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Research Article

Aegle Marmelos Bark Mediated Synthesis of CdS nanoparticles for Potent Applications in Pharmacological Studies

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Abstract
The antibacterial and antifungal activities of biosynthesized cadmium sulfide nanoparticles (CdS NPs) were evaluated against several microorganisms, including *Salmonella typhimurium*, *Shigella flexneri*, *Clostridium perfringens*, *Enterococcus faecalis*, *Aspergillus flavus*, and *Candida albicans*. The CdS NPs demonstrated significant antimicrobial effects, with a maximum zone of inhibition of 18 mm at 150 µg/mL against E. faecalis and inhibitory zones of 11 mm and 14 mm against *A.flavus* and *C.albicans*, respectively, at 400 µg/mL. The antimicrobial mechanism of CdS NPs is hypothesized to involve ionic interactions that disrupt microbial DNA and induce oxidative stress, ultimately leading to cell lysis. Furthermore, CdS NPs showed anticancer activity against MCF-7 breast cancer cells, achieving an IC_{50} value of 52.34 µg/mL, likely through interference with cellular redox status and mitochondrial Ca^{2+} signaling pathways. These results suggest that CdS NPs have promising therapeutic potential as antimicrobial and anticancer agents.

Keywords: Cds NPs; green synthesis; anticancer activity; antibacterial and antifungal activities.

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1. Introduction

A disease is a functional abnormality in the body caused by biological agents or toxic foreign particles. Diseases are generally categorized as short-term or chronic (long-term) and are further classified as communicable or non-communicable, including infectious, hereditary, physiological, maternal, neonatal, and nutritional diseases [1-2]. Despite the prevalence of pathogens, human immunity often prevents infections; however, when pathogens bypass this immunity, therapeutics such as drugs, vaccines, and antibiotics are required to support the immune system. These therapeutics inhibit foreign agents, enhance immunity, and include chemical, biological, and physical interventions like exercise, which promotes self-healing [3-6].

Nanotechnology has revolutionized therapeutic delivery, particularly for chronic diseases like cancer [7]. Nanoparticles (NPs) enable targeted drug delivery, enhancing efficacy by crossing biological barriers and promoting drug accumulation in tumor tissues through enhanced permeability and retention (EPR) effects [8]. Cadmium sulfide nanoparticles (CdS NPs), for example, show promising biomedical applications due to their optical properties, antimicrobial, anticancer capabilities, and compatibility with biological systems. Biogenic

synthesis of CdS NPs is particularly advantageous due to its eco-friendliness and biocompatibility. These NPs have potential in imaging, drug delivery, and diagnostics, underlining their valuable role in modern medicine [9-10].

In recent years, green synthesis approaches using plant extracts have emerged as a sustainable and eco-friendly alternative for synthesizing metal and metal oxide NPs. These methods utilize the bioactive compounds present in plant extracts as reducing and capping agents, resulting in NPs with enhanced biocompatibility and reduced toxicity [9, 11].

A.marmelos has a long history of use in traditional medicine, particularly for its radioprotective, antidiabetic, and anticancer properties. Various parts of the plant, including the fruit, leaves, and bark, are utilized for their medicinal benefits. These applications range from managing common ailments like asthma and diarrhea to more serious conditions such as fractures and anemia. The extensive medicinal potential of *A.marmelos* has been well-documented in previous studies [12-13]. This is due to *A.marmelos* is reported to contain chemical composition like alkaloids (aegeline, fragrine, aegelenine), coumarins (Marmin, Marmelide, Psoralen, Imperatonin), and terpenoids (cineol, Caryophyllene), etc [14].

This study explores the potential of *Aegle marmelos* bark extract as a green and efficient reducing and capping agent for the synthesis of CdS NPs. The desirable CdS NPs will be characterized using various techniques to investigate their structural, morphological, and optical properties. To extend, the antimicrobial and anticancer activities of the synthesized CdS NPs were evaluated to assess their potential for biomedical applications.

2. Experimental

2.1 Chemicals and Reagents

All chemicals classified as analytical grade, such as sodium sulfide, methanol, and cadmium chloride, were obtained from Merck company, India and utilized without additional purification. We used Milli-Q water for all of the experiments.

***Aegle marmelos* bark (AMB)** was collected and repeatedly cleaned with distilled water to get rid of any dust and debris. They were then grinded into a fine powder, dried under shade, and kept in an airtight glass containerand stored. One gram of powder was added to a 250 mL Erlen Meyer flask along with 100 mL of Milli-Q water, and the mixture was heated to 60° for 25 minutes to create 1% aqueous leaf, bark, or peel extract. Next, Whatman paper was used to filter the extract after it had cooled. The filtrate was used in a subsequent procedure after being refrigerated at 4°C.

2.2. Preparation of CdS nanoparticles

To a 50 mL solution of 0.1 M cadmium chloride, 5 mL of plant extract (leaf, bark, or peel) was added. While stirring magnetically, 50 mL of 0.1 M sodium sulfide dissolved in deionized water was gradually introduced to the cadmium chloride mixture. The combined solution was then transferred to a rotary orbital shaker and agitated at 200 rpm and 30 ºC for 12 hours in darkness. Further, an aliquot (3 mL) was sampled to monitor particle formation using UV-Vis spectrophotometer. The optical density was measured from 250 to 700 nm to identify the absorption maximum.

2.3. Characterization techniques

The crystallite sizes and phase structures of the prepared materials were investigated using the RIGAKU powder X-ray diffractometer with Cu-Kα radiation and applied current, accelerating voltage, and wavelength maintained at 30 kV, 40 mA, and 1.54, respectively. Fourier-Transformation Infrared Spectrometer (Bruker ALPHA-E) with a resolution of 4 cm^{-1} was used to investigate the functional groups present in prepared materials.

2.4 Antimicrobial assay

Nutrient agar medium (High-Media) was dissolved in water in a 100 mL conical flask and autoclaved at 121ºC, 15lbs for 15 minutes before being poured into sterilized petri plates. As a positive control for antibacterial activity, chloramphenicol was used. Agar well diffusion was used to test the antibacterial activity of plant extract. With a sterile glass spreader, inoculum was spread across the surface of agar plates. Using a sterile cork borer, four wells were made at equal distances. The plant extract was made to a final concentration of 100mg/mL to test its antibacterial activity. Aliquots of the compound (80µg/mL, 100µg/mL, and 150µg/mL) were poured into each well, and the plates were incubated for 24 hours at 37ºC in an incubator.The diameter (mm) of the clear inhibitory zone that formed around the well was also measured.(Willey, 2008)

2.5 Cytotoxic Effects

The NRU Assay was used to determine the cytotoxicity of the provided samples on the MCF-7 cell line. At 37 °C with 5% CO_2, the cells (5000-8000 cells/well) were cultured in 96 well plates for 24 hours in DMEM medium (AT149-1L) supplemented with 10% FBS (HIMEDIA-RM 10432) and 1% antibiotic solution. The medium was removed the next day, and fresh culture medium was added to each well of the plate. Treatment dilutions (of various concentrations) were added to the defined wells, and the plates were incubated for 24 hours. 100 µl of NRU (40 µg/ml in PBS) was added to the wells and incubated for 1 hour (Heal Force-Smart cell CO_2 Incubator-Hf-90). After removing the medium, NRU was dissolved in 100 µl of NRU Destain solution. Finally, the plates were read at 550/660 nm.(John K. Buolamwini)

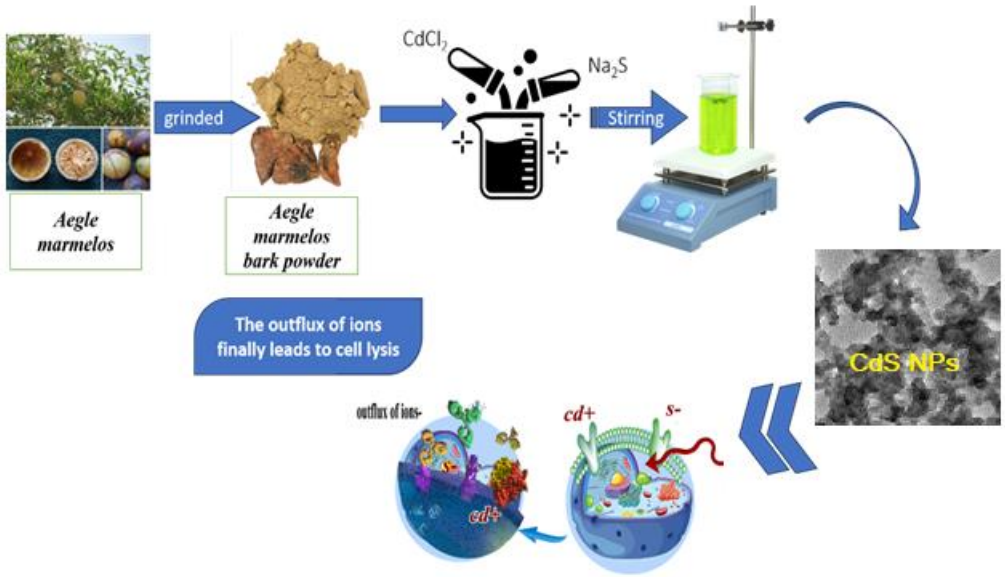

Figure 1: Graphical representation of antimicrobial activity over pathogens

3 Results and Discussion

3.1. Characterization analysis

The crystalline structure and phase of plant extract-coated CdS nanoparticles (CdS NPs) were analyzed using XRD, as shown in Fig.2. Diffraction peaks at 2θ values of 26.8°, 43.8°, and 51.8° corresponded to the (111), (220), and (311) planes, respectively, indicating a cubic phase consistent with standard JCPDS File No. 42-1411, confirming successful coating of CdS NPs on the plant extracts [15]. Using the Debye-Scherrer formula ($k\lambda/\beta\cos\theta$) [16], the average crystallite size of CdS NPs was estimated to be approximately 3.7 nm. As shown in Fig.3, the FTIR spectrum suggests the presence of several functional groups, including O-H, C=O, and C=C corresponds to the 3706, 1562 and 1503 cm^{-1} respectively.

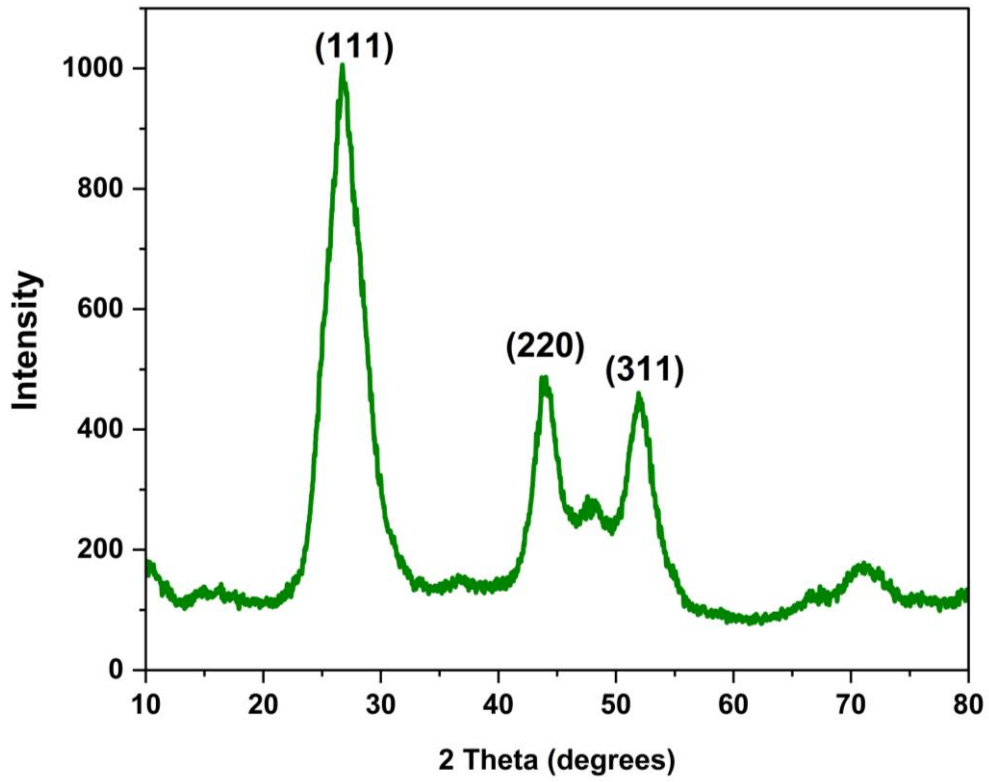

Fig.2: XRD patterns of prepared CdS NPs using *A.marmelos* bark extract

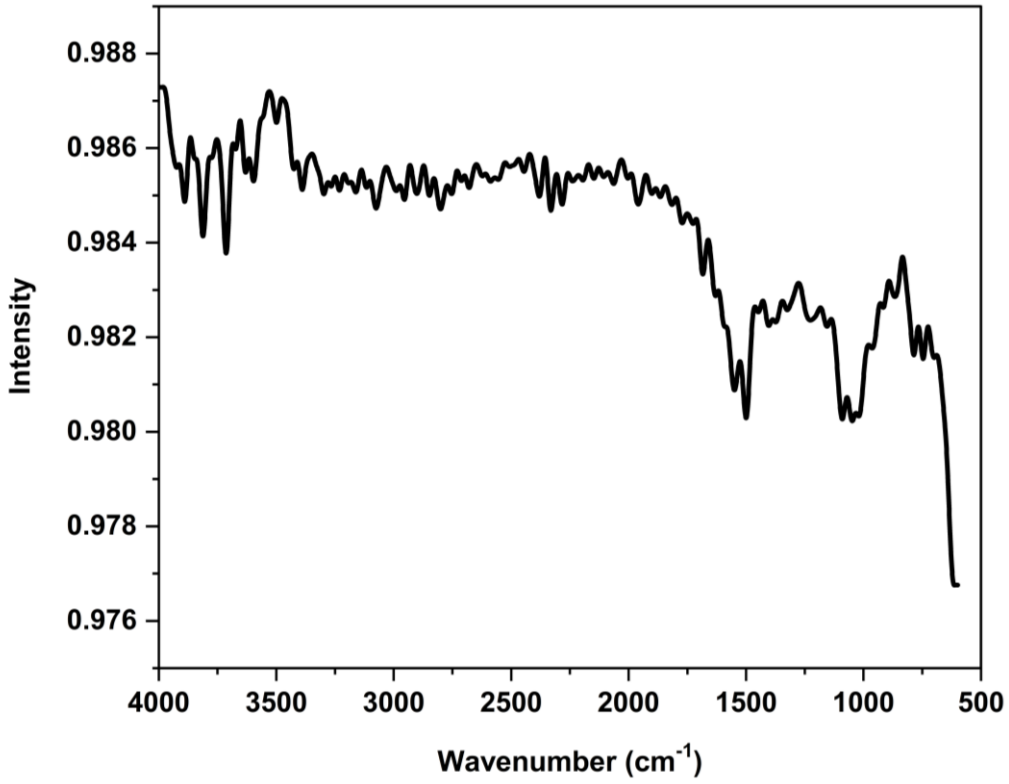

Fig.3: FTIR spectrum of CdS NPs using *A.marmelos* bark extract

3.2. Pharmacological activities

The anti-bacterial activity of biosynthesized CdS NPs was performed against the different types of microorganisms including *S.typhimurium, S.flexneri, C.perfringens, and E.faecalis*. The zone of inhibition appeared on the test microorganism which is somewhere parallel to the Control (Fig.4). At 150µg/ml the CdS NPs shows the highest zone of inhibition with a diameter of 18mm for *E.faecalis* as they can be used as therapeutic agents for bacterial diseases.

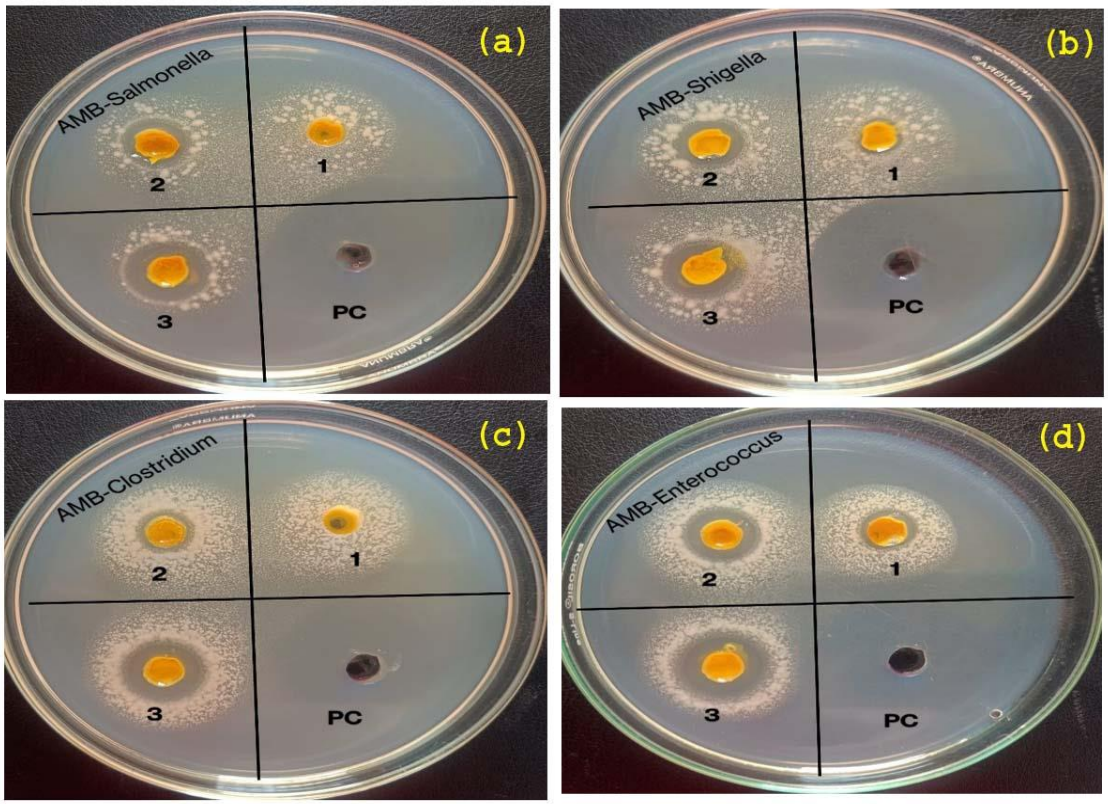

Fig.4: Antibacterial activity of AMB mediated CdS NPs over (a) *S.typhimurium,* (b) *S.flexneri,* (c) *C.perfringens,* and (d) *E.faecalis*

Table 1: Antibacterial activity of the AMB Plant Extract

S.No.	Test Organism	Zone of inhibition (mm) AMB extract (µg/mL)			
		80	100	150	Standard 30µg/mL
1	*Salmonella typhimurium*	-	12	14	22
2	*Shigella flexneri*	8	13	15	22
3	*Clostridium perfringens*	-	10	14	20
4	*Enterococcus faecalis*	10	15	18	21

The CdS NPs are examined for the antifungal activity against the *A.flavus and C.albicans*, as the CdS NPs shows maximum inhibitory zone with a diameter of 11mm & 14mm (Figure 5) at the concentration of 400 µg/ml. The CdS NPs creates internal stress in the hyphae of the fungal species which can cause the degradation of the fungal cells. Hence, AMB mediated CdS NPs were used to treat the fungal infections caused by species of *A.flavus*and *C.albicans*.

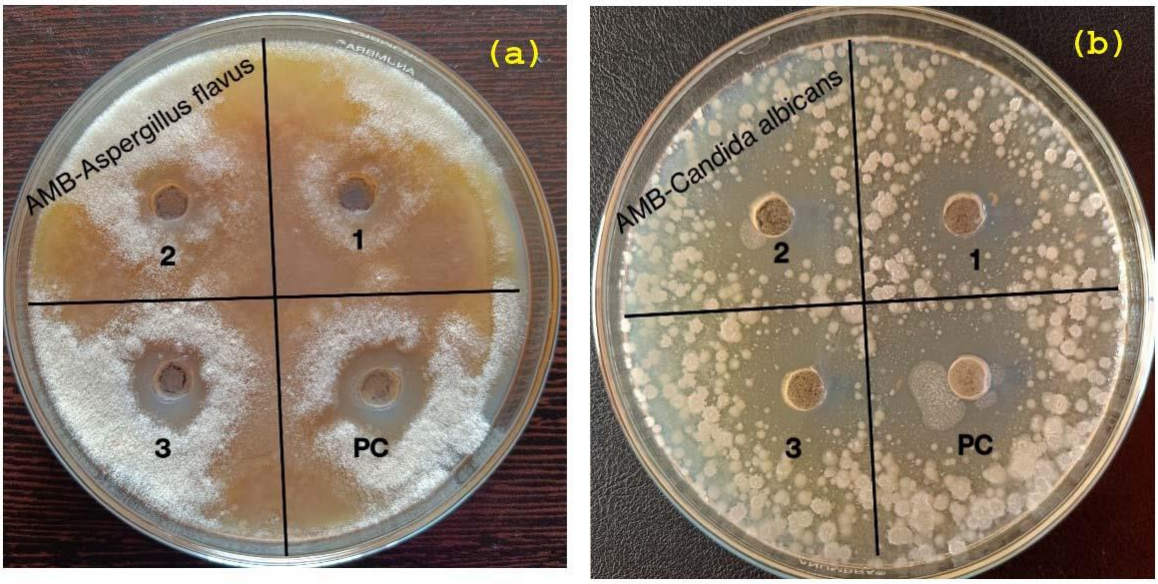

Figure 5: Antifungal activity of CdS NPs with (a)*A.flavus* and *(b) C.albicans*

Table 2: Showing the zone of inhibition of CdS NPs with fungal species

Test Organism	Zone of inhibition (mm) AMB (µg/mL)			
	200	300	400	Standard 50µg/mL
A. flavus	**8**	**10**	**11**	**14**
C.albicans	**12**	**13**	**14**	**16**

Plausible mechanism

The mechanism on antimicrobial activity of NPs not yet reported. Based on previous reports, we have drawn the attention on inactivation of pathogens using CdS NPs. As the CdS NPs are biomedically stable and also more ionic in nature hence it creates ionic flow with the bacterial cytoplasm which results in influx of ions (Cd and S) and these ions particularly the S^{2-}, because of its electronegativity nature it mimics the function of DNA and eventually disturb the DNA function and the Cd^{2+} drastically create ionic transfer in the cytoplasm which finally leads to oxidative stress followed by cell lysis as shown in Figure.1 (Alireza Ghasempour, 2023).

The synthesized CdS NPs using AMB extract were examined for the anticancer activity with the MCF-7 cell line which the major and most common type of cancer seen in most of the women and also in men. But men are being less effected compared to women. The biosynthesized CdS NPs were examined for anticancer activity with MCF-7 cell line by using NRU Assay as the cells are harvested for a period of 24 hrs and after incubation period the highest zone of inhibition was seen at 52.34µg/mL which can be taken as IC_{50} value as shown in Fig.6. As the CdS NPs alter the cellular redox status by reacting with exogenous and endogenous antioxidants such as GSH (glutathione) and also damage the Mitochondria by interfering the Ca^{2+}signalling.

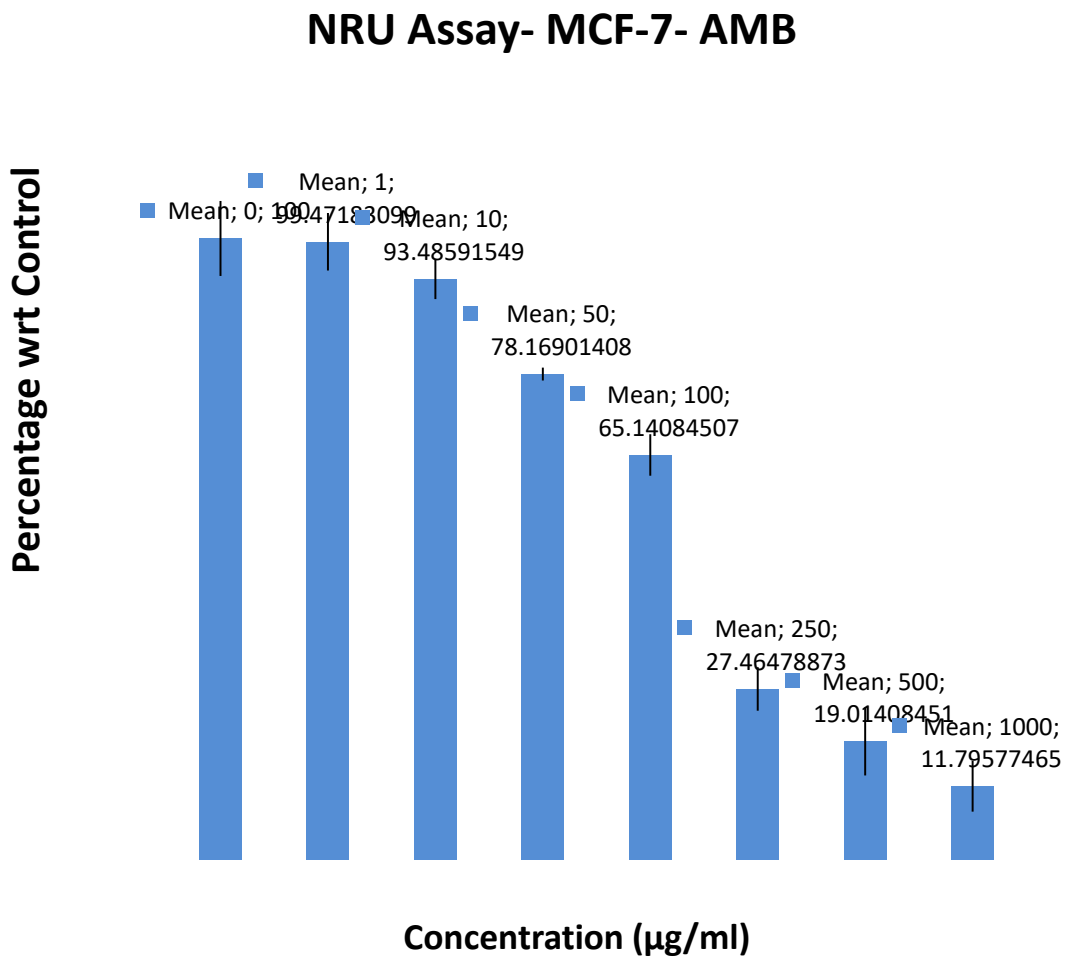

Figure 6: Cytotoxic report of synthesised CdS NPs against MCF-7 Cell line

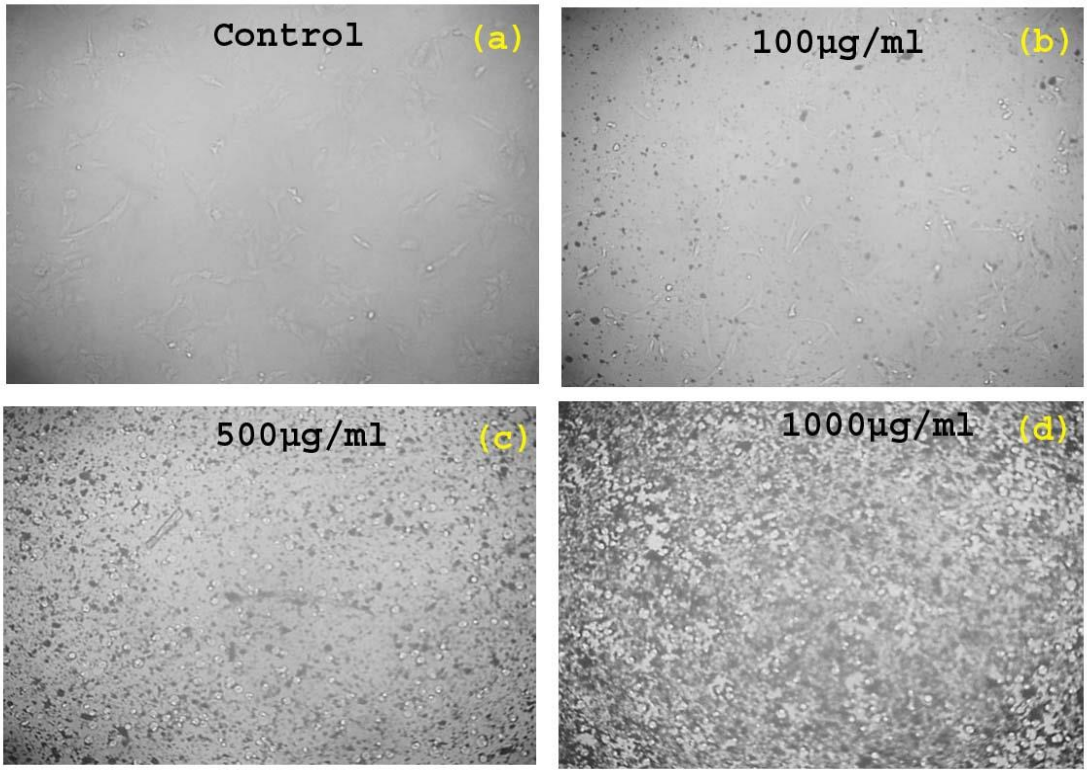

Figure 7: Cytotoxicity of CdS NPs over MCF-7 Cell lines

Conclusion

The biosynthesized CdS nanoparticles demonstrated potent antibacterial and antifungal activities against multiple microbial strains, showing maximum inhibition in *E.faecalis*, *A.flavus*, and *C.albicans*. These nanoparticles disrupt microbial cell structure by interfering with DNA functions and inducing oxidative stress, a mechanism supported by previous findings. Additionally, CdS NPs exhibited substantial anticancer effects on MCF-7 breast cancer cells, likely by altering redox states and impairing mitochondrial function. Thus, the AMB-mediated CdS NPs could serve as effective therapeutic agents against bacterial, fungal, and cancerous cells, supporting their biomedical applicability in treating infections and malignancies.

References

[1] Saravanan, A.; Kumar, P.S.; Karishma, S.; Vo, D.V.N.; Jeevanantham, S.; Yaashikaa, P.R.; George, C.S. A Review on Biosynthesis of Metal Nanoparticles and Its Environmental Applications. Chemosphere 2021, 264, 128580.

[2] Botsa, S.M., Basavaiah, K. Fabrication of multifunctional TANI/Cu_2O/Ag nanocomposite for environmental abatement. Sci. Rep., 2020, 10, 14080.

[3] Ma, N., Cai, R., & Sun, C. Threonine dehydratase enhances bacterial cadmium resistance via driving cysteine desulfuration and biomineralization of cadmium sulfide nanocrystals. Journal of Hazardous Materials, 2021, 126102.

[4] X. Gao, Y. Cui, R.M. Levenson, L.W. Chung, S. Nie In vivo cancer targeting and imaging with semiconductor quantum dots Nat. Biotechnol., 2004, 22, 969-976.

[5] B.A. Rzigalinski, J.S. Strobl Cadmium-containing nanoparticles: perspectives on pharmacology & toxicology of quantum dots Toxicol. Appl. Pharmacol., 2009, 238, 280-288.

[6] Peana, M.; Pelucelli, A.; Chasapis, C.T.; Perlepes, S.P.; Bekiari, V.; Medici, S.; Zoroddu, M.A. Biological Effects of Human Exposure to Environmental Cadmium. Biomolecules 2023, 13, 36.

[7] Kumar Suranjit Prasad, Tapan Amin, Saloni Katuva, Madhu Kumari, Kaliaperumal Selvaraj. Synthesis of water soluble CdS nanoparticles and study of their DNA damage activity. Arabian Journal of Chemistry, 2017, 10, S3929-S3935.

[8] B. R. Bhattarai, A. Regmi and S. K. Gautam, "Cadmium Sulfide Nanoparticles: Synthesis and Characterization," 2019 International Conference on Computer, Communication, Chemical, Materials and Electronic Engineering (IC4ME2), Rajshahi, Bangladesh, 2019, pp. 1-4,

[9] Ghasempour, A.; Dehghan, H.; Ataee, M.; Chen, B.; Zhao, Z.; Sedighi, M.; Guo, X.; Shahbazi, M.-A. Cadmium Sulfide Nanoparticles: Preparation, Characterization, and Biomedical Applications. Molecules 2023, 28, 3857.

[10] K.K. Kommanapalli, P. Lyot, J.R. Sunkara, P. Cheucle, A.V.L.N.S.H. Hari Haran, P. Mulukutla, Synthesis and characterization of maleic acid and sodium methallyl disulfonate new copolymer: application as a barium sulfate scale inhibitor, Journal of Petroleum Exploration and Production Technology, 9(1) (2019) 223-232.

[11] Dehghan, H.; Sedighi, M.; Jafari-Nozad, A.M.; Jafari, S.; Alemzadeh, E.; Farkhondeh, T.; Samarghandian, S. Gold Nanoparticles and Wound Healing in Rodents: A Systematic Study. Curr. Nanosci. 2023, 19, 1–10.

[12] Sanghi SB, Mushtaq S. *Aegle marmelos* a potential medicinal tree: an overview. Int. J. Res. Granthaalayah 5(8), 63–66 (2017).

[13] Monika S, Thirumal M, Kumar PR. Phytochemical and biological review of *Aegle marmelos* Linn. Future Sci OA. 2023, 9(3), FSO849.

[14] Gautam M, Ramanathan M. Ameliorative potential of flavonoids of Aegle marmelos in vincristine-induced neuropathic pain and associated excitotoxicity. Nutr. Neurosci. 24(4), 296–306.

[15] D. Adinarayana, N. Annapurna, B. Sathish Mohan, Paul Douglas. Enhanced photocatalytic removal of Cr(VI) and Rhodamine B from water using plant-mediated CdS nanoparticles: Mechanistic insights and environmental applications. Desalination and Water Treatment, 2024, 320, 100593.

[16] K.K. Kommanapalli, P. Lyot, J.R. Sunkara, P. Checule, Synthesis and Performance Evaluation of Linear Polycarboxylate Dispersant of Glacial Acrylic Acid-Maleic Acid-Sodium Methallyl Disulfonate for Ceramics, Journal of the Korean Ceramic Society, 55(2) (2018) 126- 134.