Green Synthesis of Fluorescent CdO Nanop

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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 reports 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

Research Article

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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 [1]. Among these nanostructures, metal oxide nanoparticles that exhibit the technological importance for solar cell, gas sensor, optical coating and photovoltaic cell [2]. Therein, cadmium oxide is a known n-type semiconductor, piezoelectric characteristics and polycrystalline in nature [3]. Cadmium oxide nanostructures are applied in solar cells, gas sensors, transparent electrodes and photodiodes, catalysts, photocatalysts and optoelectronic devices [4]. There are several techniques to prepare these materials such as sonochemical, micro-emulsion, hydrothermal and plant mediated method [1,2,4]. However, currently plant extract mediated nanomaterial synthesis is getting lot of attention to the several numerous advantages offered by chemical and physical methods [5,6]. 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 ($[Cd(NO_3)_2].4H_2O$ 98%, Analytical grade, Sigma-Aldrich), sodium bicarbonate ($NaHCO_3$, 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 during the synthesis. 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 250 mL beaker containing 100 mL deionized water. The mixture were refluxed at 80-90 °C for 20 minutes and cooled at room temperature followed by filtered through ordinary filter paper. Then, 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. 2.0 g of Cadmium nitrate tetrahydrate was added in 100 ml of the *Leucaena leucocephala L.* water extract solution. The solution was mixed homogeneously using magnetic stirrer at 400 rpm for 60 min (Figure 1). After time of period the color of solution turns to yellow. The solid deposit was purified by centrifugation at 4000 rpm for 30 min. It was then dried in oven at 300 °C. The resulted powder was obtained and packed for characterization purposes.

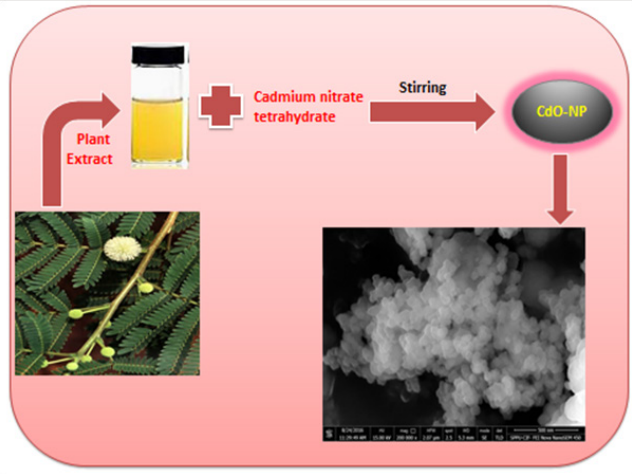

Figure 1: Schematic diagram of green synthesis of CdONPs.

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 6I30). 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 FLUROLOG-3-11, Spectroflurimeter).

Phytochemical Screening

The fresh aqueous extract of *Leucaena leucocephala L.* leaves were investigated for the presence of phytochemicals viz. coumarins, volatile oils, tannin, flavonoids, saponins, sterols and phenols by using protocol of standard biochemical methods [7].

Antimicrobial activity of synthesized CdONPs

The antimicrobial activities of synthesized CdONPs were examined by using Disc diffusion method. This method was employed against selected human pathogens i.e. *Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus,Escherichia coli, Candida alicans* and *Aspergillus niger* obtained from Institute of Microbial Technology, Chandigarh, India. The nutrient agar medium (g/l) plates were sterilized, prepared and inoculated. After inoculation, bacterial cultures spread evenly over the plate, and then various concentration of CdONPs solution was poured into each plate. These plates were incubated in incubator at 37 °C for 24 hrs and zone of inhibition against bacterial strains were measured.

In vitro antimalarial screening of synthesized CdONPs

The *in vitro* antimalarial assays were carried out in 96 well microtitre plates according to the protocol [8]. The cultures of Plasmodium falciparum strain were maintained in medium of RPMI-1640 supplemented with 25mM HEPES, 0.23% NaHCO3, 1% D-glucose and 10% heat inactivated human serum. The asynchronous parasites of Plasmodium falciparum were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was resolved by Jaswant Singh Bhattacharya (JSB) staining [9] to evaluating the percent parasitaemia and uniformly maintained with 50% RBCs (O+ve). The culture plates were incubated at 37 °C. After 36 hrs incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopicallyobserved to record maturation of the ring stage parasites intoschizonts and trophozoites in the presence of synthesized CdONPs. The synthesized CdONPs concentration which inhibitedthe complete maturation into schizonts was recorded asthe minimum inhibitory concentration (MIC). Chloroquineand Quinine were used as the reference drugs [10].

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 [11]. Stock solutions of primary 1000, 500, 250 and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 µg/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. J. medium were harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37 °C for 24 hrs. These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days 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 [10].

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 36-57 nm. It is noticed that green synthesis of CdONPs produces the small and uniform size of spherical particles.

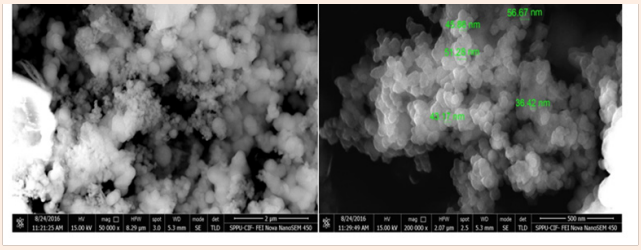

Figure 2: FE-SEM microphotographs of CdONPs deposited on a carbon strip.

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 C-grid onwhich the sample was coated while peaks for Phosphorous, Nitrogen, Iron, Aluminium, Silicon and Sulphur correspond to the phenols, flavonoids, coumarins and enzymes capping over the synthesized CdONPs.

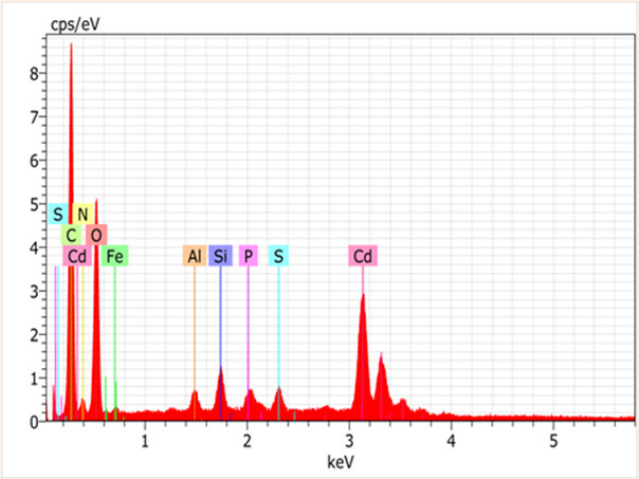

Figure 3: EDS spectrum of green synthesized CdONPs.

Vibrational properties

Figure 4 represents the FTIR spectrum of CdONPs synthesized from leaves of *Leucaena leucocephala L*. The broad peak at 3352 cm^{-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 2924 cm^{-1}. The peaks around 1614 cm^{-1} is corresponding to C=C in CdO backbone, and those at 1371 cm^{-1} correspond to wagging of CH_2 vibration. The FTIR results confirm 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 L*.

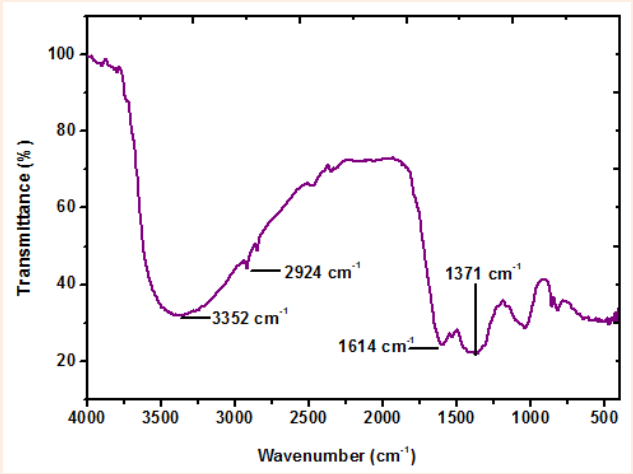

Figure 4: FT-IR spectrum of green synthesized CdONPs.

Phytochemical screening

The results of qualitative pharmocognostic assess of aqueous leaf extract of *Leucaena leucocephala L*. are shown in Table 1. Phytochemical profile of *Leucaena leucocephala L*. leaves revealed [12] and highlighted the presence of phenols, flavonoids, volatile oils, saponins, coumarins, tannins and sterols which may be accountable for the efficient capping and stabilizing agent of nanoparticles and this was further confirmed by FTIR spectrum.

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	+		

Photoluminescence study

Biosynthesized CdONPs are reported to exhibit visible photoluminescence and their fluorescence spectra are shown in Figure 5. The optimized CdONPs were found to be luminescent with four emissions at 301, 394, 508 and 602 nm for an excitation at 250 nm. When CdONPs were excited at 300 nm, it showed excitation at 605 nm, the excitation of 300 nm is of high intensity in comparison to other one. The luminescence at 250 and 300 nm may be due to presence of phytoconstituents in the plant extract. The CuONPs synthesized using *Leucaena leucocephala L*. leaf extract are also reported to be luminescent with emission band at 509 nm and 606 nm [12].

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 pyogenus, 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, 500 µg/ ml.) were tested for antimicrobial activity against these selected pathogen with amplicilline has positive control. The plates were then kept at 4-5 ˚C for 1 hr, followed by incubated in incubator at 37 °C for 24 hrs. After 24 hrs, 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.

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 *Micobacterium tuberculosis* H_{37}RV completely (99%) at the MIC of 125 µg/ml (Table 4).

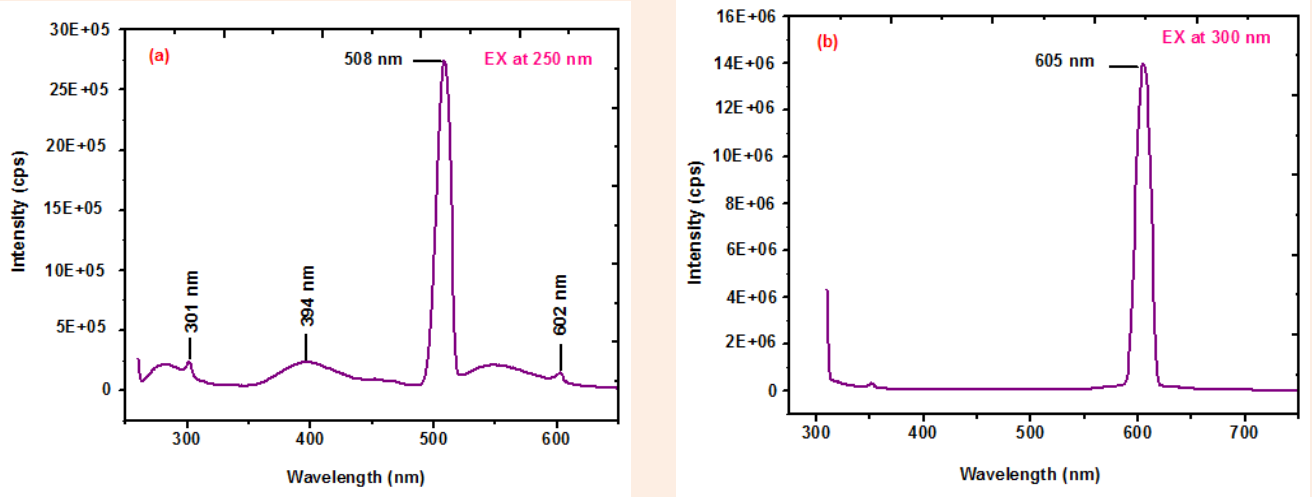

Figure 5: Fluorescence spectra of green synthesized CdONPs formed with excitation at (a) 250 nm and (b) 300 nm.

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	15	17	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_{50} Values
	CdONPs	0.95 µg/ ml
	Chloroquine (Standard)	0.020 µg/ ml
	Quinine (Standard)	0.268 µg/ ml

Table 4: Minimum inhibition concentration (MIC) of green synthesized CdONPs against *Micobacterium tuberculosis.*

Sl. No	Compound Name	MIC (µg/ ml)
	CdONPs	125µg/ ml
	Isoniazid (Standard)	0.20 µg/ 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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