Green synthesis of fluorescent CdO nanoparticles using *Leucaena leucocephala* l. extract and their biological activities

**Abstract**

The synthesis of metal oxide nanoparticle is in vogue due to their miraculous application in diverse fields. In this study, we report the facile green synthesis of cadmium oxide nanoparticles (CdONPs) synthesized by an implicitly environmentally benign process using *Leucaena leucocephala* L. aqueous plant extract as an effective stabilizing and capping agent. The characterization of green synthesized CdONPs were done by using field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform Infrared (FTIR) and Photoluminescence. Moreover, CdONPs evinced potent antimicrobial, antimalarial and antimycobacterial activity against selected human pathogens.

**Keywords:** nanotechnology, green synthesis, *leucaena leucocephala* l., CdONPs, biological activities

**Abbreviations:** CdONPs, cadmium oxide nanoparticle; FESEM, field emission scanning electron microscopy; EDX, energy-dispersive x-ray spectroscopy; FTIR, fourier transform infrared

**Introduction**

Over the past few decades, the use of nanostructured material is becoming more widespread due to its curious and miraculous application in the areas of chemistry, pharmacy, agriculture, textile sizing, optoelectronics, physics and so on. Among these nanostructures, metal oxide nanoparticles that exhibit the technological importance for solar cell, gas sensor, optical coating and photovoltaic cell. Therein, cadmium oxide is a known n-type semiconductor, piezoelectric characteristics and polycrystalline in nature. Cadmium oxide nanostructures are applied in solar cells, gas sensors, transparent electrodes and photodiodes, catalysts, photocatalysts and optoelectronic devices. There are several techniques to prepare these materials such as sonochemical, microemulsion, hydrothermal and plant mediated method. However, currently plant extract mediated nanomaterial synthesis is getting lot of attention to the several numerous advantages offered by chemical and physical methods. Herein, we investigate the cost effective, safe and ecofriendly green synthesis of CdONPs using plant extracts of *Leucaena leucocephala* L. and their antibacterial, antimalarial and antimycobacterial activity against bacterial pathogens has been evaluated. Hence it is proposed that the biosynthesized CdONPs have significant biomedical applications.

**Materials and methods**

**Materials**

Cadmium nitrate tetrahydrate ([Cd(NO₃)₂]·4H₂O 98%, Analytical grade, Sigma-Aldrich), sodium bicarbonate (NaHCO₃, Analytical grade, 99.7%, Sigma-Aldrich) and dimethyl sulfoxide (DMSO, ACS reagent, 99.9%, Sigma-Aldrich) were used. All chemicals were used as such without any further purification. All the solutions were prepared using deionized water. The fresh leaves of *Leucaena leucocephala* L. were collected from Chandwad college campus, Nashik, Maharashtra, India. The collected leaves were washed with deionized water, cut into small pieces. All glassware’s are washed with deionized water and acetone and dried in oven before use.

**Green synthesis of CdONPs**

5g powder of *Leucaena leucocephala* L. leaves were transferred into 250ml beaker containing 100ml deionized water. The mixture were refluxed at 80–90°C for 20minutes and cooled at room temperature followed by filtered through ordinary filter paper. The resultant filtrate was again filtered through Whatmann No. 1. The filtered extract is stored in refrigerator at 4°C and used for synthesis of CdONPs. 2.0g of Cadmium nitrate tetrahydrate was added in 100ml of the *Leucaena leucocephala* L. water extract solution. The solution was mixed homogeneously using magnetic stirrer at 400rpm for 60min (Figure 1). After time of period the color of solution turns to yellow. The solid deposit was purified by centrifugation at 4000rpm for 30min. It was then dried in oven at 300°C. The resulted powder was obtained and packed for characterization purposes.

**Characterization techniques**

The morphology and composition of the synthesized CdONPs were examined by field emission scanning electron microscopy (FESEM, FEI, Nova Nano SEM 450), FESEM coupled energy-dispersive X-ray spectroscopy (EDS, Bruker, XFlash 6130). The Fourier transform
Infrared (FTIR) spectrum was recorded by JASCO 4100 in the range of 4000–400 cm$^{-1}$. Photoluminescence studies were evaluated by using fluorescence spectrophotometer (JOBIN YVON FLUOROLOG-3-11, Spectrofluorimeter).

**In Vitro antimycobacterial screening of synthesized CdONPs**

The antimycobacterial screening for synthesized CdONPs was obtained for Mycobacterium tuberculosis H37RV, by using L J (Lowenstein and Jensen) MIC method. Stock solutions of primary 1000, 500, 250 and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25μg/ml of CdONPs in DMSO were added in the liquid L J Medium and then media were sterilized. A culture of Mycobacterium tuberculosis H37RV growing on L. I. medium were harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37°C for 24hrs. These tubes were then incubated at 37°C. Growth of bacilli was seen after 12days, 22days and finally 28days of incubation respectively. Tubes having the CdONPs were compared with control tubes where medium alone was incubated with Mycobacterium tuberculosis H37RV. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain Mycobacterium tuberculosis H37RV was tested with known drug isoniazid.

**Results and discussion**

**FE-SEM microphotographs**

From the FESEM image as shown in Figure 2 the synthesized CdONPs present uniform and define spherical morphology. Each CdONPs possesses the average particles size of 36-57nm. It is noticed that green synthesis of CdONPs produces the small and uniform size of spherical particles.

**EDS studies**

The composition of green synthesized CdONPs has been analyzed by investigating the energy-dispersive X-ray spectroscopy (EDS), as shown in Figure 3. EDS spectrum displays the Cd and O peaks. Other peaks corresponding to C in the EDS is an artifact of the phenols, flavonoids, coumarins and enzymes capping over the synthesized CdONPs. The antimycobacterial screening for synthesized CdONPs was carried out in 96 well microtiter plates according to the protocol.

**Vibrational properties**

Figure 4 represents the FTIR spectrum of CdONPs synthesized from leaves of Leucaena leucocephala L. The broad peak at 3352cm$^{-1}$ reveals the presence of an O-H functional group on the surface of nanoparticles. The corresponding to C-H asymmetric stretching vibration occurs at 2924cm$^{-1}$. The peaks around 1614cm$^{-1}$ is corresponding to C=C in CdO backbone, and those at 1371cm$^{-1}$ correspond to wagging of CH2 vibration. The FTIR results confirm

**Figure 1 Schematic diagram of green synthesis of CdONPs.**

**Figure 2 FE-SEM microphotographs of CdONPs deposited on a carbon strip.**

**Figure 3 EDS spectrum of CdONPs synthesized**

**Figure 4 FTIR spectrum of CdONPs synthesized**
the presence of phytochemicals in the plant extract such as, which further act as capping agents for the synthesis of CdONPs and is in good agreement with the phytochemical screening of aqueous leaves extract of *Leucaena leucocephala*.

Table 1 Phytochemical screening of aqueous extract of *Leucaena leucocephala* L.

| Phytochemical       | Test | Phytochemical       | Test |
|---------------------|------|---------------------|------|
| Tannin              | +    | Saponins            | +    |
| Coumarins           | +    | Emodins             | -    |
| Proteins            | -    | Flavonoid           | +    |
| Cardial Glycoside   | +    | Anthraquinone       | -    |
| Anthocyanosides     | -    | Steroid             | +    |
| Phenol              | +    | Amino acids         | +    |
| Carbohydrate        | +    |                     |      |

Antimicrobial activity of CdO-NPs

In this context, we decided to investigate antimicrobial activity of green synthesized CdONPs against selected human pathogens viz *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Candida alicans* and *Aspergillus niger*. These bacterial and fungal strains were poured into nutrient agar plate and spread evenly over the plate with the help of glass spreader and the “well” was made with the help of disc diffusion method. The different concentrations of synthesized CdONPs (25, 50, 100, 250, 500µg/ml) were tested for antimicrobial activity against these selected pathogen with ampicilline has positive control. The plates were then kept at 4-5°C for 1hr, followed by incubated in incubator at 37°C for 24hrs. After 24hrs, exact zone of inhibition was measured with respect to positive controls (Table 2).

Antimalarial activity

The green synthesized CdONPs were screened using *in vitro* antimalarial activity against *Plasmodium falciparum* by measuring
the MIC (µg/mL) against standard Quinine and Chloroquine, as shown in Table 3.

Table 2 Zone of inhibition (mm) of green synthesized CdONPs against selected bacterial pathogens

| Test pathogens | Inhibition zone (mm) of CdONPs (µg/mL)control |
|----------------|---------------------------------------------|
|                | 25 | 50 | 100 | 250 | 500 |
| E. coli        | 12 | 17 | 18  | 18  | 22  |
| P. aeruginosa  | 14 | 16 | 17  | 18  | 20  |
| S. pyogenus    | 13 | 15 | 17  | 19  | 21  |
| S. aureus      | 13 | 17 | 19  | 20  | 23  |
| C. albicans (Fungi) | 12 | 14 | 15  | 19  | 20  |
| A. niger (Fungi) | 15 | 16 | 18  | 20  | 24  |

Table 3 Minimum inhibition concentration (MIC) of green synthesized CdONPs against Plasmodium falciparum

| Sl. no | Compound name | Mean IC\textsubscript{50} values |
|--------|---------------|---------------------------------|
|        | CdONPs        | 0.95µg/ ml                      |
|        | Chloroquine (Standard) | 0.020µg/ ml |
|        | Quinine (Standard) | 0.268µg/ ml |

Antimycobacterial activity of CdONPs

The antimycobacterial screening of green synthesized CdONPs were performed using L J MIC method and it is worthwhile to note that CdONPs were the only evinced inhibition of *Mycobacterium tuberculosis* H37RV completely (99%) at the MIC of 125µg/ml (Table 4).

Table 4 Minimum inhibition concentration (MIC) of green synthesized CdONPs against *Mycobacterium tuberculosis*

| Sl. no | Compound name | MIC (µg/mL) |
|--------|---------------|-------------|
|        | CdONPs        | 125µg/ ml   |
|        | Isoniazid (Standard) | 0.20µg/ ml |

Conclusion

A facile, safe and green approach has been developed to synthesize CdONPs by using extract *Leucaena leucocephala* L. as both reducing and stabilizing agents. The green synthesized CdONPs exhibit potent biological activity against selected human pathogens. Overall, we conclude green synthesized CdONPs as a potential candidate for biomedical applications because of their absorbing properties.

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Conflict of interest

The authors declare no conflicts of interest in this work.

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