

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF CUO NANOPARTICLES

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ABSTRACT

Objective: The present study was done to see the effect of biologically synthesized CuO-NPs (Copperoxide nanoparticles) on the growth of bacterial strains.

Methods: Physico-chemical characterization of CuO-NPs was done by UV-Vis-spectrophotometer, XRD, FE-SEM, and EDS. The disc plate diffusion assay was used to evaluate the anti-bacterial effect of CuNPs.

Results: This study has shown a promising anti-bacterial activity of biosynthesized CuO-NPs at different concentrations ranging from 10 to 100 µg/ml against *Escherichia coli* and *Staphylococcus aureus* bacteria.

Conclusion: Nanoparticles (NPs) are small size particles between range 1 to 100 nm which expand their physical and chemical properties due to high surface area. The present study reveals that there may be possible utilization of biosynthesized CuO NPs for the treatment of bacterial infectious disease in near future.

Keywords: Biosynthesis, Nanoparticles, Characterization, Anti-bacterial

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INTRODUCTION

Nanotechnology is the utilization of nanoscale materials between size range of 1-100 nm with specific physical, chemical and biological applications to benefit the mankind [1]. Currently, efforts are being made to develop ecofriendly methods for nanoparticle (NPs) synthesis. Plants and microbial based biosynthesis of NPs are considerably adopted and appreciated in different scientific areas. Copper oxide nanoparticles (CuO-NPs) are one among the category of various NPs with broad spectrum of biological activities and therapeutic effects. The green synthesis of CuO-NPs may overcome various drawbacks over pre-existing other chemical synthetic methods. The synthesis of Copper oxide nanoparticle by biological method provide high yield as compared to chemical method [2]. The biosynthesis of CuO-NPs has been reported from various plant-based extracts including *Tridax procumbens*, *Bifurcaria bifurcate*, *Aloe barbadensis*, soybeans, Magnolia, *Euphorbia nivulia*, and *Punica granatum* [3-10].

In the present study, green synthesis of CuO-NPs was carried out from Fenugreek (*Trigonella oenum graecum*) and Indian cherry (*Malpighia emarginata*), the oldest traditional medicinal plant found in various parts across the globe [11-14]. Previously, various researchers have noticed the reducing agent ability of Fenugreek and West Indian cherry to synthesize ecofriendly NPs of silver and gold [15-17]. Recent studies using Fenugreek and West Indian cherry as resource for CuO-NPs synthesis, has shown their several properties including anti-tumor [11, 13]. Number of deaths due to several bacterial infections is increasing every year. One major problem of existing therapies is resistance development which results in the failure of treatment. Therefore, there is an urgent need to explore novel antibacterial approaches with well-defined targets in microbial cells. Present study was designed to synthesize CuO-NPs using Fenugreek leaves and West Indian cherry fruits extract and to evaluate their antibacterial effects.

MATERIALS AND METHODS

Materials

Fenugreek leaves and West Indian cherry fruits were purchased from local fruit shops of Ambala, Haryana (India). Experimental

chemicals were procured from Sigma Aldrich Chemicals, India and E-Merck India. Bacterial Cell cultures were acquired from MTCC, Chandigarh, India.

Preparation of reducing extract

The phenolic components enriched extract of both the plant sources were prepared as described by Kim and Lee (2002) [18] with necessary modifications. Briefly, 100 g of freshly fenugreek leaves and edible portion of fruit were mixed in 100 ml of methanol separately. The prepared contents were transferred into blender and macerated at high speed for 3 min under controlled temperature. The crushed materials were sonicated in 50 ml of 80 % methanol (Aq.) for 20 min. Further, both the mixtures were passed through two strainers of varied pore sizes. The collected residues were re-extracted in 100 ml methanol followed by filtrations using Whatman no. 2 filter paper. The filtrates from both the plant sources were pooled and transferred to a 1000 ml capacity rotary evaporator with 80 ml of methanol (Aq.). Under vacuum, methanol was evaporated and the aqueous concentrated extract was resuspended in 100 ml of deionized water and kept at -20 °C for further experimentations.

Synthesis of CuO NPs

0.1 M of CuSO₄ solution in 30 ml of deionized water was treated with 25 ml of reducing plant extract. The solution was mixed well followed by the addition of 10 ml of NaOH (0.1 M). The mixture was stirred continuously at 55 °C for 2 h., centrifuged and obtained pellet was air dried. A dark black tone powder was stored in the sterile under condition [3, 5].

Analysis of CuO NPs

The synthesized NPs were initially examined by UV-visible absorption spectrum at of 250-800 nm using Perkin Elmer Lambda 20 UV-visible spectrophotometer. The XRD analysis using PAN alytical X'Pert Pro diffractometer with Cu K_α (λ= 1.5406 Å) radiation at 45 kV and 40 mA over the 2 θ range of 30-80 °, was performed to determine the phase purity of the synthesized NPs. Further, the crystallite sizes (D) of sample were calculated using Scherrer

formula (equation 1). Morphological and size associated features of synthesized NPs were determined by Field Emission Scanning Electron Microscopy (FE-SEM) Sigma from Carl Zeiss equipped with an Energy dispersive Spectroscopy (EDS) setup.

Assessment of anti-bacterial activity

Bacterial Cell culture and disc diffusion assay

Antibacterial activity of the synthesized copper nanoparticles was investigated for bacterial strains of Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). These human pathogenic bacteria were grown in microbiology labs. Each bacterium was cultivated on individual Petri dishes. Further, the bacteria were incubated on a nutrient agar slant (Stationary culture) for 24 h at 37 °C. To use bacterial cell culture in experiment, cultures were inoculated in fresh nutrient agar medium overnight in shaker incubator. Then 1 ml of these cultures of

bacterial strains were transferred to solidified nutrient agar. Once the plates were ready, various concentrations (20, 40, 60, 80 and 100 µg) of CuONPs loaded discs were placed over the Nutrient agar plates. All the plates were left to diffuse the sample and kept in an incubator at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured.

RESULTS AND DISCUSSIONS

Analysis (UV-Vis, XRD, FESEM and EDS) of CuO NPs

The UV-visible spectrum for green synthesized CuO-NPs is shown in the fig. 1. The biosynthesized CuO-NPs have shown one absorption peak at 265 nm and another weak but broad resonance centered peak at about 670 nm, indicating the formation of CuO-NPs. The peak at 265 nm is due to inter-band transition of core electrons of copper metal, while that of peak around 670 nm, and corresponds band edge transition of CuO [16].

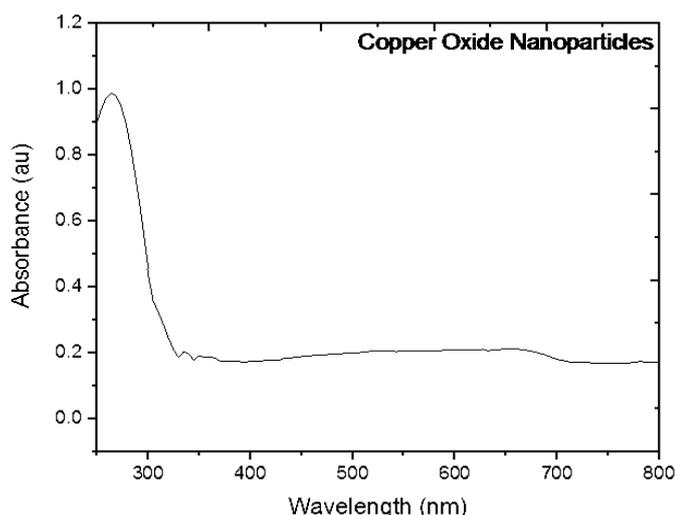


Fig. 1: UV-visible spectrum for CuO-NPs. CuO-NPs shows absorption peaks at 265 nm and 670 nm. The peak at 265 nm is due to inter-band transition of core electrons of copper metal, whereas peak at 670 nm corresponds to band edge transition of CuO

An XRD diffractogram for CuO-NPs is shown in fig. 2 having clear and strong peaks corresponding to 2θ values of 32.10, 35.40, 38.20, 48.40, 53.50, 58.10, 61.20, 65.50, 67.30 for the respectively marked indices of (110), (002), (111), (202), (020), (202), (113), (022), (113) respectively. These results are clearly indicating the formation of highly crystalline CuO-NPs [19]. The field emission electron microscopic (FESEM) image of

as-synthesized CuO-NPs (fig. 3) revealed the formation of slightly agglomerated spherical NPs. The diameter of the NPs was in the range of 20-80 nm. The energy dispersive X-ray analysis (EDX) of CuO-NPs (fig. 4) had prominent peaks of Cu and O confirmed the synthesis CuO-NPs. A peak of carbon had also been observed due to the carbon tape used for mounting the sample on aluminum stub before analysis.

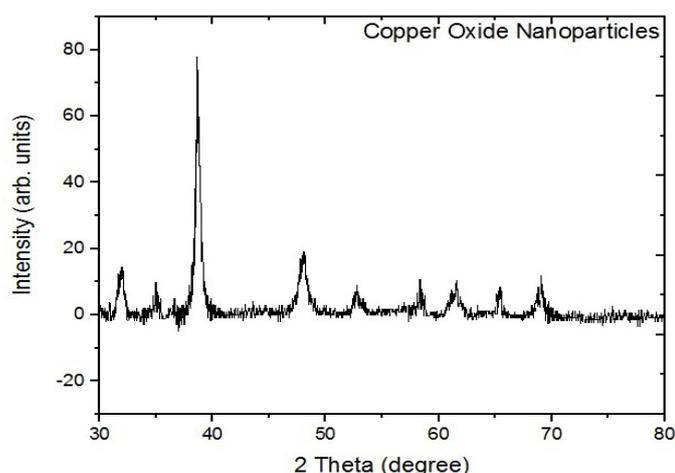


Fig. 2: XRD spectrum of CuO-NPs. The clear and strong peaks corresponding to 2θ values of 32.10, 35.40, 38.20, 48.40, 53.50, 58.10, 61.20, 65.50, 67.30 for the respectively marked indices of (110), (002), (111), (202), (020), (202), (113), (022), (113) respectively and indicating the formation of CuO NPs

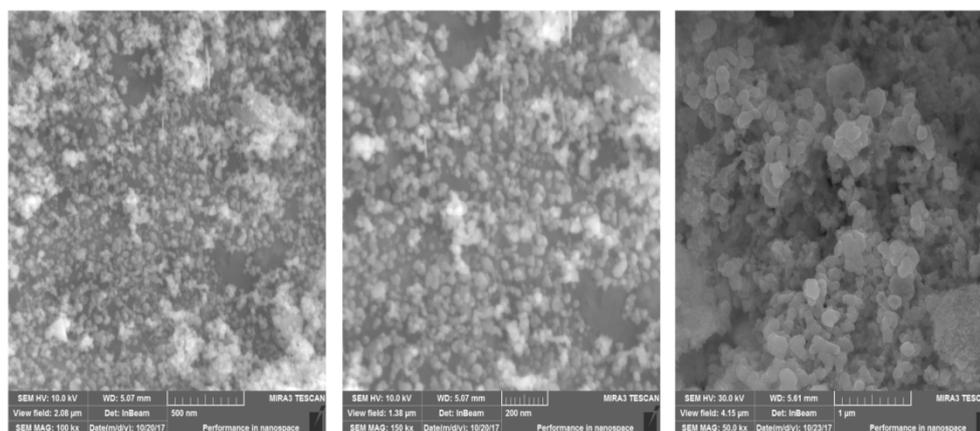


Fig. 3: FE-SEM images of CuO-NPs. (a) 500 nm (b) 200 nm the results revealed the formation of slightly agglomerated spherical NPs

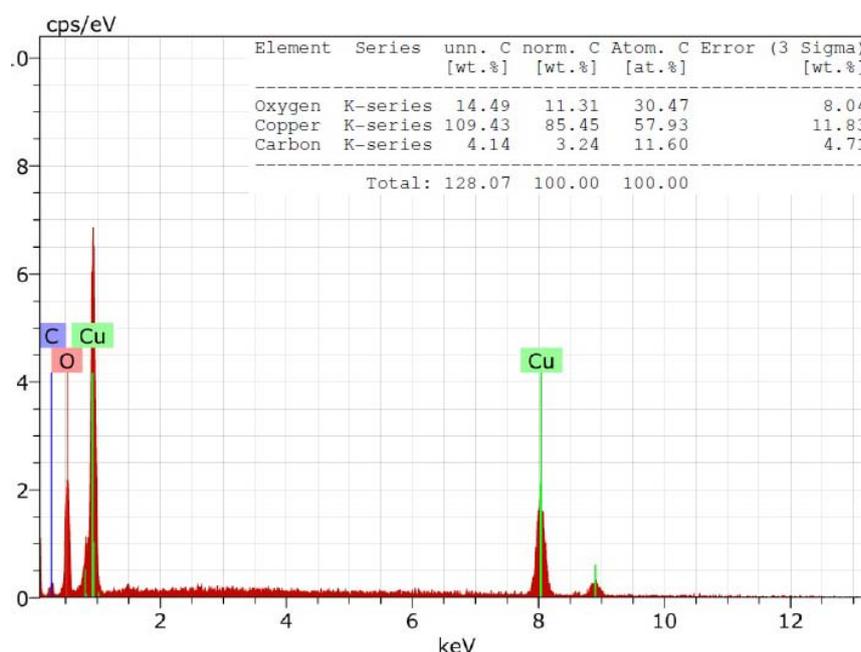


Fig. 4: EDX spectrum of CuO-NPs. The EDX of CuO-NPs had prominent peaks respective to Cu and O which confirming synthesis CuO-NPs. The peak for carbon was due to the carbon tape used for mounting the sample on aluminum stub before analysis

Antibacterial activity of copper oxide nanoparticles

The bacterial cells were exposed to different concentrations of synthesized CuO-NPs for 24 h and whereas control was without drug expose. A dose dependent anti-bacterial effect of CuO-NPs was observed on both the bacterial strains with IC_{50} 56 (*E. Coli*) and 46 (*S. aureus*) $\mu\text{g/ml}$. Maximum growth inhibitory effect of CuO-NPs was at 100 $\mu\text{g/ml}$ concentration.

DISCUSSION

The present study was performed to synthesize CuO-NPs and to check their anti-bacterial activities using Gram positive and Gram negative bacterial cultures. The CuO-NPs were successfully synthesized from Fenugreek and West Indian cherry extract. Multiple therapeutic effects of Fenugreek and West Indian cherry have already been reported [12,13]. Fenugreek Leaves and West Indian cherry extract have shown promising role as reducing agent for synthesizing of gold and silver nanoparticles [16]. Therefore, this is first report in best of our knowledge to synthesize biologically active CuO-NPs using fenugreek Leaves and West Indian cherry extract. The analytical results of synthesized nanoparticles were in good agreement with previously published reports. It has been

reported that metallic copper nanoparticles that are surrounded with copper oxide shells are characterized by absorption peak at 670 nm [5]. Similarly, the d-spacing values of the X ray diffraction planes are also in agreement with Cu (JCPDS no. 71-4610) structures [5, 20]. A broad diffraction peak of cuprite (111) was observed at a diffraction angle of 36.3° .

Further, cytotoxicity activity of NPs including Ag, Au, Cu, and Zn has already been a strong area of interest for treatment options [1]. In terms of biological activities, the synthesized CuO-NPs are found to show promising antibacterial potential. Statistic confirmed that infectious diseases are spreading most widely and there is urgent requirement for new drugs to reduce the mortality rate. CuO-NPs had shown significantly growth inhibitory effect on bacterial cell cultures upto the range of 100 $\mu\text{g/ml}$ [1]. It was observed that CuO-NPs disturb bacterial cell membrane which ultimately results in bacterial cell death. Bacterial cell membrane damage is known to be an important mechanism of action exhibited by a variety of nanoparticles [1,21]. Due to smaller size, a nanoparticle provides greater surface area and associated with cellular generation of ROS including superoxide anion, hydroxyl radical and hydrogen peroxide [14]. Furthermore, the CuO-NPs are also known to inhibit the

activity of B-lactamase enzyme that is responsible to impart antibiotic drug resistance character to the bacterial cell [22-23]. Earlier reports using bacterial cell cultures demonstrated that higher toxicity of NPs can also be related with their ability to block drug efflux pumps[15]. Recently CuO-NPs were synthesized by using *Desmodium gangeticum* root extract in economical and friendly manner. XRD analysis has confirmed the crystalline nature with cubic structure and average diameter of 1.46 nm. In another report CuO-NPs were prepared by sol-gel method and characterized by XRD and TEM techniques. Fluorescence quenching confirms the interaction of CuO-NPs with bovine serum albumin [16]. The results of present disc diffusion assay have revealed the broad spectrum of anti-bacterial activity of CuO-NPs towards *E. coli* and *S. aureus* strains. Our anti-bacterial results were consistent with previously published data [1].

CONCLUSION

The present study demonstrates that CuO-NPs can be successfully synthesized from Fenugreek Leaves and West Indian cherry extract. Results concluded that the biologically synthesized CuO-NPs possess potent anti-bacterial activity against human pathogenic bacterial strains. The growth inhibitory effect of CuO-NPs can be due to bacterial cell membrane, inactivating the β -lactamases and efflux pumps. Therefore, CuO-NPs could be considered as potent and inexpensive anti-bacterial agent. Off course, good studies are still required to be done before their use in clinical setting.

AUTHORS CONTRIBUTION

P Kumar, A G Nene, and F Thakral: Participated in the nanotechnology part; S Punia, M Kumar and Z Abbas: Contributed in paper writing and experimentation; H S Tuli: Experimentation, Results analysis and Proof reading [9].

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