

## Green Synthesis of Copper Nanoparticles Using *Syzygium polyanthum* Extract and Their Antioxidant Potential via DPPH Assay

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### Abstract

Copper nanoparticles (CuNPs) have gained attention for their cost-effective synthesis and potent antioxidant properties, offering a promising approach to address the bioavailability challenges of herbal medicines. This study investigates the green synthesis of CuNPs using bay leaf (*Syzygium polyanthum* (Wight.) Walp.) extract as a bioreductor and evaluates their antioxidant activity via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Bay leaf extract, rich in flavonoids and phenolic compounds, was prepared through heating extraction and mixed with copper nitrate ( $\text{CuNO}_3$ ) at varying ratios (1:1, 1:2, 1:3, 1:4). The formation of CuNPs was confirmed by a color change from yellow to greenish-brown after 24 hours, with particle sizes ranging from 527.48 nm to 3256.81 nm, as determined by Particle Size Analysis (PSA). UV-Vis spectrophotometry verified the reduction of  $\text{Cu}^+$  ions, with Cu concentrations decreasing post-synthesis. The antioxidant activity of CuNPs exhibited an  $\text{IC}_{50}$  value of 15.49 ppm, classified as very strong, compared to 72.09 ppm for the bay leaf extract alone (strong) and 13.82 ppm for vitamin C (very strong). These results demonstrate that bay leaf extract-mediated CuNPs possess enhanced antioxidant potential, highlighting the efficacy of green synthesis in producing bioactive nanoparticles for potential therapeutic applications.



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## Introduction

The utilization of herbal medicines is often hindered by the limited bioavailability of their active compounds, primarily due to their large molecular size and poor aqueous solubility, which impede effective penetration of lipid membranes in body cells (1,2). Consequently, many plant-derived compounds that exhibit promising in vitro bioactivity fail to translate into effective in vivo therapeutic outcomes (3). To address these challenges, nanotechnology has emerged as a transformative approach, with metal nanoparticles gaining significant attention for

their unique physicochemical properties and potential biomedical applications (4). Among these, copper nanoparticles (CuNPs) have garnered considerable interest due to their cost-effective synthesis, as well as their antimicrobial, antiviral, and antioxidant properties (5,6).

The synthesis of CuNPs can be achieved through various methods, including physical, chemical, and biological approaches (7). Traditional chemical and physical methods, while effective, often involve toxic reagents and high energy consumption, posing environmental and health risks (8). In response, the "green synthesis"

approach has emerged as an eco-friendly alternative, utilizing plant extracts as bioreductants to reduce metal ions into nanoparticles (9). This method not only minimizes environmental impact but also enhances nanoparticle stability and morphology, while conferring additional therapeutic benefits due to the bioactive compounds present in the extracts (10,11).

Bay leaf (*Syzygium polyanthum* (Wight.) Walp.), a widely available plant in tropical regions, is rich in bioactive compounds such as flavonoids, alkaloids, saponins, quinones, phenolics, triterpenoids, and steroids (12). Flavonoids, in particular, are polyphenolic compounds known for their antioxidant properties, which operate through hydrogen atom donation or metal chelation, neutralizing free radicals and mitigating oxidative stress-induced damage to cellular components such as DNA, proteins, and lipids (13,14). These compounds, present in bay leaf extract either as glycosides or aglycones, serve as effective bioreductants, making the plant a promising candidate for the green synthesis of CuNPs (15,16).

The antioxidant activity of CuNPs is a critical parameter for their potential biomedical applications, as oxidative stress is implicated in numerous pathological conditions, including cancer, cardiovascular diseases, and neurodegenerative disorders (17,18). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely adopted method for evaluating antioxidant activity due to its simplicity, cost-effectiveness, rapid execution, and reliable accuracy (19,20). By measuring the ability of a compound to scavenge DPPH free radicals, this method provides a robust assessment of antioxidant potential (21).

Previous studies have demonstrated the efficacy of plant-mediated CuNP synthesis using extracts from various species, such as *Azadirachta indica* and *Camellia sinensis*, highlighting the role of phytochemicals in stabilizing and functionalizing nanoparticles (22,23). However, limited research has explored the potential of

*Syzygium polyanthum* for CuNP synthesis and its associated antioxidant properties. Given the rich phytochemical profile of bay leaves and the advantages of green synthesis, this study aims to synthesize CuNPs using *Syzygium polyanthum* extract and evaluate their antioxidant activity via the DPPH method. This research seeks to contribute to the growing body of knowledge on sustainable nanoparticle synthesis and its applications in combating oxidative stress-related disorders.

## Method

### Materials

The equipment used in this study included: pipette bulb, stirring rod, beaker glass, blender, evaporating dish, funnel, Erlenmeyer flask, measuring cylinder, hot plate, volumetric flask, magnetic stirrer, oven, Particle Size Analyzer (PSA), graduated pipette, volumetric pipette, test tube rack and test tubes, spatula, UV-Vis spectrophotometer, and analytical balance.

The materials used in this study were distilled water (aquadest), hydrochloric acid (HCl), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), copper nitrate (CuNO<sub>3</sub>) 0.01 N, bay leaves (*Syzygium polyanthum* (Wight.) Walp.), hydrochloric acid (HCl) 10%, hydrochloric acid (HCl) 0.1 N, nitric acid (HNO<sub>3</sub>), Whatman filter paper No. 1, chloroform, methanol, ferric chloride (FeCl<sub>3</sub>) reagent 1%, Bouchardat reagent, Dragendorff reagent, Mayer reagent, Molisch reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent, and toluene.

The sample used in this study was green bay leaves (*Syzygium polyanthum* (Wight.) Walp.) collected from Binjai Utara District, Binjai City. The powdered bay leaves were extracted using the heating extraction method with distilled water. A total of 5 g of finely powdered bay leaf *simplicia* was added to 100 mL of distilled water, heated at 80 °C for 30 minutes, and stirred using a magnetic stirrer. The extract obtained was then filtered to separate the filtrate from the residue. The residue consisted of solid bay leaf material, while the

filtrate was a greenish-brown solution, which was used as the stock solution of the bioreductor.

### **Synthesis of Copper Nanoparticles from Bay Leaf (*Syzygium polyanthum*) Extract by the Reduction Method**

A total of 25 mL of bay leaf extract solution was diluted to 1000 mL. The diluted bay leaf extract solution and 0.01 M  $\text{CuNO}_3$  solution were then mixed at ratios of 1:1, 1:2, 1:3, and 1:4 to obtain a final volume of 50 mL. The mixture of plant extract and  $\text{CuNO}_3$  solution was subsequently stirred for 30 minutes. After stirring, the mixture was allowed to stand for 24 hours, during which a color change occurred and the formation of copper nanoparticles began, indicated by suspended nanoparticles. After 24 hours, copper nanoparticle precipitation was observed, characterized by a change in color to greenish-brown. The mixture was then briefly stirred using a magnetic stirrer and subsequently analyzed using a Particle Size Analyzer (PSA) to determine the particle size of the copper nanoparticles (24).

### **Particle Size Analyzer (PSA)**

The synthesized copper nanoparticles were characterized using Particle Size Analyzer (PSA) to determine particle size. The process began with centrifugation of the biosynthesis solution that had undergone a color change. The sample was then homogenized using a vortex mixer. A 3 mL aliquot of the copper nanoparticle solution was transferred into a cuvette, which was subsequently placed into the instrument and exposed to visible light, causing diffraction. The particle size distribution was analyzed based on the maximum particle size obtained in relation to the percentage volume of the sample. The distribution data were recorded on a computer connected to the instrument.

### **Antioxidant Activity Test Using the DPPH Method**

The antioxidant activity test using the DPPH method was carried out through several stages,

including: the principle of the DPPH free radical scavenging method, preparation of the DPPH stock solution, preparation of the blank solution, determination of the maximum absorption wavelength of DPPH, determination of operating time, measurement of DPPH absorbance, preparation of vitamin C standard solution, preparation of vitamin C concentrations, and measurement of antioxidant  $\text{IC}_{50}$  values.

## **Result and Discussion**

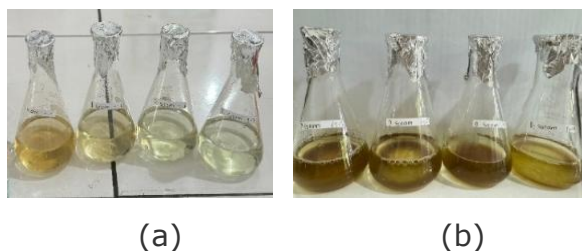
### **CuNPs from Bay Leaf Extract**

According to Wara at al., the formation of copper nanoparticles is indicated by a color change from yellow to greenish-brown (24). This color variation confirms the successful synthesis of copper nanoparticles from bay leaf extract. The solution was then briefly stirred using a magnetic stirrer, after which the copper nanoparticle solution was analyzed using a Particle Size Analyzer (PSA) to determine the particle size distribution.

The color change observed before and after the 24-hour resting period is shown in Figure 1. The figure clearly demonstrates a color transformation in the solution, confirming that bay leaf extract is capable of reducing  $\text{CuNO}_3$  at all ratios. The formation of copper nanoparticles was indicated by the reduction of  $\text{Cu}^+$  ions to metallic Cu. The appearance of a brown color verified the formation of nanoparticles, with the solution becoming increasingly darker brown and accompanied by precipitate formation over time. The final outcome of the copper nanoparticle synthesis was thus a dark brown solution.

During the synthesis process of copper nanoparticles (CuNPs) using bay leaf extract, a color change was observed when the aqueous extract was mixed with  $\text{CuNO}_3$  solution. The mixture was then stirred for 2 hours, during which a further color change occurred. This change provided strong evidence of CuNPs formation. The reduction of Cu ions by the bay leaf extract is likely facilitated by the presence of phenolic

compounds, which enable the reduction of  $\text{Cu}^+$  to  $\text{Cu}^0$ .



**Fig 1.** CuNPs Synthesis from Bay Leaf Extract (a) Before standing for 24 hours (b) After standing for 24 hours

To confirm the reduction of  $\text{Cu}^+$  ions into CuNPs, UV-Vis spectrophotometry was employed, and a standard curve was prepared using copper solutions at concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. The absorbance of each solution was measured at a wavelength of 324.8 nm. Using the operating time determined for the 4 ppm solution, a stable absorbance of 0.860 was observed between the 11th and 17th minutes during a 60-minute measurement period. Therefore, this time interval was considered optimal for measuring samples at various concentrations.

Before the reaction, the concentration of  $\text{CuNO}_3$  was 0.03. After the synthesis of copper nanoparticles, the concentration decreased as follows: 0.006 for the 1:1 ratio, 0.011 for 1:2, 0.009 for 1:3, and 0.015 for 1:4. This decrease in Cu concentration indicates that  $\text{Cu}^+$  ions were converted into copper nanoparticles. The reduction in Cu concentration is presented in Table 1.

**Tabel 1.**  $\text{Cu}^+$  Concentration Before and After Formation of CuNPs

| Ratio | $\text{Cu}^+$ Concentration Before (M) | $\text{Cu}^+$ Concentration After Conversion (M) |
|-------|----------------------------------------|--------------------------------------------------|
| 1:1   | 0,03                                   | 0,024                                            |
| 1:2   | 0,03                                   | 0,019                                            |
| 1:3   | 0,03                                   | 0,021                                            |
| 1:4   | 0,03                                   | 0,015                                            |

**Particle Size of CuNPs.** The particle measurement method using PSA is considered more accurate for determining particle size distribution. The particle size data obtained include three distributions: intensity, number, and volume, which together provide a comprehensive representation of the sample conditions.

**Tabel 2.** Particle size of CuNPs

| Various Formulations | Particle Size |
|----------------------|---------------|
| 1:01                 | 3256,81 nm    |
| 1:02                 | 2093,86 nm    |
| 1:03                 | 773,72 nm     |
| 1:04                 | 527,48 nm     |
| Bay Leaf Extract     | 1535,36 nm    |

The particle size trends for various formulations were analyzed to observe the particle size of copper nanoparticles synthesized from bay leaf extract. The ratio between the bioreductor (bay leaf extract solution) and the  $\text{CuNO}_3$  precursor significantly influenced the size of the copper nanoparticles. A higher ratio of bioreductor to copper nitrate, such as 1:1, resulted in reduced aggregation, producing smaller and more homogeneous nanoparticles with relatively uniform average diameters. According to Ningsih et al., nanoparticles are generally defined as having particle sizes below 1 micron (1000 nm), with sizes below 500 nm exhibiting superior characteristics (25).

### Antioxidant Activity of CuNPs

The antioxidant activity of bay leaf extract nanoparticles was measured at concentrations of 40, 60, 80, 100, and 120  $\mu\text{g/mL}$ . Each sample was then mixed with 200  $\mu\text{g/mL}$  DPPH solution and incubated for 15 minutes. The samples were incubated because the reaction proceeds slowly, requiring time for the sample to interact with DPPH free radicals. The reaction process is indicated by a color change of the bay leaf extract, from purple to yellow. This color change demonstrates that each concentration exhibits antioxidant activity. DPPH free radicals, which

possess an unpaired electron, display a purple color. When the electron becomes paired, the solution changes to yellow. The decrease in purple intensity to yellow occurs due to the scavenging of free radicals by the hydrogen atoms released from the sample molecules, forming diphenyl picrylhydrazyl compounds. This reaction causes the color to fade from purple to yellow, resulting in a decrease in absorbance with increasing sample concentration.

Based on Table 3, it can be concluded that the lower the concentration of the test solution, the lower the percentage of DPPH scavenging. The level of antioxidant activity is influenced by several factors, including the susceptibility of the compounds to degradation when exposed to oxygen, light, temperature, and drying processes.

**Tabel 3.** Free radical scavenging of CuNPs and Vitamin C

| Sample Solution                            | Concentration of the Sample Solution (ppm) | % Radical Scavenging |
|--------------------------------------------|--------------------------------------------|----------------------|
| Copper Nanoparticles from Bay Leaf Extract | 0 (Blanko)                                 | 0                    |
|                                            | 40                                         | 9,32%                |
|                                            | 60                                         | 17,72%               |
|                                            | 80                                         | 25,90%               |
|                                            | 100                                        | 36,58%               |
|                                            | 120                                        | 43,41%               |
| Bay Leaf Extract                           | 0 (Blanko)                                 | 0                    |
|                                            | 60                                         | 19,27%               |
|                                            | 100                                        | 25,22%               |
|                                            | 140                                        | 34,81%               |
|                                            | 180                                        | 58,02%               |
|                                            | 220                                        | 61,05%               |
| Vitamin C                                  | 0 (Blanko)                                 | 0                    |
|                                            | 1                                          | 30,91%               |
|                                            | 2                                          | 37,62%               |
|                                            | 3                                          | 50,27%               |
|                                            | 4                                          | 60,17%               |
|                                            | 5                                          | 77,88%               |

### IC<sub>50</sub> Value Analysis Results

Based on Table 4, it is shown that the antioxidant activity of copper nanoparticles synthesized using bay leaf extract has an IC<sub>50</sub> value of 15.49 ppm, categorized as very strong. The extract alone, without copper nanoparticles, has an IC<sub>50</sub> value of 72.09 ppm, categorized as

strong. As a reference, vitamin C (13.82 ppm) exhibited very strong antioxidant activity.

**Tabel 3.** Linear regression and IC<sub>50</sub> results of CuNPs and Vitamin C

| No. | Sample Solution                            | IC <sub>50</sub> (ppm) | Category      |
|-----|--------------------------------------------|------------------------|---------------|
| 1   | Copper Nanoparticles from Bay Leaf Extract | 15,49                  | Highly Potent |
| 2   | Bay Leaf Extract                           | 72,09                  | Potent        |
| 3   | Vitamin C                                  | 13,82                  | Highly Potent |

This demonstrates that the IC<sub>50</sub> value obtained for the extract alone, without copper, is classified as strong, whereas when combined with copper, it becomes very strong, with a difference of 56.6 ppm. The IC<sub>50</sub> value of CuNPs is higher because the extract is already combined or oxidized with Cu, reducing the effective concentration of the extract. Therefore, the extract incorporated into copper nanoparticles shows a higher IC<sub>50</sub> value compared to the extract alone without any treatment. A smaller IC<sub>50</sub> value indicates stronger antioxidant activity.

## Conclusion

The synthesis of copper nanoparticles (CuNPs) using bay leaf extract (*Syzygium polyanthum* (Wight.) Walp.) as a bioreductor proved effective, as evidenced by the color change from yellow to greenish-brown after 24 hours, indicating the successful formation of CuNPs through the reduction of Cu<sup>+</sup> ions. The antioxidant activity evaluation via the DPPH method revealed that the synthesized CuNPs exhibited a very strong antioxidant capacity with an IC<sub>50</sub> value of 15.49 ppm, closely comparable to vitamin C (13.82 ppm), which also demonstrated very strong activity. In contrast, the bay leaf extract alone displayed a strong antioxidant activity with an IC<sub>50</sub> value of 72.09 ppm. These findings highlight the potential of bay leaf extract-mediated CuNPs as a potent antioxidant agent, underscoring the efficacy of green synthesis in

producing bioactive nanoparticles with enhanced therapeutic properties.

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