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Biosynthesis of CdoNanoparticles using *Citrus reticulata*Peel Extract and their Biomedical Applications

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ABSTRACT

In the current study, *Citrus reticulata* peel extract was used to synthesize cadmium oxide nanoparticles (CdO NPs). The CdO NPs were characterized through XRD, SEM, and UV-DRS analyses. The CdO NPs have a single face-centeredcubic phase confirmed by XRD study. . The UV-DRS was utilized to calculate the band gap of bio-fabricated NPs and was found to be 2.58 eV. Moreover, diverse biological applications such as antibacterial, antioxidant, studies on the effects of *Citrus reticulata* peel extract-mediated CdO NPs on DNA damage were disclosed. The CdO NPs' antibacterial efficacy shown improved bactericidal performance when evaluated against human pathogens. The antioxidant potential of CdO NPs was assessed using the DPPH assay, which demonstrated strong antioxidant activity. Furthermore, CdO NPs induced by *Citrus reticulata* peel extract demonstrated DNA damage. Therefore, these biogenically generated NPs operate as effective therapeutic agents because of their biological functioning.

Keywords: CdO nanoparticles, *citrus reticulata*, DPPH, DNA cleavage, antimicrobial

INTRODUCTION

Plant extract is used in the bio-mediated synthesis of metal oxide nanoparticles, which is a potential replacement for conventional chemical synthesis. This paper describes the biological production of CdO nanoparticles. In a variety of device applications and oxide nanomaterials have demonstrated a remarkable impact. (Sundrarajan *et al*. 2015). Because of its large surface area, the creation of metal and metal oxide nanoparticles has garnered significant interest in the physical, chemical, biological, medicinal, optical, mechanical, and engineering sciences. The intriguing characteristics of metal oxides, including their antibacterial, magnetic, electrical, and catalytic activity, are caused by their high atom content. Cadmium oxide is an n-type II-IV semiconductor with a direct and indirect band gap of 2.3-2.5 eV, respectively (Somasundaram *et al*. 2019). Because of their exceptional UV filtering, antibacterial, and fungal qualities as well as their high catalytic and photochemical activity, cadmium oxide nanoparticles have drawn a lot of interest

(Skheel*et al*. 2021). Plant extracts offer a more environmentally friendly biological method for the synthesis of various metallic nanoparticles, allowing for a regulated synthesis with precisely defined nanoparticle size and form (Bhardwaj *et al*. 2020). To avoid the use of toxic organic solvents and severe reaction conditions for the synthesis of nanomaterials(Paramasivam *et al*. 2021). Lately, scientists have discovered that they can use capping and stabilizing agents to prepare nanomaterials in an aqueous media (Sundrarajan*et al*. 2015).*Citrus retuclata*contains a variety of biologically active compounds such as carotenoids, flavonoids, limonoids, terpenes, proteins, ascorbic acid, fatty acids, phytosterols, tocopherols and dietary fibers.*Citrus reticulata* peels was chosen because of its functional properties like anti-inflammatory, antioxidant, antifungal, antimicrobial, anti-cancer(Saini *et al*. 2022). Furthermore, the importance of usage of natural, renewable and low cost material *C.reticulata* peels could able to produce the metal oxide nanoparticles with aqueous medium by avoiding the presence of hazardous substance and toxic solvents.The aim of this work, to synthesize theCdO nanoparticles using *C.reticulata*. The crystal structure and surface morphology were characterized by X-ray diffraction (XRD) [BRUKER-binary V4 (.RAW)] , scanning electron microscopy (SEM) [VEGA3 TESCAN]. The antibacterial, antioxidant, DNA cleavageof CdO NPs were also investigated.

MATERIALS AND METTHODS

2.1. Preparationofaqueouspeelextract

Fresh peels of *C.reticulata*fruits were cut into tiny pieces and washed, boiledfor 15 min, filtered. The resulting *Citrus* peel extract was placed inarefrigeratorat4°Cforsubsequentwork.

2.2. Bio-inspiredsynthesisofCdONPs

About6.0gofCd$(NO_3)_2 \cdot 4H_2O$wasmixedwith100mLofpeelextract,constantlyagitated. The reaction solution was dried in a hot oven, the resultant powder was calcined at 500 °Cfor1h.After crushing, theresultantbrown-coloredCdOnano-material was characterized.

2.3. Antioxidantefficacy

Theantioxidant efficacyofbio-fabricatedCdONPswasdeterminedbytheDPPHradicalassay.Theentireprocedure for the antioxidant capacity of both assays was described inpreviousreport(Barwant*et al*. 2022).Thisstudy,useddiverseconcentrations(20-100 µg/mL) of CdO NPs and positive control (ascorbic acid). ThescavengingperformanceofDPPHassaywascomputedasfollows(equation(1)):

$$\text{Scavenging capacity (\%)} = \frac{\text{OD(blank)} - \text{OD(sample)}}{\text{OD(blank)}} \times 100 \quad (1)$$

2.4. DNAdamageactivity

The agarose gel electrophoresis was performed by applying the *E. coli*pBR322 plasmid as a target to assess the DNA cleavage efficiency of thebio-fabricatedCdONPs(Barwant*et al*. 2022).Thetargetplasmidwasblendedwithdifferentconcentrationsofsynthesized CdONPsdissolvedinDMSO(dimethyl sulfoxide) solvent. The mixture was then incubated for 30 and90 min at 37 °C. The tracking dye wasmixed with the plasmid and CdONPsmixtureafterincubation.Afterthat,itwasaddedtothe0.8%agarose gel. The bands were photographed(Gulbagca*et al*., 2021, Jadhav *et al.* 2018).

2.5.Assay of Antibacterial Activity

The bacterial strains of *Escherichia coli(gram negative)* and *Bacillus subtilis (gram positive)* were obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh. Antibacterial activity was determined by disc diffusion method as described by Bauer *et al*. (1966). By inoculating a loopful of strain in Mueller Hinton broth separately and incubated at 37°C on a rotary shaker for 12 hrs. Then 0.1 ml of fresh inoculum (containing around 1 - 2 × 10^6 CFU/ml as per McFarland standards) was spread onto the surface of sterile Mueller Hinton agar plates using a sterilized spreader. The zone of inhibition was measured in mm scale.

RESULTS AND DISCUSSION

2.5. XRDanalysis

XRD profile was applied to analyze the phase, crystal structure, andcompositionoftheas-fabricatedCdONPs.TheresultispresentedinFig. 1. The crystalline nature of the synthesized CdONPs were confirmed by strong and narrow XRD peaks. The diffraction peaks were noticed at diffraction angles (2θ values)of 31.8°, 38.4°, 54.9°, 64.5°, and 68.8° corresponding to the reflectionpeaksbelongingtothe(*hkl*)valuesof(110),(200),(220),(311),and(222)(asmarkedinFig.1),respectively. Therefore, this studyobtainedinformation regarding thehighlycrystalline nature andpurityoftheas-preparedCdONPs.

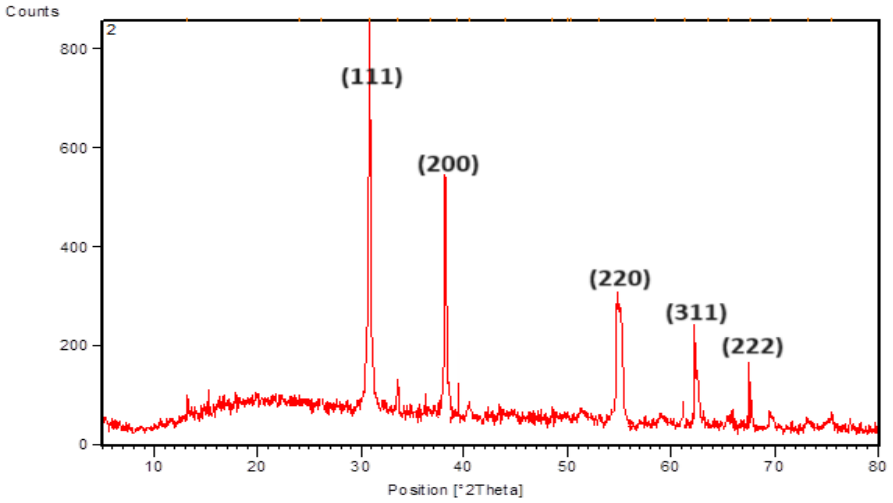

Fig.1: X-ray diffraction pattern of synthesized CdO NPs

3.2.UV-Visspectralstudy

The UV-Vis spectrum of CdO NPs was analyzed .Fig. 2(a) displays the UV-Vis spectrum of CdONPsin250nm-600nm.The absorption of CdO NPs was observed to be at 430 nm. In order tocalculate the band gap energy of as-prepared CdO NPs, the Tauc plot wasplotted and presented in Fig. 2(b), was found to be 2.58 eV which is consistentwiththereportedliterature(Thovhogi*et al.* 2016).

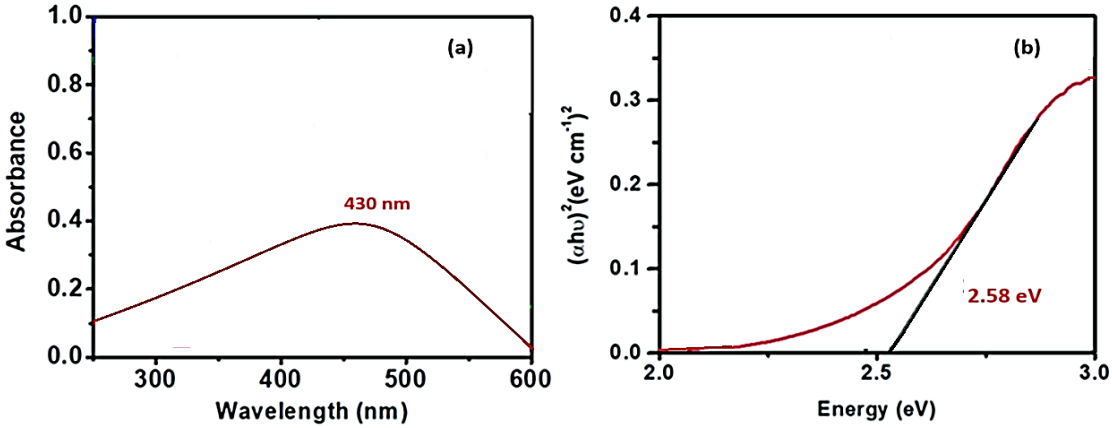

Fig.2(a)UV-Visspectrum,and(b)CorrespondingTaucplotofbiogenicallyfabricatedCdONPs.

2.6. DNAcleavageactivity

A gel electrophoresis technique was performed for the DNA damageability.The reason for the CdO NPs' superior cleavage performance above the control is their effective DNA cleavage capacity.The electrophoresis experiment amply demonstrated the action ofCdONPsonplasmidDNAmolecules.Fig. 3depictsthefindings.ComparedtocontrolDNA,thebandsofLanes2-5differ,asseeninFig. 3. In Lanes 2-4, Plasmid pBR322 was converted from Form I toFormII.CdONPsactedaschemicalnucleases,cleavingDNAFormIintoFormIII.ItmaybeinferredthatCdO NPsinhibitthegrowthofpathogenic organismsandcancer cellsbycleaving thegenome(Gulbagca*et al.*, 2021, Jadhav *et al.* 2018).

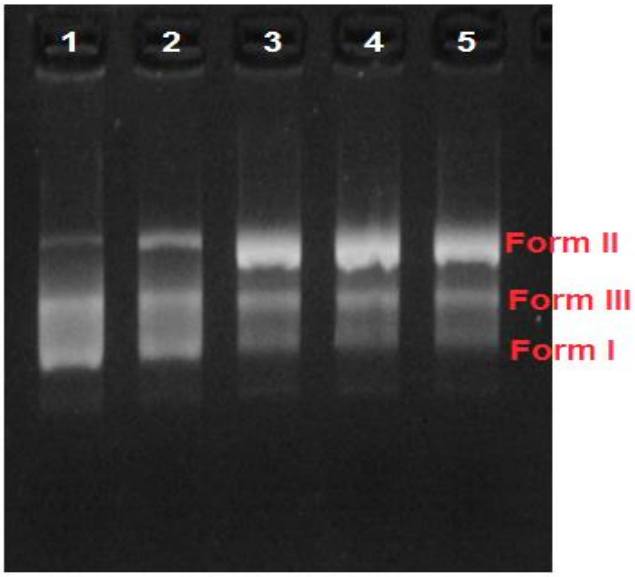

Fig.3: DNA damage study of biosynthesized CdO NPs, Lane 1- DNA (control);Lane 2- DNA + H_2O_2 (10 mM); Lane 3- DNA + H_2O_2+CdO NPs (1 µL); Lane 4- DNA+H_2O_2+CdONPs(2µL);Lane5-DNA+H_2O_2+CdONPs(3µL).

2.7. SEManalysis

SEM analysis was used to carry out the morphological investigation, and the outcome is shown in Fig. 4. The image illustrates that, despite the majority of the particles having aggregated, it is still possible to distinguish distinct boundaries between the individual grains of the particles. Moreover, few particles exhibit spherical forms, Even so, given that the majority have irregular morphology.

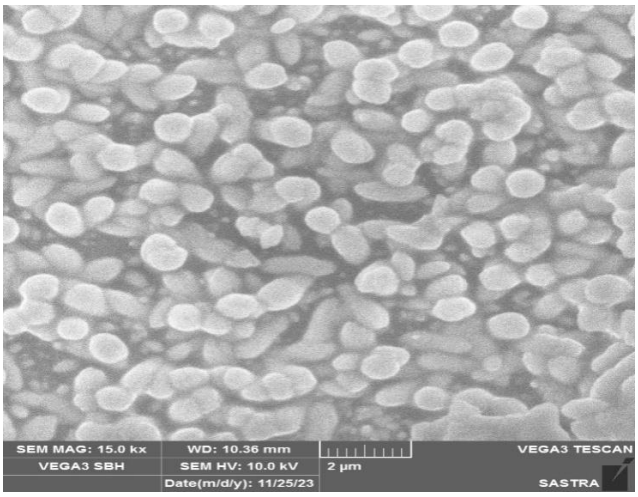

Fig. 4: SEM image of CdO nanoparticles

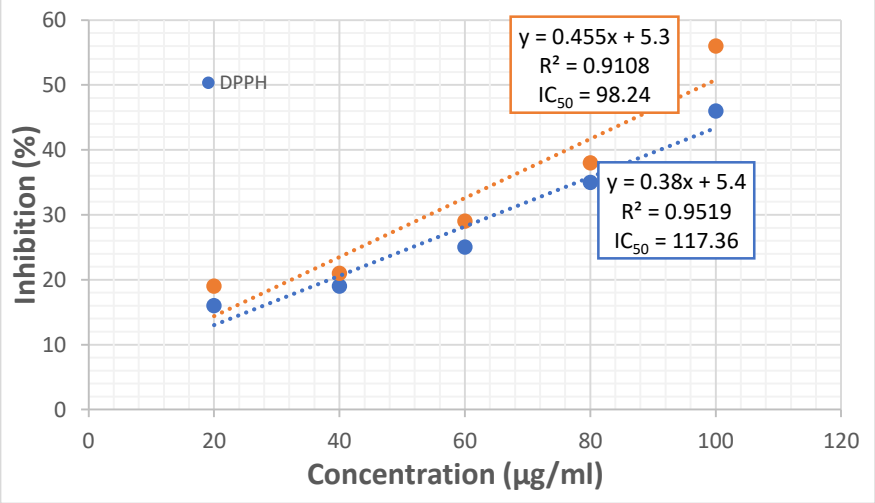

Fig.5: DPPH radical scavenging activity of CdO NPs compared with ascorbic acid

2.8. Antioxidant assay

Using the DPPH scavenging assay, the antioxidant properties of CdO NPs were compared to that of ascorbic acid (Fig. 5).Accordingtocalculations, theIC_{50}valuesforCdONPs'abilitiestoscavenge DPPH and Ascorbic acid are 117.36 and 98.24µg/mL, respectively. ThemaximalscavenginginhibitionfortheDPPHassayforCdONPs was 46.21%, and for Ascorbic acid 56.7 % . Due to the presence of bioactive components,green-producedNPshavestrongantioxidantcapabilities(Pagar *et al*. 2023).

3.5.Antibacterial activity

Synthesized CdO NPs displayed excellent antibacterial efficacy against *B. subtilis* and*E.coli*(Fig.6, Table1). Generally speaking, the mechanism of NPs—such as the production of ROS and the release of heavy metals—determines their antibacterial capability. (Dabhane*et al.* 2023, Aswini *et al.* 2021, Karthik et al. 2018).

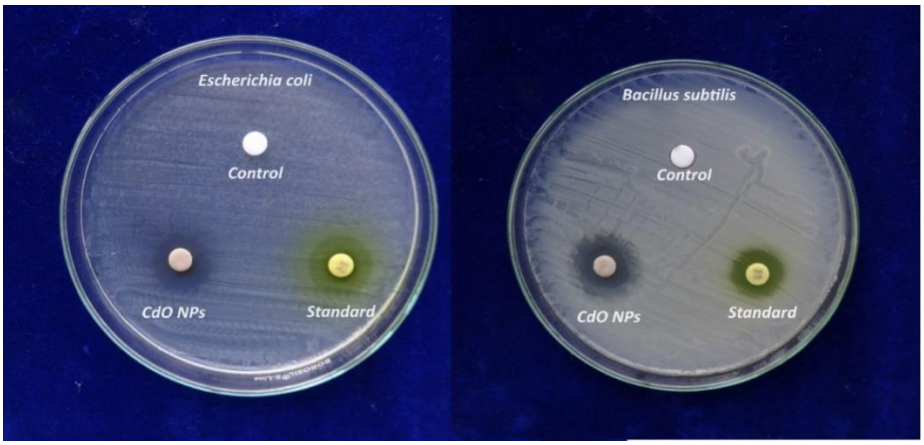

Fig.6: Antibacterial activity of CdO NPs against *E.coli* and *B.subtilis*

Table 1: Assay of antibacterial activity

S.No.	Bacteria Name	Zone of Inhibition (mm in diameter)		
		Control	Standard*	Sample
1	*Escherichia coli*	–	19	16
2	*Bacillus subtilis*	–	15	17

* Nitrofurantation (300 mcg)

3. CONCLUSION

Citrus reticulata peel extract has been shown in this work to be a potential source for CdO NP production in situations that are low time consuming, economically feasible, and environmentally benign. The spherical form of the CdO NPs was confirmed by the SEM investigation. When used against *E. coli* and *B. subtilis*, the CdO NPs demonstrated exceptional antibacterial activity. Furthermore, CdO NPs have a strong antioXidant capability against the scavenging of DPPH radicals. The ability of CdO NPs to damage DNA showed significant promise, and it may be a good idea to leverage the existing process to create other metal oxide NPs with appropriate size and structure. The results of this study collectively imply that CdO NPs can be further studied for therapeutic purposes due to their potent antibacterial, antioxidant, and DNA-damaging characteristics.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest

REFERENCES

1. Aswini, R., Murugesan, S. and Kannan, K., Bio-engineeredTiO_2nanoparticlesusingLedebouriarevolutaextract:larvicidal,histopathological,antibacterialandanticanceractivity,*Int.J.Environ.Anal.Chem.*, 101(15), 2926-2936, (2021). DOI: 10.1080/03067319.2020.1718668
2. Barwant, M., Ugale, Y., Ghotekar, S. Basnet, P., Nguyun, V-H. and Pansambal, S. . Eco-friendly synthesis and characterizations of Ag/AgO/Ag_2O nanoparticles using leaf extracts of *Solanum elaeagnifolium* for antioxidant, anticancer, and DNA cleavage activities. *Chem. Pap.,* **76**, 4309-4321 (2022). https://doi.org/10.1007/s11696-022-02178-0
3. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M., Antibiotic susceptibility testing by a

standardized single disk method, *Am. J. Clin. Pathol.*, 45(4),493-6 (1966).

4. Bhardwaj, B., Singh, P., Kumar, A., Kumar, S. and Budhwar, V., Eco-Friendly Greener Synthesis of Nanoparticles. *Adv. Pharm. Bull.*, 10(4), 566-576 (2020). doi: 10.34172/apb.2020.067.
5. Dabhane, H., Ghotekar, S., Zate, M., Lin, K. A., Rahdar, A., Ravindran, B., Bahiram, D., Ingale, C., Khairnar, B., Sali, D., Kute, S., Jadhav G. and Medhane V. ANovelApproachtowardtheBio-InspiredSynthesisofCuONanoparticlesforPhenolDegradationandAntimicrobialApplications,*BiomassConver. Biorefinery.*,1-16, (2023). Doi:10.1007/s13399-023-03954-y
6. Gulbagca, F., Aygün, A., Gülcan, M., Ozdemir, S., Gonca, S. and Şen, F.Green synthesisofpalladium nanoparticles:preparation,characterization,andinvestigationofantioxidant,antimicrobial,anticancer,andDNAcleavageactivities,*Appl.Organomet.Chem.*,35(8),e6272, (2021). https://doi.org/10.1002/aoc.6272
7. Jadhav, M.S., Kulkarni,S., Raikar, P., Barretto, D.A., Vootla, S.K. and Raikar, U.S., Green biosynthesis of CuO & Ag-CuO nanoparticles fromMalus domestica leaf extract and evaluation of antibacterial, antioxidant andDNAcleavageactivities,*NewJ.Chem.*,42(1), 204-213 (2018). http://dx.doi.org/10.1039/C7NJ02977B
8. Karthik,K., Dhanuskodi, S., Gobinath, C., Prabukumar, S. and Sivaramakrishnan, S., NanostructuredCdO-NiOcompositeformultifunctionalapplications,*J.Phys.Chem.Solid.*, 112, 106-118, (2018). https://doi.org/10.1016/j.jpcs.2017.09.016
9. Pagar, K., Chavan, K., Kasav, S., Basnet, P., Rahdar, A., Kataria, N. Bio-inspiredsynthesisofCdOnanoparticlesusing*Citruslimetta*peelextractandtheirdiversebiomedicalapplications, *J.DrugDeliv.Sci.Tech.*, 82, 104373, (2023). https://doi.org/10.1016/j.jddst.2023.10437322222.....22
10. Paramasivam, G., Palem, V.V., Sundaram, T., Sundaram, V., Kishore, S.C. and Bellucci, S., Nanomaterials: Synthesis and Applications in Theranostics. *Nanomaterials (Basel),* 11(12), 3228 (2021). doi: 10.3390/nano11123228.
11. Saini, R.K., Ranjit, A., Sharma, K., Prasad, P., Shang, X., Gowda, K.G.M. and Keum, Y.S., Bioactive Compounds of Citrus Fruits: A Review of Composition and Health Benefits of Carotenoids, Flavonoids, Limonoids, and Terpenes. *Antioxidants (Basel).*, 11(2), 239, (2022). doi: 10.3390/antiox11020239.
12. Skheel, A.Z., Jaduaa, M.H. and Abd, A.N., Green synthesis of cadmium oxide nanoparticles for biomedical applications (antibacterial, and anticancer activities), *Mat. Today: Proceedings*, 45 (6), 5793-5799 (2021). https://doi.org/10.1016/j.matpr.2021.03.168
13. Somasundaram,G., Rajan, J., Sangaiya, P. and Dilip, R., Hydrothermal synthesis of CdO nanoparticles for photocatalytic and antimicrobial activities, *Results Mat.*, 4, 100044, (2019). https://doi.org/10.1016/j.rinma.2019.100044
14. Sundrarajan, M., Ambika, S. and Bharathi, K., Plant-extract mediated synthesis of ZnO nanoparticles using Pongamia pinnata and their activity against pathogenic bacteria, *Adv. Powder Tech.*, 26 (5), 1294-1299 (2015). https://doi.org/10.1016/j.apt.2015.07.001
15. Thovhogi, N., Park, E., Manikandan, E., Maaza,M. and Gurib-Fakim, A., Physical properties of CdO nanoparticles synthesized by green chemistry via Hibiscus Sabdariffa flower extract, *J.Alloys Comp.,* 655, 314-320, (2016) . https://doi.org/10.1016/j.jallcom.2015.09.063