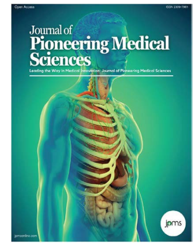

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Preparation of CuO and CdO Nanoparticles Using Cauliflower Leaf Extract, Evaluate and Compare their Bioactivities Against the MCF-7 Breast Cancer Cell Line

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Abstract Extracts from cauliflower leaves were used to biosynthesise metal oxide nanoparticles. Copper and cadmium oxide nanoparticles (CuO and CdO) are among the most important metal oxide nanoparticles (MONPs) because of their physiochemical and anticancer characteristics, which aid in molecular diagnostics and therapy. Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FE-SEM) and X-ray diffraction (XRD) were used to investigate the physicochemical characteristics of copper and cadmium oxides. One promising option for creating a novel class of anti-cancer drugs is metal oxide nanoparticles. Because of their small size, metal nanoparticles and their oxides have biological activity; they may approach, interact with and come into contact with bio-objects. The bioactivity of copper oxide and cadmium oxide nanoparticles against the MCF-7 breast cancer cell line was examined in this study and the results indicated that the copper oxide nanoparticles exhibited higher activity than the cadmium oxide nanoparticles.

Key Words Plant Extracts, Green Synthesis, CuO NPs, CdO NPs, Anticancer

INTRODUCTION

Because of their growing applications, metal oxide nanoparticles have drawn more attention from researchers in recent years [1]. It was discovered that the various properties of these oxides change dramatically at the nanoscale as opposed to traditional measurement, eventually changing completely [2,3]. Because copper, cobalt and cadmium oxide nanoparticles are super-active metal oxides with several uses in cutting-edge technology, they are utilised extensively in a variety of fascinating and significant sectors, including biology and medicine. Because plant extracts are readily accessible, safe, nontoxic and contain a wide range of metabolites (phytochemicals) that facilitate the rapid reduction of metal ions, they are frequently utilised in the manufacture of metal oxide nanoparticles [4,5]. According to Ismat Bibi and colleagues, cobalt-oxide nanoparticles (NPs) were created utilising a low-temperature extract of Punica granatum peel from cobalt nitrate hexahydrate and utilised for photo-catalytic processes [6]. According to many researchers, various plant extracts were successfully employed as a natural ligand in the manufacture of cadmium oxide nanoparticles [7,8]. Numerous studies have documented the chemical production of copper oxide (CuO) nanoparticles from $Cu(NO_3)_2$ and $CuSO_4$ and the electrocatalytic efficiencies of CuO nanoparticles and their use in the manufacture of CuO-water nanofluid have been studied [9-11]. By causing apoptosis and rupturing cell membranes, copper oxide nanoparticles (CuO NPs) have cytotoxic effects against the MCF-7 breast cancer cell line, which reduces colony formation and cell proliferation. According to research, CuO, CdO NPs and extract of the root bark of *Securidaca long pedunculata* led to the isolation of one new xanthone derivative, which may have a size-dependent anticancer impact that is amplified when used in conjunction with other treatments like hyperthermia or other nanocarriers [12-14].

As far as we are aware, there has never been a publication on the green production of Cu and Cd oxide nanoparticles using plant leaf extracts at room temperature and using them for anticancer treatment. In order to produce and evaluate the copper and cadmium oxide nanoparticles utilising leaf extracts from local plants (cauliflower leaves), as well as to investigate their anti-cancer efficacy, the current work was conducted.

Experimental

Chemicals and Materials Used: We acquired cadmium (II) nitrate ($CdNO_3$) and copper (II) chloride hexahydrate (CuCl2·6H2O) from the Department of Chemistry, College of Education for Pure Science, University of Diyala, Iraq. The cauliflower leaves were from Iraqi farms. The XRD spectra were recorded using an XRD-6000 Cukα (λ = 1.5406 A°), 220/50, HZ, SHIMADZU, (Japan). The product's morphology was determined using field emission-scanning electron microscope (FE-SEM) techniques on the ZEISS model: Sigma VP., Transmission Electron Microscopy (TEM) on the Philips model: CM120 and Atomic Force Microscopy (AFM) Scanning Probe Microscope, AA 3000 SPM 220 V-Angstrom Advanced Inc, AFM contact mode, (USA).

Preparation of the Cauliflower Leaf Extract

In order to prepare the green reducing and stabilising agent, cauliflower leaves were gathered from Iraqi farms in Diyala. To get rid of any contaminants, wash the cauliflower leaves with deionised water. Slice the cauliflower leaves into tiny bits. Put 25 g of chopped leaves in 100 mL of deionised water, then set the temperature to 80°C. After a while, the water will become green, which means that leaf extract has formed in the water. The extract will now be utilised as a reducing agent for the manufacture of CuO and CdO nanoparticles after being filtered out and poured into the burette.

Synthesis of Metal Oxides Nanoparticles

Set the temperature to 60-70°C and add 2 g of cadmium (II) nitrate (CdNO3) or copper (II) chloride hexahydrate (CuCl2·6H2O) to 100 millilitres of deionised water. Add the cauliflower leaf extract very slowly, drop by drop, until the solution changes colour. When the colour changes, stop adding more cauliflower leaf extract because this shows that CuO and CdO nanoparticles are forming in the water.

Characterization

Using Cu Kα radiation in the range of 2θ = 20-80° at a scanning rate of 5° min^{-1}, XRD analysis (Bruker, Germany) verified the quality of the produced CuO and CdO nanoparticles. Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM) and Field Emission Scanning Electron Microscopy (FE-SEM) were used to analyse the structural morphology.

RESULTS AND DISCUSSION

In order for us to complete this study, it was important to prepare the plant extract of cauliflower leaves, which acts as a reducing agent and stabiliser for nanoparticles prepared from salt precursors (CuCl2·6H2O and CdNO3). The metal hydroxides nanoparticles were first product, follow that the metal oxides nanoparticles were synthesized by calcination process at 650°C of metal hydroxides nanoparticles. CuO and CdO nanoparticles are shown in the following equations in their first and second parts, followed by the diagnosis of the prepared nanoparticles by physical spectroscopic methods.

X-Ray Diffraction Analysis

Metal oxide nanoparticles (CuO and CdO NPs) were produced and analysed using the X-ray diffraction method. By comparing the created metal oxides, we can demonstrate in Figure 1 and 2 that the type of metal's action appears to have a distinct impact on the positions of the acquired peaks.

Displays the CdO nanoparticles X-ray diffraction pattern. The nanoparticles' strong orientation along the (111), (200), (220), (311) and (222) planes is demonstrated. A polycrystalline nature with a cubic (face-centred) crystal structure is demonstrated by the presence of diffraction peaks (JCPDS 5-0640) (Figure 1). CdO NPs' average crystalline size, determined by the Debye Scherrer equation, was 37.29 nm. However, Figure 2, which shows the generated CuO nanoparticles' XRD diffraction, revealed a number of the particles' fundamental peaks inside the X-ray diffraction spectrum. The pattern closely resembles the reference patterns for CuO (Joint Committee for Powder Diffraction Studies (JCPDS) File No. 00-101-1148) and is consistent with the monoclinic crystal phase of the copper oxide nanoparticles with cell parameters a = 4.6530 Å, b = 3.4100 Å, c = 5.1080 Å, β = 99.480°. It is well indexed

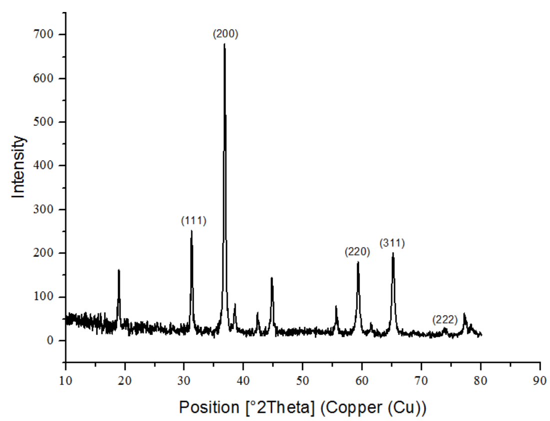

Figure 1: XRD Pattern of CdO Nanoparticles

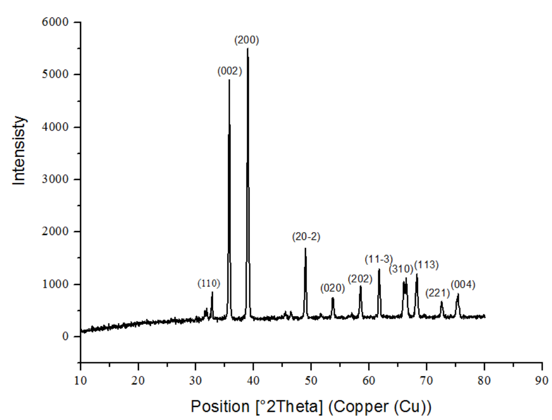

Figure 2: XRD Pattern of CuO Nanoparticles

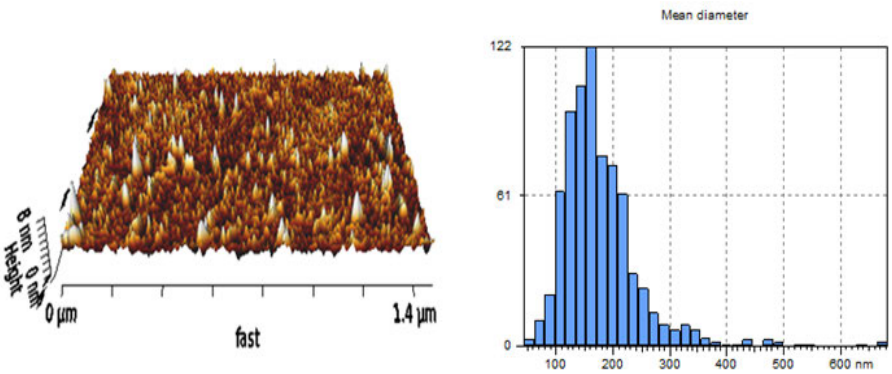

Figure 3: 3D AFM Image and Distribution Map of CdO NPs Granularity Accumulation

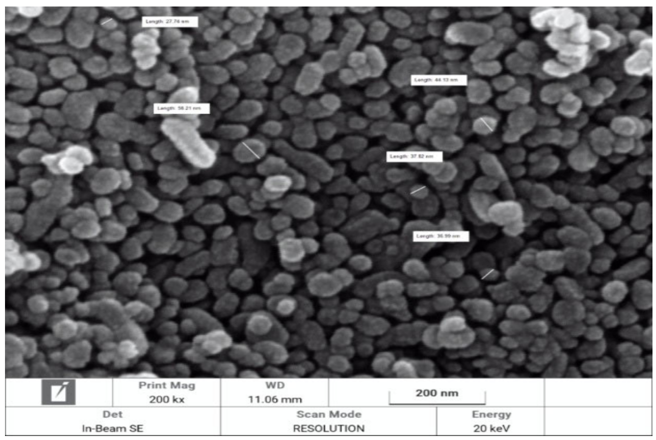

Figure 4: FE-SEM Image of Green Synthesis CdO NPs

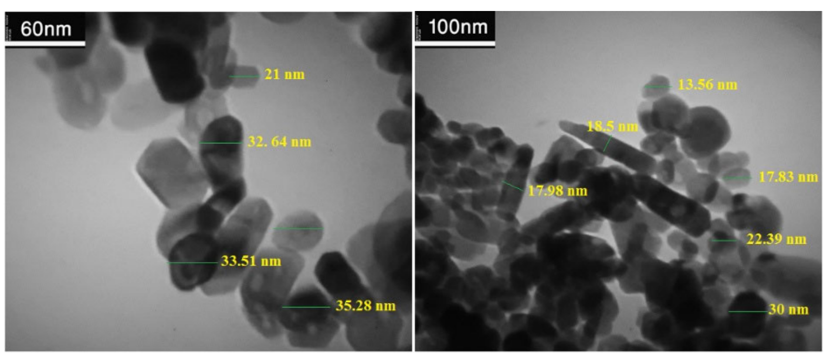

Figure 5: TEM Images of Green Synthesis CdO NPs (Scale = 60 and 100 nm)

at values of 2θ at 32.68°, 35.60°, 39.22°, 49.06°, 53.71°, 58.66°, 61.86°, 66.98°, 68.27°, 73.38° and 75.40°, which correspond to the 110, 002, 200, 20-2, 020, 202, 11-3, 310, 113, 221 and 004 planes, respectively. The average crystalline size of CuO NPs was 31.43 nm, which was calculated using the Debye-Scherrer equation.

Surface Morphological Study

Figure 3-5 display the AFM, FE-SEM and TEM pictures of CdO nanoparticles, respectively. The granularity distribution and three-dimensional AFM of CdO NPs are displayed in Figure 3. The CdO NPs are semi-ball shaped, feature uniform grains, a vertical orientation and good dispersibility. These are the approximate values of the surface's average diameter (53.67 nm), average determined roughness (2.81 nm) and root mean square (3.12 nm).

Figure 4 shows a FE-SEM picture of CdO nanoparticles that have been green-synthesized. Sizes of CdO nanoparticles range from 44.99 nm. Additionally, a cluster of CdO NPs particles is the oval and spherical form of these nanoparticles.

The particle size distribution was shown in Figure 5 by the TEM picture of the CdO nanoparticles, which matched the identical sample of XRD pattern in Figure 1 and FE-SEM in Figure 4. According to TEM (at 60 and 100 nm magnification), the average particle size is between 13 and

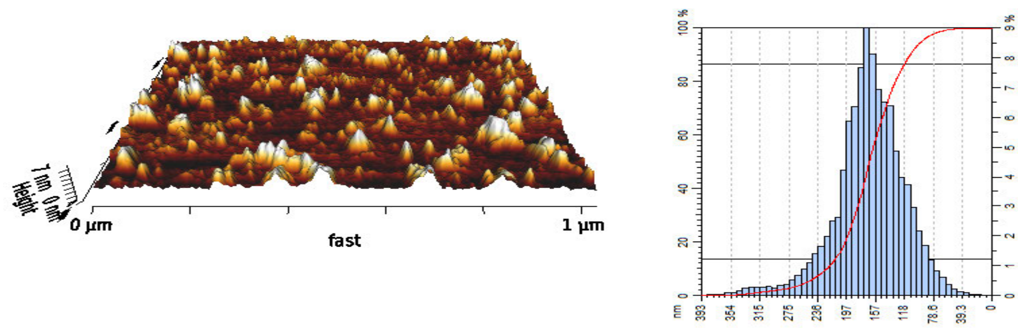

Figure 6: 3D AFM Image and Distribution Map of CuO NPs Granularity Accumulation

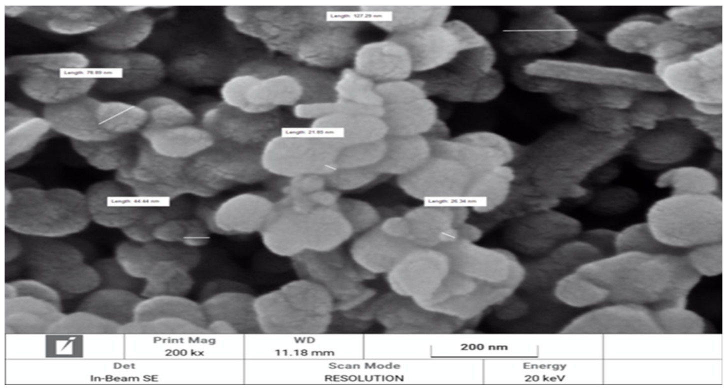

Figure 7: FE-SEM Image of CuO NPs

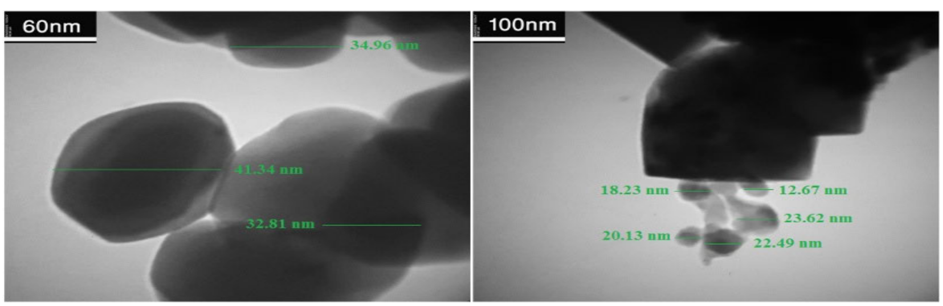

Figure 8: TEM Image of CuO NPs

35 nm. The homogeneous cubic structure of the produced cadmium oxide nanoparticles is seen from the particles.

In the realm of nanotechnology, Atomic Force Microscopy (AFM) is a tool used to determine and depict the topography of nanoscale surfaces with a high analytical capability. The spherical CuO NPs employed in this investigation were 99% pure and less than 50 nm in size (Figure 6).

FE-SEM was used to examine the shape of CuO NPs (Figure 7). Figure 7 showed that the CuO NPs had an average diameter of 35.76 nm and were shaped like spherical sheets.

The size, shape and crystalline structure of CuO nanoparticles may be seen in photos captured by a Transmission Electron Microscope (TEM). These images typically show spherical, self-assembled particles with different levels of aggregation and fusion depending on the synthesis and annealing conditions. The result is composed of spherical nanoparticles with a consistent shape and a relatively narrow size distribution from 2-40 nm, according to the TEM images in Figure 8. A tiny proportion of the nanoparticles shown in the micrographs are elongated, whereas the majority are almost spherical. Additionally, they are agglomerated and not widely distributed, with an average particle size of 40 nm, which is in good agreement with the XRD data.

Anticancer Activity

Using the MTT test, the cytotoxicity effects of CdO and CuO NPs were investigated against the MCF-7 (Figure 9, Table 1). Cytotoxicity activity on tumour cells was examined at two concentrations: Normal cells and control cells (50 and 100 µg/mL). The phytoconstituted CdO and CuO NPs IC50 values against the MCF-7 cell line were 124.5 and 94.6 µg/mL, respectively. According to these data, the

Table 1: The Proportion of Cells Remaining and the Percentage of Killing on LAMA-87 After 24 hours

	CdO NPs (IC50 = 124.5 µg/mL)		CuO NPs (IC50 = 94.6 µg/mL)	
Concentration (ppm)	Cell Death (%)	Cell Viability (%)	Cell Death (%)	Cell Viability (%)
0	9.44	90.56	4.08	95.92
20	19.82	80.18	12.01	87.99
60	39.57	60.43	32.71	67.29
100	48.91	51.09	46.82	53.18
140	65.53	34.47	59.37	40.63
180	80.27	19.73	80.19	19.81
220	87.07	12.93	86.11	13.89
260	89.13	10.87	90.68	9.32
Blank	96.3	3.7	93.3	6.7

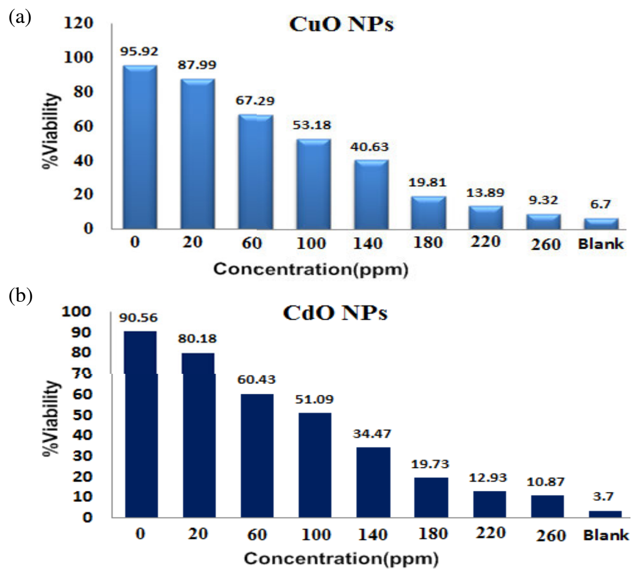

Figure 9: Anticancer Activity of CuO NPs and CdO NPs

lowest dosage exhibited a good cytotoxic effect. An illustration showing how well biosynthesised copper oxide and cadmium oxide nanoparticles work against MCF-7 (Figure 9) cells at various. Based on these results, it can be said that CuO NPs have a strong inhibitory effect on MCF-7 cells, with the percentage of cancer cells killed being higher at the lowest concentration. This is superior to CdO NPs, depending on the nanoparticle concentration.

CONCLUSIONS

Green chemistry, often known as environmentally friendly chemistry, is a cost-effective and ecologically safe approach to creating nanoparticle materials. Additionally, the produced material nanoparticle may be reused several times without losing its chemical activity and is distinguished by its chemical stability and lack of anxiety. Excellent *in vitro* anticancer and apoptotic activity against human breast cancer cells was demonstrated by the effective production of CuO and CdO NPs using cauliflower leaf extracts, which was verified by XRD, AFM, FE-SEM and TEM.

Compared to CdO NPs, CuO NPs showed more cytotoxic action and apoptosis induction against breast cancer cells. Additional in vivo studies may clarify CuO NPs' potential for use as a therapeutic agent in cancer treatment.

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