Nanoscale Reports

RESEARCH ARTICLE

DOI:10.26524.nr.3.9

3(2) (2020) 1-8

OPEN ACCESS

Appraisal of Innovative Phytosynthetic Cdo np's from Leaf extract of Ocimum Sanctum and their Bactericidal Activity against Different Strains

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Abstract

The phytosynthesis of n-type Cadmium Oxide Nanoparticles reduces the toxicity of the substance and makes it Eco-friendly. This Eco-friendly biosynthesis of CdO NP's was synthesized for the first time from the Queen of herbs, Ocimum Sanctum (holy basil). The biosynthesized Cadmium oxide was prepared using Ocimum leaf extract as a reductant and Cadmium Chloride and hydroxide as cadmium and oxide source materials by Co- Precipitation method. Thus obtained Cadmium Oxide Nanoparticles were characterized by different techniques such as X-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM), Energy dispersive X-ray spectroscopy(EDS) to study the structural and morphological properties. XRD pattern exhibited the formation of face centered cubic structure of CdO NP's with an average crystalline size of 11.51nm .The chemical bond formation of CdO NP's were confirmed by FTIR spectrum in the range of (400-4000cm^{-1}). The SEM micrographs revealed the predominant formation of Cauliflower shape with a particle size in the range of 61-142nm. The high purity of the biosynthesized nanoparticles were confirmed by EDS analysis. Further it was tested against gram positive and gram negative bacterial strains and showed significant antibacterial activity. This biosynthetic research study opens an innovative window to progress our understanding of how CdO NP's shows resistance to different bacterial strains.

Keywords: Bio Synthesis, Ocimum Sanctum, CdO NP's, XRD, Bactericidal activity

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How to cite this article: R. Aarthi, A.P. Sudha, K. Sujatha, 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), 1-8. Retrieved from https://nanoscalereports.com/index.php/nr/article/view/47

Source of support: Nil

Conflict of interest: None.

Received: 2 July 2020 **Revised:** 1 August 2020 **Accepted:** 3 August 2020

1. Introduction

The Nano world is the intermediary between the atom and the solid and it has large surface area.[1] The word Nano meaning 'Dwarf' was evolved from Greek. It is a multi- disciplinary area of applied science and engineering that helps to create extremely small components and systems. Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, hence it can use in various applications. The nano material is called as single phase solids. Nano biomaterials is used in dental which can improves mechanical properties, superior abrasion resistance, lower shrinkage and enhanced optical and esthetic properties. Bacteria are microorganism which belongs to prokaryotic type. The micro resistance problem is growing and antibacterial drug is necessary. Antibacterial activity is that it incubates the growth of bacteria such as Escherichia coli, Salmonella paratyphi,(gram negative), Staphylococcus aureus, Bacillus substilis (gram positive).[1-9] Therefore, many researches had been carried out using Mg, TiO, ZnO, CdO for inhibiting the bacteria and make them eco friendly. In analogize with noble metals, such as silver and gold, cadmium (Cd) nanoparticles was legitimately unstable in aqueous solution. From this view, therefore it was challenging role in synthesizing CdO nanoparticles. So far, many scholars have intended the environmental and biological risks of noxious cadmium oxide nanoparticles and investigated many applications of CdO.

CdO is an inorganic compound and it is the most important n-type semiconducting material having direct band gab of about 2.2-2.5 eV and indirect band gab of about 1.36 ev- 1.981eV. Because of the n- type, there will be a deficiency in oxygen. CdO is a transparent conductor as it

was noted in 1907 by Karl Baedeker. And it was crystalline in nature. The CdO nanoparticles finds various application in catalyst, solar cells, LCD, gas sensors, cadmium plating bath, electrodes or storage batteries, photodiodes, photovoltaic cells, , photo detectors etc due to its wide band gap energy. There are many methods for synthesis of cadmium oxide such as Precipitation method, Sol gel method, hydrothermal method, green synthesis method.[13] Among them green synthesis play a vital role in synthesis because it is eco friendly, cost effective and easily available. The products which are synthesized by this method are called biogenic nanoparticles. Green chemistry makes the toxic products to eliminate its hazardous substance from the sample. Photo synthesis of nanoparticles is a green chemistry approach that connects nanotechnology with plants.

In the recent years, Many researchers has great appeal in making ecofriendly environment by espousing the green mediated synthesis for making CdO nanoparticles using reductant such as Hibiscus Sabdariffa flower extract, Green tree extract, Achilleawilhelmsii flowers leaf extract. The investigation of antibacterial activity to eradicate the bacteria using Leucaenaleucocephala L. Extract shows significant effect. Among them CdO nanoparticles plays great potential and powerful role in antibacterial activity. Since it has excellent antibacterial effect, it plays a vital role in biomedical application.

To the best of our knowledge, so far no report is available for the exploitation of phytosynthesis of CdO nanoparticles using Ocimum sanctum (basil leaf) which is easily available in all markets. In the present work, phytosynthesis of cadmium oxide nanoparticles from ocimum sanctum is carried out at room temperature by using simple and cost effective co- precipitation method and it is not necessary to use high pressure, time, energy, temperature. The reason for taking Queen of herbs "Ocimum Sanctum" is that, it is the traditional medicinal plant and the different parts of plants from root to leaves has many applications. It cures malaria, eyediseases, insect diseases and so on.In addition it has pharmacological actions such as anticancer, adaptogenic, antidiabetic effects, anti-inflammatory, antifertility and it also cures HIV-I.[5-9] In this biosynthetic research, different characteristics of nano –sized cadmium oxide were studied and the results are explored in detail. The Picture of Ocimum Sanctum plant and their scientific description were showed in Figs 1a and 1b.

2. Experimental Procedure

2.1 Chemicals used

All the chemicals used in this study were of interpretive and utilized in purified form. Cadmium chloride ($CdCl_2$) and ammonium hydroxide (NH_4OH) were the precursors for biosynthesis of CdO NP's and the aqueous solution was prepared using distilled water.

2.2 Assortment of Species

Fresh leaves of Ocimum Sanctum (Basil leaves) were collected from local areas in Erode. It was thoroughly washed for many times in tap water followed by distilled water to remove the dust particles and other foreign reagents and it was further dried for 10 days to remove water molecules. The resultant product is almost powder in nature.

2.3 Preparation of plant extract

Ocimum Sanctum aqueous extract was prepared in reference with.[10] About 20g of dried leaves(powder) were weighted and transferred into beaker containing 100 ml distilled water and heated for 1hr at 80°C. The broth was then cooled at room temperature and filtered using Whatman filter paper and refrigerate for further use. Fig. 2a and 2b shows visual observation and graphical representation of Extract preparation.

2.4 Biosynthesis of cadmium oxide nanoparticles

CdO nanoparticles were successfully synthesized for the first time using infusion of Ocimum Sanctum by co-precipitation method. In this bio-synthesis of cdO nanoparticles, 0.5M (6g) of Cadmium Chloride ($CdCl_2$) was dissolved in 60 ml of distilled water stirred continuously using Magnetic stirrer at room temperature till a clear and homogeneous solution is obtained. To this solution 60ml of

Fig. 1a: Ocimum Sanctum

Class:	Magnoliopsida
Sub Order:	Asteranae
Order:	Lamiales
Family:	Lamiaceae -mints
Genus:	Ocimum
Species:	Cimumteuniflorum
Kingdom:	Plantae

Fig. 1b: Scientific Description of Ocimum Sanctum

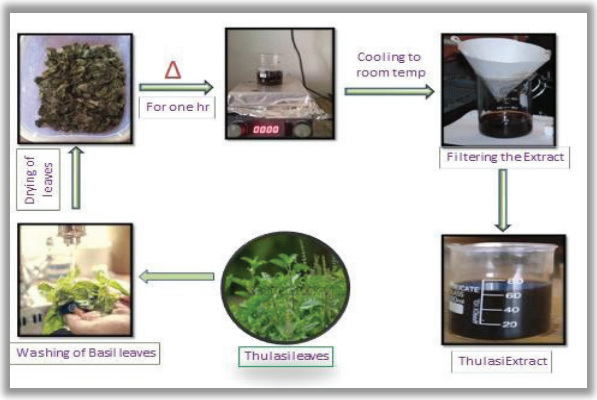


Fig.2a: Visual Observation of Extract Preparation

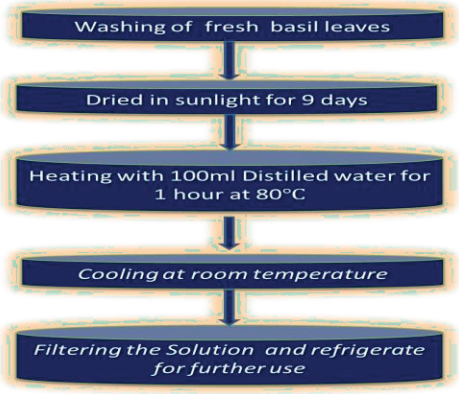


Fig. 2b: Graphical Representation of Extract Preparation

Ocimum Sanctum extract was added and stirred for 5mins. Finally to this complex mixture ammonium hydroxide (NH_4OH) solution was added drop wise under constant stirring until pH of the solution was reached to 8 where the colour of the solution turns to light green. Thus obtained resultant product is incubated for the time interval of 15 mins. Thus obtained $Cd(OH)_2$ precipitate were purified by repeated centrifugation at 2000rpm for 10 mins followed by repeated washing with distilled water to remove further foreign agents and dust particles. Then the prepared sample was dried in laboratory oven at 100°C for 3 hrs and calcinated at 400°C to remove hydroxide. Finally, the obtained precipitate was grounded to fine powder using agate mortar and light brown coloured cadmium oxide was obtained and it was stored in air tight container for further characteristics. Fig. 3a and 3b shows the schematic representation and the graphical representation of preparation of CdO NP's.

2.5 Characterization Techniques

CdO NP's were successfully characterized by different techniques for Clear understanding of the sample. The structural parameters of CdO NP's were carried out using X-ray diffraction (Bruker diffractometer) with monochromatic Cukα1 (wave length λ=1.5406Å) as a source operating at a voltage of 40 k and a current of 30mA at scanning rate of (10 deg/min) with the time constant of 0.6 sec. The bio-molecules were explored by FTIR spectroscopy (Perkin- Elmer System 2000). The surface morphology of the sample can be observed using MODEL PHILIPS XL-30 Scanning Electron Microscope with EDAX. Elemental Composition were recognized using EDS.

2.6 Antibacterial Assay

Antibacterial activity was screened against different pathogens. In this, Liquid Mueller Hinton agar media and the Petri plates were sterilized by autoclaving at 121° C for about 30 minutes at 15 lbs pressure. Under sterile conditions in the laminar airflow chamber, about 20ml of the agar medium was bestowed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, 18 hrs culture of Gram positive microorganisms such as Bacillus cereus (MTCC 430), Staphylococcus aureus (MTCC 3160),the surface of Agar plates were wiped Gram negative microorganisms such as E.coli (MTCC 1698) and Klebsiellapneumoniae (MTCC10309) obtained from IMTECH, Chandigarh .Well was prepared by using cork borer followed with loading of 50 µl and 100 µl of each sample to the distinct well with sterile distilled water as negative control and gentamycin(30mcg/disc) as positive control. The negative controller water is to identify the growth of bacteria. bacteriaThe sample loaded plates were then incubated at 37°c for 24 hours to observe the zone of inhibition. This zone of inhibition helps to identify how much bacteria is killed. In this report opens an innovative window to show how CdO NP's inhibits the growth.

3. Results and Discussions

Visual observation

The formation of CdO NP's from Ocimum sanctum leaf extract was preliminarily confirmed by the colour change. The colour change from light green to light brown confirmed the formation of cadmium oxide nanoparticles. This colour changes mainly owing to the reduction of precursor solutions by reluctant. Fig. 3c shows the visual observation of colour change of phytosynthetic CdO NP's from Ocimum sanctum.

3.1 Structural and Crystallographic studies

In the present work, CdO NP's were examined under X-Ray diffraction to analyze the crystalline structure, formation and quality of compounds. XRD pattern reveals that the obtained CdO NP's were crystalline in nature and the peak positions were in good agreement with the standard cubic CdO structure (Joint Committee for powder diffraction studies JCDPS CARD NO 78-1125-104).[11] The well-defined characteristics peaks obtained at$2\theta = 30°$, 33.9°, 37° were indexed at (111), (200), (210) planes respectively. The miller index of these reflection planes reveals the formation of cubic (FCC) phase of CdO NP's. However, a close investigation of XRD plot reveals that no additional secondary phases were

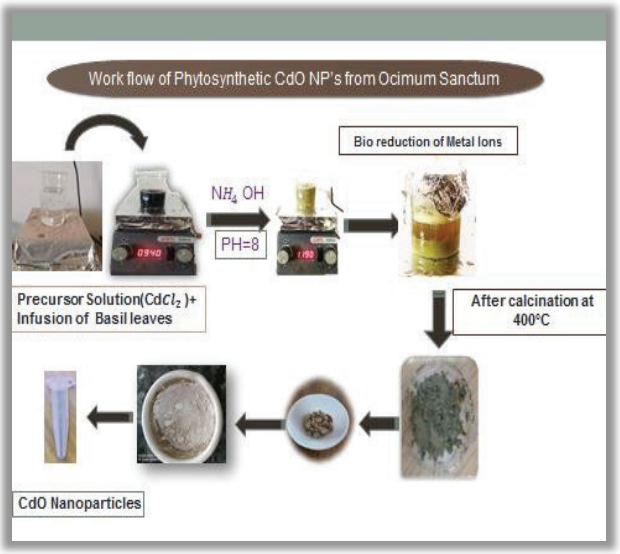


Fig. 3a: Schematic representation of CdO NP's

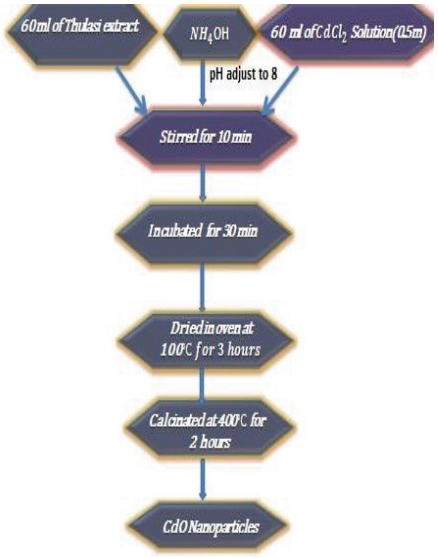
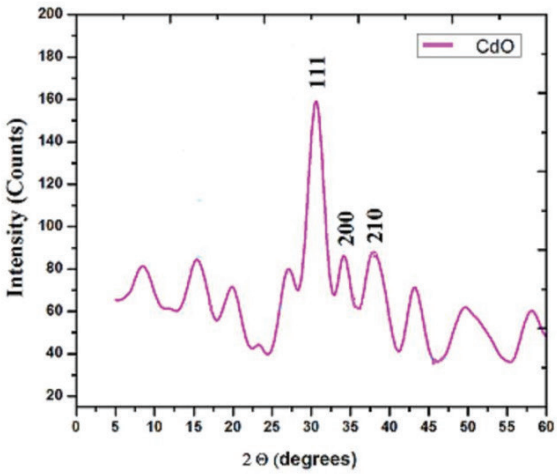


Fig. 3b: Graphical Representation of CdO Preparation

Fig.3c: Visual observation of CdO NP's

Fig.3c: Visual observation of CdO NP's

present which confirms the pure crystalline nature of the CdO NP's. The XRD Pattern and the cubic phase of CdO were displayed in Figure 4a.



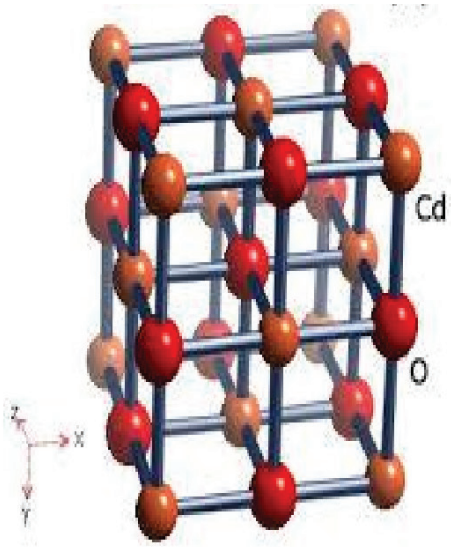


Fig . 4a: XRD pattern of Synthesized CdO NP's

$$D = k\lambda / \beta \cos\theta \ (Å) \quad (1)$$

where, D is the mean crystallite size, λ is the wavelength of the incident beam, β is the full width at half maximum and θ is the Bragg angle.

The crystalline size estimated from the standard formula is 11.5nm. It was nearly contests with the crystalline size obtained by green synthesis of CdO NP's using green tea which was investigated by Suresh Ghotekar.

The interplanar spacing is calculated from the Bragg's equation,

$$2\, d_{hkl} \sin\theta = m\lambda \quad (2)$$

Where, d_{hkl} is the interplanar spacing (m=1), (hkl) lattice plane index, 2 is the lattice constant and h, k and l are the integers. Table 1 gives the crystallite size and the spacing between the consecutive planes along with the dislocation density and micro strain.

The lattice constants were calculated from the equation of cubic system using the method of least squares

$$1/ d^2 = (h^2 + k^2 + l^2)/\alpha^2$$

Where,

$$a^2 = (h^2 + k^2 + l^2)/d^2 \quad (3)$$

Substituting (2) in (3), we get

$$a = \frac{n\lambda \pm \sqrt{h^2 + k^2 + l^2}}{2\sin\theta}$$

Dislocation density is

$$\delta = 1/ D^2 \ \ Lines/m^2 \quad (4)$$

Micro strain arises due to the lattice misfit and then calculated by,

$$\varepsilon = \beta \cos\theta / 4 \quad (5)$$

The average value of the lattice constant 'a' is found to be 5.2526Å for the biosynthesized CdO Nanoparticles exhausting the above formula was virtually synchronized to the standard value(JCDPS CARD NO 78-1125-104).The calculated structural parameters of the biosynthesized CdO NP.s were depicted in Table 1.

3.2 FTIR analysis

Fourier transform infrared spectroscopy (FTIR) used to evaluate various functional groups, various possible biomolecules and to validate the monteponite nature of the synthesized CdO NP's. Fig. 5a depicts the FTIR spectrum of the powdered CdO NP's in the spectral range of 400-

Table 1: Structural parameters of CdO Nanoparticles

2θ (deg)	FWHM (rad) (10^{-3})	D spacing Å	Crystal. Size D (10^{-9})m	Micro Strain ε (10^{-3})m	Dislocation density (10^{15}) lines/ m^2	Lattice Parameters Å	Unit Cell Volume (Å)3
30.71	21.50	2.9084	6.9589	5.2037	0.2065	5.0382	127.88
37.28	10.66	2.4094	14.333	2.5253	4.8653	4.8200	111.98
33.95	11.59	2.6383	13.4186	2.7735	5.5531	5.8996	205.33

Table 2: Various Functional groups analyzed by FTIR spectrum

S. No.	Region (Cm^{-1})	Interpretation
1	433.98,	
	514.99,	Cd-O modes
	736.81	
2	3519.16	O-H stretching (phenolic compound)
3	2997.38	C-H Stretching (alkane compound)
4	1851.66	C=H Bending (aromatic compound)
5	1598.99	N-H Bending (amine)
6	1516.05	N-O Stretching (Nitro Compound)
7	1417.68	O-H Bending (alcohol)
8	1170.79	S=O Stretching (Sulfate)
9	1056.99	C-O Stretching(Primary alcohol)

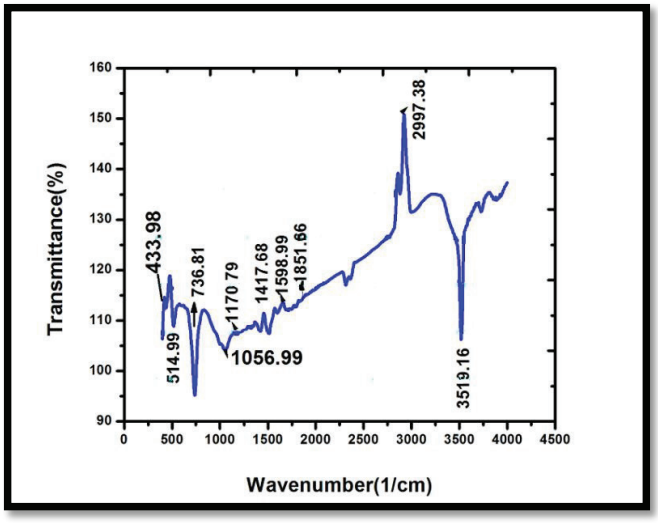


Fig. 5a: FTIR spectrum of Synthesized CdO NP's

4000 cm^{-1}. The graph conspired between IR transmission and wave number shows the major absorption at different wavenumber. The vibrational peaks obtained at 433.98, 514.98 and 736.81 cm^{-1} are all associated with Cd-O vibration mode.[12] These peaks indicates the pure monteponite nature of CdO NP's. The other vibrational bonds associated with different modes of CdO NP's and their group is given in Table 3.

3.3 Morphological analysis

Surface topography and its morphology can be identified by using Scanning Electron Microscope. The sample is mounted on a sample holder and it was coated with gold or using some other wafer. The beam of electrons is focused on the sample. The secondary electron emitted by the sample used to find the surface characteristics. The morphology of CdO calcinated at 400°C shows the distribution CdO particles with predominant cauliflower morphology[13] having a particle size in the range of 61-142nm[14] showed in Fig. 6a. In addition, no distinguishable shape particle was observed indicating homogeneous distribution of CdO NP's.

3.4 EDS analysis

The elemental composition of materials of the sample can be identified using Energy dispersive X-ray Spectroscopy Fig. 7a shows the EDS spectrum of CdO NP's synthesized from Ocimum Sanctum plant extract. The presence of cadmium and oxide in the prepared CdO NP's confirmed by the presence of respective peaks in the EDS spectrum. The purity of the sample can also be detected from the absence of other elements. The quantitative analysis of elemental composition of atomic and weight percentage of Cadmium and Oxide is shown in Fig.7b. The deficiency of oxygen is helpful in improving antimicrobial activity and photocatalytic activity.[13]

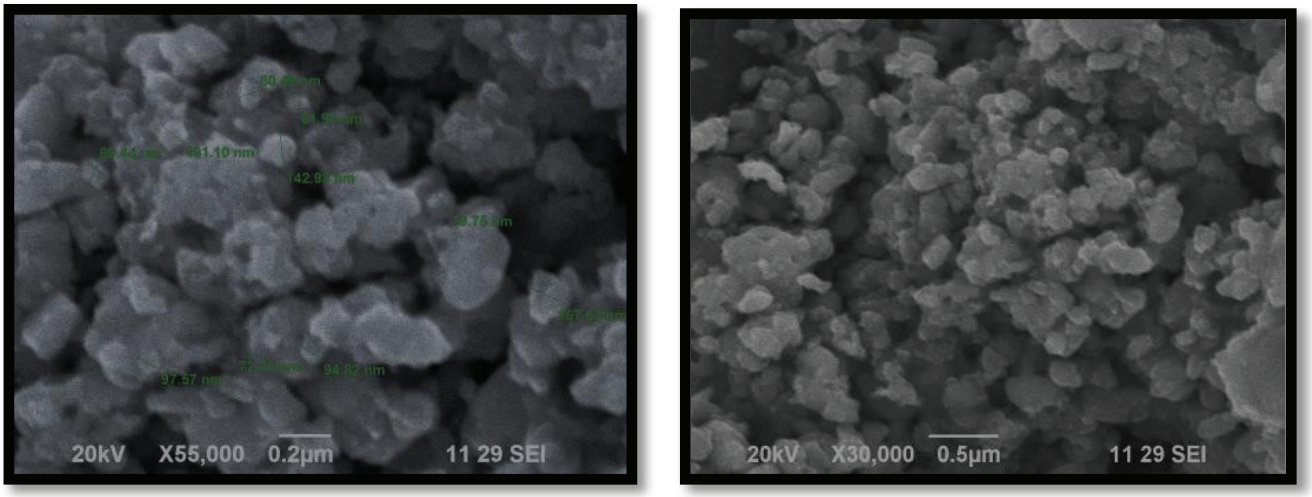


Fig. 6: SEM image of Synthesized CdO NP's

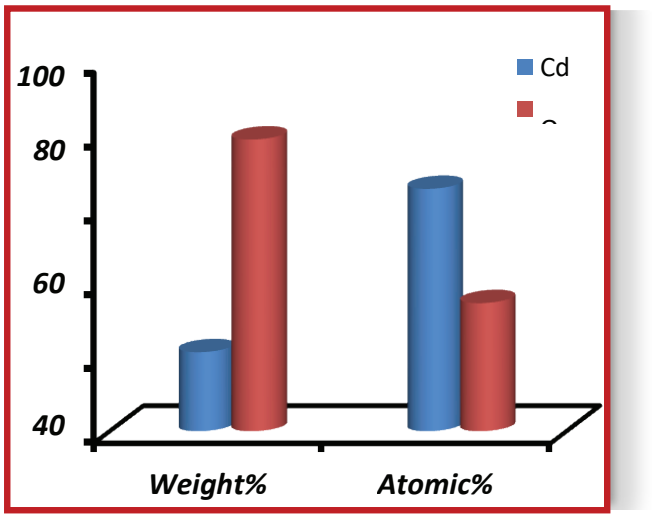


Fig. 7a: Quantitative analysis of CdO NP's

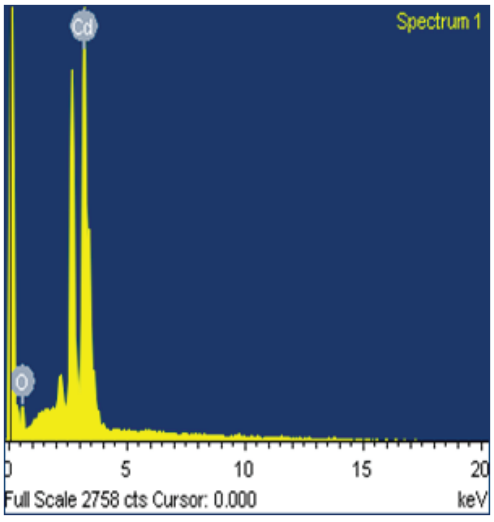


Fig. 7b: EDS spectra of Synthesized CdO NP's

3.5 Applications

Antibacterial activity

Antibacterial activity of nano-sized cadmium oxide nanoparticles using ocimum sanctum leaf extract were screened for their incubation effect against different pathogenic gram positive and gram negative bacteria. The zone of inhibition of CdO Nanoparticles at different rates of 50µl and 100 µl is compared with standard positive controller and showed in Table 3. Here the CdO nanoparticles are more effective inhibitor then the positive controller and it is shown in bar graphFig.8b.In the concentration of 100 µl of CdO NP's inhibition effect was increased. This is because the reductant Ocimum Sanctum leaves had effective reticence of different pathogens meanwhile it has medicinal property. The main goal of using nanoparticles as an antibacterial activity is to target the bacteria in alternate to antibiotic. In addition bacteria are negative charge and nanomaterials are positive charge so that they can be attracted with surface trends leading to tarnishing the microbe and finally bacteria will perish. Nanomaterials in addition with antibiotic can make the material harmless and improves antibacterial activity. Thus the disease produced by the bacteria such as E.Coli (gastroentetis) can be cured by CdO nanoparticles. These results were in good argument with Zahra assessment of antibacterial activity.[14] Several other reviews of antibacterial activity also agree with this result. Different pathogenic bacteria incubation of cadmium oxide is shown in Fig.8a.

4. Conclusion

Thus, we explode the successful synthesis of sustainable biosynthetic single phase highly crystalline CdO nanoparticles using leaf extract of Ocimum sanctum plant. This method asserts that it's economical, the procedure is simple and the preliminary materials are easily available in local areas to carry out in any laboratories. The monteponite CdO NP's were corroborated by the characteristics such as XRD, FTIR, SEM, EDS and antibacterial property. The XRD pattern confirms the formation of cubic CdO nanoparticles without any secondary phase. The average crystalline size and lattice parameter (a) were calculated as 11.3 nm and 5.2526 Å respectively using X-ray diffraction data. The vibrational mode at 433.98-736.21cm^{-1} confirms the presence of Cd-O bond. The predominant shape of cauliflower was identified by SEM. The quantitative analysis was determined by

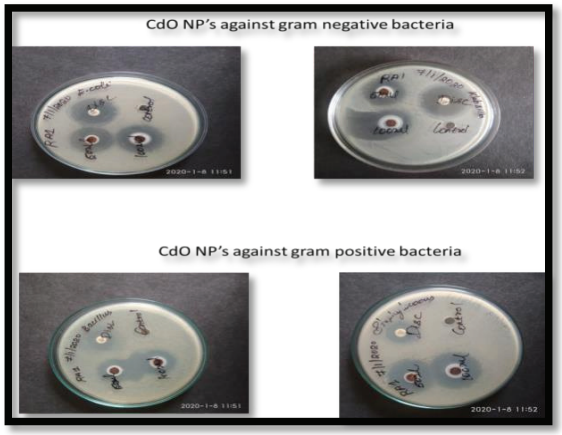


Fig. 8a: Antimicrobial activity of biosynthesized CdO NP's from O. sanctum L. against Gram-negative bacteria

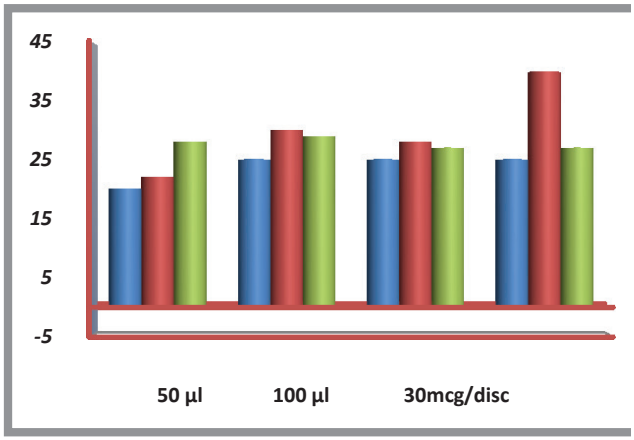


Fig. 8b: Bar graph indicating the maximum inhibition of pathogens by CdO NP's at 100 µl

Table 3: Antibacterial activity of CdO NP's against different pathogens

S. No.	IP Bacteria	Zone of incubation (mm)			Std. Antibiotic (Gentamycin)
		Control (Water)	(50 µl)	100 µl	30 mcg/disc
1	Bacillus cereus	Nill	20	22	28
2	Staphylococcus aureus	Nill	25	30	29
3	Escherichia coli	Nil	25	28	27
4	Klebsiella neumoniae	Nill	25	40	27

EDS. Antibacterial analysis showed that the synthesized cadmium oxide nanoparticles were effective against different pathogens. Further this technique was carried out in room temperature at faster rate without the effect of external parameters. Thus in upcoming years, to eliminate environmental pathogens cadmium oxide nanoparticles can be used as substitute for traditional antibiotics and result shows that it is better alternative to chemical process. Thus in future, these green mediated syntheses of nanotechnology may open the new applications of various metal oxides.

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