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Short Communication

# Antifungal activity of biosynthesized CuO nanoparticles using leaves extract of *Moringa oleifera* and their structural characterizations

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**ABSTRACT**

Copper oxide nanoparticles (CuO NPs) were synthesized using *Moringa oleifera* leaf extract *via* a simple green chemistry approach. The prepared CuO NPs were characterized using X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FT-IR), UV-visible diffuse reflectance spectroscopy (UV-DRS), and photoluminescence (PL) analysis. The CuO NPs showed antifungal activity against *Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*, *Trichophyton mentographytes*, and *Epidermophyton floccosum*. The results revealed the successful synthesis of CuO NPs by simple green chemistry approach may provide a useful tool in the field of nanotechnology.

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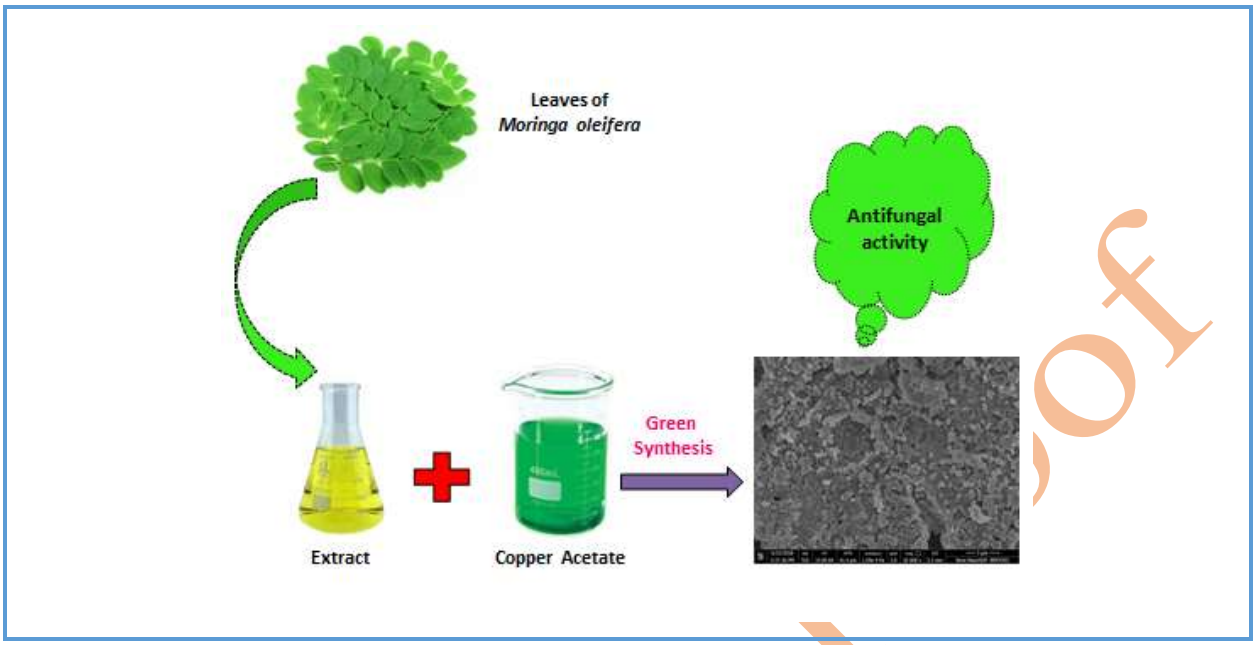
**Graphical Abstract**




## Introduction

In recent years, nanotechnology has attracted a great attention from interdisciplinary fields of research in science and technology. Metal oxide nanoparticles (NPs) have widespread popularity due to their remarkable application in variety fields including biomedical sciences, food technology, electronics, energy science, chemical industry, space industry, drug-gene delivery, and biosensor [1–14]. Among the various noble metal oxides, copper oxide (CuO) is an important p-type semiconductor and is extensively used for wide range myriad applications, such as solar cells [22], high-temperature superconductors [23], hydrogen storage materials [24], gas sensors [25], lubricant [26], catalytic applications [27], optical applications [28], and medical applications [29]. Hitherto, several methods have been used for the synthesis of CuO NPs, including electrochemical [30], hydrothermal route in the presence of PEG [31], ionic liquid assisted [32], microwave irradiation [33], precipitation [22], sol-gel [34], solid state reactions [35], solution combustion methods [36], thermal decomposition [37], ultrasonic mixing and self-assembly methods [38] and sonochemical method [39]. Nevertheless, these methods are suffered from some drawbacks such as the use of high temperature, pressure and perilous, toxic chemicals absorbed on the surface of NPs may cause adverse medical effects [1, 3, 4]. Therefore, it is a challenge to develop a simple, clean, convenient, swift, non-toxic and environmentally benign route to produce metal oxide NPs in an aqueous environment. Therein, Bacteria, fungi, actinomycetes, yeast and plants are used for NPs preparation [40], but a synthesis of NPs from plants is more stable, inexpensive, and is faster than that of the microorganisms. Hence, green synthesis of nanoparticles has lot of advantages over chemical and physical synthesis. Recently, green synthesis of CuO NPs by plants such as *Rauvolfia serpentina* [41], *Leucaena leucocephala* [42], *Calotropis*

*gigantean* [43], *Ziziphus mauritiana* [44, 45], *Aloe barbadensis* [46], *Gloriosa superba* [47], *Ficus religiosa* [48], *Albizia lebbeck* [49] and *Acanthospermum hispidum* [29] have been reported.

*Moringa oleifera* is native to Northern India; however, currently it is widely distributed in the Africa, Asia, Americas, Oceania and Europe. Leaves, seeds, pods and flowers of this tree are considered a food source of high nutritional value in many countries. Amongst them, leaves have high nutritional and medicinal value [50]. A scrutiny of the literature revealed some notable pharmacological activities of the drug like antiatherosclerotic, antioxidant, hypolipidaemic, anti-inflammatory, anticancer, antimicrobial, hepatoprotective, hypocholesterolemic, hypoglycaemic, immunomodulatory, nephroprotective, and neuroprotective activity [50].

In this work, we report the biogenic synthesis of CuO NPs using an aqueous leaves extract of *Moringa oleifera* and evaluated its antifungal activities by employing against some selected fungal strains. It was found that rapidly biosynthesized CuO NPs manifested potent biomedical application in the field of nanomedicine.

## Experimental

### *Materials and methods*

Copper acetate monohydrate [$Cu(CH_3COO)_2.H_2O$, 98%, LR grade, Sigma-Aldrich] was used without purification, and solutions were prepared using deionized water during the synthesis of CuO NPs. The fresh leaves of *Moringa oleifera* were provided from Chandwad, Maharashtra, India. The collected leaves were washed with deionized water and chopped into small pieces. All glassware's were washed with distilled water and acetone, and dried in oven.

### *Green synthesis of cuo NPs*

5 g small wizened pieces of *Moringa oleifera* leaves were transferred into 250 mL beaker containing 100 mL deionized water. The mixture were refluxed at 80-95 °C for 15 min and cooled at room temperature followed by filtered through ordinary filter paper. Then, resultant filtrate was again filtered through Whatmann No. 1 filter paper. The filtered extract is stored in refrigerator at 4 °C and used for biosynthesis of CuO NPs. The aqueous solution of 0.01 M copper acetate monohydrate was prepared in deionized water. *Moringa oleifera* leaf extract was mixed to 0.01 M aqueous copper acetate solution in 1:1 ratios in a 250 mL beaker with constant stirring on magnetic stirrer at 500 rpm (25 min). After time of period the color of solution turns to dark yellow. The resultant solution was centrifuged at room temperature by using cooling centrifuge machine at 4000 rpm (15 min) and the residue was collected after discarding the supernatant liquid. The obtain nanocrystalline CuO powder was kept in a muffle furnace at 400 °C (1 h) and subjected for combustion. A fine dark black colored material was obtained and this was carefully collected and packed for characterization purposes.

### *Characterization*

The crystal phases, purity and grain size of the CuO NPs were characterized by X-ray diffraction (XRD, Brukar, D8-Advanced Diffractometer) pattern measured with Cu-Kα Radiation (λ= 1.5406 Å) in the range of 10–80° at 40 kV and 40 mA. The shape, morphology and composition of the synthesized CuO NPs were determined by field emission scanning electron microscopy (FESEM, JEOL JSM-6701), equipped with energy-dispersive X-ray spectroscopy (EDX, Bruker, XFlash 6I30). Functional group and chemical composition were examined by

Fourier transform Infrared (FT-IR) spectrum (FT-IR 4600). UV-vis DRS absorption spectra of CuO NPs were performed by using JASCO spectrophotometer V-770. Spectral analyses of photoluminescence of CuO NPs were measured on FP-8200 Spectrofluorometer.

*In-vitro antifungal activity of synthesized CuO NPs*

Antifungal activity of synthesized CuO NPs examine against fungal strains (*Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, *Aspergillus clavatus* MTCC 1323, *Trichophyton mentographytes* MTCC 8476 and *Epidermophyton floccosum* MTCC 7880) using the agar dilution protocol [51]. To determine the minimum inhibitory concentration (MIC), a stock solution of the synthesized CuO NPs was prepared in dimethyl sulfoxide and then were incorporated in a specified quantity of molten sterile agar, dextrose agar for antifungal screening. The inoculums were prepared by taking a stock culture to about 100 mL of nutrient broth, in 250 mL clean and sterilized conical flasks. The flasks were incubated at 27 °C for 24 h before use. The plates were kept in aseptic condition at room temperature to allow diffusion of the solution properly into potato-dextrose-agar medium. Then the cultural plates were incubated at 25 °C for 48 h. The highest dilution displaying at least 99 % inhibition zone is taken as MIC and Greseofulvin were used as a standard reference drug for antifungal activity experiments. The experiments were performed in triplicate to minimize the errors of whole method of antifungal activity.

## Results and Discussion

*Structural and crystallographic analysis*

Figure 1 shows the XRD results which is used for the phase determination of the CuO NPs. Powder XRD of fabricated CuO NPs was carried out using monochromatic CuKα1 radiation (wavelength 1.5406Å) in the angular range 2θ of 10-80 deg. XRD profile exhibited a series of diffraction peaks at 32.37°, 35.48°, 38.7°, 48.9°, 58.18°, 66.03°, and 75.18°, corresponding to (110), (11-1), (111), (20-2), (202), (022) and (004) crystal planes of monoclinic CuO NPs, so obtained, is then confirmed by comparison with the data provided in MATCH! Software (card no. 96-901-5925). The average size of the CuO NPs were calculated using Debye–Scherrer's equation which was around 35-95 nm indicating its good crystalline in nature.

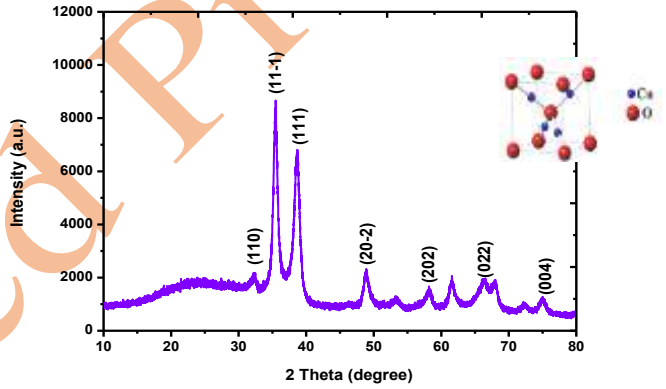



**Figure 1.** X-ray diffraction profile of synthesized CuO NPs at room temperature

*FESEM analysis*

Morphology and shape of biosynthesized CuO NPs was evaluated using FESEM. It can be seen that the average crystal grain size of the CuO NPs was mainly 35-95 nm having quasi-spherical shape except slightly agglomeration (Figure 2). This result exceed to the literature result which monoclinic structure of CuO NPs was prepared by green synthetic method [43].

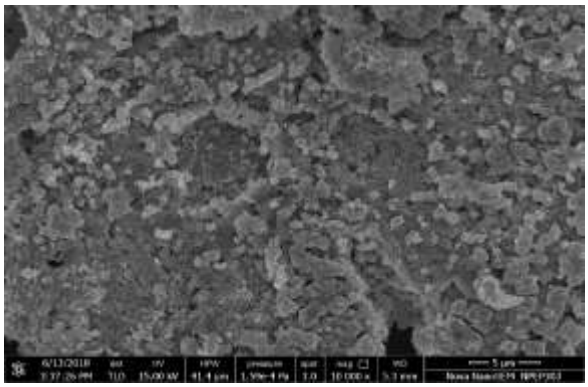


**Figure 2.** FESEM image of the synthesized CuO NPs

*EDX analysis*

Elemental composition of the biosynthesized CuO NPs is shown in Figure 3. This was carried out to understand the elemental composition of the copper and oxygen in the prepared CuO nanomaterial. There was no unidentified elemental peak observed in EDX. This quantitative data affirms the NPs purity, composition and formation of CuO NPs.

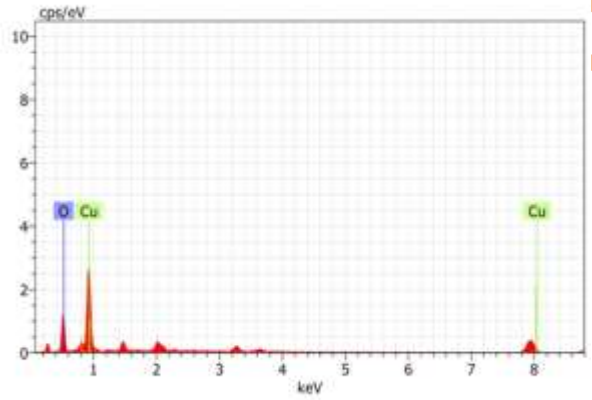



**Figure 3.** EDX spectrum of biosynthesized CuO NPs

*UV–Vis diffuse reflectance spectrum and photoluminescence of CuO NPs*

Figure 4 demonstrates UV-Vis DRS of CuO NPs. It can be seen that the CuO nanomaterial has good absorption capacity in the visible region. Therewithal, the band gap energy is important for the photocatalytic activity because the energy of incident light must be greater than or equal to the band gap energy is the criteria of material selectivity for the photocatalyst. The plot of $(\alpha h\upsilon)^2$ versus photon energy ($h\upsilon$) was obtained to determine band gap of CuO NPs (Figure 5). The band gap was found to be 3.38 eV [52], which suggest that the synthesized nanomaterial using green chemistry approach is useful for photocatalytic applications. Figure 6 exhibites the fluorescence spectrum of CuO NPs with an excitation wavelength of 290 nm. The spectrum showed broad band peak of emission at 288 nm and 580 nm.

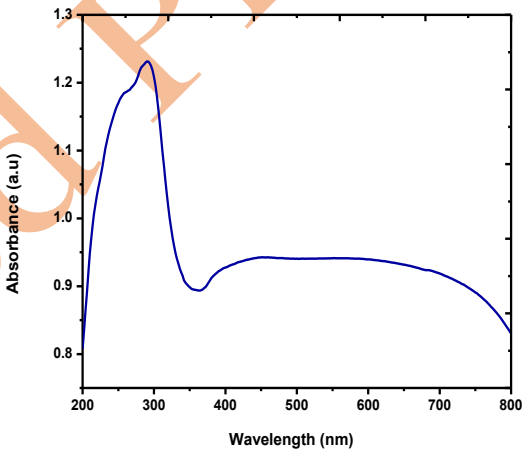



**Figure 4.** UV-vis DRS spectrum of synthesized CuO NPs

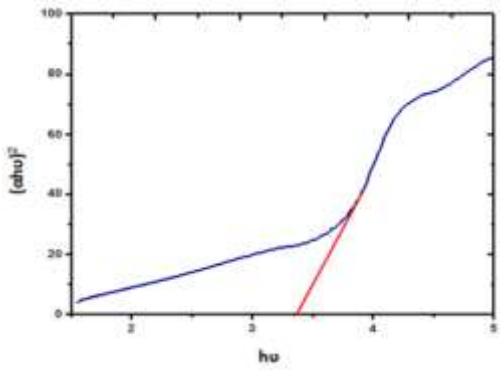



**Figure 5.** A plot of $(\alpha h\upsilon)^2$ versus photon energy ($h\upsilon$)

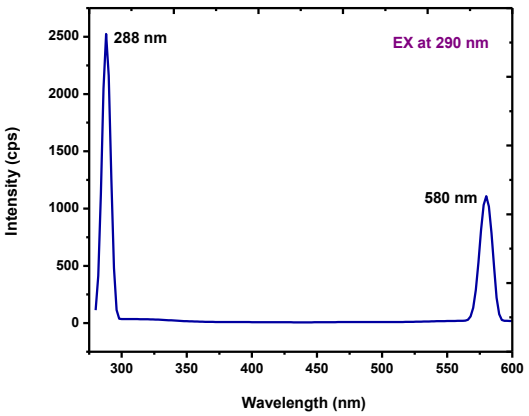
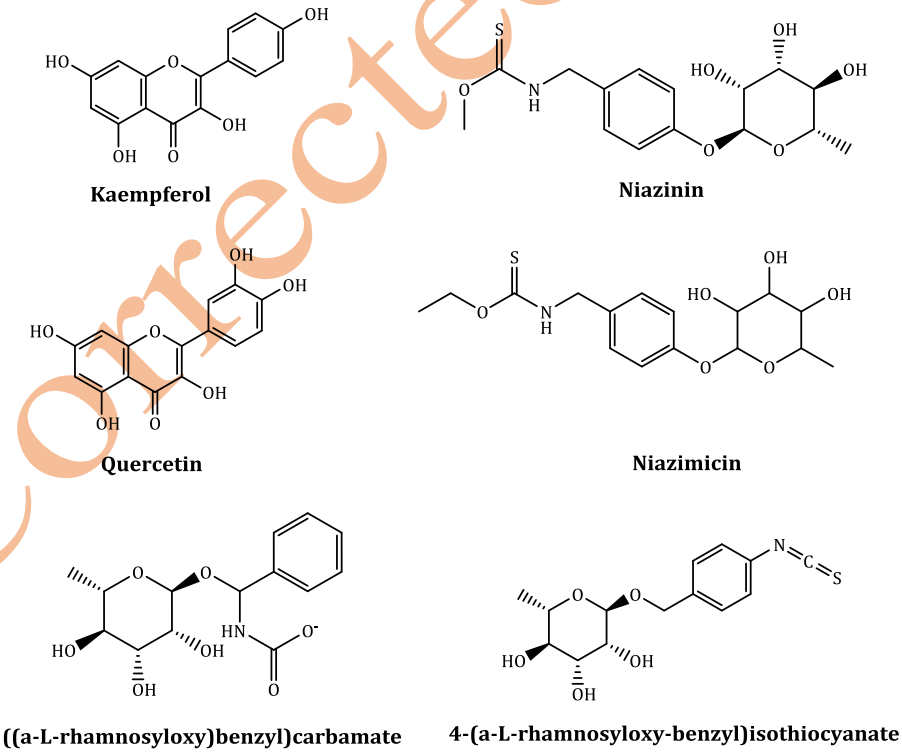



**Figure 6.** Fluorescence spectra of biosynthesized CuO NPs

*Vibrational properties*

The aqueous extract of *Moringa oleifera* contains a lot of phytochemicals such as alkaloids, glycosides, phenols, tannins, saponins, glucosinolate, and flavonoids. These compounds will act as an encapsulating agent and can be reduced from copper metal salt precursor to CuO NPs [43]. To further affirm the formation of the CuO NPs crystal structure using FT-IR spectroscopy as shown in Figure 7. The strong IR band located at 535 $cm^{-1}$ can be attributed to vibrations of CuO, confirming the formation of CuO NPs. The broad peak observed at 1100 $cm^{-1}$ corresponds to C-O stretching frequency of phytochemicals in the *Moringa oleifera* aqueous leaves extract. The peaks around at 1384 $cm^{-1}$ correspond to the O-H bend of flavonoids and polyphenols, confirm the presence of an aromatic group. The absorption peak appearing at 1577 and 3347 $cm^{-1}$ could be correlated to the bending and stretching vibrations bands of adsorbed water and residual -OH group. The biomolecules in leaf extract (Scheme 1) of *Moringa oleifera* are responsible for the capping and stabilization of CuO NPs [50].




**Scheme 1.** Major bioactive compounds in the natural leaves extract of *Moringa oleifera*

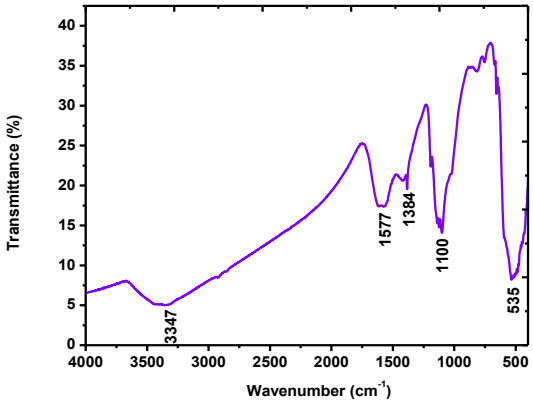



**Figure 7.** FT-IR spectrum of biosynthesized CuO NPs

*Antifungal activity of CuO NPs*

The results of antifungal activity of the biosynthesized CuO NPs are presented in Table 1. The antifungal activity of the synthesized CuO NPs was determined *in-vitro* using an Agar plate method against selected strains (*C. albicans*, *A. niger*, *A. clavatus*, *T. mentographyte* and *E. floccosum*) at different concentration ranging between 100 µg/mL to 1250 µg/mL. Biosynthesized CuO NPs exhibited a moderate activity against *T. mentographyte*, *E. floccosum* and showed excellent activity against *C. albicans* at concentration of 250 µg/mL reference standard Griseofulvin at concentration 500 µg/mL.

**Table 1.** MIC of biosynthesized CuO NPs against fungal pathogens

| Test pathogens | MIC (µg/ ml) of CuO NPs | MIC (µg/ ml) of Reference drug |
|---|---|---|
| *C. albicans* (MTCC 227) | 250 | 500 |
| *A. niger* (MTCC 282) | *250* | *100* |
| *A. clavatus* (MTCC 1323) | 250 | 100 |
| *T. mentographytes* (MTCC 8476) | 100 | 100 |
| *E. floccosum* (MTCC 7880) | 100 | 100 |

## Conclusion

We have successfully synthesized monoclinic CuO NPs via green synthetic route to obtain biologically active nanomaterial. The synthesized CuO NPs were quasi-spherical in shape as observed in FESEM analysis. The UV-DRS spectrum confirmed the synthesized CuO NPs has high absorption with 3.38 eV band gap. The synthesized CuO NPs has shown excellent antifungal activity and hence it may be useful for the treatment various fungal disease. The CuO NPs synthesized using plant extract may be implemented as nanomedicine in near future.

## Acknowledgements

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## Disclosure statement

No potential conflict of interest was reported by the authors.

## Orcid

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