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# Green synthesis of Cu nanoparticles using *Curcuma longa* extract and their application in antimicrobial activity

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ABSTRACT

In the present report, for the first time aqueous extract of *Curcuma longa* powder is used for the synthesis of Copper nanoparticles (Cu NPs) using a simple and cost effective method. Morphology, size, crystallinity, composition and microstructure of the synthesized Cu Nps are studied. Size of the particles are in the range of 5–20 nm. In addition to the above, antibacterial activity of the obtained Cu NPs is tested for both gram-positive and gram-negative microorganisms. Zone of inhibition of Cu NPs for gram positive bacteria is more compared to gram negative bacteria. The current work on green synthesis of metallic nanoparticles can be considered as an alternative method to avoid the usage of hazardous compounds and bitter reaction conditions in the production of metal nanoparticles. The obtained Cu NPs with their distinctive structural properties and effective biological effects can be used in applications viz. antimicrobial, antifungal, anticancer activity.



## 1. Introduction

Discovery of nanomaterials is considered as one of the scientific revolution of twenty first century, evidenced by the elaborate research around the globe. Among all the nanomaterials, nanoparticles have attracted lot of attention due to their various applications such as antimicrobial, antifungal, anticancer activity, agriculture, food, medical and cosmetics. Several synthesis methods have been developed for nanoparticles production which includes sonochemical reduction [1] thermal deposition [2] chemical reduction [3] and microwave methods [4]. All these synthesis methods involve hazardous compounds and bitter reaction conditions. Therefore, research has been focused on green synthesis of nanoparticles. Recently, many researchers reported the green synthesis of nanoparticles [5]. Green synthesis has advantages such as avoiding hazardous chemicals, clean process, nontoxic, environmental friendly, easy preparation, cost effective and control over size and shape [6].

Among all the metal nanoparticles, Cu NPs gained lot of attention due to their significant antifungal and antibacterial properties [7]. The advantages of Cu NPs are less expensive, shorter reaction time over conventional catalysts [8], acceptable replacements for conductive and expensive noble metals such as gold and silver in chemical and metallurgical processes, easy mixing with the polymers [9]. The major challenge in the synthesis of Cu NPs is to avoid the oxidation of Cu NPs during synthesis as well as storage. One method to avoid oxidation is conducting synthesis in the inert atmosphere which increases the complexity of the procedure and cost [8]. Therefore it is important to prepare Cu NPs using green synthesis method to prevent any oxide formation.

In the present work a yellow orange dye obtained from *C. longa* is used for the Cu NPs synthesis for the first time. The advantages of Curcumin are - arrests the formation of reactive-oxygen species, possesses anti-inflammatory properties due to the inhibition of cyclooxygenases (COX), anti-inflammatory, anti-cancer [10], antioxidant [11], wound healing and anti-microbial effects [12].

In the current manuscript, a novel green synthesis method for Cu NPs production is employed using aqueous extract of curcumin longa powder for the first time. Synthesized Cu NPs are characterized for their size, composition, microstructure and also their antibacterial activity towards gram +ve and gram −ve microorganisms.

## 2. Materials & methods

### 2.1. Extraction of c. longa solution

The *C. longa* tubers were collected from green house and thoroughly washed to remove the adhering mud particles and possible impurities. Later they were dried under sunlight for a week to

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completely remove the moisture. The final sieved powder was used for all further studies. For the production of extract, 10 g of *C. longa* tubers powder was dissolved in 100 ml of ethanol in a 200 ml flask and then stirred for 4 h at 70 °C temperature on a hot plate. The extract of *C. longa solution* was then filtered using Whatman 40 mm filter paper. The resulting solution will act as reduction and capping agent for the synthesis of Cu NPs.

### 2.2. Green route synthesis of Cu NPs

A microwave irradiation (Sharp R-219 T (W), 2.450 GHz) was used for the synthesis of Cu NPs. Copper acetate dihydrate solution (0.1 M/100 ml) was taken in 200 ml beaker and 50 ml of *C. longa* extract solution was added to it. The mixed solution was kept in the microwave oven for 180 s at 200 W power. The color of the solution changed from yellow to brick brown, which in turn affirms the formation of Cu NPs. (Supplementary information (SI) S1). Synthesized Cu NPs powder further characterized for their morphology, crystallinity, microstructure, size of the particles, stability and functional groups (SI S2).

### 2.3. Antibacterial activity

The Gram-negative and Gram-positive bacterial strains used for the present study were obtained from the Department of Microbiology, Osmania General Hospital, Hyderabad. The two strains were tested for purity by standard microbiological methods. The bacterial stock cultures were maintained on Mueller-Hinton agar slants and stored at 4 °C. An agar-well diffusion method was employed for the evaluation of antibacterial activities of test compounds. The bacterial strains were reactivated from stock cultures by transferring into Mueller-Hinton broth and incubating at 37 °C for 18 h. A final inoculums containing 106 colonies forming units ($1 \times 106$ CFU/ml) were added aseptically to MHA medium and poured into sterile Petri dishes. The test compounds at different concentrations were added to wells (8 mm in diameter) punched on the agar surface. Plates were incubated overnight at 37 °C and the diameter of inhibition zone (DIZ) around each well was measured in mm.

## 3. Results and discussion

Fig. 1(a) shows the morphology of the Cu NPs at different magnifications. It is clear from the figure that, nanoparticles are in agglomerated manner. FE-SEM images of the agglomerated Cu NPs are similar to the reported images in the literature for other nanoparticles also [13]. High resolution FE-SEM image confirms the sizes of the particles are in the range of ~5–20 nm. Composition of the Cu NPs powder was obtained from the EDS spectrum as shown in Fig. 1(c). EDS spectrum of Cu NPs confirms the presence of Cu, C and Oxygen.

Negligible amount of Oxygen observed in the EDS spectrum confirms the purity of the Cu NPs. The prepared Cu NPs powder was used for X-ray diffraction studies and the corresponding XRD pattern is shown in Fig. 1(d).

The presence of diffraction peaks at 43.26°, 50.32° and 74.05° are indexed as (1 1 1), (2 0 0) and (2 2 0) planes of Cu NPs respectively (JCPDS # 04-014-0265) and confirms the crystalline nature of nanoparticles. Impurity peaks, other phases such a CuO, $Cu_2O$ are not observed in the diffraction pattern concludes the purity of the prepared nanoparticles. The average crystallite size of the

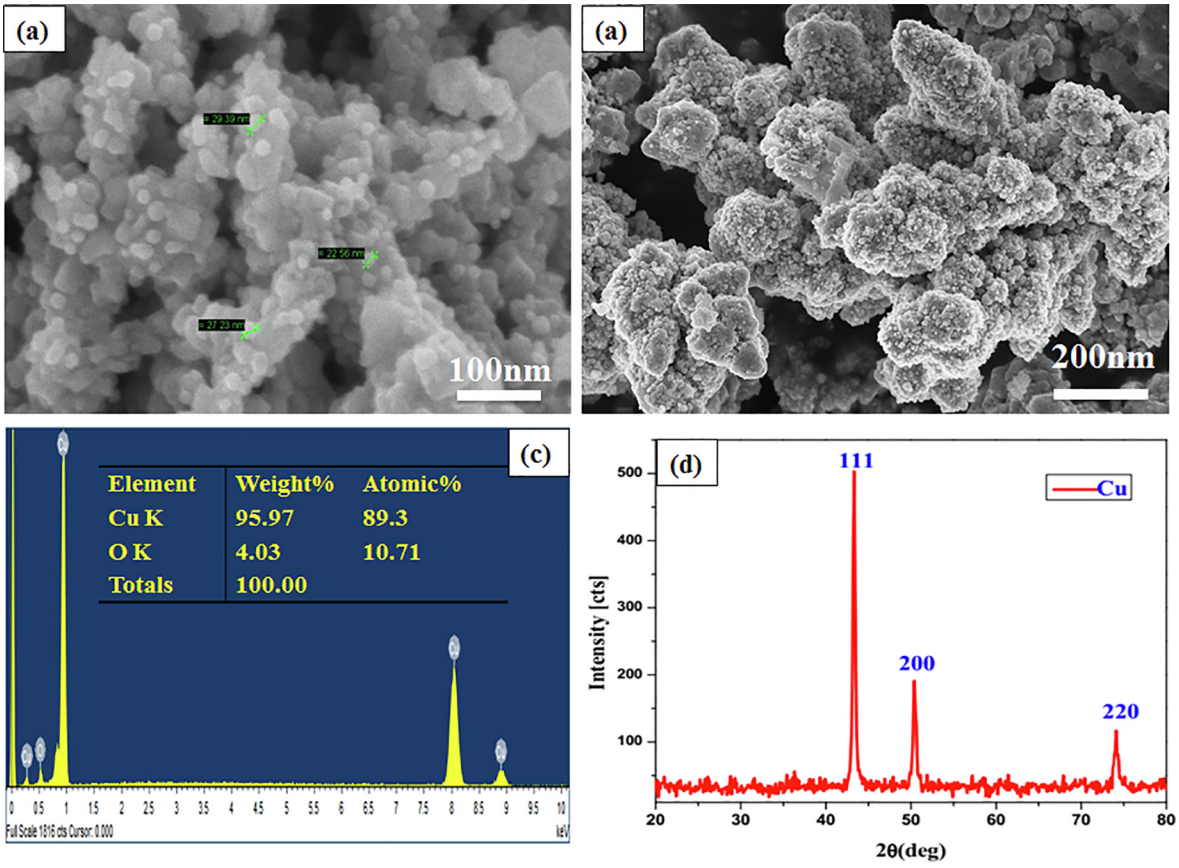


**Fig. 1.** (a)-(b) FE-SEM images Cu NPs (c) EDS spectrum of Cu NPs (d) XRD pattern of Cu NPs powder.

copper nanoparticles calculated using Debye Scherer formula is 16.77 nm.

Fig. 2(a) shows the TEM image of the Cu NPs at high magnification. Size of the particles are measured from high magnification image and found that in the range of 5–20 nm. Particle size distribution histogram of the Cu NPs is shown in the Fig. 2(b). Sizes of the nanoparticles are distributed in the range of 5–25 nm. Particle size measurement with TEM is in agreement with XRD, SEM data (SI S3). FTIR analysis was performed to pinpoint the bio molecules that are responsible for capping and stabilization of copper nanoparticles (SI S4).

Fig. 2(d) shows the UV absorption spectra of Cu NPs dispersed in ethanol solution. The maximum absorbance of copper nanoparticles is observed at a wavelength of 524 nm. This absorption band is attributed to Surface Plasmon Resonance (SPR) of Cu NPs and which is coinciding with the reported literature value [14]. It is observed a blue shift of 25–35 nm in copper nanoparticles synthesized using curcumin relative to that of bulk counterpart.

Antibacterial activity of Cu NPs was tested for gram positive and gram negative organisms. Photographs of the Zone of inhibition of Cu NPs against Gram +ve and Gram −ve organisms are show in the Fig. 3(a) and (b). Zone of inhibition diameter of Cu NPs against

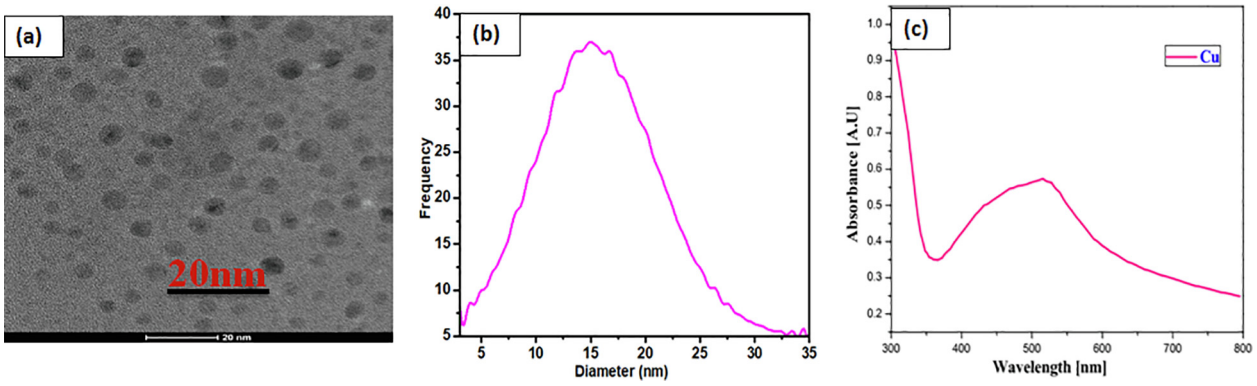


**Fig. 2.** (a) TEM image of Cu NP, (b) particles distribution histogram, (c) UV–vis analysis of Cu NPs.

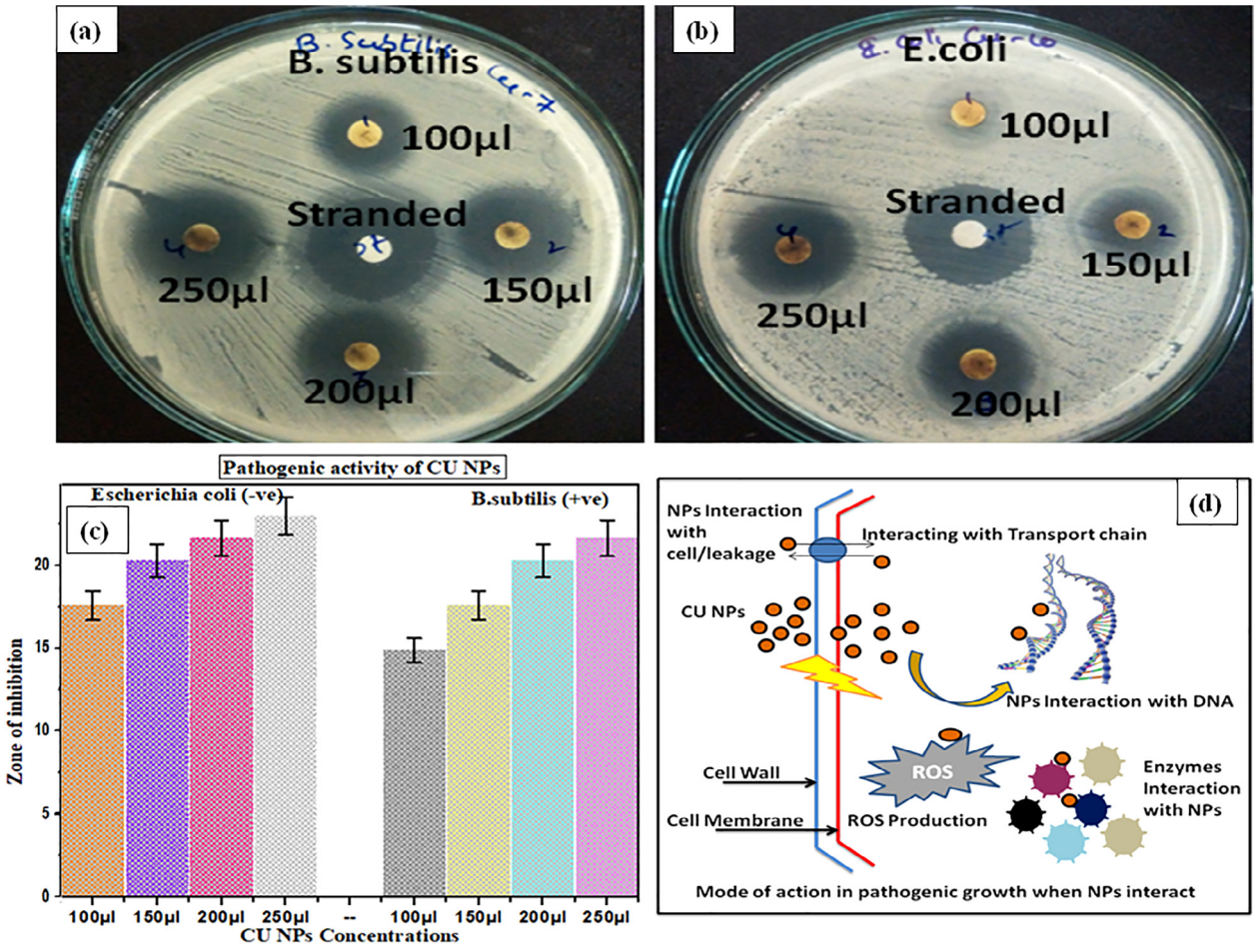


**Fig. 3.** antibacterial activity of Cu NPs towards (a) *B. subtilis* (+ve) (b) *E. coli* (−ve) (c) Zone of inhibition graph (d) mechanism of Cu NPs.

**Table 1**
Zone of inhibition effected by Cu NPs against *B. subtilis* (+ve) and *E. coli* (−ve).

| S.No. | Compound | Zone of inhibition (Diameter in mm at stock conc. 3 mg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | NPs | | | | | | | |
| | | *Basilus subtilis* (Gram +ve) | | | | *Escherichia coli* (Gram −ve) | | | |
| | | 100 μl | 150 μl | 200 μl | 250 μl | 100 μl | 150 μl | 200 μl | 250 μl |
| 1 | Cu NPs | 15 ± 0.18 | 19 ± 0.24 | 21 ± 0.57 | 23 ± 0.89 | 14 ± 0.15 | 18 ± 0.35 | 20 ± 0.55 | 22 ± 0.76 |

gram positive and gram negative organisms were presented in the Table 1. It is observed that Cu NPs demonstrated excellent antibacterial activity against both bacteria. The diameter of zone of inhibition reflects magnitude of susceptibility of microorganisms. Gram positive bacteria showed the larger zone of inhibition growth than Gram −ve bacteria that indicate the Gram +ve bacteria were more susceptible to Cu NPs when compared with the gram negative bacteria.

The Cu NPs possess antibacterial property which is useful in the treatment of various topical diseases caused by different microorganisms. The antibacterial activity of green synthesized Cu NPs is bind with ions that are liberated from nanoparticles. In general, smaller nanoparticles have the properties of high surface/volume ratio, high dispersion which allow more interaction with microorganism surfaces. Antibacterial activity is due to tendency to alternate between its cuprous and cupric oxidation states [15]. Differentiating Cu NPs from other trace metals, results in the creation of hydroxyl radicals that consequently bind with DNA molecules and lead to disorder of the DNA structure by cross-linking within and between the nucleic acid stands and damage essential proteins by binding to the sulfhydryl amino and carboxyl groups of amino acids [16,17]. The Cu ions inside the bacterial cell membrane also disrupt the biochemical process. Nano Cu quickly interact with bacteria cell membrane eradicate the respiratory system which create death of organisms. Finally, the present study clearly indicate the *C. longa* extract mediated Cu NPs exhibited the excellent antibacterial activity against Gram +ve and Gram −ve organisms. Previous reported studies shown smaller size has higher active penetration energy which killing the organism activity [18–22]. In present research article Cu NPs is shown to be more efficient towards Gram +ve and Gram −ve bacteria due to different cell wall morphology. The maximum zone of inhibition was found in 250 μl of Cu NPs against *B. subtilis* and *E. coli*.

## 4. Conclusion

A novel green route synthesis of Cu NPs was reported in this article using aqueous extract of *C. longa* solution for the first time. The synthesized particles are spherical in shape and crystalline in nature with average sizes between 5 and 25 nm. The *C. longa* extract-capped Cu NPs have exhibited attractive antibacterial activity with both Gram-positive and Gram-negative microorganisms. Cu NPs synthesized in this report can be used as an efficient antibacterial additive in textile coatings, disinfectants, and in antiseptic creams in areas such as food, medical and cosmetics applications.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2019.126813.

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