



GREEN SYNTHESIS AND CHARACTERIZATION OF COPPER OXIDE NANOPARTICLES USING AMALA FRUIT EXTRACT AND EVALUATION OF THEIR ANTI-MICROBIAL ACTIVITY

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Abstract:

Green nanotechnology replaces toxic synthesis with ecofriendly biological methods, producing nanomaterials for gas sensors, energy storage, optical devices, and water treatment. Copper nanoparticles attract attention due to high surface area, stability, conductivity, catalytic and biological activities. The current study reports a straight forward and environmental friendly approach for the preparation of Cu-NPs using amala (*Emblica officinalis*) fruit extract in aqueous medium. The synthesized copper oxide nanoparticles were investigated for their structural validation using different analytical techniques. The highest absorption peak at 348.nm on UV-Vis spectra provides strong supports towards the formation of Copper nanoparticles. Orientation and crystal structure was studied by using X ray diffraction analysis and was found to be monoclinic crystalline structure for CuO NPs. Peaks on FTIR clearly indicates presence of polyphenols, alcohols and ascorbic acid in the fruit extract of amala which acts as capping and stabilizing agents on the surface of CuO nanoparticles. Antimicrobial activity screening results revealed the synthesized CuO nanoparticles exhibits potential antibacterial activity against *S. aureus* and *E. coli*. In order to synthesize CuO nanoparticles on large scale, the present study provides cost effective, better green approach, free from toxic chemicals which can be used as antibacterial agents.

Keywords: Nanoparticles, Amala Fruit Extract, Reducing Agent, Anti-Microbial Activity.

1. Introduction

Nanotechnology is fast growing broad area of research where different brains from various scientific corners are continuously sharing their outputs in order to create something newer and novel [1]. Nanotechnology is a multidisciplinary approach and it provides new opportunities for the creation of new functional nanomaterials with specified qualities [2-3]. Due to smaller dimensions of nanomaterials within 1-100 nanometer range, the physicochemical properties like shape, size and chemical constitution alters significantly which increases the surface area and efficiency of nanomaterial as catalyst in various reactions [4]. In last few years due to the unique sizes and exceptional physicochemical characteristics of metal oxide-based NPs, majority of researchers are engaged in more in-depth investigation of some metallic nanoparticles and CuO nanoparticles are found to be one of the most researched nanomaterials [5]. Because of its low cost, stability, and potent antibacterial action, copper oxide (CuO) has become one of the most promising metal oxide nanoparticles for biomedical, agriculture, cosmetics, paints, textiles, photo catalysis and environmental applications [6-11]. The particle size and shape of CuO nanoparticles also influences the optical properties. Considering this broad spectrum of applications, day by day scientific efforts are goes on increasing to synthesize and investigate the physicochemical properties of copper nanoparticles. Traditional chemical synthesis involves use of toxic chemicals and also it releases some environmentally hazardous by-products. As compared with newer green synthetic methods both physical and chemical synthetic methods for nanoparticles have some disadvantages. A sustainable substitute to conventional chemical synthesis of nanoparticles is green biological synthesis in which different plant extract are utilized for synthesis of nanoparticles [12-15]. In such method the toxic reducing and capping agents are replaced by some green phytochemicals which makes this method eco-friendly [16-19]. Various plant parts including leaves, fruits, stems, roots, flowers and latex etc. are used to prepare biological extract and by adding this extract into the solution of suitable precursor solution under required reaction conditions, large variety of nanoparticles has been synthesized [20]. Different phytoconstituents like flavonoids, alcohols, phenols, amines present in plant extract plays important role as stabilizing or binding agent on the surface of nanoparticles. CuO nanoparticles synthesized by using plant extract found to be more biocompatible as well as chemically stable due to biomolecule capping and found to be more precious in their applications. Such CuO nanoparticles show enhanced antimicrobial activity against bacteria, fungi and viruses [21-23]. Also they exhibit remarkable antioxidant activity and explores some potential therapeutic uses like wound healing, anticancer, anti-diabetics and drug delivery [24-27]. Some of them are also used in energy storage devices while other finds use in photo degradation of dyes [28-31]. In this context we report here a synthesis of CuO nanoparticles by using amala fruit extract by rapid, ecofriendly, cost effective and simple synthesis method.

2. Experimental section

2.1 Materials

The solid copper sulphate required for the synthesis of CuO nanoparticles was purchased from Sigma-Aldrich and used as received without any further purification.

2.2 Preparation of amla fruit extract

Fresh Amala fruits (50 gm) purchased from local market was first washed, dried, deseeded and then cut into small pieces. These pieces were blended with 100 mL distilled water and resultant extract was taken into 250 mL beaker and heated over a water bath for about 30 minutes at 70°C-80°C temperature. The fruit extract was cooled at RT

and filtered through muslin cloth to obtain a clear juice extract which was then stored in refrigerator for the further use.

2.3 Preparation of Copper oxide nanoparticles (CuO-NPs)

For the synthesis of CuO nanoparticles, 50 mL (0.02M) CuSO₄ solution was prepared in 250 mL beaker and heated to 60°C with continuous stirring on magnetic stirrer equipped with hot plate. 50 mL previously prepared amala juice extract was then added drop wise to the CuSO₄ solution maintaining the temperature at 60°C for about 30 minutes. Change in color of solution from blue to dark brown suspension indicated the formation of CuO nanoparticles (Change in color of solution was due to the reduction of Cu ions by the phytochemical reducing agents in amala fruit extract). The suspension was centrifuged for 15 minutes, then filtered, washed with distilled water and ethanol and finally dried in oven at 80°C for overnight.

After structural validation by various analytical techniques, the synthesized nanoparticles then tested for their catalytic applications. Schematic presentation of synthesis of CuO-NPs shown as in figure 1.



Figure 1: CuO-NPs synthesis from copper sulphate solution and amala fruit extract

2.4 Characterization CuO nanoparticles

After successful synthesis, next task was to validate the structure of synthesized nanoparticles. The physicochemical structural properties of synthesized CuO-NPs were investigated through different characterization techniques. The absorbance in UV-Visible range was measured using a Shimadzu double beam UV-visible spectrophotometer in 250–800 nm. The FTIR performed recorded using Lambda 7600 PC with wavelength range between 4000 cm⁻¹ and 400 cm⁻¹. X-ray diffraction (XRD) measurement was performed using Bruker Ltd., Germany, D2 Phaser X-ray diffractometer instruments.

2.5 Biological activity

The antimicrobial activity of synthesized CuO-NPs was determined against one gram positive and one gram negative bacterial strains, *Staphylococcus aureus* and *Escherichia coli* respectively by using the well diffusion method. Both microorganisms were cultured overnight on nutrient agar plates. A solution of CuO-NPs 50 mg/mL was prepared for antimicrobial study. The 100 µL of microbial suspension dispensed with the help of sterile spreader. The plates were then incubated for one day at 30°C. The DMSO was used as negative control. The zone of inhibition in millimeters on the agar surface surrounding the well was measured and used to calculate the antimicrobial potential. The zone of inhibition by CuO nanoparticles was compared with conventional antibiotics kanamycin.

3. Result and Discussion

Synthesis of CuO nanoparticles by using plant extract is reliable, rapid green method which replaces the tedious chemical method. Phytochemicals like flavonoids, polyphenols, alcohols, amines, proteins, alkaloids, starch, tannins, sterols, etc. present in the plant extract acts as green alternative for toxic reducing or capping agents. Hence different plant parts like leaves, roots, latex, bark, stem, and seeds are utilized to extract the phytochemical in aqueous solution and are used to prepare different nanoparticles. Such phytochemicals also have inbuilt biological properties as a result, in order to study in depth and to explore more the biological plant extracts. we reported here the synthesis of amala fruit extract catalyzed synthesis of CuO nanoparticles and screened for their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

3.1 Characterization CuO nanoparticles

3.1.1 Uv-visible spectroscopy

The CuO nanoparticles synthesized by using amala fruit extract was isolated by filtration and washed thoroughly with water and ethanol. After oven dried (at 80 °C overnight) the absorbance of copper nanoparticles were measured on a UV-Visible spectrophotometer and the highest absorption peak at 348 nm strongly supports the formation of CuO-NPs.

3.1.2 FTIR analysis

The stabilizing functional groups on the surface of CuO-NPs prepared from amala fruit extract were determined from FTIR spectral analysis. Peaks on FTIR at 3300-3500 cm^{-1} , 1600-1650 cm^{-1} , 1000-1000 cm^{-1} , 400-600 cm^{-1} clearly indicates presence of polyphenols, alcohols and ascorbic acid in the fruit extract of amala which acts as capping and stabilizing agents on the surface of CuO nanoparticles. The presence of carboxylate group in the fruit extract of amala plant is responsible for the binding of proteins with the surface of Cu leading to the stabilization of the biosynthesized nanoparticles.

3.1.3 X-Ray Diffraction (XRD) analysis

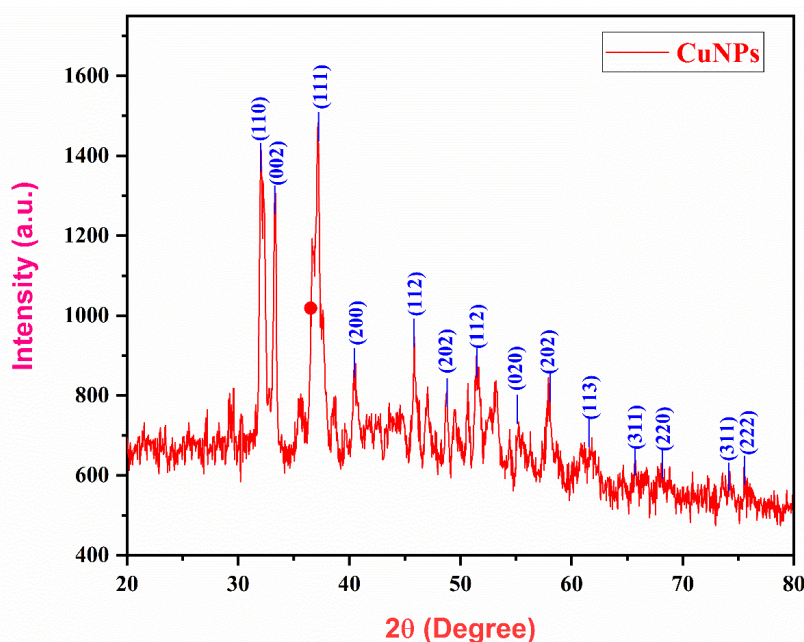


Figure 2: Characterization of Silver nanoparticles (Cu-NPs) by XRD

The X-Ray Diffraction peak pattern for Copper nanoparticles from amala fruit extract shown distinct peaks at 2θ values approximately at 32.7° , 35.4° , 38.6° , 40.1° , 45.7° , 48.8° , 51.5° , 53.3° , 58.2° , 61.6° , 66.3° , 68.0° , 72.3° , 75.2° corresponding to (110), (002), (-111), (200), (-112), (-202), (112), (020), (202), (-113), (-311), (220), (311) and (-222) crystal planes which confirms monoclinic structure for crystalline CuO-NPs. To check successful green synthesis, the XRD data were compared with the pure crystalline structure database of the JCPDS (JCPDS card number, 04-0783). The diffraction peak at 38.6° had a robust diffraction intensity indicating the preferential orientation of CuO crystal along the (111) plane. The other peaks shown in the figure might be due to metabolites of amala fruit that were still attached to the surface of CuO nanoparticles. The XRD graph of CuO-NPs shown in figure 2.

3.2 Biological activity

The anti-microbial activity of synthesized CuO-NPs were investigated against *Staphylococcus aureus* and *Escherichia coli* using the well diffusion method. The screening results revealed that CuO nanoparticles exhibited significant antimicrobial activity against both tested organisms. The diameter of inhibition zones (in millimeters) around the different antibiotic disks with or without CuO-NPs against test strains are shown in **Table 1**. The effects of CuO-NPs on the antibacterial activity of the aforementioned antibiotics for *E. coli* were lower than *S. aureus*.

Table 1: Bioactivity information of synthesized compounds (1-4) and Reference Compounds

Compounds	Inhibition zone (in mm)	
	Gram +ve (<i>Staphylococcus aureus</i>)	Gram -ve (<i>Escherichia coli</i>)
1. CuO-NPs	13	10
2. Kanamycin	21	20

Conclusion

This study demonstrates the biological synthesis of CuO-NPs using amala fruit extract is a rapid, simple, eco-friendly, cost effective synthesis. This green approach involves use of phytochemicals in aqueous fruit extract as stabilizing agent. The nanoparticles were characterized by various techniques to explore the physic-chemical properties. The antibacterial activity of amala fruit extract mediated CuO-NPs against *Staphylococcus aureus* and *Escherichia coli* was screened by using well diffusion method. The maximum zone of inhibition was observed against *S. aureus* which showed potent antimicrobial activity. Green synthesis using Amala offers a sustainable route for producing bioactive nanomaterials suitable for biomedical and environmental applications.

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