Bio Synthesis of Copper oxide Nanoparticles from *Jatropha Curcas* Leaf Extract and Their Antibacterial Activity Study

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Abstract. Ecofriendly and cost effective technologies in the synthesis of nanomaterials are receiving special attention due to their wide range of applications in environmental remediation, medicine, and bio sensing. Green nanotechnology has emerged as a novel scientific research in the synthesis of nanoparticles by a simple, cost effective and ecofriendly manner. The current research focused on the green synthesis of copper oxide nanoparticles employing environmentally friendly and sustainable technique using *Jatropha curcas* leaf extract as reducing and capping agents. The synthesized nano particles were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM), and Energy Dispersive X-Ray (EDX) analysis. The fabricated nanoparticles were successfully employed in the antibacterial activity study by disc diffusion technique. The green synthesized copper oxide nanoparticles showed excellent antibacterial activity.

1 Introduction

Metal and metal oxide nanoparticles are receiving considerable attention in recent times owing to their improved and modifiable surface morphology, physico-chemical characteristics, extremely small size, and huge surface area [1, 2]. Most of the nanoparticle synthesis techniques employ physical, biological and chemical methods, which are expensive and release harmful chemicals resulting into severe environmental pollution. Copper oxide nanoparticles are monoclinic semiconducting compounds with diverse applications. Copper oxide is the simplest copper compound exhibiting unique properties such as high-temperature superconductivity, electron correlation effects, and spin dynamics, making it suitable for a wide range of applications. These nanoparticles have a wide and direct band gap of 1.2–2.1 eV and are applied in solar energy systems, batteries, gas sensors, antimicrobials, water purifiers, algaecides, fungicides, and anti-fouling agents. Syntheses of rod-shaped copper oxide from Anabaena cylindrica extract are used for the disinfection of drinking water [3]. *Brassica oleracea* algal extract derived copper oxide

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showed distinct functional groups of hydroxyl, carbonyl, and amine in polyols and polysaccharides and played significant roles in nanoparticle reduction, capping, and stabilization [4]. Another study by Araya-Castro et al employed protein fractions from *Macrocystis pyrifera* extract for copper oxide nanoparticle synthesis [5]. Using UV/VIS diode arrays and TEM-EDS analysis, they observed spherical nanoparticles formed by low- and high-molecular-weight proteins. Similarly, the biosynthesis of spherical copper oxide nanoparticles, averaging about 22.6 nm in size, using di terpenoids from Bifurcaria bifurcata. Kappaphycus alvarezii seaweed extracts, in which sulfated polysaccharides acted as stabilizers, forming copper oxide nanoparticle with a mean size of 53 nm [6]. They exhibit low toxicity, improved specific surface area, variable morphology at the nanoscale, and low cost. Hence these nanoparticles have become a potential candidate for environmental applications [7, 8]. The commonly employed chemical and physical synthesis methods are not effective with elevated cost, and lengthy processing time with multi-step experimental procedures. Hence, it is required to develop non-toxic and cost-effective methods to synthesize copper oxide nanoparticles employing ecofriendly techniques [9, 10]. Green synthesis methods are practiced by researchers to produce NPs from natural plant extracts which are rich in phytochemicals such as alkaloids, polyphenols, proteins, vitamins, flavonoids etc, which act as both reducing and capping agents [11]. copper oxide nanoparticles have received great attention in wastewater treatment applications due to their impact on the environment [12]. Many research studies have been focused on the application of copper oxide nanoparticles in antimicrobial activity study [13-15]. Very limited studies have been carried out in the ecofriendly synthesis of copper oxide nanoparticles from biomaterials for antibacterial studies. In this research, a simple and cost-effective green synthesis method was used to produce copper oxide nanoparticles using *Jatropha curcas* leaf extracts for the application of antibacterial activity study.

2 Materials and Methodology

2.1 Materials

The *Jatropha curcas* leaves were collected from local plantation in Muscat, Oman. The leaves were washed with pure water followed by drying, grinding and sieve analysis to get the required size (less than 0.75 µm). Anhydrous copper sulfate (Analytical grade with 95% purity) procured from sigma Aldrich was used for the experiment. For the preparation of the extract and the solutions, distilled water was utilized, and pH adjustment was performed using 0.1 M Hydrochloric Acid (HCl) and 0.1 M Sodium Hydroxide (NaOH). Millipore water of 18.2MΩ resistivity was used in all experiments. The experiments were repeated three times to ensure the accuracy and reproducibility of the results. Green synthesis was employed in the preparation of copper oxide nanoparticles. The main characterization tools employed are FT-IR, SEM, EDX and XRD.

2.2 Methodology

The methodology employed in the synthesis of copper oxide nanoparticles is green synthesis technique. The leaves of the *Jatropha curcas* plant leaves were cleaned using distilled water to remove dust particles and then dried in an oven at 60 $\pm$ 10 °C for 24 hours. The dried leaves were ground into fine powder. The extract was prepared by boiling 20.0 g of dried powder in 1L of distilled water for about 2 hours to get a homogenous dark green grassy color mixture. After 2hours, the resulting solution was centrifuged at 6,000 rpm for 15 min. The nanoparticle was synthesized by mixing the extract with 1.0 M copper sulphate solution in the ratio 1:1and the reaction was carried out for 30 hr. Subsequently, the reaction mixture was centrifuged to separate the nanoparticles followed by three to five washings stages with double-distilled water to ensure the purity of the synthesized particles. The bottom layer showing blue or red color indicates the formation of particles. The remaining residue was calcined in a muffle furnace. The synthesized nanoparticles were employed in the antibacterial activity study.

3 Results and Discussion

The synthesized nanoparticles were tested for surface morphological characterization and elemental composition analysis using SEM and EDX. The Figure 1 (a) and Figure 1 (b) indicates the images of fresh and dried leaves. The powdered leaves are shown in Figure 2. The microstructural characteristics of the nanoparticles were examined through SEM operated at 2000 X magnification and 15.0 keV as shown in Figure 3. The SEM image indicates the nanoparticles are well formed with high yield and purity demonstrates the successful synthesis. The ensuing product was examined using a range of analytical tools, SEM, EDX, FTIR spectroscopy and XRD analysis. The leaf extract contains phytochemical component, which aid in the development of nanoparticles. The leaf extract act as reducing and capping agent during the green synthesis.

Fig. 1(a). Fresh *Jatropha* leaves.

Fig. 1(b). Dried *Jatropha* leaves.

Fig. 2. Powdered leaves.

The elemental composition of the final product by EDX analysis is shown in Figure 4, revealing 63.9 % copper, 18.7% oxygen, 8.5% Si, 5.5 % C, and 3.4 % Al. The EDX analysis demonstrates the high purity of the synthesized nanoparticles. The particles are seen in the clustered form with aggregation of particles. The produced NPs are nanoscale in size, according to SEM and XRD data.

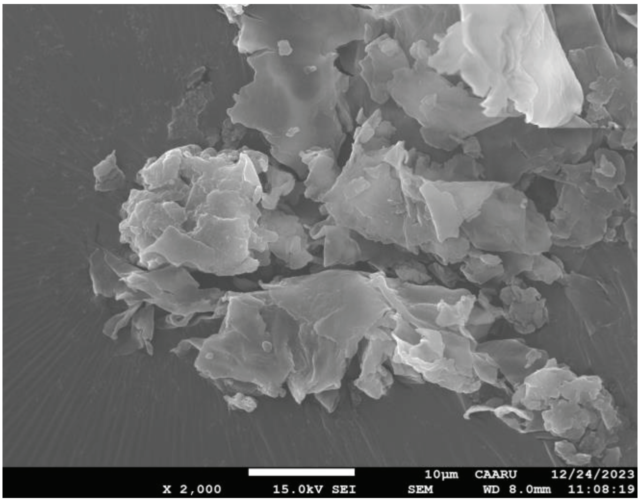

Fig. 3. SEM image of copper oxide nanoparticles.

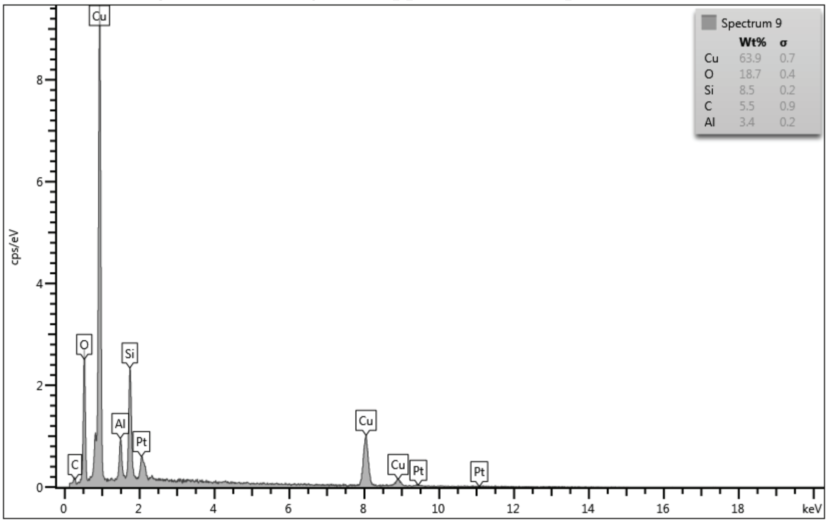

Fig. 4. EDX spectra of copper oxide nanoparticles.

The XRD analysis was performed to check the purity and phase identification of the CuO nanoparticles as displayed in Figure 5. The XRD analysis was carried out at a diffraction angle from 0 to 90 degree. One single peak in the spectrum shows amorphous nature of the particles.

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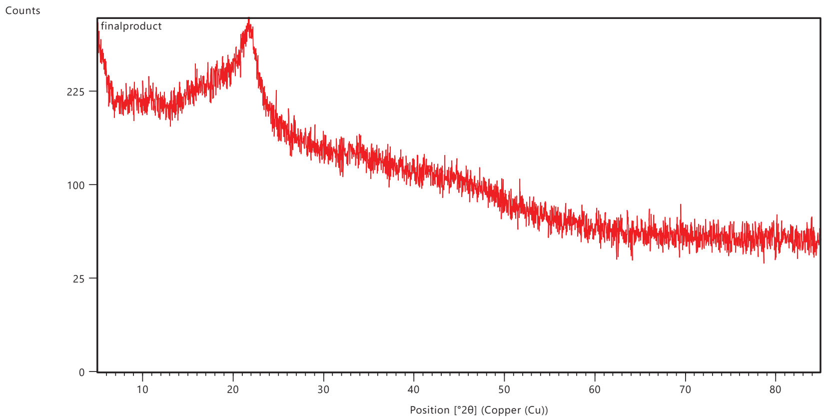

Fig. 5. XRD spectrum of Copper oxide nanoparticles.

The Brunauer-Emmett-Teller (BET) analysis was performed to describe the surface area and pore size distribution of the synthesized nanoparticles. The BET analysis was accomplished by plotting the amount adsorbed (cm^3/g STP) versus Relative Pressure (P/Po) as presented in Figure 6. The BET surface area of the nanoparticles was found to be 0.1000 m^2/g, then after increasing the mass of test sample the surface area increased to 0.1117 m^2/g. The difference in surface area value indicates the total surface available for possible adsorption and provides valuable information about the reactivity and nature of surface interactions. A higher surface area value suggests greater potential for catalytic activity, adsorption capacity, and other surface-dependent phenomena. Figure 7 displays the BET Isotherm Plot.

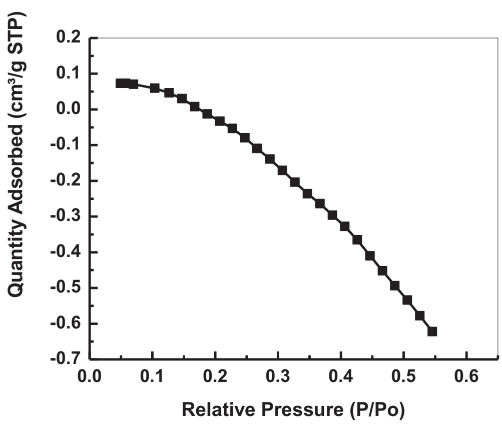

Fig. 6. BET surface area plot.

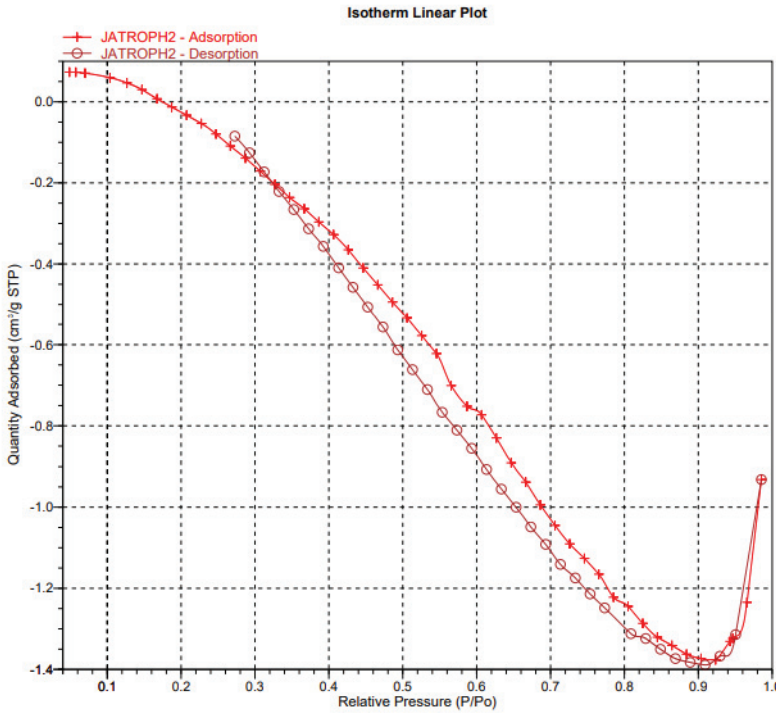

Fig. 7. BET Isotherm Plot.

The molecular level interaction between *Jatropha curcas* leaf extract and copper sulfate is assessed by the FTIR analysis as portrayed in Figure 8. The various peaks appear in the FTIR spectra denote the functional groups present on nanoparticle surface. The representative peak between 1650 cm^{-1} and 1800 cm^{-1} in the FTIR spectra is shown in the spectrum confirms carbonyl groups (C=O) in biomolecules. However, there is a noticeable shift to a lower wavenumber (1050 cm^{-1} –1200 cm^{-1}) in the final product. This implies that the functional groups represent the successful synthesis of copper oxide nanoparticles.

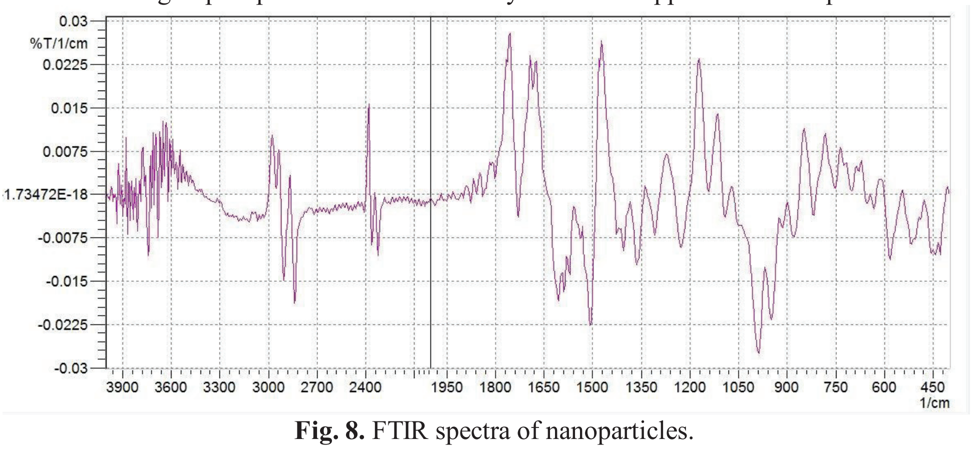

Fig. 8. FTIR spectra of nanoparticles.

3.1 Antibacterial activity of copper oxide nanoparticles

The copper oxide nanoparticles were tested for the antibacterial activity by disc diffusion method. The stock solutions were prepared at different dilutions and then permeated into a sterile blank disc. A 2 µl nanoparticle suspension was marked alternately on both sides of the discs and allowed to dry and inoculated with agar media. The antibacterial activity results are tabulated in Table 1.

Table 1. Antibacterial assay of copper oxide nanoparticles.

Sample	Sample Name	Value
1	Extract	211×10^{6} cfu/ml
2	after 12 days	413×10^{5} cfu/ml

4 Conclusion

Copper oxide nanoparticles are evolving as a promising antibacterial agent. In this research, safe and sustainable green extraction technique was employed in the synthesis of copper nanoparticles without using toxic and costly chemicals. The nanoparticles were characterized by SEM, FTIR, EDX and XRD. The phytochemical component present in the leaf extract aided the creation of the nanoparticles. The produced NPs are at nano size range and high yield as demonstrated by SEM and XRD. The facile synthesis of copper oxide nanoparticle has attracted considerable attention in environmental, medical and chemical engineering. It has been anticipated as a cheaper and ecofriendly substitute to conventional chemical and physical method. The outcome of the research shows that copper nanoparticles have excellent potential as antibacterial agent. The current research offers a green and sustainable way to synthesize copper oxide nanoparticles from *Jatropha curcas* leaf extracts, in a simple and cost effective technique for the antibacterial study.

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