract of chaya leaves, C = $CuSO_4 \cdot 5H_2O$

After the determination of optimal conditions based on the bioreductant-to-precursor volume ratio, further pH optimization is performed to obtain nanoparticles with smaller size, uniform morphology, and better stability.

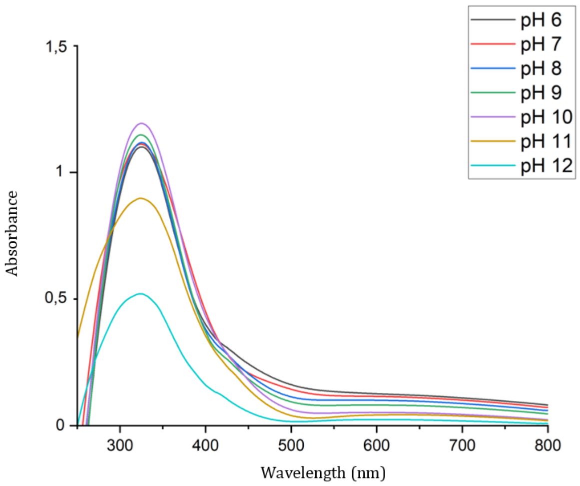

Figure 3. Optimization pH variation of CuNPs using chaya leaves extract UV-Vis spectrophotometer

As shown in Figure 3., the lowest absorbance was recorded at pH 12, with a value of 0.5205 at a wavelength of 325 nm, while the highest absorbance was observed at pH 10, reaching 1.1933 at 325 nm. Therefore, pH 10 is the optimum pH for the synthesis of copper nanoparticles using chaya leaves extract as a bioreductant. The results of the measurement of the maximum wavelength of the UV-Vis spectrophotometer with some pH variation are presented in Table 2.

Table 2. Results of UV-Vis Spectrophotometer Optimization of CuNPs Using Chaya Leaves Extract with pH Variation

pH	Wavelength (nm)	Absorbance
6	325	1.1001
7	325	1.1118
8	325	1.1177
9	325	1.1477
10	325	1.1933
11	325	0.8979
12	325	0.5205

Characterization of CuNPs Using Chaya Leaves Extract

Copper nanoparticles obtained from the synthesis results under optimum conditions (volume ratio and pH) were then characterized using a UV-Vis spectrophotometer, FTIR spectrophotometer, PSA, and XRD.

Spectrophotometer UV-Vis

Characterization using UV-Vis was conducted to identify the formation and monitor the optical stability of CuNPs. Measurements were made in the wavelength range of 200–800 nm.

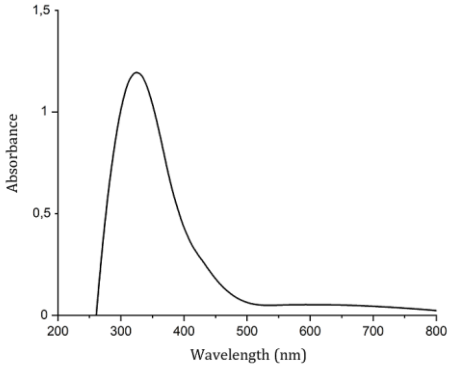

Figure 4. Characterization of CuNPs using chaya leaves extract with UV-Vis spectrophotometer

As shown in Figure 4., the UV-Vis spectrum shows an absorbance of 1.1933 at a wavelength of 325 nm, which corresponds to the typical peak of copper.

Spectroscopy FTIR

FTIR characterization was used to find the molecules and functional groups present in chaya leaves extract, which play a role in reducing Cu^{2+} ions and stabilizing the CuNPs formed. As well as to find out the functional groups of Cu contained in CuNPs.

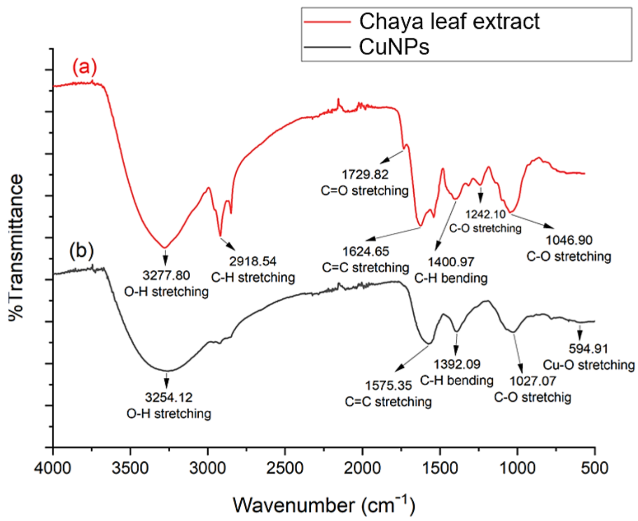

Figure 5. (a) FTIR spectrum of chaya leaves extract; (b) FTIR spectrum of CuNPs using chaya leaves extract

As shown in Figure 5., the FTIR spectrum shows the presence of phenol (O-H), carbonyl (C=O), and ether (C-O) functional groups in chaya leaves extract, which play a role in the CuNPs reduction and stabilization process. In CuNPs, a typical Cu stretching vibration appears at 594.91 cm^{-1}, which indicates the formation of the Cu phase.

Particle Size Analyzer (PSA)

For particle size determination, the CuNPs were characterized using Particle Size Analyzer (PSA).

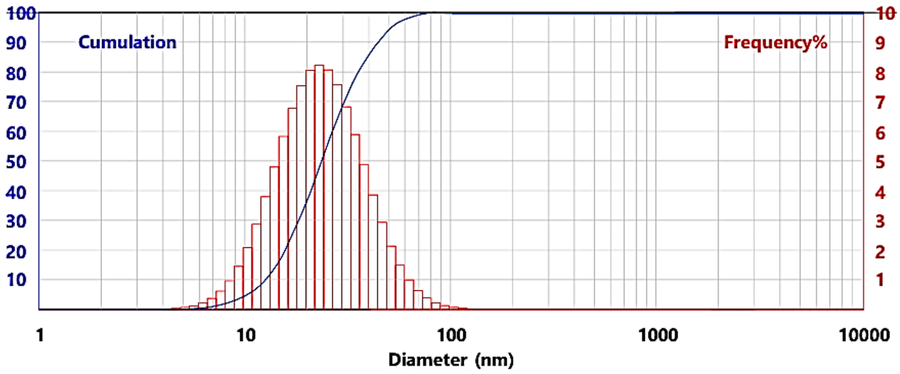

Figure 6. Characterization of CuNPs using Chaya Leaves Extract with PSA

A substance is categorized as a nanoparticle when its size ranges from 1–100 nm (Sharma et al., 2021). As shown in Figure 6, the PSA histogram of the synthesized CuNPs revealed an average particle diameter of 35.17 nm, which is in the range of the nanoscale.

X-Ray Diffraction (XRD)

The synthesized CuNPs were further characterized using X-ray diffraction (XRD) to confirm their crystal structure. The analysis was performed using Cu Kα radiation with wavelength λ = 1.54060 Å.

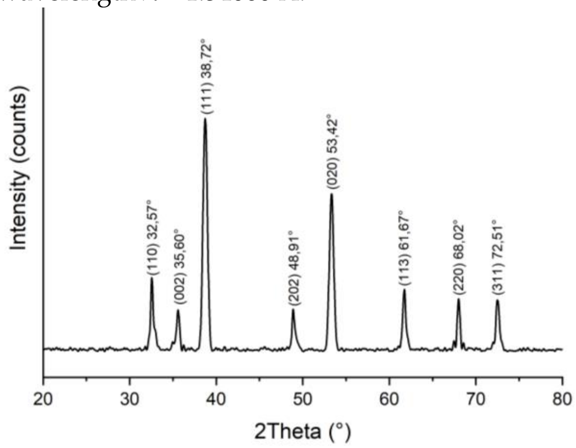

Figure7. Results of Characterization of CuNPs using Chaya Leaves Extract with XRD

As shown in Figure 7., the XRD results result in a diphragtogram consisting of a series of peaks of varying intensity and position. This diffraction peak, identified by its 2θ value, was compared with reference patterns from the *Joint Commite on Powder Diffraction Standars* (JCPDS) database to determine the phase composition of the synthesized CuNPs (Prabhu et al., 2022).

Discussion

Optimization of CuNPs Using Chaya Leaves Extract

Copper nanoparticles (CuNPs) of chaya leaves extract (*C. aconitifolius*) optimized based on bioreductant-to-precursor ratios and pH variations. In this study, the optimum synthesis condition was determined based on the highest absorbance observed in the UV–Vis spectrum. According to Sulistiorini et al., (2024) at the maximum absorption wavelength, the absorbance reaches its peak, reflecting the highest concentration of nanoparticles formed under those conditions.

At Figure 2., the highest absorbance is known to be recorded at a ratio of 1:1 (E1C1), with an absorbance of 0.9919 at 314 nm. This observation is in line with previous findings by Aziz et al. (2024), which reports maximum wavelength at 308 nm for CuNPs synthesized using *Niallia sirkular* G9. In addition, the study by Javani & Sutoyo (2023) also demonstrated that CuNPs synthesized using *Garcinia mangostana L.* peel extract reached optimal conditions at a bioreductant-to-extract volume ratio of 1:1.

Excessive amounts of reducing substances can inhibit the nucleation of nanoparticles, leading to decreased absorbance. This suggests that the formation of nanoparticles does not solely depend on the quantity of the extract, but rather on the balanced ratio between the extract and the precursor (Setiani & Suyatno, 2024). Therefore, optimizing the bioreductant-to-precursor volume ratio is essential to determine the proportions that result in the highest nanoparticle production.

After the determination of optimal conditions based on the bioreductant-to-precursor volume ratio, further pH optimization is performed to obtain nanoparticles with smaller size, uniform morphology, and better stability. pH plays an important role in influencing the reduction reaction rate, particle surface charge, and colloidal stability. Therefore, identifying the most optimal pH conditions is essential to support the effective formation and stability of nanoparticles.

At Figure 3.,the highest absorbance is observed at pH 10, with an absorbance of 1.1933 at 325 nm. These results are in line with previous findings, which reported that CuNPs were synthesized using *Anredera cordifolia* have maximum wavelength at 325 nm (Munandar et al., 2022). These results are also in line with research reported by Alahdal et al., (2023) which showed that CuNPs synthesized using extracts *Phragmanthera austroarabica* has an optimal pH of 10. Variations in wavelength and absorbance are affected by the pH of the reaction medium.

At higher pH levels, nanoparticles tend to acquire more negative surface charges, resulting in increased electrostatic repulsion between particles, which prevents aggregation. Thus, the nanoparticles that are synthesized will be smaller in size compared to those that are formed at a lower pH level (acid). This phenomenon is associated with reduced activity of carboxyl-containing compounds in the extract under acidic conditions, which weakens its function as a capping agent during the formation of nanoparticles (Villagrán et al., 2024; Wu et al., 2020).

Characterization of CuNPs Using Chaya Leaves Extract

Spectrophotometer UV-Vis

The synthesized CuNPs with the most optimal conditions were then characterized using UV-Vis spectrophotometry. This technique serves as an initial method for confirming the formation of copper nanoparticles by identifying characteristic wavelengths and maximum absorbance, as well as determining the optimal bioreductant-to-precursor ratio based on absorbance values. Figure 4. showed characterization was carried out in CuNPs that was synthesized under the most optimal conditions, specifically at a volume ratio of 1:1 bioreductants-to-precursors (E1C1) and pH 10. The UV-Vis spectrum shows an absorbance of 1.1933 at a wavelength of 325 nm. These findings are in line with previous research, which reported that copper nanoparticles were synthesized have optimal formation at pH 10 and peak absorption at 325 nm (Munandar et al., 2022; Putri & Sutoyo, 2025).

Spectroscopy FTIR

As shown in Figure 5., The FTIR spectrum of chaya leaves extract showed a peak absorption at 3277.80 cm⁻¹ according to the stretching of the phenolic compound O-H, indicating its role as a reducing and stabilizing substance. The peak at 2918.54 cm^{-1} represents an aliphatic C-H stretch, while the sharp band at 1729.82 cm^{-1} is associated with a C=O stretch, indicating the presence of carbonyl groups such as carboxylic acid or aldehydes. Absorption at 1624.65 cm^{-1} is associated with stretching C=C in the aromatic ring, typical of flavonoids. Peaks at 1400.97 cm^{-1} indicate the bending of the C-H alkyl group. Finally, peaks at 1242.10 cm^{-1} and 1046.90 cm^{-1} correspond to the C-O stretch, related to alcohols, ethers, or esters involved in the stabilization of nanoparticles (Al-Faouri et al., 2021; Fentie et al., 2022).

Whereas, the FTIR spectrum of synthesized CuNPs produces several absorption band characteristics. The vast peak at 3254.12 cm^{-1} corresponds to the O-H stretching vibrations of phenolic compounds, indicating the involvement of plant-derived biomolecules in the process of reduction and stabilization. The absorption band at 1575.35 cm^{-1} was associated with a C=C stretch in the aromatic structure, while the peak at 1392.20 cm^{-1} was associated with the bending vibration of the C-H alkyl. In addition, the band at 1027.07 cm^{-1} corresponded to the C-O stretch, indicating the presence of alcohols, esters, or ether groups. In particular, a distinct peak at 594.91 cm^{-1} was observed, whose spectrum showed a number of peaks in the region of 500–800 cm^{-1}, which was associated with Cu stretching vibrations, indicating the presence of a Cu phase in the synthesized nanoparticles (Kushwaha, 2021; Sharma et al., 2021).

The observed change in the wavenumber value and absorption intensity showed a significant interaction between the functional groups in the extract and Cu^{2+} ions, which supported the bioreduction process. The phenolic -OH group, along with other functional groups found in flavonoids, tannins, and alkaloids, most likely play a role in reducing Cu^{2+} ions to Cu^{0} (Letchumanan et al., 2021; Sugiyarti et al., 2021). The mechanism of reduction of Cu^{2+} ions from the $CuSO_4.5H_2O$ precursor by phenolic compounds is illustrated in Figure 10. The phenolic -OH group in flavonoid compounds is oxidized into a quinon group, while the Cu^{2+} ion is reduced to a copper (Cu^{0}) nanoparticle. In addition, flavonoid compounds also act as capping agents to stabilize copper nanoparticles (CuNPs).

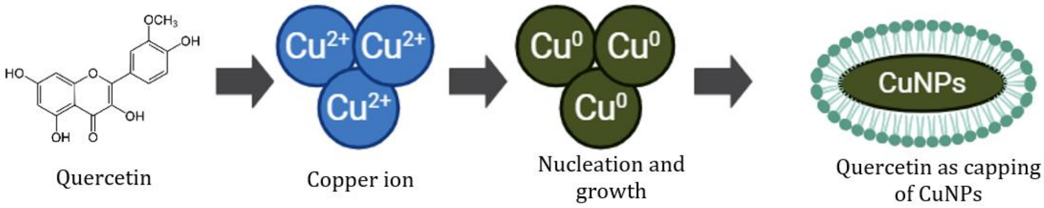

Figure 10. Reduction Reaction between Plant Bioactive Compounds and Cu^{2+} Ion

Particle Size Analyzer (PSA)

As shown in Figure 6, the PSA histogram of the synthesized CuNPs revealed an average particle diameter of 35.17 nm that was in the nanoscale range indicating the successful synthesis of copper nanoparticles. In this research, Poly Dispersion Index (PDI) obtained is 0.2656. According to Mardiyanto et al., (2023) PDI value below 0,5 indicated a homogeneous particle distribution and a relatively high degree of size uniformity. These results are in line with previous findings, which achieved copper nanoparticles with an average size of 30-75 nm using *Persea americana* seed extract (Ibrahim et al., 2023).

Likewise, synthesis uses *Cinnamomum zelanicum* leaves extract which produces particles with an average diameter of 19-69 nm (Liu et al., 2021). The average particle size is larger than the crystallite size calculated from XRD data. This difference is expected because PSA measures the hydrodynamic diameter of nanoparticles, which includes the contribution of capping agents and possible particle agglomeration in suspension. In contrast, XRD analysis estimates the size of individual crystalline domains (Velayati et al., 2025).

X-Ray Diffraction (XRD)

At Figure 7., the XRD results result in a diphragtogram consisting of a series of peaks of varying intensity and position. This diffraction peak, identified by its value of 2θ, is compared to the reference pattern of the database *Joint Commite on Powder Diffraction Standards* (JCPDS) to determine the phase composition of synthesized CuNPs (Prabhu et al., 2022). When matched with JCPDS card no. 76-1467 for metallic copper (Cu), the observed diffraction peaked at 2θ = 32.57°, 35.60°, 38.72°, 48.91°, 53.32°, 61.67°, 68.02°, and 72.51° respectively corresponding to the crystal planes (110), (002), (111), (202), (020), (113), (220), and (311). This reflection shows a face-centered cubic crystal structure (FCC) (Liu et al., 2021; Prabhu et al., 2022).

The crystal size of Cu nanoparticles is estimated using the Scherrer equation, $D = K\lambda/ \beta \cos\theta$, where d is the average crystal size, K is the Scherrer constant (0.9), λ represents the wavelength of the X-ray, β is the full width at the maximum half (FWHM) of the diffraction peak, and θ is the Bragg angle (Fentie et al., 2022; Mobarak et al., 2022). Based on these calculations, the average crystal size of CuNPs is determined to be about 15.5 nm. These results are in line with those reported in previous studies, CuNPs synthesized from *Ephedra alata* were reported to have a particle size of 15.21 nm (Atri et al., 2023).

The crystallite size calculated using the Scherrer is smaller than the particle size obtained from PSA analysis. This discrepancy indicates that each particle may consist of several crystalline domains or be coated with organic compounds from the extract (Velayati et al., 2025).

CONCLUSION

Copper nanoparticles (CuNPs) synthesized using chaya (*C. aconitifolius)* leaves extract were optimized using UV-Vis spectrophotometry, with the best absorbance observed at a 1:1 bioreductant-to-precursor volume ratio and pH 10. The resulting CuNPs exhibited a particle size of approximately 35.17 nm and a maximum wavelength at 325 nm. FTIR analysis confirmed the presence of characteristic Cu functional groups, XRD analysis showed the typical 2θ peaks for copper. Therefore, this study expands existing green synthesis approaches by introducing chaya (*C. aconitifolius*) leaves extract as a natural bioreductant for the synthesis, optimization, and characterization of CuNPs. The findings support the application of green chemistry principles and provide a scientific reference for the development of sustainable nanomaterials using widely available plant resources.

RECOMMENDATION

Further studies are suggested to evaluate the activity and potential applications of the synthesized copper nanoparticles. Additionally, optimizing other synthesis parameters and exploring different stabilizing agents could provide deeper insights into improving particle stability and performance.

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