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Green Synthesis of CuO Nanoparticles using Phyllanthus Amarus Leaf Extract and their Antibacterial Activity Against Multidrug Resistance Bacteria

Green synthesis of CuO nanoparticles

N. P. S. Acharyulu, R. S. Dubey, V. Swaminadham
Department of Nanotechnology, Swarnandhra College of Engineering and Technology, Narsapur – 534 280, India.

R. L. Kalyani
Srinivasa Rao College of Pharmacy, P. M. Palem, Visakhapatnam, A.P-530041, India.

Pratap Kollu
[b]DST- INSPIRE Faculty, Department of Metallurgical Engineering & Materials Science, Indian Institute of Technology Bombay, Mumbai, India

S. V. N. Pammi*
Advanced Analytical Laboratory, DST-PURSE Programme, Andhra University, Visakhapatnam 530003, India.

Abstract*— In the present study, we report biologically oriented process for green synthesis of CuO nanoparticles by using eco friendly and non-toxic *Phyllanthus amarus* leaf extract. Powder X-Ray Diffraction (XRD) analysis revealed that synthesized CuO nanoparticles are in monoclinic phase with average particle size of 20 nm. The antibacterial activity of these nanoparticles was tested against various multidrug resistance bacteria viz. both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E.coli* and *P. aeruginosa).* Further, these nanoparticles show higher antibacterial activity against *B. subtilis* followed by *S. aureus, P. aeruginosa* and *E.coli.

Keywords*— *nanoparticles, plant extract, multidrug resistance bacteria, Phyllanthus amarus

I. INTRODUCTION

Cupric oxide (CuO) nanoparticles are of significant technological interest and have attracted more attention due to its unique properties. This transition metal oxide with a narrow band gap (E_g= 1.2 eV) forms the basis of several high temperature superconductors[1-4], gas sensors[5-7], giant magneto resistance materials[8-9], solar energy transformation and preparation of organic–inorganic nanostructure composites[10]. Applications include as an antimicrobial, anti-fouling, anti-biotic and anti-fungal agent when incorporated in coatings, plastics and textiles[11]. Copper and copper-based compounds, due to their potent biocidal properties[12], are now routinely used in pesticidal formulations[13] and several health related areas applications are being explored and/or implemented. Therefore, on the basis of the fundamental and practical importance of CuO nanomaterials, well-defined CuO nanostructures with various morphologies have been fabricated. There are several routes through which CuO nanoparticles can be formed, like Sonochemical[14], microwave irradiations[15], alkoxide based route[16], sol–gel[17] technique, one step solid-state reaction method at room temperature[18], electrochemical methods[19], precipitation-pyrolysis[20], thermal decomposition of precursor [21] or by combination of electro deposition and self-catalytic mechanism etc. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical application. Synthesis of high-quality nanomaterials with respect to chemical purity, phase selectivity, crystallinity, and homogeneity in particle size with controlled state of agglomeration in a cost-effective procedure is still a challenge to material chemists. Moreover chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical application. Increasing awareness towards green chemistry and other biological processes has led to a desire to develop an eco-friendly approach for the synthesis of nanoparticles. The use of environmentally benign materials like plant leaf extract[22], bacteria[23], fungi[24] and enzymes[25] for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. There are various reports on metal nano particles and very few reports on metal oxide nano particles using plant extracts and other biological methods[26]. However, studies on the synthesis of CuO nanoparticles by biological method are sparse.

In this study we report the green synthesis of CuO nanoparticles using *Phyllanthus amarus* leaf extract and study their antibacterial activity against various bacterial pathogens viz. both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E.coli* and *P. aeruginosa).*

II. EXPERIMENTAL SECTION

Preparation of CuO NPs using Phyllanthus amarus leaf extract.

In a typical procedure, *Phyllanthus amarus* leaf extracts were diluted to optimized concentration (25%), with distilled water and the volume was made up to 250 ml. Later, analytical grade copper sulfate was dissolved in the *Phyllanthus amarus* solution under constant stirring using magnetic stirrer. After complete dissolution of the mixture, the solution was kept under vigorous stirring at 130 ^{o}C for 7 h, allowed to cool at room temperature and the supernatant was discarded. The black solid product obtained was centrifuged twice at 3500 rpm for 20 min after thorough washing and dried at 120 ^{o}C for 6 h. The resulting dried precursor was crushed into powder and stored in air tight container for further analysis.

Determination of Antimicrobial Activity by the Well-Diffusion Method

Antimicrobial activities of the biosynthesized CuO nanoparticles were determined using Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*B. subtilis* and *S. aureus*). In brief, the bacteria were cultured in Müller–Hinton broth at 35°C ± 2°C on an orbital shaking incubator (Remi, India) at 160 rpm. A lawn of bacterial culture was prepared by spreading 100 µL culture broth, having 10^6 CFU/mL of each test organism on solid nutrient agar plates. The plates were allowed to stand for 10–15 minutes to allow for culture absorption. The 8 mm size wells were punched into the agar with the head of sterile micropipette tips. Using a micropipette, 50 µL (100 µg) of the nanoparticles solution sample was poured into each of wells on all plates after labeling. After incubation at 35°C ± 2°C for 24 hours, the size of the zone of inhibition was measured. A solvent blank was run as a negative control whereas the antibiotic (rifampicin) was used as a positive control.

III. RESULT AND DISCUSSION

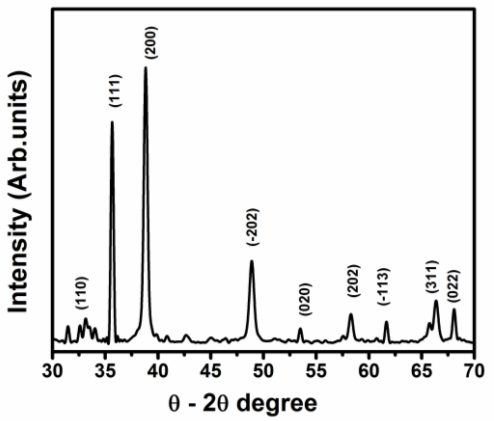

Fig. 1 XRD Pattern of green synthesized CuO NPs using *Phyllanthus amarus* leaf extract.

XRD analysis shows a series of diffraction peaks at 2θ of 32.41, 35.61, 38.81, 48.91, 53.31, 58.21, 61.61, and 66.31, which are assigned to the (110), (111), (200), (-202) ,(020), (202), (-113), and (022) planes of monoclinic CuO (JCPDS 45-0397), respectively (Fig.1). All diffraction peaks can be indexed as typical monoclinic structure and except for these CuO peaks, no other peaks corresponding to Cu or Cu_2O were observed. The average crystallite size of CuO nano particles was calculated using Scherrer formula, and was found to be about 22 nm indicating nano crystalline natures. The results of the present study indicated the successful preparation of CuO nano particles from *Phyllanthus amarus* leaf extract.

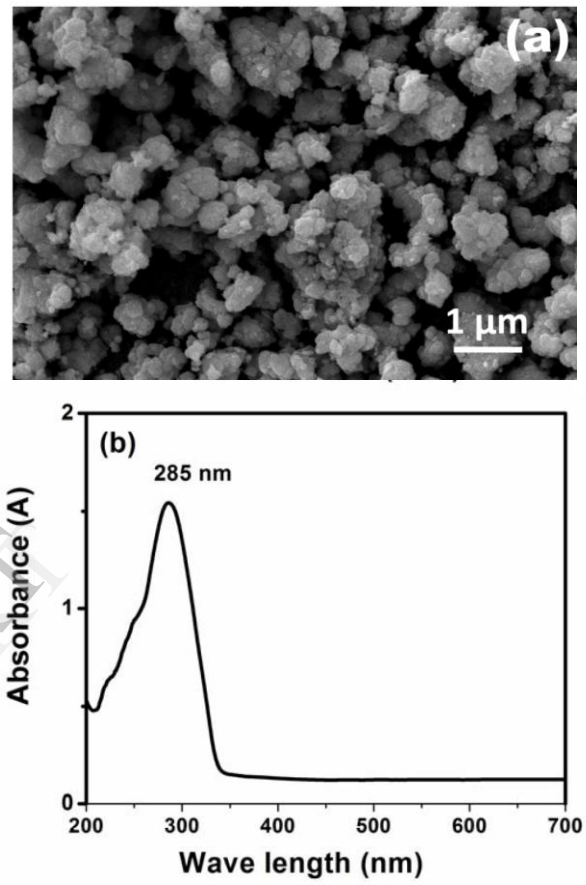

Fig. 2 SEM and UV-Vis Spectra of biosynthesized CuO using *Phyllanthus amarus* leaf extract.

SEM image (Fig. 2(a)) of CuO nano particles shows the presence of some large particles which can be attributed to aggregation or overlapping of smaller particles with sizes around 50 nm. Fig. 2 (b) showing UV-spectra of CuO particles indicated that the CuO SPR bands are obtained around 285 nm with absorption edge at 436 nm. The estimated optical band gap was about 2.39 eV (not shown here) which is much larger than that of bulk CuO and close to reported value 2.1 eV for CuO nano particles of 5-10 nm.

Antibacterial Activity of biosynthesized CuO nanoparticles using Phyllanthus amarus leaf extract:

Copper can also be used as an antimicrobial agent, and CuO nanoparticles have been investigated previously for enhancing antibacterial properties [11,12]. The bactericidal property of such nanoparticles depends on their size, stability, and concentration added to the growth medium, since this provides greater retention time for bacterium nanoparticles interaction. Furthermore, the antibacterial activities of CuO nanoparticles against multi drug resistance bacteria i.e., two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus*

subtilis) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were investigated. The zone of inhibition values of CuO nanoparticles and standard antibiotic (rifampicin) were represented in table 1.

CuO nanoparticles showed significant inhibitory effect against both Gram-positive and Gram-negative bacteria and is observed that maximum zone of inhibition has been noticed in case of *B.subtilis*, which was 55% more than he zone of inhibition of Rifampicin (positive control). It is clear from Table 1 (maximum zone of inhibition against B. subtilis and S. aureus) that CuO nanoparticles have shown greater antimicrobial activity against *B. subtilis* and *S. aureus*. The variation in the sensitivity or resistance to both Gram-positive and -negative bacteria populations could be due to the differences in the cell structure, physiology, metabolism, or degree of contact of organisms with nanoparticles. For example, greater sensitivity among Gram-positive bacteria such as B. subtilis and S. aureus to the CuO nanoparticles has been attributed to the greater abundance of amines and carboxyl groups on their cell surface and greater affinity of copper towards these groups[27]. Alternatively, Gram-negative bacteria like E. coli have a special cell membrane structure which possesses an important ability to resist antimicrobial agents[28]. Furthermore, other factors such as nanoparticle diffusion rates may also affect bacterial strain differently.

In conclusion, for the first time we report green synthesis of CuO nanoparticles using *Phyllanthus amarus* leaf extract. From XRD analysis we have calculated the average crystallite size 20 nm with monoclinic phase CuO. The effective antibacterial activity of biosynthesized CuO NPs against both Gram positive and Gram negative bacteria was examined. The biosynthesized CuO nanoparticles using *Phyllanthus amarus* leaf extract showed significant antibacterial activity against various multidrug resistance bacteria. In particular, these nanoparticles exhibited greater antibacterial activity towards gram positive bacteria when compared to gram negative bacteria.

Micro organisms	***E.coli***	***P.aeruginosa***	***B.subtilis***	***S.aureus***
CuO NPs-Zone of inhibition (mm)	24	25	31	28
Rifampicin – Zone of inhibition(mm)	20	18	20	20

Table 1: Anti Bacterial Activity and MIC of CuO Nanoparticles against Two Gram-Positive and Gram -Negative bacteria

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