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Research Article

COMPARATIVE STUDIES ON BIO AND CHEMICALLY SYNTHESIZED CUO NANOPARTICLES AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Copper oxide (CuO) nanoparticles are known as multifunctional inorganic nanoparticles with a wide range of applications. This work mainly concentrated on the comparative study of antibacterial properties of CuO nanoparticles using two different methods. The green CuO nanoparticles were synthesized from *Cajanus Cajan* plant leaf extract. The CuO nanoparticles were visually confirmed by the change of color after addition of leaf extract into the copper acetate solution. The synthesized CuO nanoparticles were also characterized by using UV-Vis analysis, Fourier Transform Infrared analysis (FTIR), and X-ray diffraction analysis (XRD), Field Emission Scanning Electron Microscopy (FESEM) Energy Dispersive X-Ray (EDX) analysis. From the results, it is suggested that bio-CuO nanoparticles show more enhanced biocide activity against various human pathogens when compared to chemical CuO nanoparticles.

Keywords: CuO nanoparticles, *Cajanus Cajan*, antibacterial activity, FESEM

INTRODUCTION

Human beings have been using metal and its composites for various purposes for centuries, such as water purifiers, algaecides, fungicides, and as antibacterial and antifouling agents(1). Inorganic antibacterial agents such as metal and metal oxides are advantageous compared to organic compound required to their stability. In particular, metal oxide nanoparticles are recognized as ZnO, MgO, SiO_2 etc have been investigated as inorganic antibacterial agents(2). Among the studied inorganic metal oxides, CuO is of **a** particular interest because they interestingly exhibit antibacterial activity and can reduce the attachment and viability of microbes on the biomedical surface (3, 4).

Metal oxide nanoparticles are usually synthesized using various chemical methods just as chemical reduction, sonochemical, solvothermal reduction, electrochemical techniques etc..(5-7) Among them, the chemical reduction is the most frequently applied method. Literatures showed that the use of a chemical reducing agent resulted in the generation of larger particles and consumed more energy and was not eco-friendly(8). Hence there is a need to develop an eco-friendly protocol to synthesize stable nanoparticles more efficiently by consuming less energy. An alternate method like biological methods, using plant extract is simplest, efficient and the environment- friendly than the conventional method(9). It is also more compatible with various biomedical and pharmaceutical applications because they do not require toxic chemicals for the synthesis(10).

This work mainly concentrated on a comparative study on antibacterial properties of CuO nanoparticles which are synthesized by two different methods. Secondly, this study aims to evaluate the bacterial activity of CuO nanoparticles against two important pathogens: *Pseudomonas aeruginosa (P.aeruginosa)* and *Salmonella typhi (S.typhi).*

MATERIALS AND METHODS

Materials

Copper acetate ($Cu(CH_3COO)_2$) and ethanol of analytical grade has been purchased from Sigma- Aldrich. Deionized water is used in all experimental works**.** The fresh leaves of *Cajanus Cajan* have been collected from Idukki district, Kerala.

Plant description under study

Cajanus Cajan is a short-lived perennial commonly known as Tuvara or pigeon peas which belong to the family of Fabaceae. The cultivation of Tuvara goes at least 3000 years back and is the most common pulse eaten all over India. The seed of this medicinal plant is used for diseases of internal organs like stomach, liver, intestines and it reduces cancer of these organs. For the green synthesis of CuO nanoparticles, plant leaf extract was used as both the reducing and stabilizing agent. The photographs of the used plants are shown in figure.1.

Figure.1 Photo of medicinal plant *Cajanus Cajan*

Experimental procedure

Cajanus Cajan plant leaves were collected, washed and separated from its stem. About 20 grams of leaves added with 100 ml distilled water and kept for 3 minutes at a constant temperature. The mixture was cooled to room temperature, filtered and stored at 4°C for further use. Then, the desired amount of copper acetate is dissolved into distilled water and stirred for 30 minutes using magnetic stirrer. The sample solutions were prepared by adding 3ml of leaf extract with 20ml of copper acetate solution and exposed to sunlight for 3 hours. After the completion of the reaction, the precipitate was centrifuged and washed with deionized water and dried in hot air oven at 60 °C.

RESULTS AND DISCUSSION

The dark brownish colored final product obtained after the reaction were characterized by various techniques such as UV, FTIR, FESEM and XRD in order to investigate their morphological and structural features.

X-ray diffraction Analysis

Figures (2a&b) show the XRD pattern of the CuO nanoparticles are synthesized by two different methods. The XRD pattern reveals the orientation and crystalline nature of CuO nanoparticles. The peak position with 2θ values is in good agreement with those of powder CuO obtained from the International Center of Diffraction Data card confirming the formation of a crystalline monoclinic structure. No extra diffraction peaks of other phases are detected, indicating the phase purity of CuO nanoparticles.

The average crystallite size of the CuO nanoparticles was evaluated by Debye-Scherrer's formula. The average crystallite size of chemical and bio CuO nanoparticles was found to be 57 nm and 35 nm respectively.

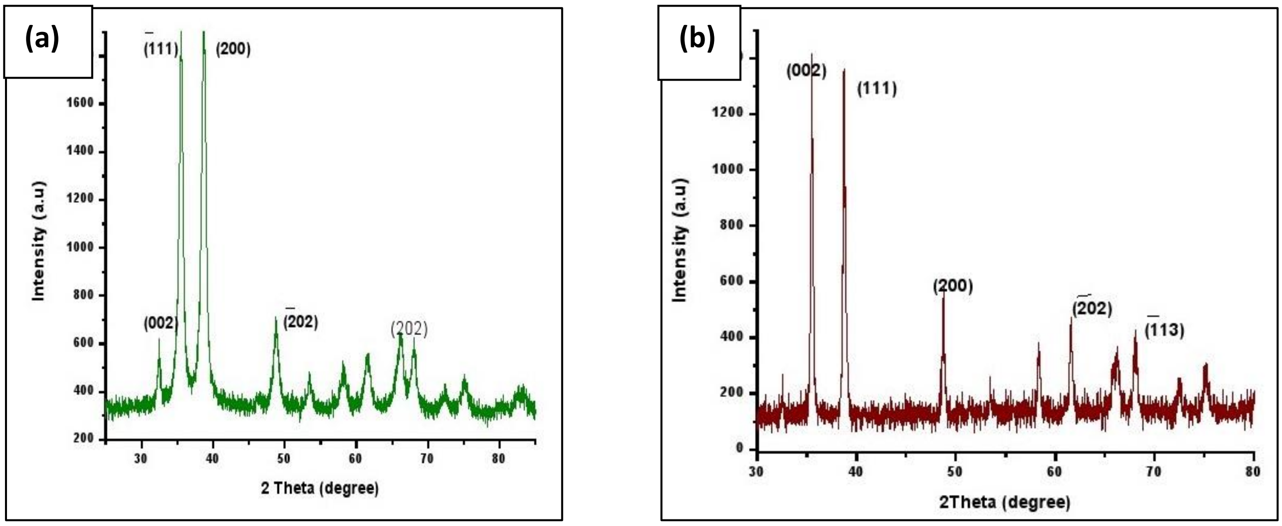


Figure.2 XRD analysis of bio (a) and chemical (b) synthesized CuO nanoparticles

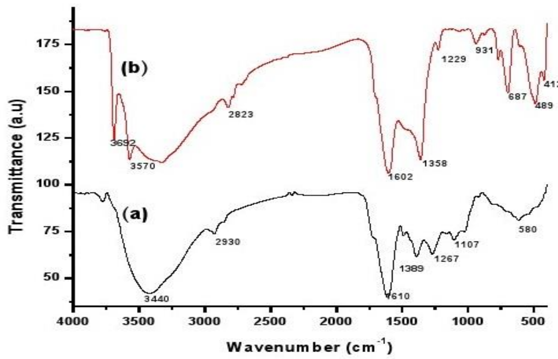


Figure. 3 FTIR analysis of bio (a) and chemical (b) synthesized CuO nanoparticles

FTIR analysis

The FTIR spectrum of chemical synthesis (Figure. 3a) and biosynthesis (Figure.3b) are given below. The FTIR analysis was used to identify and get an approximate idea of the possible biomolecules that are responsible for capping and stabilization of the CuO nanoparticles.

UV-Vis spectroscopy analysis

The results obtained from UV-Visible spectroscopy analysis of the CuO nanoparticles are presented in Figure (4a &b). The chemically synthesized CuO nanoparticles show the absorption peak near to 382 nm in the UV region. Figure 4(b) shows the sharp absorption peak appears at 365 nm which is slightly shifted to the shorter wavelength region.

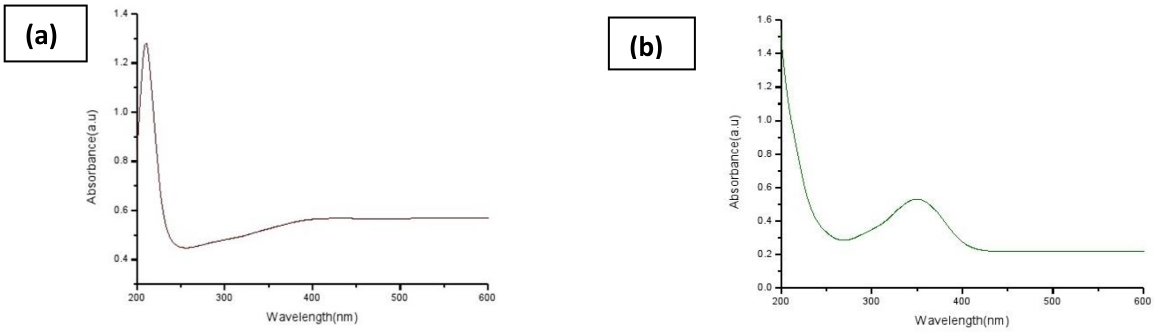


Figure 4. UV analysis of chemical (a) and bio (b) CuO nanoparticles

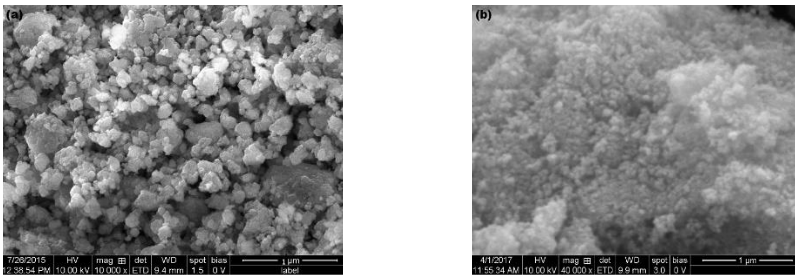


Figure. 5 FESEM image of (a) bio and (b) chemical synthesized CuO nanoparticles

P. aeruginosa ***Salmonella typhi***

Figure. 6 Zone of inhibition produced by bio-CuO nanoparticles

P. aeruginosa ***Salmonella typhi***

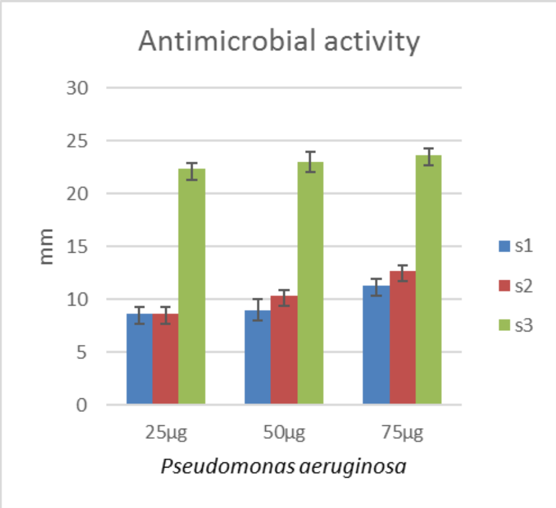
Figure. 7 Zone of inhibition produced by chemically synthesized CuO nanoparticles

FESEM Analysis

The surface morphology of the prepared CuO nanoparticles is shown in figure [5(a& b)]. Figure (5a) shows that the bio CuO nanoparticles are cubical in shapes and homogeneously distributed. Whereas the chemically synthesized CuO nanoparticles small sphere like in shape with some aggregation.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the CuO nanoparticle was examined against two important pathogenic bacteria such as *Pseudomonas aeruginosa* and *Salamonella typhi* using disc diffusion method. The zone of inhibition for different pathogen at different concentration CuO nanoparticles is displayed in Figure 6 &7. The presence of the clear zone of inhibition indicates the biocidal action of the CuO nanoparticles against bacterial strains. The histogram data confirms the strong antibacterial inhibitory effect of bio CuO nanoparticles against both pathogens (Figure 8).



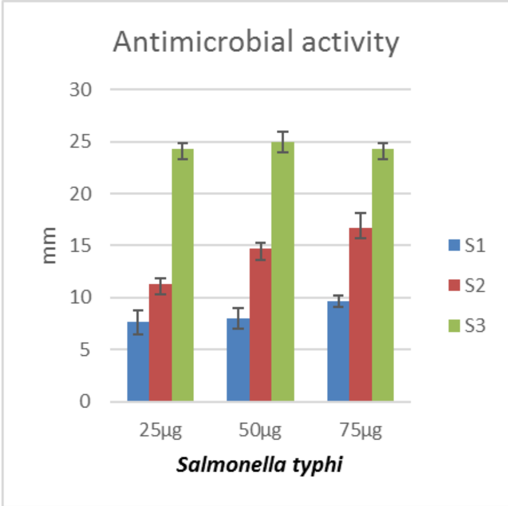


Figure. 8 Antibacterial activity of chemical (S1) and bio (S2) synthesized CuO nanoparticles

CONCLUSION

The study acclaims that CuO nano particles are successfully synthesized by two different methods. All characteristic studies such as XRD, FTIR, and UV-Visible confirmed the formation of CuO nanoparticles. The surface morphology of the prepared CuO nanoparticles was revealed through the FESEM image. It is found that *Cajanus Cajan* plant leaves are the good source for the synthesis of CuO nanoparticles. It is suggested that bio-CuO nanoparticles show more enhanced biocide activity against various human pathogens when compared to chemical CuO nanoparticles.

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