

Green Synthesis, Characterization and anticancer activity of CdO nanoparticles synthesized using aqueous leaf extract of *Nelumbo nucifera* (lotus) and *Pistia Stratiotes* (water lettuce)

M.Kalaivani^{1*}, S.Ravi^{1&2}.

¹Research scholar, Department of Physics, Annamalai University, Annamalai nagar
Chidambaram, Tamil Nadu, India-608002

²Associate Professor, PG & Research Department of Physics,
Thiru Kolanjiappar Govt. Arts College(Grade-I), Viruthachallam, Tamil Nadu, India-606001.

Abstract

To reduce the potential hazards of these chemicals in safer environments, research is being performed into environmentally friendly ways of producing metal oxide particles (NPs). In this study, *Nelumbo nucifera* (lotus) and *Pistia Stratiotes* (water lettuce) leaf extract was used to successfully synthesize simple, ecologically friendly Cadmium oxide (CdO) nanoparticles from two plants. The structure and shape of the calcined oxide nanoparticles were examined using X-ray diffraction (XRD), scanning electron microscopy (SEM) with energy dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR). It was discovered that the CdO nanoparticles produced by plants were elliptical and evenly distributed in suspension. Since cadmium oxide particles are valuable in a variety of applications, these green synthetic CdO NPs have anticancer properties.

Keywords: CdO, Green synthesis, Nanoparticles, Plant extracts.

1.Introduction

Nanotechnology has gained significant attention for the synthesis of nanoparticles due to the increasing demand for rapid, simple, cost-effective, and eco-friendly methods. The development of processes that produce nanoparticles with controlled size, shape, and dispersity holds great importance in advancing various fields.[1]. Nanoparticles, typically ranging from 1 to 100 nm, have proven to be transformative across numerous industries such as food, healthcare, cosmetics, optics, drug gene delivery, energy science, optoelectronic photochemical, agriculture, among others.[2]. Cadmium (Cd), for instance, is a well-known toxic element and environmental pollutant. [3]. Its ecotoxicity is well documented, with metallic Cd being one of the most harmful

metallic particles due to its cytotoxicity and bio-accessibility. [4]. Cadmium oxide (CdO) nanoparticles stand out as an n-type II–IV semiconductor with a direct band gap of 2.5 eV and an indirect band gap of 1.98 eV, making it valuable in applications like optoelectronics, though its toxicological implications necessitate careful handling and further study. [5]. CdO has potential uses in photovoltaic cells [6], detectors [7], catalysts [8], and solar cells [9], etc., as a versatile semiconductor. The synthesis of CdO nanoparticles in various shapes and sizes is crucial for optimizing their use in different applications. [10]. There are several techniques to prepare these materials such as sonochemical, micro-emulsion, hydrothermal and plant mediated methods [11]. Green synthesis stands out due to its ecofriendly, cost effective and easily accessible nature. Nanoparticles produced through this method are known as biogenic nanoparticles.[12]. The Synthesis of nanoparticles (NPs) using plant-based materials presents an innovative and environmentally sustainable approach, adhering to the principles of green chemistry. This method eliminates the need for high pressure, energy, elevated temperatures, or hazardous chemicals, cost-effective and eco-friendly. Various plant parts, such as leaf or milky extracts from (*E-tirucalli*, *Mimosa Pudica*, *Aloe vera gel*, *EGCG*, *Leucas Aspera* *Calatropis gigantia* etc.) have been successfully utilized to synthesize nanoparticles. These eco-friendly nanoparticles have applications across diverse fields, including pharmaceuticals, White LEDs' radiation dosimetry and biomedical uses. [13]. Among different types of nanoparticles, transition metal oxide NPs like ZnO, FeO, CuO) are especially effective against bacteria as these comprise the most reduced state, and consequently enter in the food chain of the ecosystem. Cadmium oxide (CdO) Nanoparticles, in particular, exhibit exceptional properties, making them highly suitable for use in biomedicine and biotechnology, where they can offer significant benefits in terms of efficiency and performance.[14]

This research is focused on the eco friendly and sustainable synthesis of CdO NPs using extracts from *Nelumbo nucifera* (lotus), *Pistia Stratiotes*, (water lettuce). The goal of study is to explore the catalytic properties of these plant extract in nanoparticle synthesis and assess the resulting CdO NPs for their anticancer application. The green synthesis method holds promise for reducing the environmental impact of nanoparticle production by utilizing naturally derived reagents instead of toxic chemicals. The synthesized NPs were assessed for their morphological and elemental properties using XRD, FTIR, SEM with EDX techniques. Plants extracts *Nelumbo nucifera* (lotus) and *Pistia Stratiotes* (water lettuce) are used as reducing and stabilizing agents in

the synthesis of CdO NPs. The study also explores the potential anticancer activity of CdO nanoparticles, which could be promising in cancer treatment due to their unique properties, such as oxidative stress induction in cancer cells.

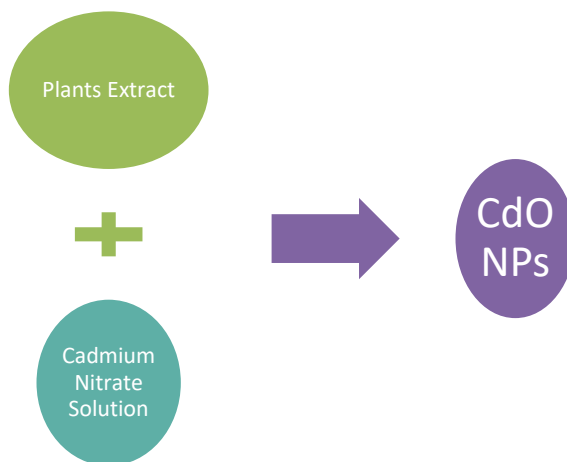


Figure.1. Schematic representation of green synthesis of Cadmium Oxide Nanoparticles using 20ml of plants leaf extract

2. SAMPLE PREPARATION

2.1. Materials

Cadmium oxide (CdO) are purchased from Sigma – Aldrich Chemicals for this study. All glassware's are washed with HNO_3 and distilled water and dried in the oven. *Nelumbo nucifera*, *Pistia Stratiotes* leaves were collected from the vadamoor village, kattumannarkovil (tk), Cuddalore District.

2.2. Preparation of leaf extract

The fresh leaves of *Nelumbo nucifera*, *Pistia Stratiotes* were collected and washed in running water and then with distilled water. 5g of dried leaf powder were added to 100 ml distilled water at 60°C , boiled in a water bath for 30 mins, and after that cooling at room temperature. Finally, this extract was filtered through Whatman No.1 filter paper and stored at

4°C for further synthesis. Similarly, all the leaf extracts were prepared and used for the synthesis of cadmium nitrate tetra hydrate, nanoparticles.

2.3. Synthesis of CdO nanoparticles

100 mL of 1M aqueous solution of cadmium nitrate tetra hydrate was taken in an Erlenmeyer flask and a quantity of 20ml of *Nelumbo nucifera* (lotus) and *Pistia Stratiotes* (water lettuce) leaf extracts was added slowly using a burette for the bio-reduction process at room temperature. After 30 min the solution was turned from light yellow, which indicates the formation of CdO NPs. The same procedure was adopted to synthesize CdO NPs using the other leaf extracts of *Nelumbo nucifera* (lotus) and *Pistia Stratiotes* (water lettuce). A pale yellow CdO NPs powder was annealed for two hours at 500°C in a muffle furnace.

2.4. CHARACTERIZATION ANALYSIS

The Cadmium Oxide nanoparticles was characterized by FTIR scrutiny using the dried powder of the synthesize CdO NPs by (Thermo Nicolet 380) FTIR spectroscopy spectra range 4000-400 cm^{-1} . The Fourier Transform Infra-Red Spectroscopy spectrum was recorded in (DLA TGS detector). The average crystalline size and purity being characterized by Powder X-ray Diffractometer (PANalytical x'pert PRO Powder X-ray Diffractometer) using Cu-K α radiation of wavelength $\lambda=1.5401 \text{ \AA}$ of synthesize CdO NPs. The shape and structures of the products were characterized by using scanning electron microscopy (SEM) with EDAX (Model JSM 6390LV, JOEL, USA).

2.4. Cell lines and culture medium

HeLa cell line was procured from NCCS, stock cell was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 $\mu\text{g/mL}$) in a humidified atmosphere of 5% CO_2 at 37°C until confluent.

The in vitro determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3- [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water-soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

3. RESULT AND DISCUSSION

Different characterization techniques were used to study the morphological, biological activity of the as-synthesized ZnO nanoparticles.

3.1.XRD analysis

The XRD pattern of CdO NPs that were annealed at 500°C in the recording spectrum range of 2θ from 20 to 80° is displayed in Figure 2. For example, the XRD pattern of the sample showed prominent diffraction peaks corresponding to CdO at this temperature. As a result, heat treatment at a higher temperature is needed to complete the reaction and enhance the crystallization. Sharp peaks were observed in the room-temperature X-ray diffraction data following a 2-hour calcination process in air at 500°C, during which CdO crystallized in the face-centred cubic phase. The diffraction peaks correspond with at $2\theta = 33.01, 38.30, 55.29, 65.90,$ and 69.27 corresponding to the (111), (200), (220), (311), and (222) planes of the CdO

NPs, respectively. The biosynthesized nanoparticles' XRD pattern corresponds to the cubic phase of polycrystalline monteponite CdO (JCPDS 05-0640). [15]. The Debye-Scherrer equation was utilized to determine the crystallite size of the biosynthesized CdO NPs, as reported by below

$$D = \frac{K\lambda}{\beta \cos \theta} \quad \text{-----(1)}$$

The average particle size of the CdO powder was determined to be, where D is the particle size (nm), K is a constant of 0.94, λ is the wavelength of the x-ray radiation (1.5406Å), β is the full width at half maximum (FWHM) of the peak (in radians), $\cos \theta$, and 2θ is the Bragg angle (degree). The average particle size in *Pistia stratiotes* is 32 nm, and the average crystallite size in *Nelumbo nucifera* is 31nm.

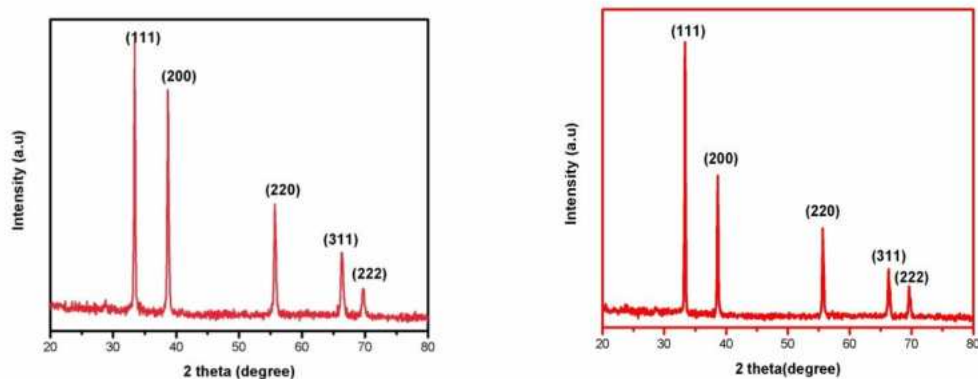


Figure. 2. XRD Patterns of CdO NPs using 20ml of *Pistia stratiotes* and *Nelumbo nucifera* leaf extract

3.2.FTIR ANALYSIS

Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the biomolecules involved in the reduction of Cd^{2+} ions using *Nelumbo nucifera* and *Pistia stratiotes* leaf extracts. The CdO nanoparticles (NPs) showed distinct vibrational modes in the 400–4000 cm^{-1} range, as depicted in Figures. 3 (a,b). A broad peak at 3440-3439 cm^{-1} corresponds to N-H symmetric and O-H stretching vibrations. The significant peaks at 2922, 2882 and 2866 cm^{-1} are due to the C-H stretching vibration of aldehydes. Additionally, the band observed between 1641–1636 cm^{-1} is associated with C=C, N-H bonds, while a weak and sharp peak near 1384 cm^{-1} corresponds to C-H stretching. The peak at 1021 cm^{-1} indicates C-O stretching vibrations. In the fingerprint region i.e., < 1000 cm^{-1} , which known for metal oxides and hydroxides, peaks at 445 cm^{-1} , 619 cm^{-1} and 961 cm^{-1} confirm the presence of Cd-O bonds in the synthesized (CdO) nanoparticles. These findings from the FTIR analysis validate the green synthesis of CdO NPs, where the extracts of *Nelumbo nucifera*, *Pistia stratiotes* serve as capping, reducing and stabilizing agents.[16].

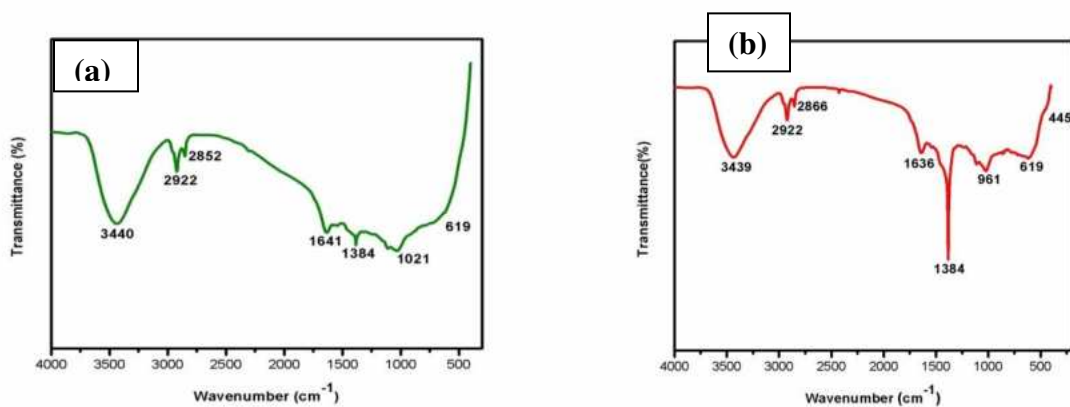


Figure.3(a,b). FT-IR spectra of synthesized cadmium oxide nanoparticles of 20ml *Pistia Stratiotes* and *Nelumbo nucifera* leaf extract

Table-1. FT-IR Stretching vibration and functional groups of synthesized CdO NPs using plant *Pistia Stratiotes* leaf extract

Wavenumber (cm ⁻¹)	Stretching	Functional group
CdO NPs		
3440	O-H	Alcohols, Phenols
2882, 2922	C-H	Alkanes
1641	C=C, N-H bend	Amines
1384	C=O, C-H	Alkanes
1021	C-N	Aliphatic amines
619	M-O	Cd-O

Table-2. FT-IR Stretching vibration and functional groups of synthesized CdO NPs using plant *Nelumbo nucifera* leaf extract

Wavenumber (cm ⁻¹)	Stretching	Functional group
CdO NPs		
3439	O-H	Alcohols, Phenols
2866, 2922	C-H	Alkanes
1636	C=C, N-H bend	1 ⁰ amines
1384	C=O, C-H	Lkanes
961	C-N	Aliphatic Amines
619, 445	M-O	Cd-O

5.5. SEM with EDX

Scanning Electron Microscopy (SEM) is used to determine the size and morphology of the cadmium oxide nanoparticles. The SEM images, which are presumably presented in figure.4-5. (a), show the form of the CdO nanoparticles synthesized using 20 ml of leaf extract from *Nelumbo nucifera* and *Pistia Stratiotes*. The nanoparticles have irregular, cubic shapes and appear aggregated. This aggregation is attributed to the polarity and electrostatic attraction

between the nanoparticles. The average size of the nanoparticles is reported to be 50 nm. Energy Dispersive X-ray Spectroscopy (EDX or EDS) is used to determine the elemental composition of the nanoparticles. The EDX spectra, as presented in figure 4-5.(b), confirm the presence of Cd and O with weight percentages of 74.00%, 86.72% and 26.00%, 13.28%, respectively. The absence of other peaks in the spectra suggests that the synthesized nanoparticles are pure CdO. The data on weight percentage and atomic percentage of oxygen and cadmium are summarized in Table-3, further confirming the elemental composition and purity of the CdO nanoparticles.[17]

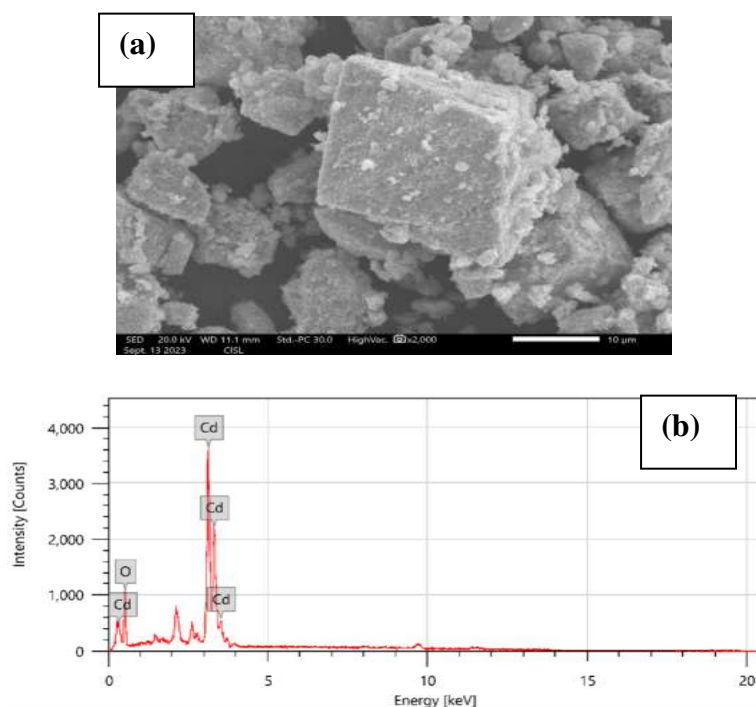


Figure 4 (a,b). SEM with EDAX images of CdO NPs using *Nelumbo nucifera* leaf extract

Table-3. Chemical composition of CdO NPs synthesized in *Nelumbo nucifera* leaf extract

Elements	Series	Weight %	Atomic %
O	K-Series	26.00	71.17
Cd	L-Series	74.00	28.83
Total		100	100

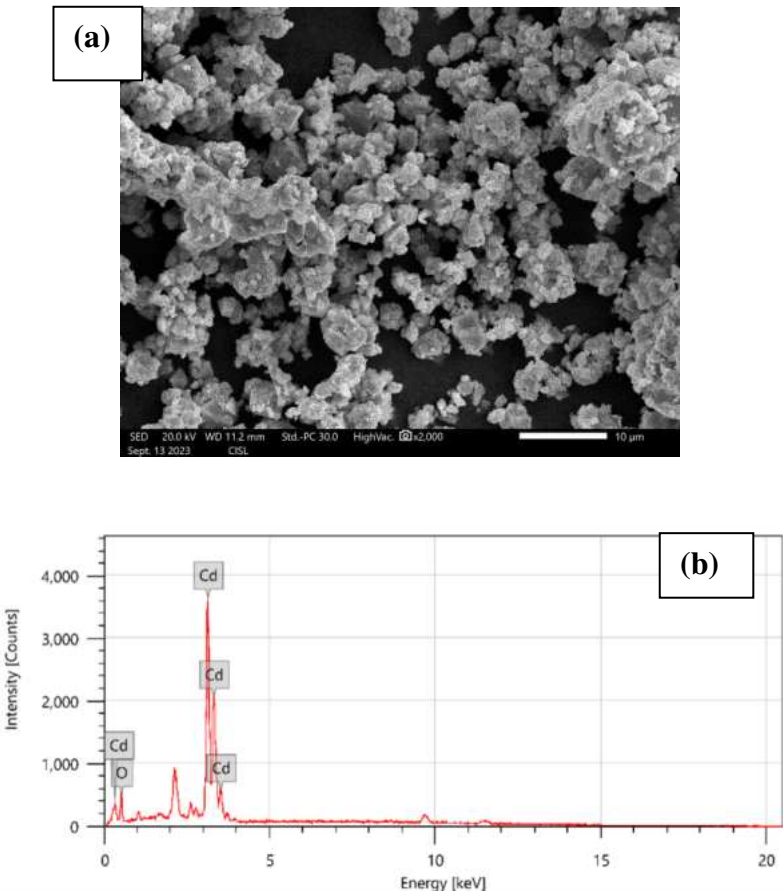


Figure 5. SEM with EDX images of CdO NPs using *Pistia Stratiotes* leaf extract

Table-4. Chemical composition of CdO NPs synthesized in *Pistia Stratiotes* leaf extract

Elements	Series	Weight %	Atomic %
O	K-Series	13.28	51.84
Cd	L-Series	86.72	48.16
Total		100	100

6. Anticancer activity

The HeLa cell line was tested for in vitro cytotoxicity of CdO NPs at various concentrations (0.25–100 µg/mL), and light microscopy was used to assess the morphological changes (Figure.6-7). As a result, as compared to the control, which displayed normal morphology and total inhibition of the cells at 25 µg/mL, there was no apparent reduction in the cells at the 0.25 µg/mL concentration. Considering that all inhibition was achieved at a dosage of 25 µg/mL, it is undeniable that concentrations greater than 25 µg/mL would likewise cause the cells to exhibit complete inhibition. The results make it evident that increasing the concentration is the primary cause of the breakdown of cells and significant cell growth. [18].

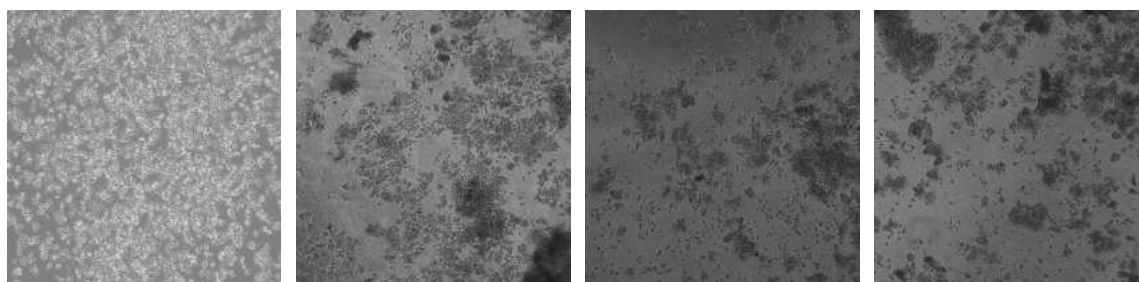


Figure 6. Light microscopic image of HeLa cell line treated with bioinspired CdONPs

Table-5. Anticancer activity of CdO NPs synthesized by *Nelumbo nucifera* leaf extract for HeLa cell line

S.No.	Concentration(µg/ml)	Cell Viability (%)	IC ₅₀
1.	6.25	95.91	47.59
2.	12.5	89.86	
3.	25	80.77	
4.	50	65.62	
5.	100	56.54	

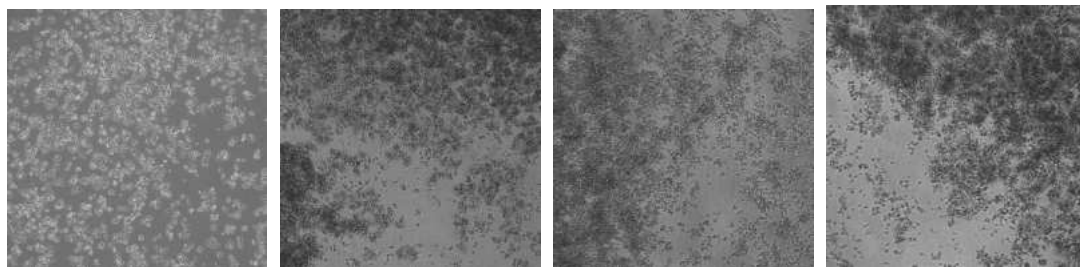


Figure 7. Light microscopic image of HeLa cell line treated with bioinspired CdO NPs

Table-6. Anticancer activity of CdO NPs synthesized by *Pistia Stratiotes* leaf extract for HeLa cell line

S.No.	Concentration($\mu\text{g/ml}$)	Cell Viability(%)	IC ₅₀
1.	6.25	87.85	41.43
2.	12.5	79.78	
3.	25	68.66	
4.	50	55.54	
5.	100	44.43	

Conclusion

In this work, green synthesis of CdO nanoparticles the use of plant extracts from *Nelumbo nucifera* (lotus) and *Pistia Stratiotes* (water lettuce) not only acts as reducing agents but also stabilizes the nanoparticles through capping. These plants contain bioactive compounds (such as flavonoids, alkaloids and phenolic compounds) which help in the formation and stabilization of nanoparticles in an environmentally benign manner. The green synthesized CdO nanoparticles have been successfully characterized using XRD this would have provided information on the crystalline structure of the CdO nanoparticles. FTIR likely helped to identify the functional groups in the plant extract that were responsible for the reduction and stabilization of the nanoparticles. SEM would have provided images of the surface morphology and size distribution of the nanoparticles, while EDX would confirm the elemental composition, verifying the presence of Cd and O in the nanoparticles. The CdO nanoparticles synthesized using these plant extracts showed significant cytotoxic activity against HeLa (human cervical cancer) cells,

indicating potential as a therapeutic agent. This is further reinforced by the fact that both *Nelumbo nucifera* and *Pistia Stratiotes* themselves have documented anticancer properties, likely due to bioactive compounds like alkaloids, flavonoids and phenolic acids.

Reference

1. Javad Karimi Andeani and Sasan Mohsenzadeh, Phytosynthesis of Cadmium Oxide Nanoparticles from *Achillea wilhelmsii* Flowers, *Journal of Chemistry*, (2013) 1-4. <http://dx.doi.org/10.1155/2013/147613>
2. K. Sujatha S. Sharmila A.P. Sudha , O.L. Shanmugasundaram , Surfactant assisted spectroscopic application of cadmium oxide nanoparticles prepared via co-precipitation method, *Materials Today: Proceedings*, (2021), 1-5. <https://doi.org/10.1016/j.matpr.2021.03.626>
3. T.S. Nawrot, J.A. Staessen, H.A. Roels, E. Munters, A. Cuypers, T. Richart, A. Ruttens, K. Smeets, H. Clijsters, J. Vangronsveld, Cadmium exposure in the population: from health risks to strategies of prevention, *Biometals*, 23 (2010) 769-782. <https://doi.org/10.1007/s10534-010-9343-z>
4. Demir E, Qin T, Li Y, Zhang Y, Guo X, Ingle T, Yan J, Orza AI, Biris A, Ghorai S, Zhou T, Chen T, Cytotoxicity and genotoxicity of cadmium oxide nanoparticles evaluated using *in vitro* assays, *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, (2020), [doi:https://doi.org/10.1016/j.mrgentox.2020.503149](https://doi.org/10.1016/j.mrgentox.2020.503149)
5. N. Clament Sagaya Selvam, R. Thinesh Kumar, K. Yogeenth, L. John Kennedy , G. Sekaran , J. Judith Vijaya , Simple and rapid synthesis of Cadmium Oxide (CdO) nanospheres by a microwave-assisted combustion method, *Powder Technology*, 211 (2011) 250–255. [doi:10.1016/j.powtec.2011.04.031](https://doi.org/10.1016/j.powtec.2011.04.031)
6. Kumari, R.; Kumar, V. Impact of zinc doping on structural, optical, and electrical properties of CdO film prepared by sol–gel screen printing mechanism. *Journal of Sol-Gel Science and Technology*, 94 (2020) 648-657, <https://doi.org/10.1007/s10971-019-05202-0>.
7. Ye, C.; Xu, F.; Wu, Z.; Gao, Z.F.; Wang, M. Ultra sensitive photo electrochemical platform with micro-emulsion-based p-type hollow silver iodide enabled by low solubility product (K_{sp}) for H₂S sensing. *Nanotechnology*, 32 (2021) <https://doi.org/10.1088/1361-6528/ac1094>.

8. Shashanka, R. Jayaprakash, G.K., Kumar, M., Kumara Swamy, B.E., Electro catalytic determination of ascorbic acid using a green synthesised magnetite nano-flake modified carbonpaste electrode by cyclic voltametric method. *Materials Research Innovations*, (2021) 1-11, <https://doi.org/10.1080/14328917.2021.1945795>.
9. Shashanka, R. Investigation of optical and thermal properties of CuO and ZnO nanoparticles prepared by Crocus Sativus (Saffron) flower extract. *Journal of the Iranian Chemical Society*, 18 (2021) 415-427, <https://doi.org/10.1007/s13738-020-02037-3>.
10. Ajay Savale, Suresh Ghotekar, Shreyas Pansambal and onker pardeshi, Green Synthesis of Fluorescent CdO Nanoparticles using *Leucaena leucocephala* L. Extract and their Biological Activities, *Journal of Bacteriology & Mycology*, 5 (2017) 1-6. DOI: [10.15406/jbmoa.2017.05.00148](https://doi.org/10.15406/jbmoa.2017.05.00148)
11. R. Aarthi, A.P. Sudha, K. Sujatha, and K.Sowmyalakshmi, Appraisal of Innovative Phytosynthetic Cdo np's from Leaf extract of Ocimum Sanctum and their Bactericidal Activity against Different Strains, *Nanoscale Reports*, 3(2) (2020) 1 – 8. DOI: [10.26524.nr.3.9](https://doi.org/10.26524/nr.3.9)
12. H. Nagabhushana, R.B. Basavaraj, B. Daruka Prasad, S.C. Sharma, H.B. Premkumar, Udayabhanu, G.R. Vijayakumar, Facile EGCG assisted green synthesis of raspberry shaped CdO nanoparticles, *Journal of Alloys and Compounds*, (2016) 1-28. doi: [10.1016/j.jallcom.2016.01.201](https://doi.org/10.1016/j.jallcom.2016.01.201).
13. Mehrina Asghar, Sadaf Habib, Wajid Zaman, Sajjad Hussain, Huma Ali, Saddam Saqib, Synthesis and characterization of microbial mediated cadmium oxide nanoparticles, *Microsc Res Tech*, (2020) 1–11. DOI: [10.1002/jemt.23553](https://doi.org/10.1002/jemt.23553)
14. S. Gowri, K. Gopinath, A. Arumugam, Experimental and computational assessment of mycosynthesized CdO nanoparticles towards biomedical applications, *Journal of Photochemistry & Photobiology, B: Biology*, (2017) 1-38. <https://doi.org/10.1016/j.jphotobiol.2018.02.009>
15. Sone BT and Maaza M, Room Temperature Green Synthesis of CdO Nanoparticles Using Aqueous Extracts of *Callistemon Viminalis*, *J Nanomater Mol Nanotechnol*, (2017) 1-6. DOI: [10.4172/2324-8777.1000205](https://doi.org/10.4172/2324-8777.1000205)
16. Prammitha R, Samson Y and Rejo Jeice, Synthesis of Cd(OH) 2 -CdO Nanoparticles Using Veldt Grape Leaf Extract: Enhanced Dye Degradation and Microbial Resistance, *Research square*, (2022) 1-34. DOI: <https://doi.org/10.21203/rs.3.rs-2332224/v1>

17. K. Karthik¹ S. Dhanuskodi C. Gobinath S. Prabukumar S. Sivaramakrishnan, *Andrographis paniculata* extract mediated green synthesis of CdO nanoparticles and its electrochemical and antibacterial studies, *J Mater Sci: Mater Electron*, (2017) 28, 7991–8001. [DOI 10.1007/s10854-017-6503-8](https://doi.org/10.1007/s10854-017-6503-8)
18. Ashutosh Dixit, Renu Bala , Bhawna Pareek, Ashun Chaudhary, Saroj Arora, Davinder Singh, Anju Bala, Vivek Sheel Jaswal, Green Synthesis of Cadmium Oxide Nanoparticles with Various Plant Extracts and their Use as an Anticancer Agent, *Biointerface Research in Applied Chemistry*, 13 (2023) 1-14. <https://doi.org/10.33263/BRIAC131.004>