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SYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES USING CARICA DIENG (*Carica pubescens*) SEED EXTRACT AS A BIOREDUCER AND TESTING THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

This study aims to create copper nanoparticles using a synthesis approach called the green synthesis method. In this method, copper sulfate pentahydrate ($CuSO_4.5H_2O$) is combined with Carica Dieng (*Carica pubescens*) seed extract, at 80 ºC, for 30 minutes. The materials were analyzed using techniques including UV-Vis spectrophotometer, FTIR, PSA, SEM, and XRD. The UV-Vis spectrum displayed absorption at a wavelength of 535 nm while FTIR analysis revealed a peak at 614 cm^{-1}. That corresponds to Cu-O bond stretching. The Cu-NPs had a size of 14.49 nm, from the SEM morphology. The X-ray diffraction (XRD) analysis confirmed the presence of crystals with 2θ peaks at 31.77° and 44.66°. Antibacterial properties were tested using *Staphylococcus aureus* and *Escherichia coli* in a paper disc diffusion assay showing inhibition zones for both bacteria species. Cu-NPs demonstrated effects with a 15.84 mm zone against *E. coli* and moderate effects with a 9.84 mm zone, against *S. aureus*.

Keywords: Antibacterial, Carica Dieng, *Carica pubescens*, Copper Nanoparticles, Green Synthesis
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INTRODUCTION

Recently, nanotechnology has experienced rapid development. This is due to its extensive application across various fields, including biomedicine and physico-chemistry, as well as its flexibility to combine with other technologies. Nanotechnology applications span sectors such as medicine, agriculture, marine science, and industry.[1] Nanomaterials ranging in size from 1-100 nanometers, commonly referred to as nanoparticles, are a major focus of current developments in nanotechnology.[2] Copper nanoparticles have garnered new interest due to their strong antibacterial properties against various microorganisms, including those resistant to multiple drugs.[3] One of the biggest health issues today is infectious diseases. Antibacterials are used to treat various infections, as they inhibit bacterial growth. However, the uncontrolled use of antibacterials can lead to bacterial resistance, which poses significant challenges in treating infectious diseases. As bacterial resistance to antibiotics increases, efforts are needed to discover alternative therapies, such as plant-based traditional medicines capable of killing bacteria such as copper nanoparticles derived from plant extracts.[4] The size and shape of nanoparticles influence various levels of antibacterial performance due to factors such as large surface area and copper ion release.[5] Therefore, efforts are needed to control the size and shape of nanoparticles through various synthesis methods. Some commonly used methods for nanoparticle synthesis include chemical and physical approaches.[6] However, these methods are time-consuming and costly, use solvents that are harmful and toxic to the environment, and produce hazardous byproducts.[7,8] Consequently, an environmentally friendly nanoparticle synthesis method is required to address these drawbacks. Biological methods, such as green synthesis, offer a better alternative for nano-material production. This method's advantages include non-toxicity, cost-effectiveness, uniform nanoparticle size, and high purity levels.[10,11] In green synthesis, biological molecules are utilized as bioreductants.[12] Plant extracts have the ability to reduce metal ions and act as stabilizers.[13] This is due to the presence of both primary and secondary metabolite compounds such as alkaloids, amines, amides, proteins, carbonyl groups, terpenoids, and phenolics.[14,15] Numerous studies have utilized green synthesis methods for the synthesis of copper nanoparticles, employing extracts from *Citrus medica* Linn. fruit[16], *Cissus arnotiana* leaves[9], *Nigella sativa* seeds[17], Neem flowers[18], *Cuscuta reflexa* leaves[19], and

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Ziziphus spina-christi fruit.[20] However, research using the aqueous extract of Carica Dieng (*Carica pubescens*) seeds has not been found so far, making it a potentially novel approach. This study will synthesize copper nanoparticles using Carica Dieng (*Carica pubescens*) seed extract as both a bioreductant and stabilizing agent. Additionally, the copper nanoparticles will be characterized using UV-Vis Spectrophotometer, Fourier Transform InfraRed (FTIR), Particle Size Analyzer (PSA), Scanning Electron Microscopy Energy Dispersive X-ray Spectroscopy (SEM), and X-ray diffractometer (XRD). Antibacterial activity tests will also be conducted against *Staphylococcus aureus* and *Escherichia coli*.

EXPERIMENTAL

Material and Methods

Sterile water, $CuSO_4.5H_2O$ (Merck), NaCl (Merck), dish paper, filter paper, Mueller Hinton Agar CM0337 (OXOID), ciprofloxacin, *Staphyloccocus aerus* (ATCC 25923) serta *Eschericia coli* (ATCC 25922), and Carica Dieng (*Carica pubencens*) seed.

Plants Sample

The seeds of *C. pubencens* are separated from the fruit pulp and then washed with distilled water to remove impurities and air-dried. Then, they are ground into a powder. A total of 25 grams of the powder of *C. pubencens* seed is combined with 100 mL of demineralized water and heated to approximately 80°C for 30 minutes using a hotplate stirrer. Once cooled, the extract is filtered using a Buchner funnel.

Synthesis of Cu-NPs

A total of 45 mL of $CuSO_4.5H_2O$ solution is mixed with 15 mL of Carica Dieng (*C. pubescens*) seed extract drop by drop while stirring with a hotplate stirrer. The solution is stirred for 30 minutes at 80°C and then allowed to stand for 24 h. The solution is then centrifuged for 30 minutes at 8000 rpm. The resulting precipitate is washed with distilled water and dried in an oven at 80°C for 3 hours until dry. The resulting Cu-NPs were further characterized using a UV-Vis spectrometer (Shimadzu UV-1800), FTIR (Shimadzu IR-Prestige-21), PSA (Biobase), SEM (Hitachi SEM S-4500), and XRD (Shimadzu XRD-6000).[21]

Antibacterial Test

Antibacterial activity is conducted using the agar disk diffusion method. Pure cultures of *Staphylococcus aureus* and *Escherichia coli* are evenly spread on Mueller Hinton Agar (MHA) media. A 6 mm diameter paper disk is aseptically immersed in Cu-NPs. The Petri dishes are then incubated at 36°C for 24 hours, and the inhibition zone is measured (in millimeters) using a caliper.[22]

RESULTS AND DISCUSSION

UV-Visible Spectroscopy Analysis

The analysis using a UV-Vis spectrophotometer is conducted in the range of 200-800 nm to determine the absorbance of the synthesized Cu-NPs. The color change of the solution to blue-green indicates that reduction has begun and Cu-NPs have formed.[23] The characteristic absorbance peak for copper nanoparticles is in the wavelength range of 500-700 nm.[17] In this study, the results of the UV-Vis spectrum can be seen in Fig.-1.

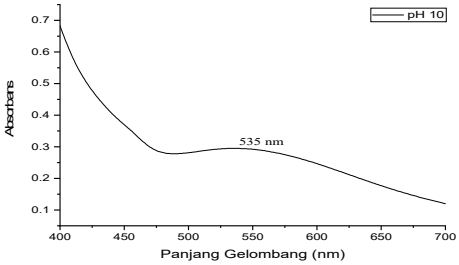


Fig.-1: UV-Vis Spectrum of Synthesized Copper Nanoparticles

Based on Fig.-1 copper nanoparticles have formed after mixing the $CuSO_4.5H_2O$ solution and the aqueous extract of Carica seeds, as indicated by the peak appearing at a wavelength of 535 nm. Previous studies have shown that Cu-NPs are spherical in shape with an absorption band in the UV-Vis Spectrophotometer around 450-600 nm. This absorption is assumed to correlate with relatively small-sized Cu-NPs.[24] The SEM images from this study confirm an almost spherical shape with slight agglomeration.

Fourier Transform Infra-Red Spectroscopy Analysis (FTIR)

The FTIR spectrum of the copper nanoparticles shows peaks at wave numbers 3278.37 cm^{-1}, 1651.43 cm^{-1}, 1541.00 cm^{-1}, 1410.96 cm^{-1}, 1080.79 cm^{-1}, and 616.41 cm^{-1} (Fig.-2). The absorption peak at 3278.37 cm^{-1} indicates O-H bond stretching vibrations. The absorption at 1651.43 cm^{-1} shows C=O carbonyl stretching vibrations, while 1541.00 cm^{-1} is associated with aromatic C=C stretching vibrations, and 1410.96 cm^{-1} indicates alkyl C-H bending vibrations. The peak at 1080.79 cm^{-1} shows C-O vibrations, and the presence of an absorption peak at 616.41 cm^{-1} indicates Cu-O stretching in the Cu_2O phase.[25,26]

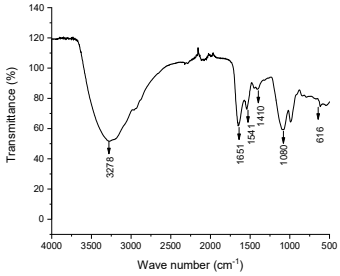


Fig.-2: Characterization Results of Synthesized Copper Nanoparticles by FTIR

Particle Size Analyzer (PSA)

Figure-3 shows a histogram of the size distribution of copper nanoparticles, indicating that the synthesized Cu-NPs have a relatively varied diameter with an average diameter of 14.49 nm. Particles with diameters of 1-100 nm are acceptable as nano-sized carriers suitable for various applications.[27]

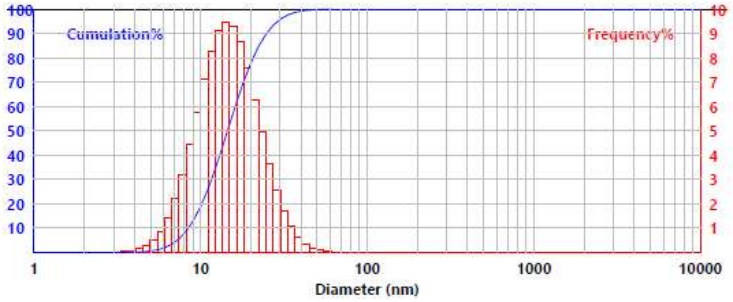


Fig.-3: Characterization Results of Synthesized Copper Nanoparticles by PSA

Scanning Electron Microscopy Analysis (SEM)

The synthesized nanoparticles were analyzed using Scanning Electron Microscopy (SEM), revealing the formation of nanoparticles within the nanometer range below 100 nm. It has been observed that copper nanoparticles from Carica Dieng (*Carica pubescens*) seed extract are nearly spherical and appear agglomerated, as shown in Fig.-4.[25,28]

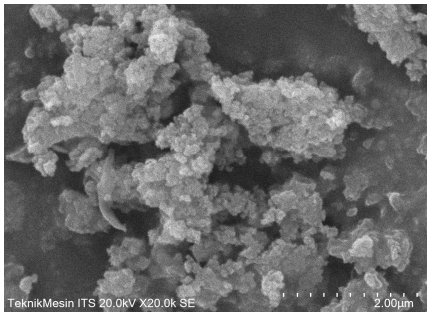


Fig.-4: Characterization Results of Synthesized Copper Nanoparticles by SEM

X-Ray Diffractometers (XRD)

The diffractogram of copper nanoparticles shows peaks at diffraction angles (2θ) that correspond to the standard Cu_2O diffraction data published by the International Centre for Diffraction Data (ICDD, file No. 01-078-2076) at 31.77° and 44.66°. The theoretical analysis of these diffraction peaks indicates Miller indices of {111} and {200} for each peak. Crystal size analysis of the copper nanoparticles using the Debye-

Scherrer equation yielded crystal sizes ranging from 17.2219 to 20.8912 nm, with an average nanoparticle size of 19.0566 nm.[29,30]

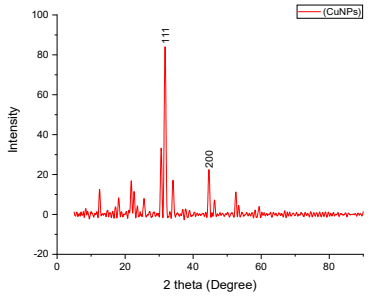


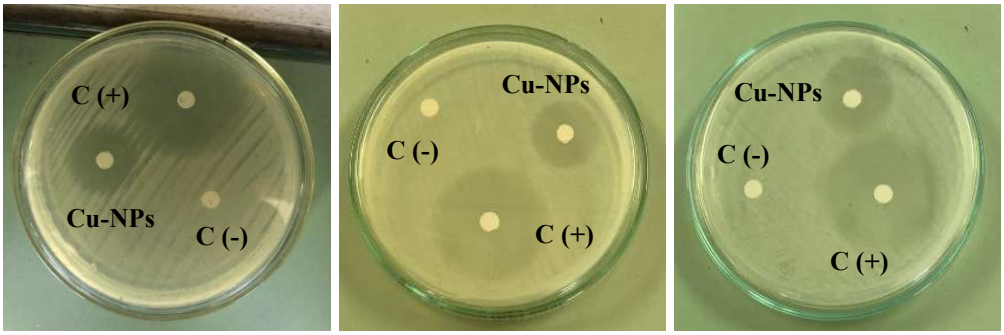
Fig.-5: Characterization Results of Synthesized Copper Nanoparticles by XRD

Antibacterial Activity Test against *E. coli* and *S. aureus* Bacteria

The antibacterial testing of mangosteen peel extract and copper nanoparticles was conducted by measuring the inhibition zone produced around the paper disc. The formed inhibition zone was measured using a caliper.

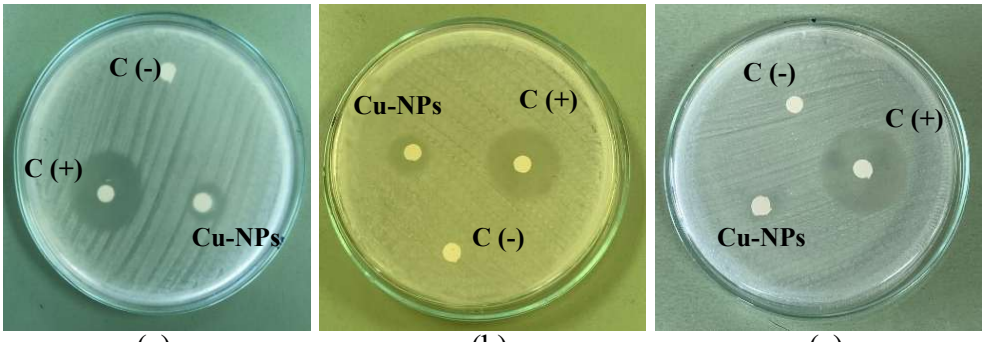
Table-1: Results of Bacterial Inhibition Zone Measurements

Types of Test Bacteria	Diameter of Inhibition Zone (mm)			Average Diameter (mm)	Inhibition Response of Growth
	I	II	III		
Escherichia coli	15.90	15.78	15.84	15.84	Strong
Staphyloccocus aureus	6.20	6.12	5.60	5.98	Moderate



(a) (b) (c)

Fig.-6: Antibacterial Activity Test Against *Escherichia coli* (a) First Replication (b) Second Replication (c) Third Replication. C(+): Positive control, C(-): Negative control



(a) (b) (c)

Fig.-7: Antibacterial Activity Test against *Staphylococcus aureus* (a) First Replication (b) Second Replication (c) Third Replication. C(+): Positive control, C(-): Negative control

The antibacterial mechanism of metal nanoparticles is due to the disruption of the helical structure of bacterial DNA by the interaction with Cu-NPs. The interaction of Cu-NPs with the bacterial cell membrane results in a decrease in transmembrane electrochemical potential, which affects membrane integrity. It is assumed that metal ions in the metal NPs are released. Copper nanoparticles and copper ions accumulate on the bacterial cell surface and create pores in the membrane, allowing cellular components to exit and enter the cell, leading to oxidative stress and cell death.[9,31]

CONCLUSION

Cu-NPs were successfully synthesized using the bioreductor from *Carica pubescens* seed extract, indicated by a color change to bluish-green, signaling the reduction process of Cu^{2+} to Cu^{0}. The maximum absorption wavelength observed by UV-Vis spectroscopy resulted in a peak at 535 nm. FTIR analysis showed the stretching of the Cu-O bond at a wavelength of 614 cm^{-1}. The average particle size was found to be 14.47 nm, as observed by PSA. SEM and XRD analyses revealed that the synthesized copper nanoparticles have a slightly rounded morphology with a cubic crystal system. Additionally, the synthesized copper nanoparticles exhibited strong antibacterial activity against *Escherichia coli* (15.84 mm) and moderate activity against *Staphylococcus aureus* (5.98 mm).

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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