Biosynthesis and characterization of CdO nanostructure and its influence on cancer cells of (HT29)

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Abstract. Due to increased drug potency and lower toxicity in the nano-sized mediated drug delivery model, the environmentally sustainable synthesis of nanoparticles by the bio route from plant extracts has a wide variety of applications in novel science. In this report, our research groups have synthesized stable and cost-effective CdO nanostructure by the Curcuma plant. The biosynthesis of CdO nanostructure by the Curcuma plant extract was confirmed by different analytical methods such as UV-Visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffractometer (XRD), Atomic force microscopy AFM and Field emission scanning electron microscopy (FESEM), in addition to MTT assay screening of the synthesized CdO nanostructure for anticancer activity on (HT29). The result was that the biosynthesized CdO nanostructure exhibited strong anticancer cytotoxicity on (HT29). The findings of the MTT shows that at a concentration of 50.81 μ, 50 percent of the cancer cell line was destroyed by the extract.

Keywords: CdO nanostructure, biosynthesis, Anticancer effects, colon cancer cell lines (HT29).

1. Introduction: Nanotechnology also includes the synthesis of nanoparticles between 1 and 100 nm in scale. Besides, there is a new branch of Nanotechnology, bio-nanotechnology, which combines biological concepts with the chemical and the physical processes to create nano-sized particles with unique functions. The bio-inspired nano-metal synthesis protocols are both environmentally and economically green because they are based on the ideals of green chemistry and are simple and relatively inexpensive. The chemical techniques available, however, are often costly, use lethal chemicals, and are comparatively complex. Therefore, in the field of nanotechnology, the biosynthesis of nanoparticles using biological agents like plant extracts has earned a ton of publicity[1]. An inorganic compound with the chemical formula CdO is cadmium oxide. It occurs naturally in the unusual mountpoint mineral. It can be found as crystals of brown or red or colorless amorphous powder.

In recent research through the conducting oxides, CdO is the promising candidate due to its low band gap (2.5eV), high conductivity (103Ω−1cm−1)[2], high carrier mobility (142 cm2/Vs), and index of refractive (n = 2.49)[3]. CdO is an II-VI binary cadmium and oxygen compound that is normally obtained with anion centers and octahedral cation in a NaCl-like cubic structure and is classified as a semiconducting n-type formed by burning elemental cadmium in the air [4]. It is used in several applications, including solar cells, phototransistors, gas catalysis, and chemical sensors[5, 6]. Nanomaterials with various structures showed good antibacterial
activity against human pathogens because of the ability to cross the cell membrane and also cell membranes in the range of (1-100) nanometers [5]. Due to their antibacterial properties, CdO nanostructure play a vital role in the biomedical area. Due to the formation of reactive oxygen species (ROS), the discharge of cadmium Cd2+ ions and the size and morphology of the product nanoparticles, these microstructures have demonstrated their important anti-bacterial and anti-fungal capabilities resistant to bacterial and fungal [7]. CdO nanostructure, because of their unique physicochemical properties, have anti-cancer properties. Interestingly, most heavy metals, including cadmium Cd, have the ability at a low concentration to eliminate cancer cells. Via destructing their cell wall as possible to another nanoparticle, it regulates cancer cells[8]. The second and third most common cancer among men and women, respectively, is colon cancer. Colon cancer care varies depending on the tumor's location. Tumor removal using surgical techniques combined with radiotherapy and chemotherapy is one of the most popular approaches to treating colon cancer. Increased resistance to chemotherapy drugs and tumor recurrence was one of the big problems with this procedure. Therefore several cancer studies have recently centered on the use of herbal remedies and natural ingredients for cancer prevention and treatment. [9]. Here the cost-effective, safe, and eco-friendly biosynthesis of CdO nanostructure is investigated using Curcuma plant extracts and their antibacterial, anti-malarial influence on cancer cells of (HT29).

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2. Experimental work

4 grams of Curcuma plant powder was thoroughly washed for 15 minutes in distilled water, air-dried, and chopped into fine pieces. The parts were mounted for an hour on the device with 50ml of distilled water at 60°C with stirring, called plant extracts. filtered by Whatman four filter paper to remove the dust particles. 1M of Cadmium nitrate (Molar mass= 236.42 g/mol) was dissolved in 50 ml distilled water and heated at 60°C for one hour with stirring followed by the addition of Curcuma plant water extract transparent solution (50 ml) The mixture was heated at 160 °C and stirred continuously. After a while, the color of the solution turns brown. The gradual change of color indicates the formation of CdO nanostructure, as demonstrated in Figure 1.

![Figure 1. The schematic diagram of the CdO NPs biosynthesis](image-url)
The following techniques calculated the exact configuration of the manufactured, phase purity, composition, average particle size, crystal morphology, and distribution of particle. Crystalline size structure was studied by X-ray diffractions using (Malvern Panalytical, UK) X-ray diffractometer system with Cu-κα radiation at a wavelength of X-ray equal to (λ= 0.154056 nm) in the 2Theta (20°-80°) range. Atomic force microscopy for size, surface topography, and granularity volume distribution of synthesized nanoparticles with AFM, (AA-680, Shimadzu-Japan). The morphology and composition of the synthesized CdO nanostructure were examined by (FESEM, TESCAN MIRA-3) under an acceleration voltage of 30–250 kV. The transmittance spectrum is taken by a spectrophotometer (Cary A hundred Cone plus Ultraviolet-Visible-NIR, split-beam optics, dual detectors) fitted with a xenon lamp with a wavelength ranging of (200 - 900 nm). FTIR spectrum was acquired by JASCO FT-IR-460 spectrometer in the range of wavenumbers 400–4000 cm\(^{-1}\). In this procedure, the cells were exposed to different concentrations of CdO nanostructure against the colon cancer cell (HT29) using the MTT assay. Approximately thousands of HT29 could fix in 96-well plate at 37°C. The different concentrations of CdO nanostructure synthesized from Curcuma plant (10, 50, 100, 150, 200, 220, 240 and 280µL) after 24, 48 and 72 hours. The drug free medium was used to wash the cells after the treatment. After the completion of the drug treatment incubation period, 10 µL of 5 mg/mL in PBS, MTT and 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2 were added to each well and incubated for 4 hours at 37°C followed by the addition of 100 µL of 0.04 mol/L hydrochloric acid in isopropanol. Then, the absorbances of the wells were read by the wavelengths of 570 nm and 630 nm for both the test and reference was measured using an ELISA plate reader. The plot was created for the percentage of live cells at each group concentration and the control cells [8, 9].

3. The Results

3.1. XRD measurement

The XRD diffraction patterns of biosynthesized CdO nanostructure films and deposited on glass substrate by the drop-casting method, three drops (Each drop equal 100 µl ) [12], as shown in Figure 2. The three main peaks of CdO face centered cubic (FCC) corresponding to (111), (200), and (220) have been compared with the standard X-ray diffraction data the card (JCPDS file no.005-0640) [13, 14]. In the present analysis, CdO nanostructure films exhibit a preferential orientation along the (200) diffraction plane formed by plant extracts of CdO nanostructure on the glass substrate, Figure 2 also shows another peaks that fits with the card (JCPDS file No. 039-1221: CdO2) [13]. The size of the biosynthesized CdO nanostructure crystallites was calculated by the formula of Debye-Scherrer [15, 16], and which are mentioned in Table 1.
\[ D = \frac{K\lambda}{\beta \cos\theta} \]  

where \( D \) is the size of the crystallite, \( \beta \) is the full half-maximum width (FWHM) and \( K \) is the form factor (0.94).

The XRD peaks suggest that the particles were polycrystalline in structure. The dislocation density (\( \sigma \)) and strain (\( \eta \)) of CdO nanostructure can be determined using the following equations [17] and are shown in Table 1, respectively:

\[ \sigma = \frac{1}{\beta s^2} \]  

\[ \eta = \frac{\beta \cos\theta}{4} \]  

Table 1 Important parameters derived from the CdO NPs XRD diffraction pattern.

| 2Theta (degree) | phases        | \( \beta \) (degree) | \( D_{nm} \)  | \( \sigma \times 10^{14} \text{ lines m}^{-2} \) | \( \eta \times 10^{-4} \text{ lines}^{-2} \text{m}^{-4} \) |
|-----------------|---------------|----------------------|---------------|-----------------------------------------------|-----------------------------------------------|
| 29.71           | CdO (111)     | 0.1476               | 58.18         | 2.95                                          | 6.22                                          |
| 33.1            | CdO (111)     | 0.2952               | 29.34         | 11.62                                         | 12.3                                          |
| 38.64           | CdO (200)     | 0.1476               | 59.60         | 2.8                                           | 6.08                                          |
| 42.02           | CdO2 (211)    | 0.1968               | 45.18         | 4.90                                          | 8.01                                          |
| 47.63           | CdO2 (220)    | 0.1968               | 46.10         | 4.71                                          | 7.85                                          |
| 54.33           | CdO (220)     | 0.1968               | 47.41         | 4.45                                          | 7.64                                          |

3.2 Optical properties
The transmittance spectra and optical band gap of CdO nanostructure biosynthesis using Cadmium nitrate with Curcuma plant are shown in Figure (3a, b). Figure (3a) demonstrates the transmittance spectra of the
biosynthesis CdO nanostructure observed an increase in the wavelength and high transmittance spectra of the visible and infrared part of the spectrum. Figure (3a) also shows the Plasmon resonance peak due to the Ultraviolet quantum size effect with wavelengths ranging from around 247 nm to 300 nm, suggesting that a Nano-scale solution. The relationship among \((\alpha (cm^{-1}) \times \hbar \nu (eV))^2\) and photon energy \(\hbar \nu\) of CdO nanostructure gives the value of bandgap is seen in Figure (3b). Taut plots are plotted among \((\alpha (cm^{-1}) \times \hbar \nu (eV))^2\) and photon energy and the linear portion of the graph was extrapolated to meet the energy axis to determine the energy band difference\[18\] as seen in Figure (3b). Results of measured band gaps \(E_g\) of the sample were obtained, that the band gaps of the prepared sample were 3.8 eV and 5 eV that the values of band gaps increased. This behavior is obeyed reported in the nano field\[19\]. Two energy gaps may be due to the fission of Fermi at the concentration of charge carriers and the increase in the permissible states due to the increase in the concentrations of charge carriers\[20\].

**Figure 3** CdONPs freshly colloidal biosynthesized by plant extract (a) Transmittance Spectra and (b) Energy gap \(E_g\).
3.3 FTIR spectrum

The attachment of biomolecules responsible for capping and stabilizing the NPs was verified by Fourier Transform Infrared Spectra. Figure 4 of the FTIR spectrum of CdO NPs indicates major peaks at (3557.21, 3038.59, 1620.48, 1344.75, 1038.57, and 828.65 cm\(^{-1}\)). The peaks of 3557.21 and 3038.59 cm\(^{-1}\) show the presence of N–H symmetric stretching and hydroxyl O–H group\(^{[2, 3]}\). The peaks of 1620.48, 1038.57, and 1344.75 cm\(^{-1}\) are due to C=C and C=O vibration modes of the aldehydes or ketones groups\(^{[22]}\). At 828.65 cm\(^{-1}\) indicates to the CdO bond\(^{[23]}\).

![FTIR spectrum of CdONPs](image)

**Figure 4.** Spectrum FTIR of CdONPs.

3.4 FESEM analysis

The synthesized CdO NPs revealed a semi-spherical shape with particle sizes ranging from 20.47 to 33.50 nm, from the FESEM picture as shown in Figure 5.

![FESEM microphotographs of CdONPs](image)

**Figure 5 FE-SEM microphotographs of CdONPs deposited on glass (10 µm and 100nm in range).**
3.5. Atomic force microscopy (AFM)

Figure 6 show the three-dimension AFM and granularity distribution of nanostructured CdO films deposited by the drop-casting method on the glass. With strong dispensability, homogenous grains, and vertically oriented, CdO NPs have a semi-ball form. The approximate values of the root mean square of the average surface roughness and the average diameter of the surface roughness were determined using special software and described in table 2.

![3D AFM picture and distribution map of granularity accumulation of CdO nanostructure deposited on glass.](image)

**Figure 6** 3D AFM picture and distribution map of granularity accumulation of CdO nanostructure deposited on glass.
Table 2 shows the values of roughness average and average of diameter of CdONPs.

| Average Diameter | Roughness Average | Root mean square |
|------------------|-------------------|------------------|
| 53.25 nm         | 4.41 nm           | 5.08 nm          |

3.6 MTT Test
At three different times, the effect of different concentrations of CdO NPs on growth inhibition of HT-29 wild type and resistant derivative cell lines was investigated, and dose and time-dependent growth inhibition were observed in HT-29 cell lines. The results for this sample showed that the relative cell viability after 24 hours of treatment is in a moderate grade and after 48 and 72 hours of treatment which decreased dramatically by the rate of 15 to 29. Therefore, this sample is dose-dependent and time-dependent manner (Figure 7). According to the results for the cancer cell HT29 IC50, 50 percent of cell death at 48h was recorded at 50.81 μl. Figure 8 is a microscopic image showing the impact of the CdO NPs on 20 μL and 200 μL treated cell viability.

![Cell Viability Graph](image)

**Figure 7** MTT assay to calculate cell viability in HT-29 by CdONPs
4. Conclusions
The current study has shown that for the green synthesis of CdO NPs, the Curcuma plant can be an unconventional resource. Analytical techniques such as UV-Vis, XRD, FESEM, FTIR, and AFM have defined the existence of synthesized CdONPs. The significant anticancer activity on cancer cell line HT29 with an IC50 value of 50.81 μL was demonstrated by the CdO NPs biosynthesized from the Curcuma plant. Besides, it will smooth the way for the development of a novel anticancer drug to recognize and extract the biologically active compound from the Curcuma plant.

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