

A Green and Facile Approach for the Synthesis Copper Oxide Nanoparticles Using *Hibiscus rosa-sinensis* Flower Extracts and Its Antibacterial Activities

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Abstract

This work is to details an environmental benign route for the fabrication of Copper oxide nanoparticles (CuONPs) using *Hibiscus rosa-sinensis* flower extracts at room temperature. This method is totally a green method, free from toxic and harmful solvent. The present study is aimed to prepare flower extract of *Hibiscus rosa-sinensis* in deionised water. This extract is added to copper acetate solution and we observed the Colour change of the solution from blue colour to sea green coloured solution, in confirmed the formation of Copper Oxide nanoparticles. The leaf extract helped in the bio reduction of Copper ions yielding CuO NPs. The CuO NPs thus biosynthesized were characterized using UV- visible spectra showed the absorbance peak of 505 nm due to the excitation of surface Plasmon vibrations. FTIR spectrum revealed that the reduction of Copper oxide nanoparticles. X-ray diffraction of the synthesized nanoparticles indicates the formation of CuO with a monoclinic structure. The synthesized nanoparticles are subjected to SEM, EDX, and antibacterial activities.

Keywords: CuO NPs; XRD; *Hibiscus rosa-sinensis*; Antibacterial activity

Introduction

In modern era, the nanoparticles synthesis causes lot of toxic by-product [1]. Therefore, the need for development of clean, reliable, biocompatible and eco- friendly processes to synthesis the nanoparticles forced many researchers to develop green synthesis because of the do not create any toxic by-product [2], there has been growing attention towards eco-friendly synthesis of nanoparticles using plants. In recent times, nanoparticles are synthesis and utilization in green synthesis way, for that reason of its wide range of applications in the attention of new technology in the field of medical, materials science etc., because of low cost, eco-friendly, nontoxic to the environment. So, Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellular using plants or their extracts in a controlled manner according to their size, shape and disparity.

Copper Oxide is a p-type semiconductor with the band gap of ~ 1.7 eV [3]. Further it can be used as an antimicrobial, anti-biotic and anti-fungal agent when incorporated in coatings, plastics textiles etc. [4]. Copper and copper-based compounds are efficient biocidal properties, which are generally used in pesticidal formulations and several health related applications. Different methods available to prepare CuO Nanoparticles the stability and the use of toxic chemicals are subjects of paramount concern [5].

Hibiscus rosa-sinensis (commonly called shoe flower, in tamil Semparuthi Poo) is wealthy in polyphenolic phytochemicals similar to tannins and phenolic proteins, triterpenoids, 2, 3-hexanediol, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and squalene [6,7]. These compounds have excellent antimicrobial, anti-oxidative and anti-proliferative activity and therefore be used to treat cancer, especially lung cancer, cardiovascular disease, asthma and pulmonary role [8,9]. In adding together, the phytochemicals are believed to act as capping agent to stabilize the synthesized Copper nanoparticles and to prevent its coalescence. Such nanoparticles may have the advantage of offering synergistic biomedical effects. For example, antibiotic-coated nanoparticles exhibited a synergistic antimicrobial effect [10,11]. Hence it can be hypothesized that Hibiscus flower extract may result in phytochemicals stabilized nanoparticles with better bio medical activity [12-14].

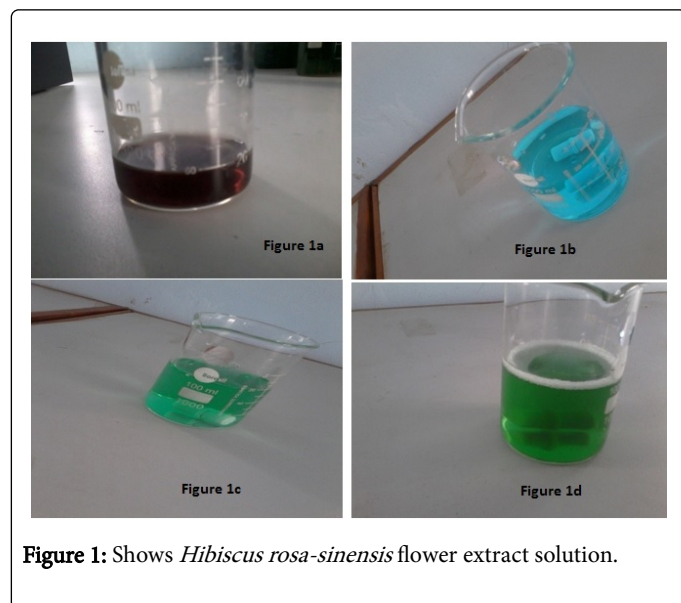
Experimental

Materials

All the chemical reagents used in this experiment were of analytical grade purchased from Merck chemicals. The *Hibiscus rosa-sinensis* flowers were collected from in and round Nehru Memorial College Puthanampatti, Tiruchirappalli, Tamil Nadu, India by using sterile polythene bags. The leaves were authenticated by a plant taxonomist, Departement of Botany, Nehru Memorial College Puthanampatti, *Hibiscus rosa-sinensis* flowers washed flower (150 g) were cut and boiled with 150 ml of deionized water for 15 min in heating mental at temperature 80°C. The extract was then filtered using Whatman's No.1 filter paper. The filtrate was collected in in a clean and dried conical flask by standard sterilized, filtration method and was stored.

Synthesis of copper oxide nanoparticles

For the CuO nanoparticles synthesis, 15 ml of *Hibiscus rosa-sinensis* flower extract was added to 200 ml of 1 M aqueous Copper Acetate solution in a 250 ml flask and then stirred three hours, the change in colour shows in Figure 1.



Thus colour change indicates reduction and reduced Copper Oxide nanoparticles were obtained. The CuO nanoparticles solution thus obtained was purified by repeated centrifugation at 8,000 RPM for 15 min followed by re-dispersion of the pellet in deionized water. Then the CuO nanoparticles were dried in oven at 75°C.

Antimicrobial screening of CuO NP on infection causing bacterium

E. coli cultures were obtained from National Chemical Industrial Microorganisms (NCIM), Pune, India. *In-vitro* antimicrobial sensitivity study was carried out using the well diffusion method to the test samples against *E. coli* on Muller Hinton Agar (MHA) medium. A sterile cotton swab was used to inoculate the diluted bacterial suspensions (test culture suspensions prepared in sterile 0.85% saline matching an optical density of 0.5 McFarland standards corresponding to 10^8 CFUs ml⁻¹) on the surface of agar plates for homogeneous growth. The 15 µl of test solution was poured into an each well separately, and then the test sample coated disks were placed in each dish by a sterilized forceps. The plates were incubated at $37 \pm 1^\circ\text{C}$ for 24-48 hrs. After incubation, the zone of inhibition was measured with ruler/Hi Antibiotic Zone Scale-C. The assays were performed in triplicate and the average values were present. All the media, standard disks and Hi Antibiotic Zone Scale-C were purchased from Hi-Media (Mumbai, India).

Characterizations

UV-Vis analysis: UV-Vis absorbance spectrum of the sample was recorded by UV-Vis spectrometer (M/s JASCO, Model-v-670, USA).

FT-IR analysis: Functional group of materials was confirmed by Fourier Transformation Infrared spectrometer (M/s JASCO, USA) in

the transmittance mode in the range of 4000 to 400 cm⁻¹ using KBr pellet.

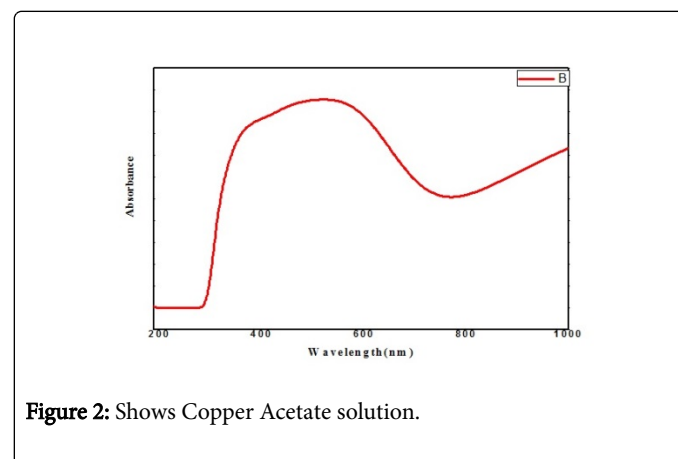
XRD analysis: From XRD measurement crystalline phase and Crystallite size of TiO₂ Np were characterized using powder X-ray diffractometer (M/s PANalytical X' pert pro, The Netherlands) operated at voltage=40 kV and Current=30 mA with Cu-ka ($\nu=1.5406$ Å) radiation.

SEM and EDX analysis: The surface morphology of as synthesized product was analysed by Scanning electron microscopy (Carl Zesis). The energy dispersive X-ray spectrometer was performed by a (Carl Zesis) EDX spectrometer to determine the elemental composition of the samples.

Results and Discussion

UV-Vis spectroscopy

The synthesis of copper oxide nanoparticles occurred during the revelation of *Hibiscus rosa-sinensis* flower extract to 1 M aqueous Copper acetate solution. The extract was mixed with the solution of the copper ion complex, it started to change the colour i.e., light green to Sea green colour due to the reduction of copper ion. The pH was determined by using Digital pH meter. The pH value of the solution reduced to 4.56 to 2.36 due to the reduction of copper ion. The colour (Figure 1) and pH changes indicated the formation of copper oxide nanoparticles. This confirmed the formation of copper oxide nanoparticles. Figure 2 shows the UV-Visible spectrum of nanoparticles using *Hibiscus rosa-sinensis* extract.



The characteristic Plasmon absorption band for copper nanoparticles was observed at 505 nm. When the particles are spherical, the surface plasmon resonance and the blue shift are affected by the size distribution. Hence it is suggested that the band observed at 505 nm.

FTIR analysis

Copper nanoparticles FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. Figure 3 shows the FT-IR spectrum of CuO nanoparticles using *Hibiscus rosa-sinensis* extract.

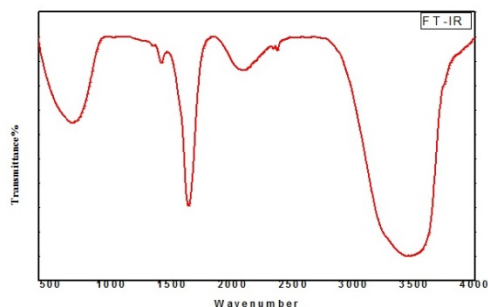


Figure 3: Shows Copper acetate+*Hibiscus rosa-sinensis* flower extract.

Different functional group is involved in synthesis of CuO nanoparticles. The FT-IR spectrum of CuO nanoparticles shows band at 3453 cm^{-1} , 2371 cm^{-1} , 1639 cm^{-1} , 1407 cm^{-1} , 1115 cm^{-1} corresponds to O-H Stretching H-bonded alcohols and phenols, carbonyl stretching, N-H bend primary amines, corresponds to C-N stretching of the aromatic amino group and C-O stretching alcohols respectively. Whereas the stretch for CuONPs were found around $650\text{--}700\text{ cm}^{-1}$. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of Copper Oxide nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of Copper Oxide nanoparticles in the aqueous medium. FTIR spectrum of CuO nanoparticles suggested that CuO nanoparticles were surrounded by different organic molecules.

Crystalline structure

Figure 4 shows the XRD pattern of CuO nanoparticles using *Hibiscus rosa-sinensis* flower extract.

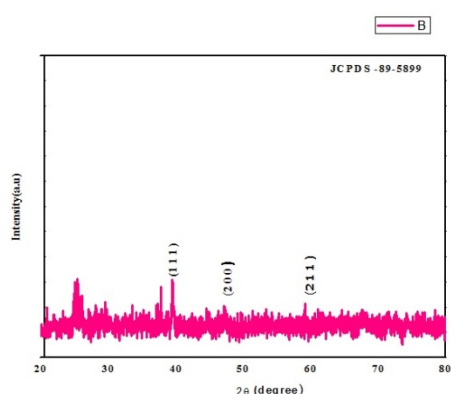


Figure 4: Shows copper acetate+*Hibiscus rosa-sinensis* after 3 hr.

The powered sample was used by a Cu $K\alpha$ - X Ray Diffractometer for confirming the presence of CuO and analyse the structure. XRD

Pattern indicated the CuO Nps had strongly crystalline structure. The XRD pattern gives a CuO NPs is monoclinic structure with the lattice parameters are $a=4.68$, $b=3.42$, $c=5.12$.

The XRD diffraction pattern peaks 16.61° , 25.91° , 39.95° corresponding reflection from (001), (010) and (-200) planes. The average particle sizes of CuO nanoparticles were approximately 26.54 nm. This was based on the full-width half maximum measurement at 2θ of the maximum diffraction peak using Scherrer's formula: $D=K\lambda/\beta\cos\theta$

In this equation, D is the crystallite size, K the Scherrer constant usually taken as 0.89λ the wavelength of the X-ray radiation (0.15418 nm for Cu $K\alpha$).

Structural analysis

Scanning Electron Microscope (SEM): Figure 5 shows the SEM Analysis of Copper oxide nanoparticles using *Hibiscus rosa sinensis* flower extract. The size and Surface morphology of the synthesized nanoparticles be obtained by Scanning Electron Microscopy (SEM) analysis. The SEM images indicated that the crystalline CuO are spherical and size of particles is ascertained from the SEM scale ranged between 45-80 nm. Energy Dispersive X-ray Spectroscopy (EDX) analysis, the quantitative and qualitative Analysis of elements may be concerned in the formation of copper oxide nanoparticles (Table 1).

Element	Weight%	Atomic %
O K	40.54	73.03
Cu L	59.46	26.97
Totals	100	

Table 1: Atomic Weight percentage of Cu and O.

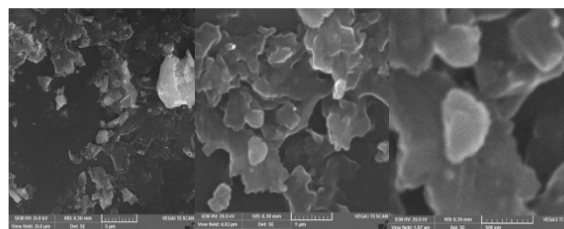
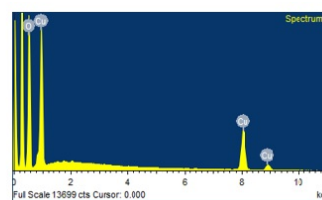


Figure 5: Shows the SEM analysis of copper oxide nanoparticles using *Hibiscus rosa-sinensis* flower extract.

Anti-bacterial activity

The Figure 6 shows antibacterial studies against *Klebsiella pneumoniae*, *E. coli*, *Shigella flexneri* and *Bacillus subtilis*. The zone of reserve observed against CuO nanopowders and the standard antibiotic is summarized samples exhibited almost similar antibacterial efficacy against both the bacteria selected in our studies. The antibacterial properties of the CuO Nps was evaluated against Gram -ve *Klebsiella pneumoniae*, *E. coli*, Gram +ve bacteria *Bacillus subtilis*

and *S. aureus* using agar well diffusion method. In agar well diffusion method the CuO Nps showed significant antibacterial activity on all the four bacterial strains. Some reports available for the mechanism behind the antimicrobial activities. In the present case, *Klebsiella pneumoniae* and *E. coli* show significant zone of inhibition by CuO Nps. Naturally, *Shigella flexneri* and *Bacillus subtilis* have abundance of amine and carboxyl groups on cell surface and high affinity of copper toward these groups.

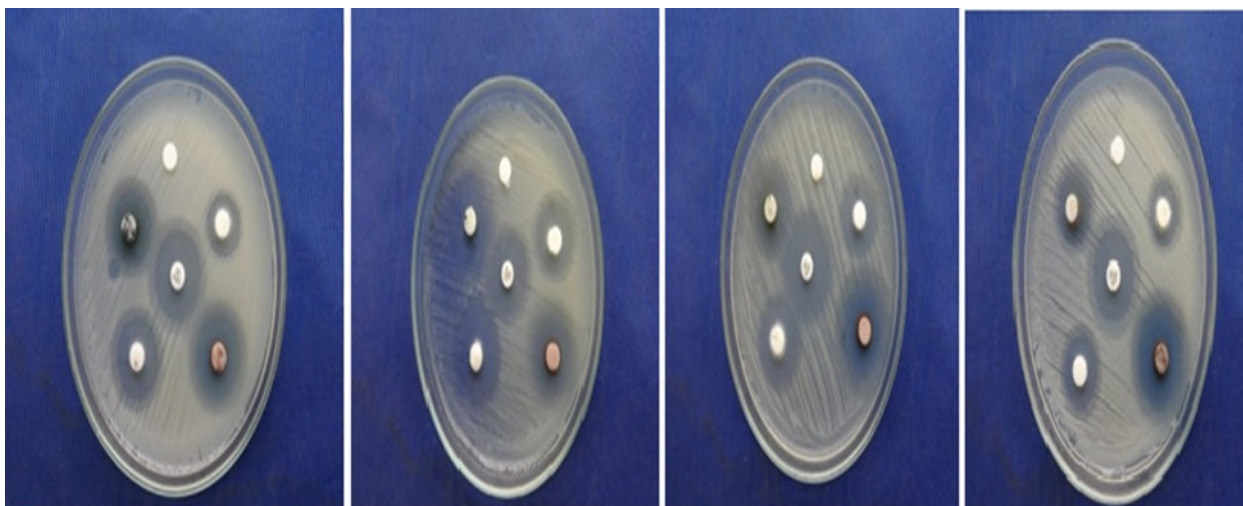


Figure 6: Shows the EDX analysis of copper oxide nanoparticles using *Hibiscus rosa-sinensis* flower extract.

Conclusion

The rapid biological synthesis of Copper oxide nanoparticles using Flower broth of *Hibiscus rosa-sinensis* provides an environment friendly, simple and efficient route. The UV-Vis absorption peak at 505 nm confirmed the presence of CuO in the nano scale. The FT-IR studies showed an absorption peak at 676 cm^{-1} (Cu-O) which indicated the formation of Copper oxide nanoparticles. The obtained product was confirmed to be Copper oxide, the crystal structure was studied and the d- spacing of CuO nanoparticles was studied using XRD. The SEM images indicated that the crystalline CuO are spherical. EDX analysis can be concluded that the sample mainly consist of copper and Oxide.

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