

Green Synthesis of CdO Nanoparticles by Olive Leaf Extract and their biological effectiveness

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Abstract

Research into green production methods for metal oxide particles (NPs) is underway to overcome the possible risks of these chemicals in safer environments. In this study, the synthesis of simple, environmentally friendly Cadmium oxide (CdO) nanoparticles was successfully achieved using olive leaf extract. The nanoparticles were further characterized by Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX) and Atomic Force microscopy (AFM).

Plant-mediated CdO nanoparticles were found to be elliptical and well dispersed in suspension. Cadmium oxide particles are used in many applications; therefore, these green synthetic CdO NPs are useful as antifungal and antibacterial agents.

Keywords: CdO, Green synthesis, Nanoparticles, Olive.

Introduction

Cadmium oxide (CdO) is one of the most attractive semiconductor materials. The n-type semiconductor has a direct band gap of 2.5 eV and an indirect band gap of 1.98 eV¹. It has a tunable band gap, which proves to be useful. Catalysts, sensors², nonlinear materials³, solar cells⁴ and other optoelectronic devices and biological applications have broad application prospects as antibacterial devices⁵⁻¹⁰. CdO NPs have been prepared using different plants that affect the morphology of the resulting nanomaterials^{11,12}. In fact, plant extracts may act as reducing and blocking agents that direct the structure of the resulting NPs¹³. Following the principles of green chemistry, here we report the construction of CdO NPs by using extracts of different vegetable (cauliflower, potato and pea) wastes, as well as their microstructure, morphology and optical properties.

Green Chemistry focuses on the production of the desired product without producing harmful intermediate by-products during the chemical reaction. The incorporation of green chemistry into nanotechnology has identified multifunctional environmentally friendly reagents because they can act as reducing agents and blocking agents^{14,15}. The synthesis of nanoparticles (NPs) can be carried out using a number of conventionally used chemical and physical methods¹⁶⁻¹⁷. The large-scale production of metallic and non-metallic nanoparticles has created risks to the human health and environment^{18,19}. Improper disposal of nanomaterial waste in laboratories and industry poses an alarming threat to ecosystems and aquatic life. From this

point of view, the researchers focus on the green synthesis of nano.

The goal is to protect the environment and human health from the toxic effects of nanomaterials and the complex compounds they arise from, while using our nano safely. Nanoparticles synthesized from *Pterospermum acerifolium* leaf extract are angiosperms of the Malvaceae family distributed in Southeast Asia²⁰. Many researchers have studied the environmental and biological risks of cadmium nanoparticles. Applications of cadmium oxide stem from their respective electrical and optical properties. So far, many scholars have applied cadmium oxide nanoparticles. Similarly, we use cadmium oxide nanoparticles to fight pathogenic bacteria²².

Material and Methods

Finely chopped material is allowed to boil for 5 minutes at 80 °C with 100 ml of de-ionized water in 250 ml Erlenmeyer bottle and then cooled to room temperature. The resulting solution is passed through filter paper to remove any solid particles and then filtered again using filter paper of 0.2 μm pore size.

It is stored at 4 °C as a stock for synthesis of CdO NPs. The solution is then centrifuged at 10,000 rpm for 10 min at room temperature (using Beckman centrifuge with Beckman JA-17 rotor) and the mixture is collected after discarding the supernatant. The collected CdO NPs were allowed to dry on a glass watch.

Methodology of inhibitory effect of Cadmium Oxide:

Pure cultures of all experimental bacteria and fungi were obtained from the Ibn Al-Nafess Center Bank – Ministry of industrial. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub-culturing frequently on the same medium and stored at 4° C before use in experiments.

Preparation of Cadmium oxide dilutions: Cadmium oxide nano compound had been prepared as described previously. From the stock different serial dilution had been used. All diluted samples were stored in sterile glass bottles at room temperature until screened.

Microbiological screening: Antimicrobial activities of different diluent were evaluated by the agar well diffusion method^{23,24} with minimum inhibitory concentration (MIC)²⁵.

Media Preparation and Its Sterilization: For agar well diffusion method^{23,24} antimicrobial susceptibility was tested on solid (Agar-agar) media in Petri dishes. For bacterial assay, nutrient agar (NA) (40 gm/L) and for fungus, PDA (39 gm/L) was used for developing surface colony growth. The minimum inhibitory concentration (MIC) was determined by serial micro dilution assay. The suspension culture, for bacterial cells growth was done by preparing 2% Lauria Broth (w/v) and for fungus cells growth, 2.4% (w/v). PDB (Potato dextrose broth) was taken for evaluation. All the media prepared were sterilized by autoclaving the media at (121°C) for 20 min.

Agar well diffusion method: Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of cadmium oxide was prepared at a concentration of 100 ppm. DMSO was used to prepare a serial dilution. Approximately 100 µL of various dilutions were added to the sterile syringe into the wells and allowed to dissolve at room temperature for 2hrs. Control experiments that make inoculums without cadmium oxide have been established.

The plates were incubated at 28 ° C for 48 h and pathogens and fungi, 37 ° C for 18-24 hours for bacterial pathogens. The diameter of the delay zone (mm) was measured and the activity index was measured, three copies remained and the experiment repeated three times. For each replicator the readings were taken in three different fixed directions and the average values were recorded.

Minimum Inhibitory concentration: The “minimal inhibitor concentration” is well defined as the last concentration able to inhibit any visible bacterial growth on culture plates. This is determined from reading on culture plates after incubation. The most common methods are tube dilution methods and agar dilution methods. Serial dilutions are made from the products in bacterial fungal media. Test organisms are then added, incubated and scored for growth. This procedure is a standard assay for antimicrobial. Minimal inhibitor concentration is important in diagnostic laboratories to confirm resistance of microorganisms to microbial agent and also to monitor the activity of new antimicrobial agents.

Preparation of Inoculum

Antifungal Activity Test: In order to investigate the antifungal activity of the extracts, a different micro dilution technique was used. The fungal spores were washed from the surface of the agar plates with a sterile 0.85% saline solution containing 0.1% tween 80 (v / v). The spore suspension was adjusted with sterile saline solution to a concentration of about 1.0 - 107 in a final volume of 100 µL per well. Inocula were stored in 4 °C for further use.

Dilutions of inocula were cultured on solid agar and potato dextrose to verify the absence of contamination to test the validity of inoculum.

Determination of MIC: The minimum inhibitory concentrations (MIC) were performed by a serial dilution technique using 96-well microtiter plates. The different cadmium oxide dilutions with potato dextrose broth for fungus, inoculum were used. Microplaters were incubated for 72 hours at 28 ° C. The lowest concentrations without visible growth (in the binocular microscope) were defined as micromails.

Characterization of Synthesized CdO NPs: The morphological, structural and chemical composition of CdO NPs were analyzed by employing SEM-EDS INSPECT S50) and XRD (PAN analytical: XPERT-PRO) equipment, AFM (SPM Scanning Probe Microscope) and and UV-Vis (Shimadzu UV-Vis 2450) spectroscopy spectral analysis.

Results and Discussion

Atomic Force Microscope: The AFM analysis provides the measurements of average grain size (Table 1). The figure 2 show typical surface AFM image (a: in tow and b: in three dimensional) and the granularity cumulating distribution for CdO. The average diameter is 72 nm for CdO.

Diffraction and Scanning electron micrograph: The XRD technique was used to determine and confirm the crystal structure of the nanoparticles. XRD analysis showed a series of diffraction peaks at 2θ of 25.41, 37.61, 48.81, 55.91, 53.31, 64.21. The XRD spectrum clearly demonstrated the crystalline nature of CdO NPs synthesized from the leaf extract of olive leaf extract. Record positions presented the one-time structure of CdO approved by the International Center for Data Dissociation (ICDD) card no. 801916 as in figure 2.

The SEM image of the CdO particles (Fig. 3) shows the presence of large particles, which can be attributed to the aggregation or overlap of small particles having a size of about 100 nm and the morphology of CdO appears in the nanorods. The EDS analysis revealed the chemical composition of the nanoparticles having atomic percent of 54 % for Cd and 45% for O (Figure 4).

Inhibitory effect of Cadmium Oxide: In this study, the inhibitory effects of different dilutions of cadmium oxide were evaluated for fungicidal and bacterial strains. The antibacterial activity was measured by agar diffusion method and micro-dilution method. The activity was quantitatively assessed on the basis of the inhibition zone and their activity index and minimum inhibitory concentration (MIC) were also calculated.

Measurement of antimicrobial activity using Agar well diffusion Method: The antimicrobial potential was evaluated based on their zone of inhibition against various

pathogens and the results (inhibition zone) were compared to the activity of the standard (10 cc). The results showed that all of the diluents for cadmium oxide were effective against *Candida albicans* and no inhibition zone was detected when *E. coli* was used. Among the different diluents for cadmium oxide studied, (25, 20 and 15 ppm) showed high inhibition, followed by (60, 50, 40 and 30 ppm).

When used (25, 20 and 15 ppm), the antibacterial activity of *Candida albicans* with the largest zone of inhibition was

obtained, diameter (18 ± 0.37 mm) and when used (60, 50, 40 and 30 ppm), obtained (15 ± 0.34mm) table 2, figure 5.

Determination of MIC value: The minimum inhibitory concentration (MIC) is defined as the highest or lowest concentration of the extract that inhibits growth of the organism. The determination of the MIC is important in diagnostic laboratories because it helps to confirm the resistance of the microorganism to the antimicrobial agent and it monitors the activity of the new antimicrobial agent. The MIC value of Cadmium oxide to *Candida albicans* is at least 60 ppm.

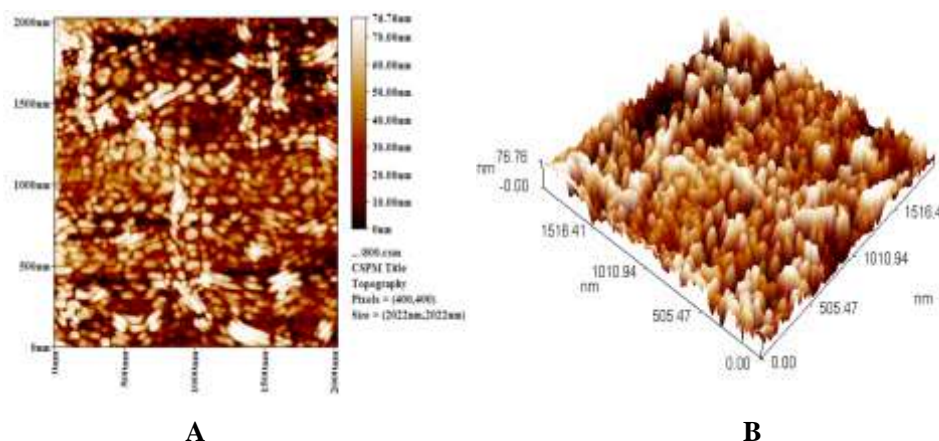
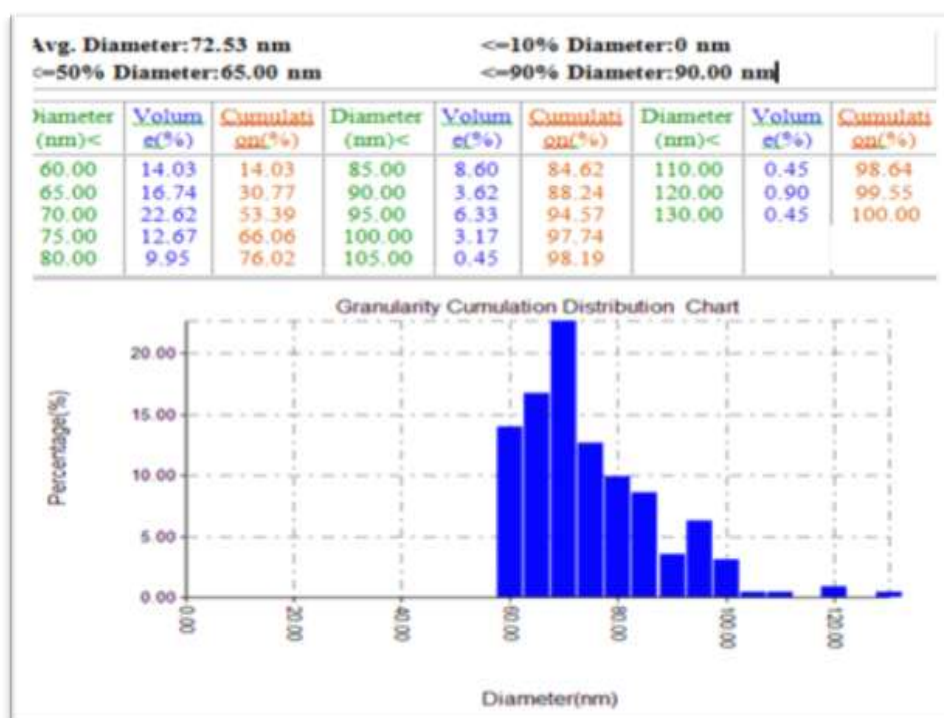


Figure 1

Table 1
The measurements of average grain size of CdO



X-ray

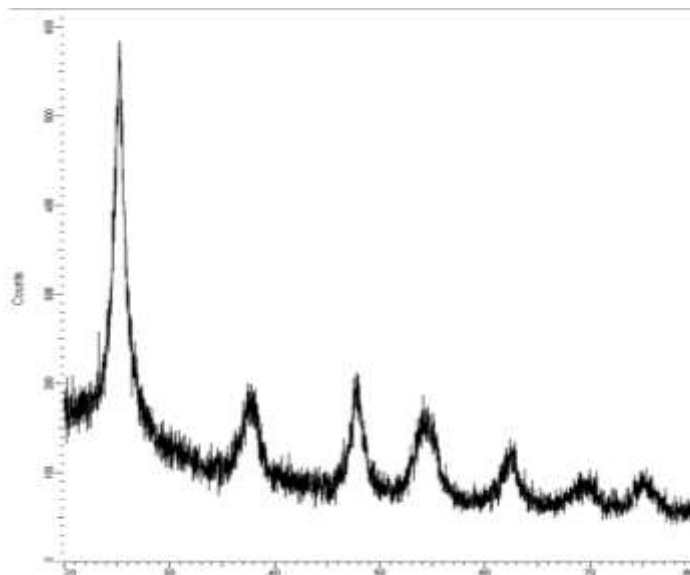


Figure 2: XRD patterns of pure CdO powder

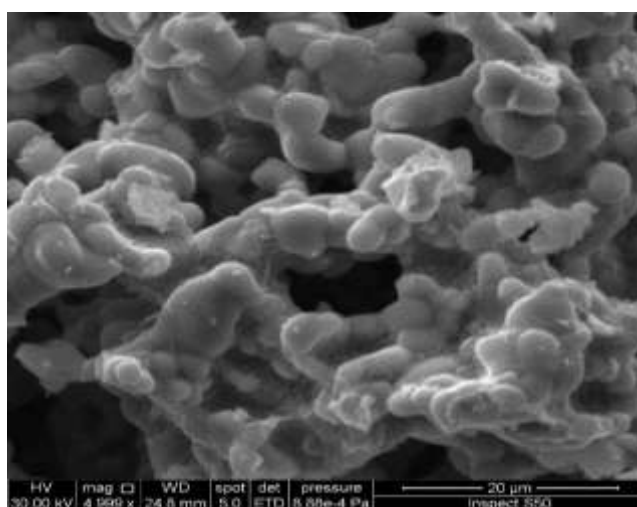


Figure 3: Scanning electron micrograph

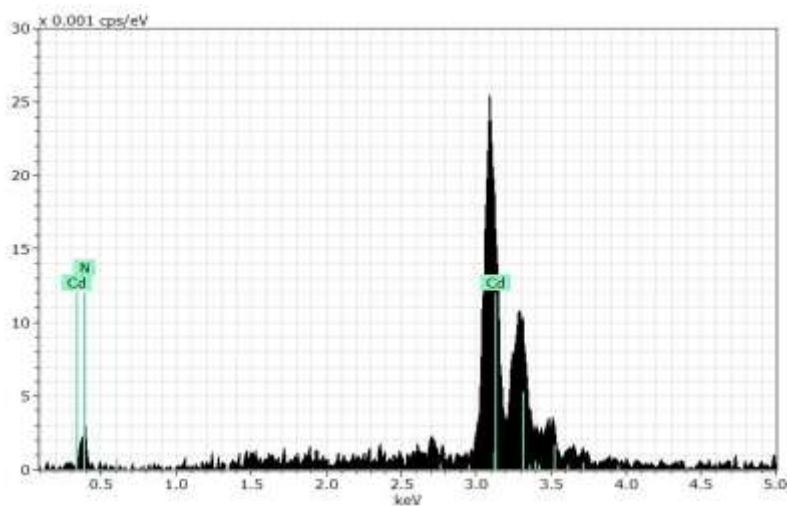
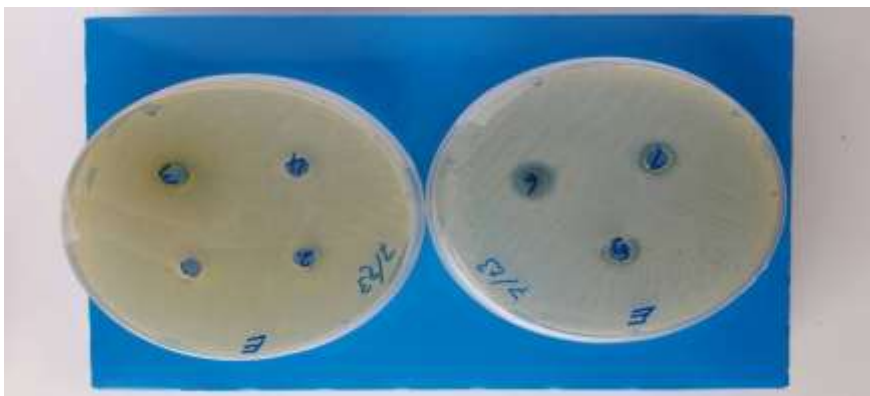
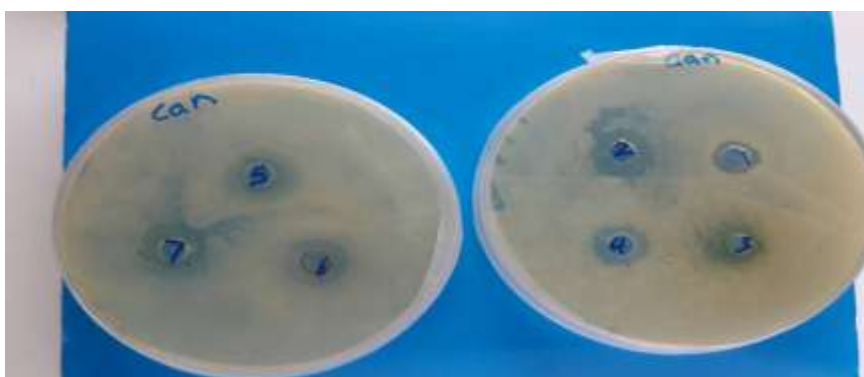


Figure 4: EDX analysis



Cadmium oxide against *Escherichia coli*



Cadmium oxide against *Candida albicans*



Cadmium oxide with control against *Candida albicans*

Figure 5: Antimicrobial activity of different diluent of cadmium oxide against clinical pathogens

Table 2

Antimicrobial activity (zone of inhibition, mm) of various diluent of cadmium oxide against clinical pathogens.

cadmium oxide	<i>Candida albicans</i>	<i>Escherichia coli</i>
100ppm	12mm	-
60ppm	15mm	-
50ppm	15	-
40ppm	15	-
30ppm	15	-
25ppm	18	-
20ppm	18	-
15ppm	18	-
10ppm	10	-

Conclusion

In summary, the green synthesis of CdO NPs was carried out by using olive leaf extract alone without any chemical reagents. XRD, SEM and AFM analysis showed that the average grain size of CdO NPs was 70 nm. These green synthetic CdO NPs have antibacterial activity in *Candida albicans* and the maximum zone of inhibition (18 ± 0.37 mm) is obtained when used 25, 20 and 15 ppm and 15 ± 0.34 mm when used 60, 50, 40 and 30 ppm.

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