

Synthesis and Characterization of Copper Nanoparticles with Bioreductor Carica Dieng (*Carica pubescens*) Seed Extract

Fifin Setiani, Suyatno*

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia

*E-mail: suyatno@unesa.ac.id

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Abstract: Secondary metabolite compounds in plants act as bioreductors in the metal reduction process and complement inorganic reductants. This study studied the characteristics of copper nanoparticles using Carica Dieng (*Carica pubescens*) seed extract. The synthesis of copper nanoparticles was carried out using the green synthesis method by reducing CuSO₄ 10 mM with distilled water extract from Carica Dieng (*Carica pubescens*) seeds. The Synthesis was carried out with the ratio of the composition of the extract and CuSO₄ solution 1:3 at pH 10. Nanoparticles were then characterized using UV-Vis spectrophotometer and PSA (Particle Size Analyzer) instruments. The characterization results using a UV-Vis Spectrophotometer showed a maximum absorption peak at a wavelength of 535 nm; the wavelength is included in the wavelength range of copper nanoparticles, which ranges from 500-700 nm. The particle size distribution analyzed using PSA shows an average size of 14.49 nm; this size is included in the range of nanoparticle sizes, which is between 1-100 nm, thus proving that nanoparticles have been successfully formed, the Poly Dispersity Index (PDI) value obtained is 0.1943 which indicates that the nanoparticle sample is categorized as homogeneous so that it has uniform size uniformity.

Keywords: Bioreductor; Carica Dieng Seeds; Copper Nanoparticles; Copper Sulfate.

Introduction

Nanotechnology is a widely developed technology because of its wide application in the biomedical and physiochemical fields [1]. Preparative material with a size of 1-100 nanometers, more commonly referred to as nanoparticles, is one of the results of nanotechnology that is being developed today due to its broad field of application. The fields of application of nanoparticles include medicine, biomedicine, sensors, antimicrobials, catalysts, electronics, agriculture, food, non-food, and health fields, and one of the benefits used in the pharmaceutical field is as an anti-bacterial [2-5]. Some applications of anti-bacterial properties are in food packaging, water management, and wound dressings [6-7]. Copper nanoparticles synthesized using plants are one of the widely developed metal nanoparticles [1]. This is due to the low cost of preparation compared to silver, gold, and platinum nanoparticles [8]. Copper nanoparticles also exhibit low toxicity, abundance, good environmental acceptance, and high antimicrobial activity through ROS generation [49].

Some methods used in the Synthesis of nanoparticles are chemical and physical [10]. However, these methods consume a lot of energy, time, and cost, use solvents that are harmful to the environment, and produce by-products that are harmful to the environment [11-15]. Therefore, an environmentally friendly nanoparticle synthesis method is needed to overcome the drawbacks of this method. Biological methods and green synthesis can be a better alternative to producing nanomaterials [16]. The advantages of this method are that it is non-toxic, cheap, uniform in nanoparticle size, and highly purified [12,14,17,18]. In the

green synthesis method, biological molecules are utilized as bioreductors. [19].

Because of its creation, there are two kinds of reductants: manufactured reductants and bioreductors. One illustration of a manufactured reductant can be utilized to incorporate nanoparticles, specifically NaBH₄. NaBH₄ is a poisonous compound [50]. This compound, if responded with water to deliver gas hydrogen (H₂), which is explosive and unstable whenever reacted with concentrated H₂SO₄ or concentrated HCl, will produce harmful diborane gas (B₂H₆) [41]. In this manner, using manufactured reductants can create an unpleasant waste climate. It is not quite the same as waste from utilizing non-unsafe bioreductors for the environment. Moreover, unrefined substances for making bioreductors are not challenging, and the cost is generally modest because it is broad in regular [50].

Synthesis of nanoparticles with the green synthesis method uses the help of plant extracts as bioreductors; bioreductors play a role in reducing metal ions to create nano sizes [51]. However, not all plant extracts can be utilized as bioreductors. This is because one of the requirements for plants is that they can be used as bioreductors. These namely plants contain secondary metabolite compounds such as alkaloids, flavonoids, phenols, tannins, and polyphenols [3].

Plant extracts can reduce metal ions as stabilizers [20,48]. This is due to the content of both primary and secondary metabolite compounds, such as alkaloids, amines, amides, proteins, carbonyl groups, terpenoids, and phenolics [21-22]. In the Dieng area, Wonosobo, Central Java, there is a plant commonly called Carica. Carica Dieng (*Carica pubescens*) belongs to the *Caricaceae* family [23]. Its area of origin is the Andean highlands of South America

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[24]. Carica fruit seeds contain flavonoids, tocopherols, terpenoids, alkaloids, saponins, papain enzymes, chymoprotein, and lysozyme [25-26]. The content of secondary metabolite compounds in Carica Dieng seeds functions as a stabilizing and reducing agent [27].

Many studies have used green synthesis methods in the Synthesis of copper nanoparticles, such as the use of Citrus medica Linn. fruit extract [28], *Cissus arnotiana* leaves [16], *Nigella sativa* seeds [29], *Neem* flowers [30], *Cuscuta reflexa* leaves [31], *Ziziphus spira-christi* fruit [32]. Research using Carica Dieng (*Carica pubescens*) seeds has not yet been found. Research using a combination of Carica Dieng (*Carica pubescens*) seed water extract has not been found and can be a novelty.

This research will synthesize copper nanoparticles using Carica Dieng (*Carica pubescens*) seed extract as a bioreductor and stabilizing agent. In addition, copper nanoparticles will also be characterized using a UV-Vis Spectrophotometer instrument to determine the maximum absorption wavelength of nanoparticles and a particle Size Analyzer (PSA) to determine particle size.

Research Methods

This research is experimental. The study was conducted at the Organic Chemistry Laboratory of Surabaya State University. The tools used in this research include a UV-Vis Spectrophotometer (Shimadzu UV-1800), PSA instrument (Biobase), analytical balance (Adventurer Ohaus), hot plate & magnetic stirrer (DLAB), Erlenmeyer 250 mL, beaker, volume pipette, measuring pipette, dropper pipette, test tube, stirring rod, spatula, and measuring cup. Meanwhile, the materials used in this study include carica fruit taken from Sembungan village, Wonosobo, Central Java, distilled water, sterile water, Copper (II) Sulfate Pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (Merck, Germany), Whatmann No. 1 filter paper.

Research Procedures

Extraction of Dieng Carica (*Carica pubescens*) Seeds

Carica seeds were separated from the pulp, collected, washed with distilled water, dried, pulverized with a blender, and stored in a closed container [33]. 25 g of powder was dissolved in 100 mL of de-ionized water and heated at 80°C for 30 minutes. The extract was filtered using the Whatmann No. 1 filter [34].

Phytochemical Screening

Phytochemical screening is the initial stage of identifying the content of a compound in simplisia or plants to be tested.

Tannin content test. 2 mL of 1% Ferric chloride solution was added to the extracts. A blue-green to black color indicates the presence of tannins. [35].

Flavonoid content test. 1 mL of extract was added with 3 mL of 70% ethanol. Then, the filtrate obtained was added with 0.1 g of Mg and 2 drops of concentrated HCl. The formation of a color change to orange indicates the presence of flavonoids [36].

Saponin content test. The extract was diluted with distilled water. The mixture was shaken vigorously. The formation of stable foam indicated the presence of saponins [37].

Terpenoid dan Steroid content test. A total of 2 mL of chloroform was added to 1 mL of extract and then with concentrated sulfuric acid (H_2SO_4). The formation of a pink or reddish brown /ring interface layer indicates the presence of terpenoid content. In contrast, the formation of a blue or bluish-green color suggests the presence of steroids [35].

Test for Alkaloid content. 2 mL of extract was heated, shaken, and filtered. The filtrate was put into 3 test tubes of 1 mL each, and 2N HCl was added, shaken, and allowed to stand. After that, a few drops of Dragendorf, Mayer, and Wagner reagents were added. The formation of each orange precipitate, white precipitate, and reddish brown precipitate indicates the presence of alkaloid content [37].

Synthesis of Copper Nanoparticles (CuNPs)

Synthesis of copper nanoparticles: 15 mL of distilled water extract of *Carica pubescens* was added to 45 mL of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 10 mM (1:3) and adjusted pH 10 by adding NaOH 0,1 M while stirring using a magnetic stirrer, for 30 minutes at 80 °C. Changes in straw yellow color to bluish-green indicate the formation of copper nanoparticles [34]. After synthesis, the solution is centrifuged at 8000 rpm for 30 minutes. Then, the residue obtained was oven at 100 °C for 4 hours until dry [6].

Characterization of Copper Nanoparticles (CuNPs)

Spektrophotometer UV-Vis

3 mL of copper nanoparticle solution was put into a cuvette; then, measurements were taken at a wavelength of 200-800 nm [38].

Particle Size Analyzer (PSA)

3 mL of CuNPs solution was put into the cuvette. Then, the cuvette is inserted into the PSA instrument and shot with visible light so that diffraction occurs. The reading results by PSA are then displayed in graphical form [39].

Results and Discussion

This study aimed to determine the characteristics of copper nanoparticles synthesized using Carica Dieng (*Carica pubescens*) seed extract, including maximum absorption wavelength and particle size.

Extraction of Dieng Carica (*Carica pubescens*) Seeds

The samples used in this study were Dieng carica seeds (*Carica pubescens*). Dieng carica seeds used in this study were obtained from Sembungan village, Dieng, Wonosobo, Central Java, in January 2023. The Dieng carica seeds obtained weighed ± 10 kg in wet conditions.

Dieng carica seed samples that will be used are first cleaned from the membranes attached by washing and soaking with water. The purpose of washing and soaking is to separate the white membrane covering the seeds [40]. Dieng carica seeds are then dried by aerating at room temperature. Drying at room temperature aims to reduce the water content while maintaining the quality of the simplisia and can be stored for further applications [40]. Then, the sample was ground using a blender, and a fine powder of brown Dieng carica seeds was obtained. This grinding process aims to minimize and damage the cell walls of

Carica Dieng seeds so that the secondary metabolite compounds in them quickly come out [40]. Figure 1. shows the stages of preparation of *simplisia* to become powder.



Figure 1. a) Wet carica seed sample b) Dried carica seed sample c) Carica seed powder

Phytochemical Screening

Phytochemical screening on Carica seed extract is essential to identify bioactive compounds contained therein [41]. The results of the phytochemical screening test on Carica Dieng seed *simplisia* can be seen in Table 2.

Table 2. Phytochemical Screening Test Results of Carica Dieng Seeds

Phytochemical Screening	Reagent	The Resulting Color	Results
	Reagen Dragendroff	Orange colored precipitate	+
Alkaloid	Reagen Mayer	The solution is white without sediment.	-
	Reagen Wagner	The residue is reddish brown.	+
Flavonoid	Etanol 70% + serbuk Mg + HCl pekat	Orange colored solution	+
Saponin	Aquades dan dikocok secara kuat	Stable foam	+
Steroid	Pereaksi Liebermann-Bouchard	The solution is reddish brown	-
Terpenoid	Pereaksi Liebermann-Bouchard	The reddish-brown solution between surfaces	+
Tanin	FeCl ₃ 1%	The solution is blackish-blue	+

Note: (+) positive (-) negative

The results of the phytochemical screening of Carica seed water extract in this study showed positive alkaloid, flavonoid, saponin, terpenoid, and tannin compounds. Water is a polar solvent, and secondary metabolite compounds with polar functional groups such as alkaloids, flavonoids, saponins, terpenoids, and *tannins*, can still be extracted. Other studies with water solvents are also able to extract phenolic compounds, polyphenols, and glycosides can be extracted [42]. Research conducted by Supono *et al.* (2014) showed that 70% ethanol extract of Carica seeds positively contained terpenoid compounds, saponins, and alkaloids. In contrast, research by Wijayanti & Febrinasari

(2013) showed that 70% ethanol extract of Carica seeds positively contains alkaloids, flavonoids, saponins, terpenoids, and tannins.

Synthesis of Copper Nanoparticles (CuNPs)

Figure 2 illustrates the synthesis of copper nanoparticles pH 10 conditioned at a solution composition of 1:3 (extract: 10 mM CuSO₄.5H₂O solution) at 80 °C, 30 minutes. The content of secondary metabolite compounds such as alkaloids, flavonoids, saponins, triterpenoids, and tannins in the aqueous extract of Dieng Carica seeds (*Carica pubescens*) acts as a reductant and stabilizer in the formation of copper nanoparticles. Flavonoid and phenolic secondary metabolite compounds act as reductants, while saponin, tannin, and triterpenoid compounds act as capping agents [43].



Figure 2. a) Carica seed extract b) Copper nanoparticle solution

It has been hypothesized that copper ions can be reduced to form copper nuclei, or CuNPs, by reactive hydrogen atoms in flavonoids released during the tautomeric transition from the enol form to the keto form (Figure 3) [44].

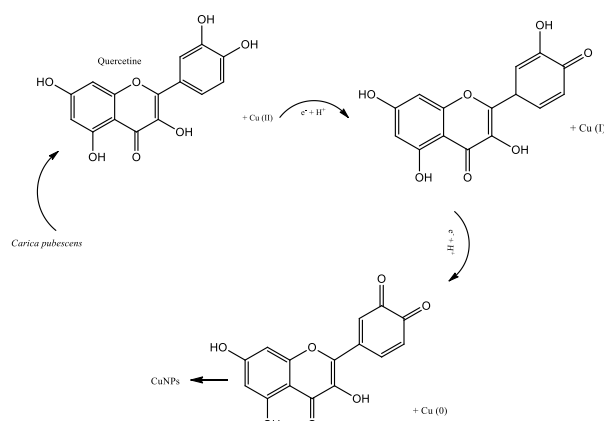


Figure 3. Mechanism of Cu ion reduction by quercetin compounds

Characterization of Copper Nanoparticles (CuNPs) Spektrofotometer UV-Vis

Initial characterization of copper nanoparticle synthesis was performed with a UV-Vis Spectrophotometer to determine the maximum absorption wavelength. Based on Figure 3. shows a maximum peak of 535 nm, which is the wavelength of copper nanoparticles. Wavelengths between 500-600 nm have been reported for synthesizing copper nanoparticles using the green synthesis method [44] [45].

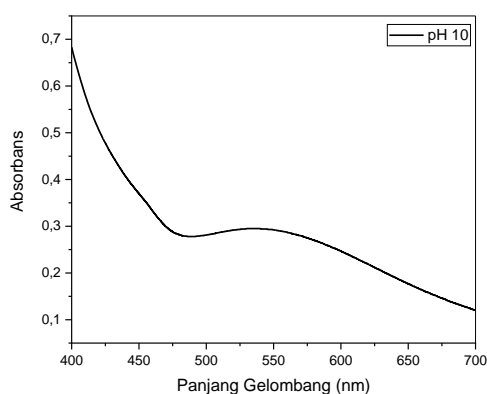


Figure 4. The UV-Vis spectrum of synthesized copper nanoparticles

Particle Size Analyzer (PSA)

The average size of the synthesized copper nanoparticles PSA confirmed their particle size distribution. Figure 4. is a histogram of the size distribution of copper nanoparticles. The results of the PSA analysis showed the presence of particles with a size range of 14.49 nm. According to [47], particles with a diameter of 1-100 nm are acceptable as nano-sized carrier substances that can be used in various applications. The Poly Dispersity Index (PDI) value obtained was 0.1943, indicating that the sample is categorized as homogeneous and has a uniform size level. PDI values less than 0.3 are classified as monodisperse [48]. The results of this study are supported by other studies that have successfully synthesized copper nanoparticles with the green synthesis method from *Eryngium caucasicum* Trautv leaf extract, which obtained nano copper with a size of less than 40 nm [47]. Meanwhile, from the *Citrus medica* Linn fruit extract, nanoparticles with a size of 10-60 nm were obtained [28].

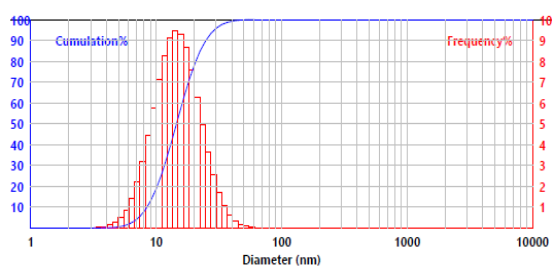


Figure 5. Results of characterization of copper nanoparticles using PSA

Conclusion

In this study, green Synthesis of copper nanoparticles using aqueous extract of Dieng Carica seeds (*Carica pubescens*) was carried out for the first time. Flavonoid and phenolic compounds contained in the aqueous extract of Carica seeds act as reductants and capping agents in the Synthesis of copper nanoparticles. Synthesis of copper nanoparticles in optimal conditions obtained λ_{max} 535 nm under pH 10 conditions, the concentration of $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ is 10 mM with the extract ratio: $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ solution (1:3) in 60 mL. Particle size analysis using PSA obtained an average size of copper nanoparticles of 14.49 nm with a PDI value of 0.1943.

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