Biogenic synthesis of CuO nanoparticles using Couroupita guianensis petal, stem, bark and leaves extract: Characterization and antibacterial applications

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Abstract

This article reports on a new rout to synthesize of copper nanoparticles with aqueous extracts from Couroupita guianensis Aubl. This is an environmentally friendly, inexpensive and time-saving method of producing nanoparticles. The aqueous extracts of petals, stems, bark and leaves were used to synthesize the nanoparticles. Plant extracts induce the reduction of Cu2 + ions into CuNP and act as protection and stabilization agents. The formation of CuNPs was monitored throughout the synthesis process by absorption spectra from the UV-Vis spectrophotometer. Fourier transfer infrared (FTIR) and Scanning Electron Microscope (SEM) were used to characterize the synthesized nanoparticles. The new synthesized nanoparticles exhibited good antibacterial activity against *Bacillus Subtilis* and *Escherichia coli*. The antibacterial properties have also proven to be a good result for bacteria reduction with using copper nanoparticles as a low cost-effective production for sustainable applications from Couroupita guianensis Aubl extract.

Keywords: Copper nanoparticles, Couroupita guianensis, UV-Vis, FTIR, X- ray, SEM antibacterial activity

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1. Introduction

Nanoparticles have become part of our life in cosmetics, drug delivery systems; Therapeutic, biosensor, and pharmaceutical materials applications can be harnessed to dramatically enhance important material properties. A discipline of nanotoxicology would play an important role in advancing safe and sustainable nanotechnology.

Advances in organizing nanoscale structures into predefined superstructures ensure that nanotechnology will have a decisive role in many technologies. Nanoparticles are of more great interest due to their extremely small size and large surface-to-volume ratio. The synthesis of copper nanoparticles were achieved physically, chemically and biologically [1]. Biosynthesized of nanoparticles will have improved constancy, biocompatibility with reduced the toxicity [2]

Copper nanoparticles have gained more concentration due to their high electrical conductivity, high melting point, low electrochemical migration and it is a cheaper metal compared to other metals as silver, gold, platinum and palladium. An additional advantage of copper nanoparticles is that they oxidize to form copper nanoparticles and are stable in chemically and physically properties.

Green nanotechnology became an interesting field in which functional nanoparticles were prepared from iron, zinc, copper and gold without the use of dangerous toxic chemicals. The green synthesis of nanoparticles is seen as a more significant and cost-effective process with a particular focus on the approach to protecting the environment and the ecosystem. The biosynthesis of nanoparticles through natural resources such as plant extracts [3-10], bacteria, fungi [11], enzymes, algae, etc. is an emerging area. Natural resources are enriched with several secondary metabolites that could be used to synthesize nanoparticles, providing benefits such as energy literacy, cost efficiency and environmentally friendly chemicals. In particular, biosynthesized NPs have good medicinal properties compared to NPs synthesized using traditional methods. Biosynthesized nanoparticles have significant antimicrobial, antioxidant, antimalarial, anti-inflammatory, anti-diabetic, and anticancer of C. guianensis Aubl [12-23]. Therefore, this study applied on the use of the active ingredients from C. guianensis extract for the biosynthesis of copper nanostructures for antimicrobial activity. Modern developments and applications of nanotechnologies provide many researches and industries processes that are actively involved in the production, preparation and discovered unique of new materials as well as numerous applicable in every major industry. There is a considerable potential for profitable applications for the future. Nanoparticles have received significant attention as an emerging class of nanomaterials used in different field.

Fig 1 Couroupita guianensis Aubl

2. Materials and methods

2.1. Collection of plants and Chemicals

Copper sulphate [CuSO4] was purchased from Aldrich. Fresh and healthy leaves, stems, petals and bark of C. guianensis were collected in the state of Tamilnadu State, India. After washing thoroughly, the petiole, petals and bark were scraped and dried in the shade for 5-7 days, and then dried and ground to a fine powder using a commercial stainless steel electric mixer.

2.2. Preparation of plant extracts

The extract was prepared by mixing 10 g of powdered leaves, stems, petals and barks in 100 ml of deionized water and boiled at 60°C for 30 minutes and filtered using Whatman No. 1 filter paper to remove the deposits. The extract was stored at 4°C for further experiments work.

2.3. CuNPs synthesis using C. guianensis extract

For the synthesis of CuNPs, 0.25 g of copper sulfate [CuSO4] was dissolved in 50 ml of distilled water; 4 ml of each C. guianensis extract was added dropwise and stirred for 10 minutes using a magnetic stirrer until a deep green color formed. The reaction mixture was further stirred at 60 ° C for 1-2 hours. The resulting reaction mixture was kept at room temperature (12-14 hours) and centrifuged at 10,000 rpm for 10 minutes. The resulting precipitates were rinsed with deionized water, followed by washing with absolute ethanol (2-3 times) to remove residual impurities, then the precipitates were dried in an oven about 70°C for 10 hours.

2.4. Characterization of fabricated CuNPs

Synthesized CuNPs were characterized by various conventional modern techniques. The optical properties of synthesized CuNPs were examined with a Shimadzu spectrometer (model UV 2600) in the wavelength range from 200 nm to 400 nm. The XRD measurement of CuNPs was performed on an XPERT-PRO operating at 30 mA and 40 kV at a 2θ angle pattern using monochromatic Cu-Kα radiation. The scanning was carried out in the range of 20°-80 °C and the crystal size was calculated from the width of the XRD peaks using the Scherrer's formula $D = 0.94\lambda / \beta\cos\theta$. Here, D is the average crystalline domain size at right angles to the resulting planes, is the X-ray wavelength (1.540 A °), β is the half-maximum of the full width, and θ is the diffraction angle. The size and morphology of copper nanoparticles were examined with a scanning electron microscope (Sigma HV-Quantan 200-Z10EDS) at 20 KV.

2.5 Antibacterial activity assay

The tested compound was prepared for their *in vitro* antibacterial activities, which are carried out against two bacterial strains *Bacillus cereus and Escherichia coli*. The results showed the antimicrobial activity ranging from moderate to good activities.

The antibacterial activity was carried out using the diffusion plate method. This was done using the agar well diffusion technique. The bacteria were kept on Czapek-Dox agar medium. The agar media were inoculated with various microorganisms. The diameter of the inhibition zone (mm) was measured for bacteria after 24 hours of incubation at 30°C. A filter paper sterilized disc saturated with measured quantity (25 µl) of the sample (1 mg/ml) is placed on a plate (9 cm diameter) containing a solid bacterial medium Czapek-Dox agar medium which has been seeded with the spore suspension of the test organism. After incubation at 30^{o}C for 24 h for bacteria the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm) / plate diameter x 100). The antibacterial activity of the tested compound was examined with gram positive bacteria, *Bacillus cereus*, and *gram negative bacteria Escherichia coli* (24-27).

3. Results and Discussion

3.1. X-ray Diffraction analysis

The formation of CuNPs is confirmed by powder X-ray diffraction (XRD). The XRD pattern of CuNPs powder is shown in (Fig. 2). All diffraction peaks are well indexed on the face-centered cubic area. The (FFC) crystalline structural phases of copper that works well with the JCPDS card No. 89-2838. All peaks positions agree with metallic copper. The sharp peaks of the XRD pattern indicate the crystalline nature of the product.

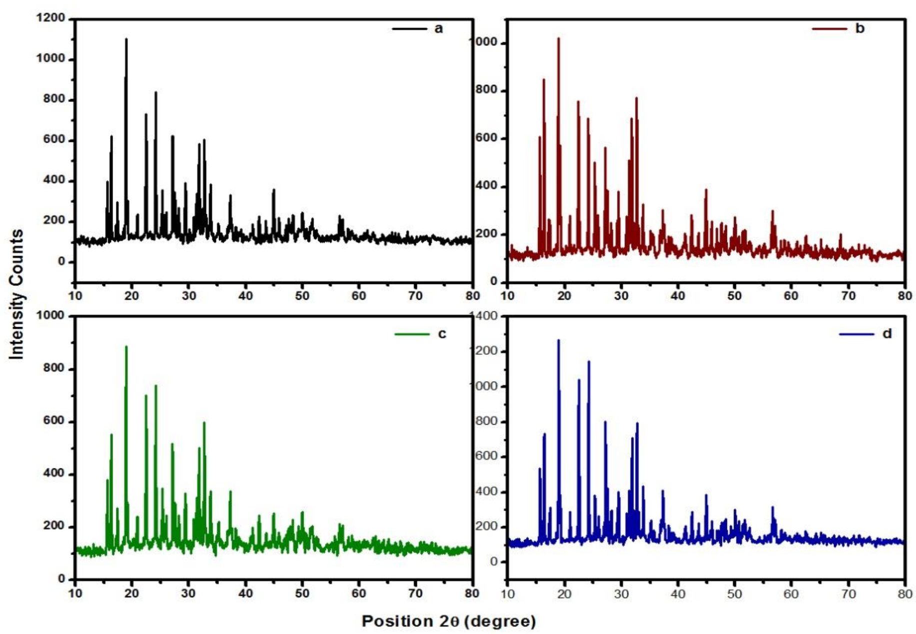

Fig. 2. XRD patterns of powdered CuNPs of C. guianensis (a). Flower Petals (b). Stem (c) Bark and (d) Leaves

3.2. Particle size distribution

In accordance with Fig. 4, the particle size distribution curve of CuNPs using plant extracts from Couroupita Guianensis Aubl has confirmed that the mean diameter size of these nanoparticles is 1.3-188 nm for petals and stems, 3.5-188 nm for bark and 1.3 nm for the case of leaves.

3.3. UV-visible absorption spectra of CuNPs

UV analysis was used to quickly confirm the formation and stability of CuNPs, as the plasmon peak of Cu is sensitive to the size and shape of the resulting NPs. The UV spectrum of biosynthesized CuNPs was measured (Fig. 3), typically; broad peaks at higher wavelengths indicate an increase in particle size, while a narrow peak at shorter wavelengths indicates the formation of smaller CuNPs. A sharp and relatively narrow absorption between 260 nm and 380 nm was observed. The leaves and petal extract turned pale yellow when administered with 1 mM copper sulphate solution within 24 hours, but stem and bark extracts changed color while the reaction mixture was heated for 10 to 15 minutes. The reaction mixture with aqueous extract from leaves and bark exhibited a maximum absorption spectral peak at 260 to 380 nm, stem and flower at 270 to 320 nm.

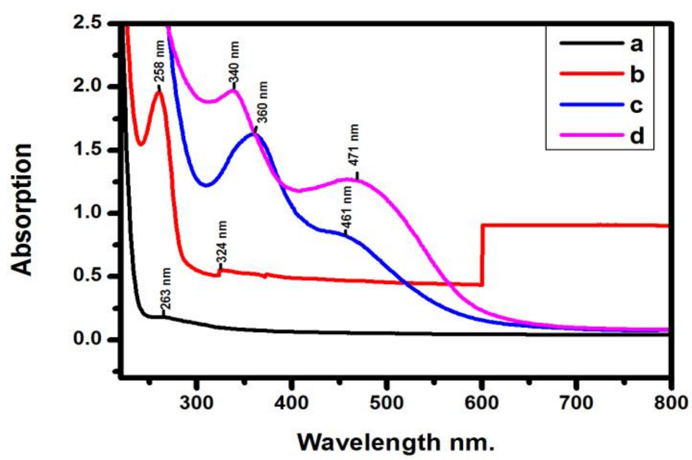

Fig.3. UV visible spectrums of CuNPs made from couroupita guianensis (a). Flower Petals (b). Stem

3.4. FTIR analysis

The synthesis solution of CuNPS each gave molecules, and some of them are adsorbed on the surface of CuNPs. FTIR analysis was performed to further demonstrate the successful conjugation of some of the molecules associated with CuNPs. (Fig. 4) shows the overlap of the FTIR spectra of plant extracts from Couroupita Guianensis Aubl and bio-reduced CuNPs.

The absorption peaks observed at 1650 and 3353 contribute to the flexural and stretching vibration of the moisture content on the surface of CuNPs. Some excellent absorption bands for petal extract have been appeared at 3196, 2452, 2079, 1650, 1102 and 871 cm^{-1}.

Main peaks at 3196 cm^{-1} (OH stretching of the phenol group), 2452 cm^{-1} (O=C=C-stretching), 2079 cm^{-1} (N= C= S-stretching), 1650 cm^{-1} (C=N Stretching), 1102 cm^{-1} (CO stretching) and 871 cm^{-1} (C=C-bending) Some known absorption bands for strain extract of CuNPs were observed at approximately 2738, 2409, 1763, 1375 and 831 cm^{-1}.

Synthesized CuNPs exhibited main peaks of 2738 cm^{-1} (aldehyde C-H stretching). 2409 (O = C = C stretching), 1763 (C = O stretching), 1375 (aldehyde C-H stretching), and 831cm $^{-1}$ for (C = C bending). Synthesized CuNPs using bark extracts show the peaks at 3655 and 3424 cm^{-1} (OH stretching of the phenolic group), 2346 cm-1 (O=C=O stretching), 1475 cm^{-1} for (OH bending), 1116 cm^{-1} (CO stretching) and 793 cm^{-1} for (C=C bending).

FTIR analysis of CuNPs using leaf extract produces strong peaks at 3353 cm^{-1} (OH stretching of the phenolic group), 2762 cm^{-1} (CH stretching), 2386 cm^{-1} for (O=C=O stretching) , 2068 cm^{-1} (C= C= C-stretching), 1763 cm^{-1} (C=O-stretching), 1626 cm^{-1} (C=C-stretching), 1375 cm^{-1} (OH bending), 999 cm^{-1} and 897 cm^{-1} for (C=C-bending).

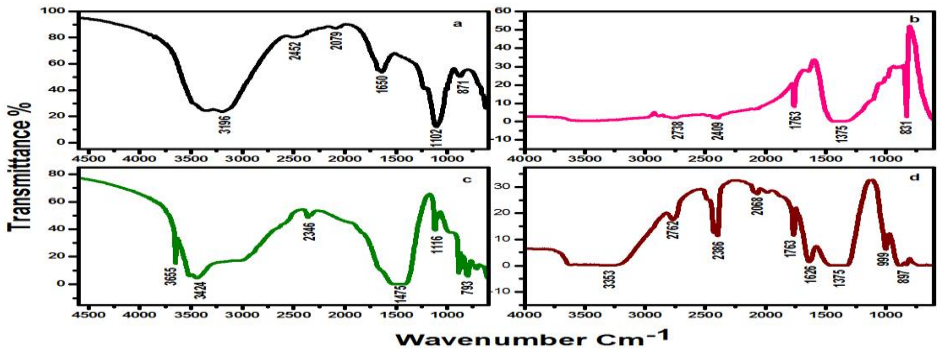

Fig.4. FTIR spectrums of CuNPs made from couroupita guianensis (a). Flower Petals (b). Stem (c) Bark and (d) Leaves

3.5. SEM analysis

SEM images (Fig. 5) showed that the synthesized CuNPs were clustered and the surfaces of the aggregates were irregular. As was also observed, the images showed that the particles were present in both mono-disperse and agglomerated forms with approximately spherical morphologies. Such a variation in particle size and shape distribution could be explained by the chemical structures of the various components contained in the plant extracts.

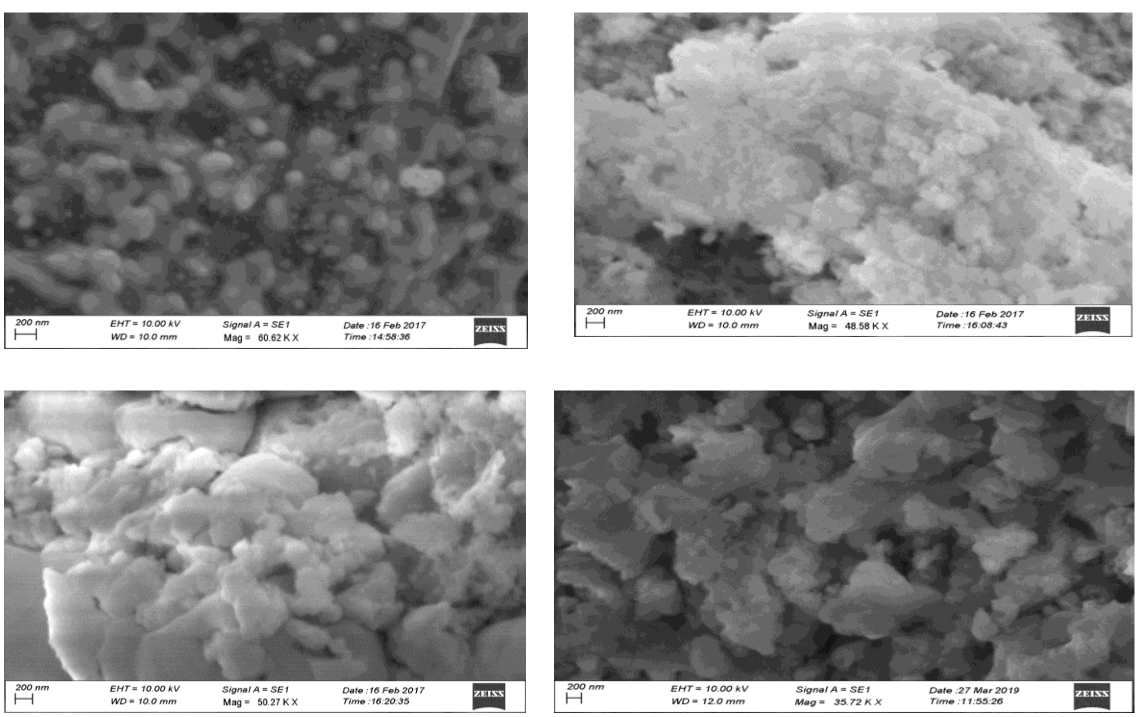

Fig. 5. SEM images of biosynthesized CuNPs of C. guianensis (a). Flower Petals (b). Stem (c) Bark and (d) Leaves.

3.6. Antibacterial Activities

The antibacterial activity of the synthesized CuNPs was tested against bacteria by the zone inhibition disk diffusion method. The copper nanoparticles showed good activity against *Escherichia Coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive). The results showed that the inhibition zone (Table 1) for the strains examined was 14, 11, 14, 13 mm for *E. coli* and 16, 16, 17, 18 mm for *Bacillus subtilis* should also be investigated on the broader range of bacterial strains (Fig. 6) .

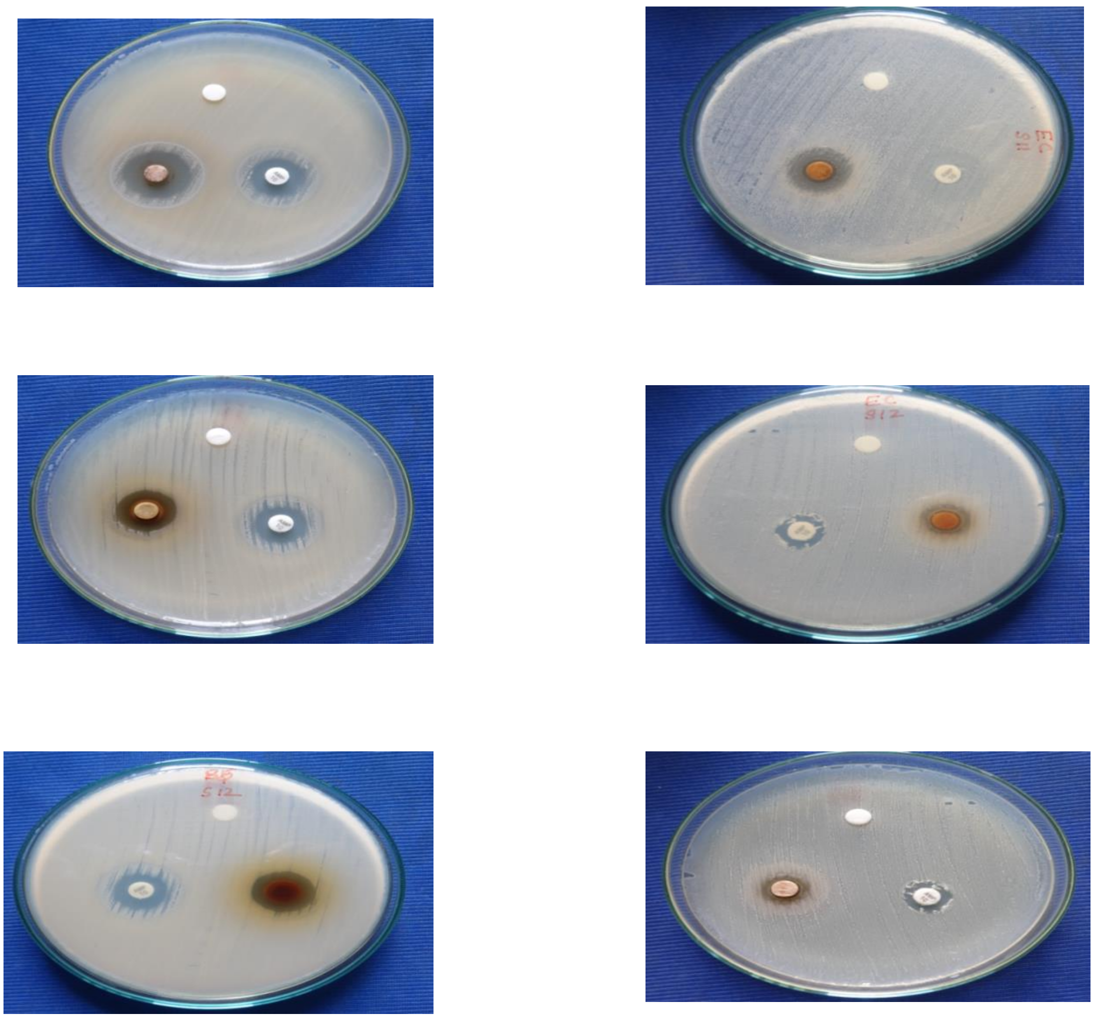

Fig. 6. Antibacterial activity of CuNPs containing C. guianensis against *Bacillus Subtillis* and *Escherichia Coli* (a). Flower Petals (b). Stem (c) Bark and (d) Leaves

Table 1. Zone of inhibition area (in mm) exhibited by the formed CuNPs against different pathogens bacterial

Name of the Organism	Flower Petals		Stem		Bark		Leaves	
	Standard	Sample	Standard	Sample	Standard	Sample	Standard	Sample
Bacillus Sutillis	15	18	16	16	15	16	15	17
Escherichia Coli	15	13	12	11	15	14	15	13

4. Conclusions

In the present work, the synthesis of copper nanoparticles was investigated using aqueous extracts of Couroupita Guianensis Aubl (flower petals, stem bark and leaves) as a stabilizer agent. A simple and feasible method is presented in this work to produce copper nanoparticles with desirable functional properties. The synthesized copper nanoparticles were characterized by UV, FTIR, XRD, particle size distribution and SEM. UV-Vis spectra of all CuNPs showed a characteristic absorption peak at 260 to 380 nm. FTIR analysis showed the presence of group features of phenols, flavonoids and other active substances in the bio extracts examined. Sharp peaks of the synthesized CuNPs also appeared in the XRD spectrum, confirming their crystalline nature. The particle size distribution of the nanoparticles confirmed the mean diameter size of 1.3–188 nm. The characterization of CuNPs were subjected to the antibacterial activity for gram-negative and gram-positive bacterial strains, and exhibited good results for *Bacillus Sutillis*, mainly due to its thin peptidoglycan layer and the electrostatic interaction between the bacterial cell wall and the surfaces of CuNPs. The CuNPs may be an effective product used for many biomedical applications, which could be a potential agent used in future research work with descriptive the functional properties.

Conflicts of Interest: The authors declare no conflict of interest

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