Chapter 5

Development of Biogenic Cadmium sulfide nanoparticles using *Daruharidra* and its application as anticancer agent.

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1.1 Background-

The emerging field of nanotechnology has opened up new avenues for biomedical research, particularly in the development of novel therapeutic agents. One promising area within this field is the synthesis of nanoparticles using green and environmentally friendly methods. These nanoparticles, such as DH-CdSNP (cadmium sulfide nanoparticles), hold great potential for various applications, including medicine and healthcare. One such approach involves the biogenic synthesis of nanoparticles, where natural extracts, like those from the *Daruharidra* plant (*Berberis aristata*), are utilized as precursors or stabilizing agents. By harnessing the bioactive compounds present in these extracts, researchers aim to create nanoparticles with enhanced biocompatibility and reduced toxicity, offering a safer alternative to conventionally synthesized nanoparticles.

In the context of cancer therapy, nanoparticles derived from plant extracts have gained significant interest due to their potential anticancer properties. These nanoparticles can be tailored to target specific cancer cells while minimizing damage to healthy tissues, thereby improving treatment efficacy and reducing side effects. Additionally, nanoparticles synthesized through green methods hold promise for combating bacterial infections, offering a potential solution to the growing problem of antibiotic resistance.

The integration of plant extracts with nanoparticles represents a novel approach in cancer therapy, offering a synergistic effect that enhances therapeutic outcomes. By combining the unique properties of plant-derived compounds with the versatility and targeting capabilities of nanoparticles, researchers can develop innovative treatment strategies for various types of cancer.

Overall, the bioinspired synthesis of nanoparticles holds tremendous potential for revolutionizing healthcare and biomedicine. Through continued research and development in this field, scientists aim to unlock the full therapeutic potential of nanoparticles derived from natural sources, paving the way for safer, more effective treatments for cancer and other diseases

1.2 Introduction

Daruharidra-based nanoformulations represent a promising approach in biomedical research, leveraging the medicinal properties of the *Daruharidra* plant (*Berberis aristata*) for therapeutic applications. These nanoformulations involve the incorporation of *Daruharidra* extracts or bioactive compounds into nanoparticles, allowing for targeted delivery and enhanced efficacy. With its rich source of bioactive molecules, including berberine and other alkaloids, *Daruharidra* exhibits various pharmacological activities such as anti-inflammatory, antioxidant, and anticancer effects. By encapsulating *Daruharidra*-derived compounds within nanoparticles, researchers aim to improve their bioavailability, stability, and targeted delivery to specific tissues or cells, offering potential solutions for the treatment of various diseases, including cancer, inflammation, and microbial infections. *Daruharidra*-based nano formulations hold promise for advancing personalized medicine and addressing unmet medical needs through innovative and sustainable therapeutic approaches.

Cadmium sulfide nanoparticles (DH-CdSNP) belong to the II-VI group of semiconductor nanoparticles. These nanoparticles possess unique optoelectronic properties due to quantum confinement, which distinguishes them from the bulk material. The exceptional properties of these low-dimensional semiconductor nanoparticles have attracted significant attention in the field of cancer treatment. [164] Nano-composed therapeutics have emerged as potential candidates for treating various diseases. These therapies provide accurate and targeted medication administration, reducing adverse effects related to cytotoxic drug systemic

dispersion. They have successfully shown that they can inhibit the formation of tumors and cancer cell growth.[165][166] Conventionally, DH-CdSNP are typically synthesized through a complex and costly chemical process that involves the use of toxic chemicals and multiple downstream steps. Hence, to enable the manufacturing of DH-CdSNP using non-hazardous materials while keeping their nano size, biocompatibility, low cytotoxicity, and physicochemical qualities, novel methodologies are thus required in this sector. [167] Biogenic synthesis methods utilizing biosurfactants derived from microbes and plants have shown promise in achieving these objectives. Recent research on DH-CdSNP produced through biogenic synthesis using biological stabilizing agents has revealed remarkable quantum confinement effects, high photoemission, and significant antibacterial activity. [168] [169], [170], [171]

This approach offers several advantages, including simplified downstream processing and efficient extraction of the nanoparticles.[167] Nanoparticles derived from plant sources have shown great potential in targeted drug delivery with reduced toxicity. [165] Using the stem of *Berberis aristata (Daruharidra*), silver nanoparticles (AgNPs) (AgNO3) were produced quickly and easily by biologically reducing AgNO3 with the plant's aqueous extract. [172] The phyto-mediated method, which makes use of various plant components and their extracts, is thought to be a novel, straightforward, and less expensive way to create metal nanoparticles. Using leaf extract from the medicinally significant plant Berberis aristata, zinc oxide nanoparticles (ZnO NPs) were biosynthesized. [173]Cadmium sulfide nanoparticles (DH-CdSNP) are familiar in the field of science for their optical activity and find applications in several fields. They can be particularly useful in drug delivery for cancer treatment and bio-imaging. However, the utilization of bioinspired cadmium sulfide nanoparticles as an anticancer drug delivery system remains relatively unexplored. Hence, this research tries to review the most current developments in the use of biogenic cadmium sulfide nanoparticles for

drug delivery in cancer treatment.[174] Since hazardous chemicals are not adapted for the generation of a protocol, using an ecologically friendly substance, such as plant extracts for CdSNPs, offers significant benefits of eco-friendliness and suitability for pharmaceutical and therapeutic practice. Chemical methods may also leave behind some harmful substances, allowing for the absorption of those chemicals into particle surfaces and potentially affecting medical functioning. Green synthesis is more effective than chemical and physical methods since it is less expensive, environmentally harmful, more straightforward to produce, and uses less energy, toxic chemicals, pressure, and temperature.

5.3 Methodology

5.3.1 Materials utilized-

The cell lines used in the study were human ovarian cancer cell line (PA1) and human breast cancer cell line (MDAMB231), acquired from NCCS Pune. The MiliQ water purifying system provided the decontaminated water for the experiment. DMEM-HG was supplemented with 10% Fetal Bovine Serum was used in maintaining cell lines. MP Biomedicals, Germany provided materials such as Dulbecco's Phosphate Buffered Saline 1X (DPBS), 0.25% Trypsin-EDTA solution, MTT reagent, and cell culture-grade Dimethyl Sulfoxide (DMSO). The PI/RNase staining solution was obtained from BD Biosciences, while the Apoptosis Detection Kit- KTA0002 with Annexin V-AbFlourTM 488 was procured from Abbkine, Inc. Chemicals such as CdSO4 (99.99% purity) and Na2S (98% purity) were sourced from SRL.

5.3.2 Preparation of solvent extract

The Daruharidra plant, including its stems and roots, was obtained from a local herb shop in Varanasi, India. To ensure its authenticity, the plant was authenticated by the Raw Materials

Herbarium and Museum in Delhi (RHMD), with an assigned authentication number of NIScPR/RHMD/consult/2022/4385-86. The plant parts were sterilized by washing them multiple times with tap water and then rinsing with MiliQ. Afterward, they were dried in the shade. The powdered plant material was then combined with 100 cc of methanol after being pounded into a powder. For 24 hours, this combination was incubated at 45 °C and 150 rpm. The macerated plant solution was then put through a Whatman number 1 filter paper for filtering. The final methanol extract was kept in a freezer at 4 °C for further use.[148]

5.3.3 Development of biogenic DH-CdSNP

The preparation of DH-CdSNP involved two main steps. In the initial step, 1 mL of 0.025 M $CdSO_4$ solution was mixed into 15 mL of plant extract. The solvent extract was incubated in the dark at room temperature without agitation for 72 hours. Moving on to the next step pre prepared Na2S solution 0.3 mL of 0.025 M was added to the mixture. The resulting solution was incubated for 96 hours at a constant temperature without any change. During this incubation period, DH-CdSNP were formed, indicated by a noticeable change in color to a bright green hue.

After the incubation period, the solution containing the nanoparticles was subjected to centrifugation at 8,000 rpm for 15 minutes, repeated twice. This process helped separate the nanoparticles from any contaminants. The recovered DH-CdSNP were then washed three times with miliQ water to remove any residual impurities. Finally, the pellet containing the nanoparticles was lyophilized to facilitate further characterization studies.[164] To break up the clusters and achieve a more uniform dispersion of nanoparticles for biological assay studies nanoparticles were subjected to ultrasonication (20KHz).

5.3.4 Surface characterization and analysis of DH-CdSNP

The FTIR system for IR imaging was given by Sophisticated Analytical Instrumentation Facility (SAIF), IIT-Bombay. It was particularly the 3000 Hyperion microscope integrated vertex 80 FTIR system model made by Bruker, Germany. The method of infrared (IR) spectroscopy is crucial for determining the functional groups that are present in an organic sample because it gives details on the rotational and vibrational phases of motion in a molecule. In this work, the effects of CdS nanoparticle production on the sample were investigated utilizing FTIR micro-ATR imaging on both the natural extract and lyophilized nanoparticles. For the determination of size distribution, the lyophilized nanoparticle sample was subjected to dynamic light scattering (DLS) using a Malvern Zetasizer Nano-S. The nanoparticles were dispersed in methanol, and the measurement was conducted at room temperature.

The formation of nanoparticles was visually examined by observing the color change, and UV-visible spectrometry was employed to further confirm the formation of DH-CdSNP. The presence of characteristic peaks within specific nanometer ranges in the UV-visible spectrum indicated the formation of DH-CdSNP. The optical density measurements were carried out using a Shimadzu UV-1800 UV-Vis spectrophotometer, covering the wavelength range of 200-800 nm. The surface changes and elemental analysis of the CdS powdered sample were investigated using FEG-SEM (JEOL JSM-7600F). To gain further insights into the structure of the particle systems, small-angle x-ray scattering (SAXS) studies were conducted using the SAXS-Xenocs SAS model Xeuss 2.0. SAXS is an analytical characterization technique used to determine average sizes, clustering, and shapes of particle systems.

High-resolution transmission electron microscopy (HRTEM) was used to examine the exact form, crystalline structure and aggregation if any, in the CdS lyophilized powder sample in greater detail. The HRTEM analysis was performed using a JEOL JEM 2100F instrument. This

technique allowed for a closer examination of the morphology and crystal structure of the DH-CdSNP.[175]

5.3.5 Bactericidal effects and minimum inhibitory concentration of DH-CdSNP

Using the Kirby-Bauer testing or, well-diffusion technique, the antibacterial capability of cadmium sulfide nanoparticles was measured against four bacterial species.: *Escherichia coli* (MTCC NO. 452), *Serratia marcescens* (MTCC NO. 86), *Staphylococcus aureus* (MTCC NO. 96), and *Bacillus subtilis* (MTCC NO. 441). The bacterial cultures were inoculated in nutrient broth (NB) and incubated at 37°C and 180 rpm for 24 hours.

The grown bacterial cultures (50 µl) were spread evenly on freshly prepared NB agar Petri dishes using a sterile glass spreader. After drying for a few minutes, wells with a diameter of 6 mm were created in the agar plates using a sterilized cork borer. These wells served as reservoirs to hold the test compound. In this assay different concentrations of DH-CdSNP (10 and 20 mg/mL) were loaded into the wells, and the plates were incubated overnight. The experiment was conducted in triplicate to ensure reproducibility.

Following the incubation period, the clear zones of antibacterial activity surrounding the wells were measured using a millimetre ruler. This allowed for the quantification of the extent of antibacterial activity exhibited by the DH-CdSNP against the tested bacterial species.

The lowest concentration of a particular antimicrobial drug that can prevent bacterial growth following an overnight incubation is known as the Minimum Inhibitory Concentration (MIC) assay. In this experiment, the McFarland standard was applied to measure the turbidity of bacterial inoculums uniformly.

To have a comprehensive evaluation for the antibacterial property of the nanoparticles, different bacterial cultures were cultured in Mueller Hilton broth, and using a UV-Vis spectrophotometer with a 600 nm wavelength; the optical density was adjusted up to turbidity

comparable to barium sulfate 0.5 McFarland standards. Bacteria were collected by centrifugation (2000 rpm, 10 min) and resuspended in phosphate buffered saline (PBS). The number of bacteria was spectrophotometrically adjusted to $O.D._{600\ nm} = 0.5$ (equivalent to 1.5×10^8 CFUs/mL) and confirmed by culturing 1/10 serial dilutions of the initial suspension. 900 µl of Mueller- Hilton broth was infused with 100 µl of diluted cell suspension at the chosen concentration of 10 and 20mg/ml of the nanoparticles. For the control, broth without DH-CdSNP was used. All the assays were performed in triplicates.

5.3.6 Anticancer potential of DH-CdSNP

5.3.6.1 MTT Assay analysis for cytotoxic activity of DH-CdSNP -

MDAMB-231 and PA1 cell lines were cultured in T-25 flasks. They were collected into a 5 mL centrifuge tube after being trypsinized. Using centrifugation at 300 x g, the cell pellet was extracted. The cell count was adjusted using DMEM-HG medium, yielding a 200 µl suspension with about 10,000 cells. Each well of the 96-well microtiter plate received 200 µl of the cell suspension, which was subsequently incubated for 24 hours at 37 °C with 5% CO_2. After 24 hours, the used media was aspirated. Various ultrasonicated nanoparticle concentrations in volumes of 200µl were added to the respective wells. Following that, the plate was incubated at 37°C with 5% CO_2 for 24 hours. The plate was removed after the incubator was opened. After that, the medium containing drugs was aspirated. 200µ l of media containing 10% MTT reagent were added to each well to reach a final concentration of 0.5 mg/mL. After that, the plate was incubated at 37 °C with 5% CO_2 for three hours. The culture media was spread out without harming the crystals that had previously formed. Subsequently, 100 µl of DMSO (10%)was added to the formazan that had been formed, and the plate was gently shaken in a gyratory shaker. [176] 570 nm and 630 nm wavelengths of the microplate reader were used to measure the absorbance. The amount of test drug required to inhibit cell growth by 50% (IC_{50})

was produced from the dose-response curve for the cell line after eliminating the background and the blank. The percentage growth inhibition was then determined.[177]

5.3.6.2 Assessment of cell cycle by flow cytometry-

Ovarian and breast cancer cell lines were cultured in a 6-well plate, with a density of 3 x 105 cells per 2 ml of culture medium, and incubated in a CO_2 incubator at 37°C for 24 hours.[178] The previously utilized media was taken out after the duration of incubation, and the cells were then rinsed with 1 ml of 1X PBS. After that, the cells were exposed to biogenic DH-CdSNP at their IC_{50} concentration in 2 ml of culture media and cultured for a further twenty-four hours. Cells that were not treated served as the negative control in the study.

For the fixation of the cells, cold 70% ethanol was gradually added while stirring gently to prevent clumping to the cells held on ice. Ethanol fixation ensures that the cells can be pelleted at higher centrifugal speeds compared to unfixed cells. The supernatant containing ethanol was carefully discarded to avoid losing the cell pellet. The pellet was washed twice with PBS.

For cell cycle analysis, 400 µl of PI-RNase solution per million cells was added to the pellet and thoroughly mixed. The cells in T-flasks were then incubated for 15 minutes at ambient temperature. Subsequently, the samples were analyzed using FACS in a PI/RNaseA solution to assess the cell cycle distribution and DNA content.[177]

5.3.6.3 Assessment of cell death by DH-CdSNP

For this study, cells were seeded in a 6-well plate at a density of 3 x 105 cells per 2 ml of culture medium and incubated overnight in a CO_2 incubator at 37°C. The cells were then rinsed using 1 ml of PBS after the used media was removed. The IC_{50} value for DH-CdSNP was applied to the culture cells in 2 ml of the medium used for culture, and the cells were then incubated for around 20 hours. As a negative control, one well was not treated at all.

The medium was extracted from each of the wells into a 5 ml centrifuge tube and then rinsed with 500 µl of PBS following the nanoparticle treatment. After the PBS had been washed away, 200 µl of trypsin-EDTA was then added to the culture cells. The cells were then treated for an additional 5 minutes at 37°C. After harvesting the cells into the centrifuge tubes, the culture medium was put back into the corresponding wells. The tubes were then centrifuged at 300 x g at 25°C for 5 minutes, and the supernatant was discarded. The cells were washed twice with PBS and decanted completely.

At a concentration of 1 x 105 cells/ml, the cells were then again suspended in 1X Binding Buffer. The cell suspension was divided into 100 µl portions and put into a 5 ml culture tube along with 5 µl of AbFlour 488 Annexin V. Following a 15-minute dark incubation period at room temperature, the cells were vortexed. The tubes were then gently stirred after adding 400 µl of 1X Binding Buffer and 2 µl of PI. Flow cytometry analysis was conducted immediately after the addition of PI to assess the results.[179]. The presented results represent the means ± SD of three independent experiments. Statistical comparisons were made between the treated groups and the untreated control, with * indicating a significant difference at a p-value of less than 0.05.

5.4. Results and discussions

5.4.1 Surface characterization of nanoparticles-

FTIR analysis provided insights into the functional group composition of the crude solvent extract of *Daruharidra* (*Berberis aristata*). The analysis revealed the presence of various compounds: halogen compound (600-650 cm-1), nitrogen compound (1500 cm-1), sulfur-containing compound (1360 cm-1), primary aliphatic amines (3300-4400 cm-1), C-O unsaturated ester (1700 cm-1), aromatic compound (2000-1600 cm-1), amines and other aromatic esters (1250-1350 cm-1), indicating an abundance of alkaloids as shown in Figure. 5.1(a).

This extract was further utilized for the synthesis of DH-CdSNP, and corresponding changes in the FTIR peaks were observed, confirming the generation of nanoparticles, as depicted in Figure. 5.1(b). The presence of specific peaks indicated the attachment of cadmium sulfide bonds, such as the presence of aromatic primary amino N-H stretch at 3406.81 cm-1, methyl C-H asymmetric stretch at 2919.19 cm-1 and 2850.38 cm-1, and carboxylic acid at 1590.24 cm-1. Notably, a peak at 562.08 cm-1 was observed, which corresponds to the stretching of the cadmium sulfide bond. The FTIR spectroscopy played a crucial role in identifying the functional groups present in the phytomolecules of the methanolic extract of *Daruharidra* (*Berberis aristata*) and assessing the bioreduction process of the synthesized DH-CdSNP.

In the initial endeavour to ascertain the size of the DH-CdSNP, a Malvern Zetasizer Nano S instrument was employed. The presence of a high concentration of nanoparticles loaded in the cuvette resulted in noticeable clustering of the nanoparticles. The dynamic light scattering (DLS) analysis in Figure 5.2 illustrates a size distribution intensity peak at a diameter of 110 nm. It is important to note that this peak may be attributed to the hydrodynamic intensity resulting from the aggregation of the particles, a characteristic that is further observed in the transmission electron microscopy (TEM) images. The optical properties of DH-CdSNP were examined by UV-Visible spectroscopy, which showed a peak at 430 nm characteristic of cadmium sulfide nanoparticles and attributed to the CdSNP-surface plasmon excitation bands. *The sample's FEGSEM images show that the DH-CdSNP have a spherical or dot-like appearance. Another research that used Chlamydomonas reinhardtii as a stabilizing agent also noted the presence of oxygen in biogenic DH-CdSNP. [180]* High-resolution transmission electron microscopy (HRTEM) was employed to study the crystal structure properties and particle range of the DH-CdSNP. The HRTEM research showed that although the nanoparticles are evenly dispersed, they have a propensity to cluster. The size of CdS particles is between 5 and 10 nm. A single crystal structure with a typical particle size of 3 to 5 nm was also discovered

by HRTEM investigation. With a diameter less than 10 nm, these tiny particles can be referenced to simply as quantum dots (QDs). Various articles have reported different size ranges for QDs, ranging from 5 to 10 nm.

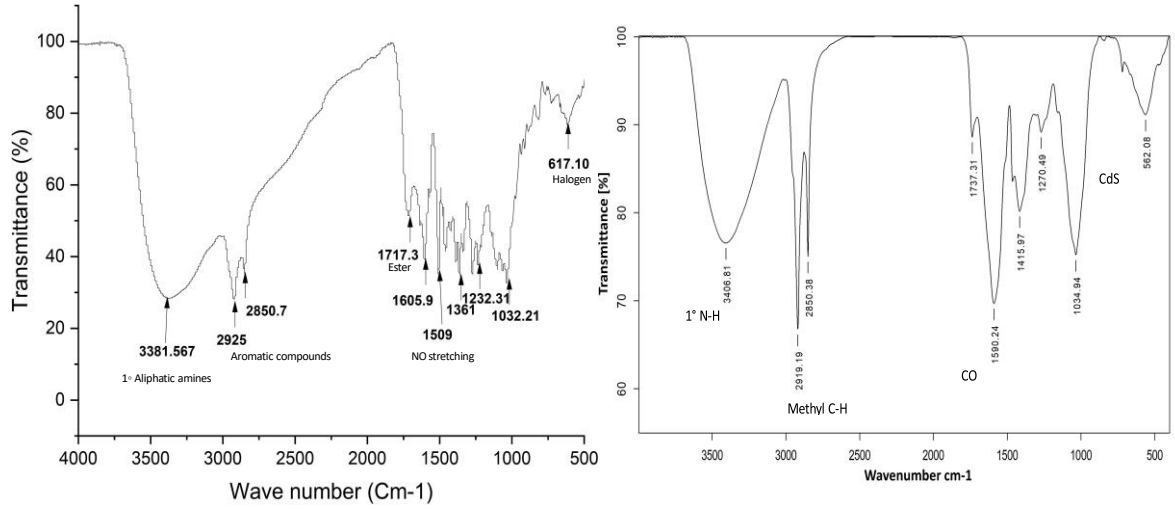

Figure. 5.1. FTIR spectra depicting the observed changes in peaks. (Figure.5.1a) represents the FTIR spectrum of the crude plant-methanol extract, while (Figure. 5.1b) demonstrates the attachment of CdS, as indicated by the peak at 562. These observations provide evidence of the synthesis of DH-CdSNP from the methanolic extract.

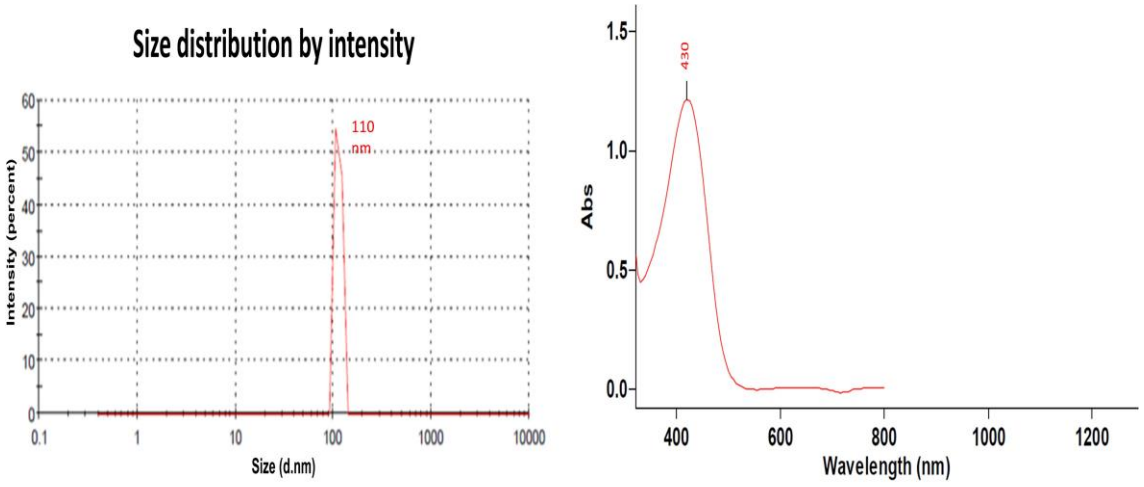

Figure. 5.2. (a) The dynamic light scattering (DLS) analysis of DH-CdSNPs, revealing an intensity peak observed at a diameter of 110 nm. (b) displays a graph of the UV-visible (UV-vis) scan, indicating a peak observed at 430 nm.

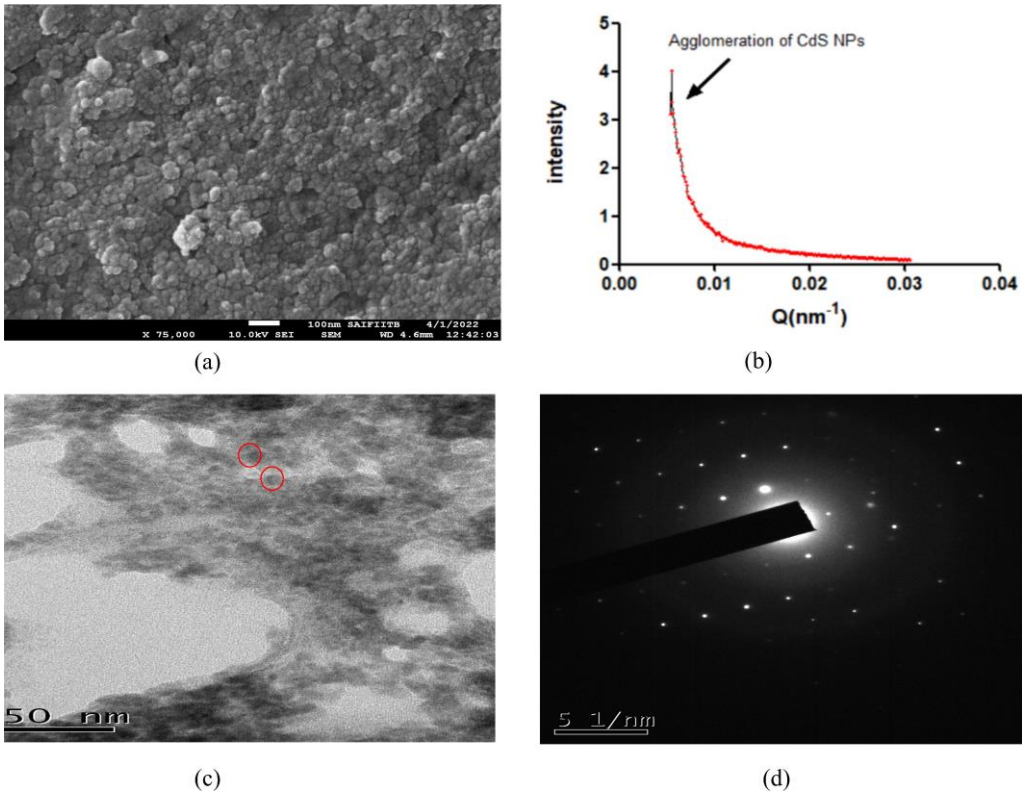

Figure.5.3. Surface characterization of DH-CdSNPs. (a) FEGSEM image showing a cluster of nanoparticles, (b) X-ray scattering observed by SAXS spectrum, (c) HRTEM image at 10 nm scale encircled shows single nanoparticles, and (d) TEM diffraction image scale 5-1nm proving single lattice structure.

According to SAXS curves, the typical size of CdS QDs is around 10 nm. Small Angle X-ray Scattering curves may be modelled using a sphere-like with a form factor with an outer radius of 25 nm in the higher Q area (Q > 0.07 Å-1), where scattering is mostly impacted by the form factor. The lowest CdS size determined by SAXS is the crystal size, which denotes that the nanoparticles made of CdS are polycrystalline or aggregated. The structural characterization is depicted in Figure 5.3.

5.4.2 Bactericidal activity and Minimum inhibitory concentration of DH-CdSNP.

The bactericidal properties of DH-CdSNP were investigated against both Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and Gram-negative bacteria (Escherichia coli and Serratia marcescens). The presence of clear areas around the wells, referred to as zones of inhibition, was observed for both Gram-positive and Gram-negative bacteria. Notably, at higher concentrations of DH-CdSNP (20 mg/mL), a clear zone with a diameter of 27 ± 0.4 mm was observed for S. aureus, while the zone of inhibition for E. coli measured 20 ± 0.1 mm in diameter as seen in Figure 5.4.

These significant findings demonstrate that DH-CdSNP exhibit favourable bactericidal activity against both Gram-positive and Gram-negative bacteria. It can be inferred that the nano-sized CdS particles have an enhanced ability to penetrate the cell walls of these bacteria, leading to evident damage. Reactive oxygen species are produced when the thiol group of a protein reacts with the Cd^{2+} ions of nanoparticles, disrupting the cells. The disruption of active transport, enzymatic activity, and dehydrogenase that occurs when nanoparticles bind to the protein layer prevents the creation of DNA, RNA, and proteins, which leads to cell death.*[181]* [182] DH-CdSNP showed broad-spectrum activity. The MIC obtained above indicated that DH-CdSNP have a bactericidal rather than bacteriostatic effect on bacteria shown in table 5.1 which in represented through histogram in Figure 5.5. According to Ansari et al., the MIC of these nanoparticles towards drug-resistant *S. aureus* was 14.5 ±0.09mg/ml, which is the lowest value obtained amongst bactericidal effects against drug-resistant *S. aureus*. Since DH-CdSNP make extensive contact with the thiol groups in crucial bacterial respiratory enzymes, they impede bacterial cell growth. As DH-CdSNP pass through the membrane, their positive charge interacts electrostatically with the negatively charged proteins upon the bacterial cells' surface, changing their internal structure. ROS are produced when the thiol groups in proteins engage with the

free ions from the NPs. This causes a breakdown of cell structure and resulting loss of cell function. ROS helps in the hyperactivation of cell death and apoptosis.

The finding that biologically stable CdS QDs have the potential for biomedical applications, including tumor therapy specifically by causing caspase-dependent apoptotic cell death, is encouraging.[183][184], [185] ROS are produced when the thiol groups in proteins interact with the liberated ions from the NPs. This disruption of cell structure and subsequent disruption of cell function results.[186]

Table 5.1 Minimum inhibitory concentration MIC dose of DH-CdSNP against gram-positive and gram-negative bacteria.

Microbe	**DH-CdSNP MIC (mg/ml)**
B. subtilis	18±0.08
E. coli	8.3 ± 0.03
S. aureus	14.5 ±0.09
S. masercens	15 ±0.1

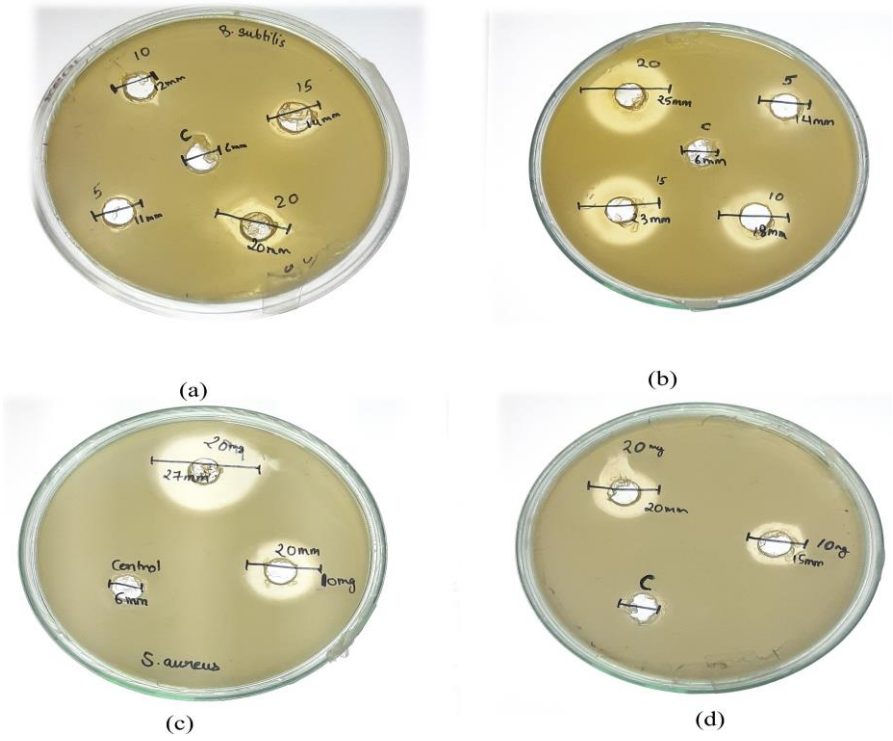

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Figure.5.4. Bactericidal effect of DH-CdSNPs against various bacterial strains: (*a) B. subtilis, (b) E. coli, (c) S. aureus, and (d) S. marcescens*. A separate well containing DMSO was used as a control for comparison.

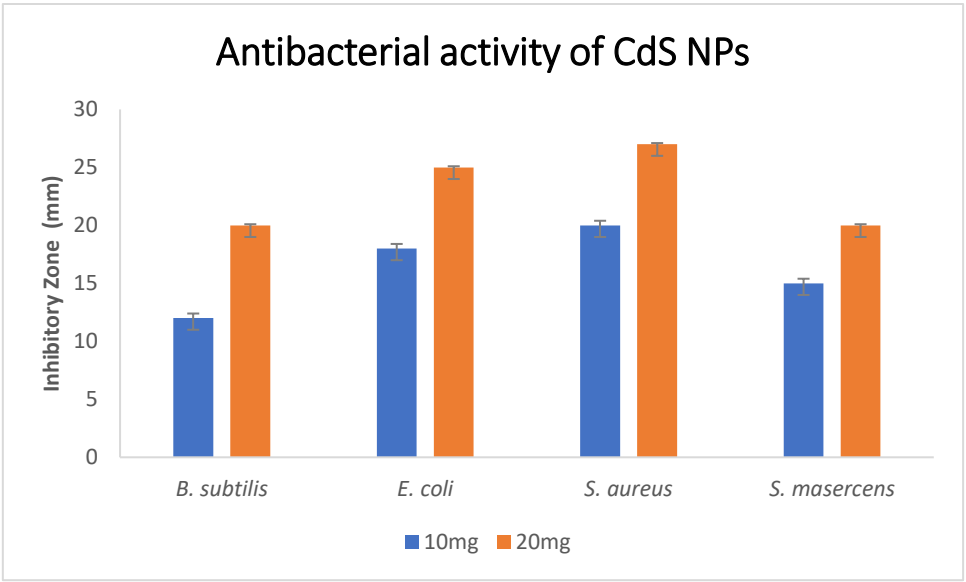

Figure.5.5. Bar chart histogram illustrating the antibacterial effect of the biosynthesized DH-CdSNPs where Inhibitory zones in mm are shown on Y axis. Using DH-CdSNPs at 10 mg/mL and 20mg/mL against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Serratia marcescens.*

5.4.3 MTT assay for testing cytotoxicity of nanoparticles on breast and ovarian cancer cell line-

The human breast cancer cell line (MDAMB-231) and the human ovarian cancer cell line (PA1) were subjected to treatment with the synthesized DH-CdSNP. Increasing concentrations of the nanoparticles were tested on both cancer cells using the MTT assay. Table 5.2 shows increasing dose and loss in cell viability percentage when treated with different samples on the two cell lines. The viability of the cells was assessed and measured over a 24-hour period. The IC_{50} values for DH-CdSNP were determined as 95.74 µg/ml for the PA1 cell line and 796.5 µg/ml for the MDAMB-231 cell line. Similarly, the effectiveness of a simple methanolic extract of Daruharidra was tested as a control on both cell lines. Daruharidra methanolic extract on the PA1 cell line had an IC_{50} of 602.49 µg/ml; on MDAMB 231, IC_{50} was 469.21 µg/ml. The interaction between biogenic DH-CdSNP and phosphorus moieties in DNA is likely to have occurred. This interaction potentially resulted in the inactivation of DNA replication, leading to the inhibition of enzyme functions and ultimately causing a loss of cell viability.*[187]*

Table 5.2 Testing Cytotoxic effect of nanoparticles and *Daruharidra* (*Berberis aristata*) methanolic extract on cell lines by MTT assay. The experiment was performed in triplicates.

Sample	**Dose (µg/ml)**	**Cell viability %**
DH-CdSNP on PA1 cell line	Untreated (blank)	100
	62.5	57.49±0.07
	125	49.22±1.569
	250	28.73±0.683
	500	10.41±0.602
	1000	1.08±1.004
	Untreated (blank)	100

DH-CdSNP on MDAMB231 cell line	62.5	97.84±0.024
	125	93.39±0.23
	250	98.92±0.84
	500	85±1.316
	1000	29.06±0.47
Berberis aristata methanolic extract on PA1	Untreated (blank)	100
	62.5	89.80±0.26
	125	80.62±0.90
	250	65.79±0.92
	500	53.67± 1.02
	1000	27.56± 1.88
Berberis aristata methanolic extract on MDAMB 231	Untreated (blank)	100
	62.5	83.66± 0.18
	125	75.72±0.11
	250	60.75± 0.32
	500	41.8± 1.26
	1000	16.54± 0.91

5.4.4 Apoptotic cell death assessment -

The treated cells exhibited a significant rise in the early apoptotic cells, increasing from 3% to 14.5% in the PA1 cell line and from 4% to 13.6% in the MDAMB-231 cell line. Simultaneously late apoptotic cells also increased from 1.57% to 5.62% in PA1 and 1.09% to 19.58% in MDAMB231. Cells exhibiting an elevation solely in Propidium fluorescence were classified as dead cells. The fluorescence of Annexin V AbFlour 488 was measured in the FL1 detector with a 525nm bandpass filter, while the fluorescence of Propidium Iodide (PI) was measured in the FL3 detector with a 620nm bandpass filter. Figure 5.6 demonstrates the impact of DH-CdSNP on cancerous cells, revealing cell death as evidenced by a notable increase in AbFlour 488 fluorescence compared to the untreated control.[188]. Inter-nucleosomal DNA fragmentation is one of the hallmarks of programmed cell death.

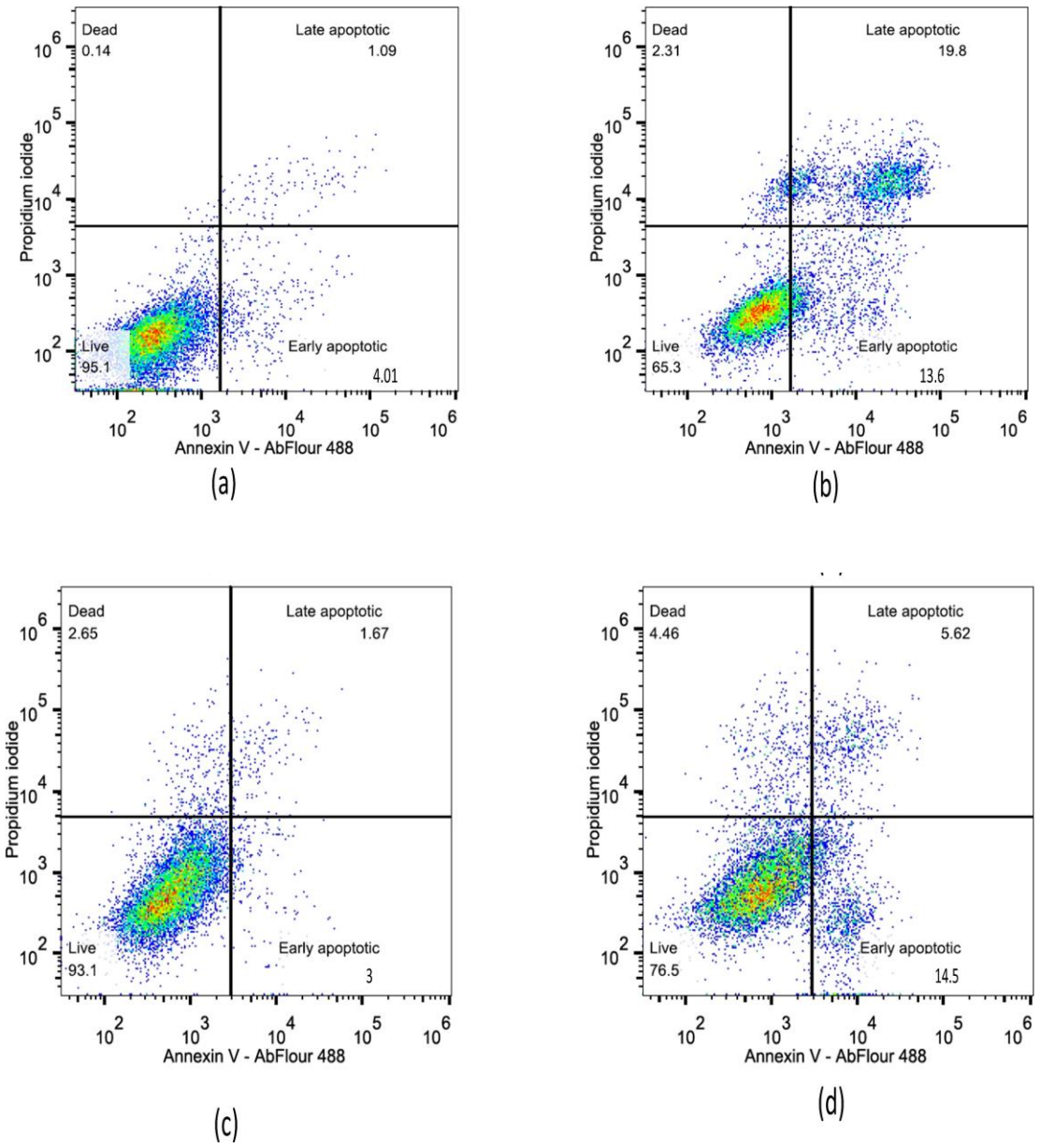

Figure. 5.6. Programmed cell death analysis or apoptosis assessment was conducted using FACS for the MDAMB-231 cell line, with (a) representing the untreated cells and (b) representing the cells treated with DH-CdSNP. Similarly, for the PA1 cell line, (c) denotes the untreated cells and (d) represents the cells treated with DH-CdSNP. Apoptotic cells are observed in the top and bottom right quadrants, indicating both early and late stages of apoptosis.

5.4.5 Flow cytometry assessment of cell cycle -

Fluorescence-activated cell sorting (FACS) was utilized to determine the cell count in different stages of the cell cycle, including G0/G1, S, and G2/M. Propidium Iodide fluorescence was collected using a 620nm bandpass filter in the FL3 detector. As depicted in Figure 5.7, the PA1 cell line treated with the IC_{50} concentration of DH-CdSNP exhibited a significant increase in the number of cells in the G2/M phase of the cell cycle, accompanied by a decrease in the number of cells in the G0/G1 phase compared to the untreated control. These findings suggest a cell cycle arrest at the G2/M stage due to the treatment with DH-CdSNP.

This arrest in the cell cycle indicated that damage in the intracellular DNA when subjected to DH-CdSNP was so significant that it was beyond repair. This damage in DNA leads to a potential anticancer mechanism of the nanoparticles.[189] A noticeable increase in cells exhibiting DNA damage in the SubG1 phase was also observed. In the G0/G1 phase, the cell population in the untreated cells accounted for 40.6%, which gradually decreased to 28.4% upon treatment with DH-CdSNP. Conversely, in the MDAMB-231 cell line, there was an increase in the cell population in the G0/G1 phase from 61.7% to 66.0%, accompanied by a decrease in the cell population in the G2/M phase from 16.7% to 11.7% in the nanoparticle-treated sample compared to the control sample. These findings suggest a cell cycle arrest in the G0/G1 stages due to the treatment with DH-CdSNP.[190] The FACS analysis suggests that DH-CdSNP inhibit the proliferation of MDAMB 231 cells during the G0/G1 phase.

The histograms display the cell cycle analysis of human breast and ovarian cancer cells after 24 hours of treatment with DH-CdSNP at their IC_{50} concentration. It is worth noting that the treatment with nanoparticles leads to an accumulation of cells in the G2/M phase of the cell cycle.

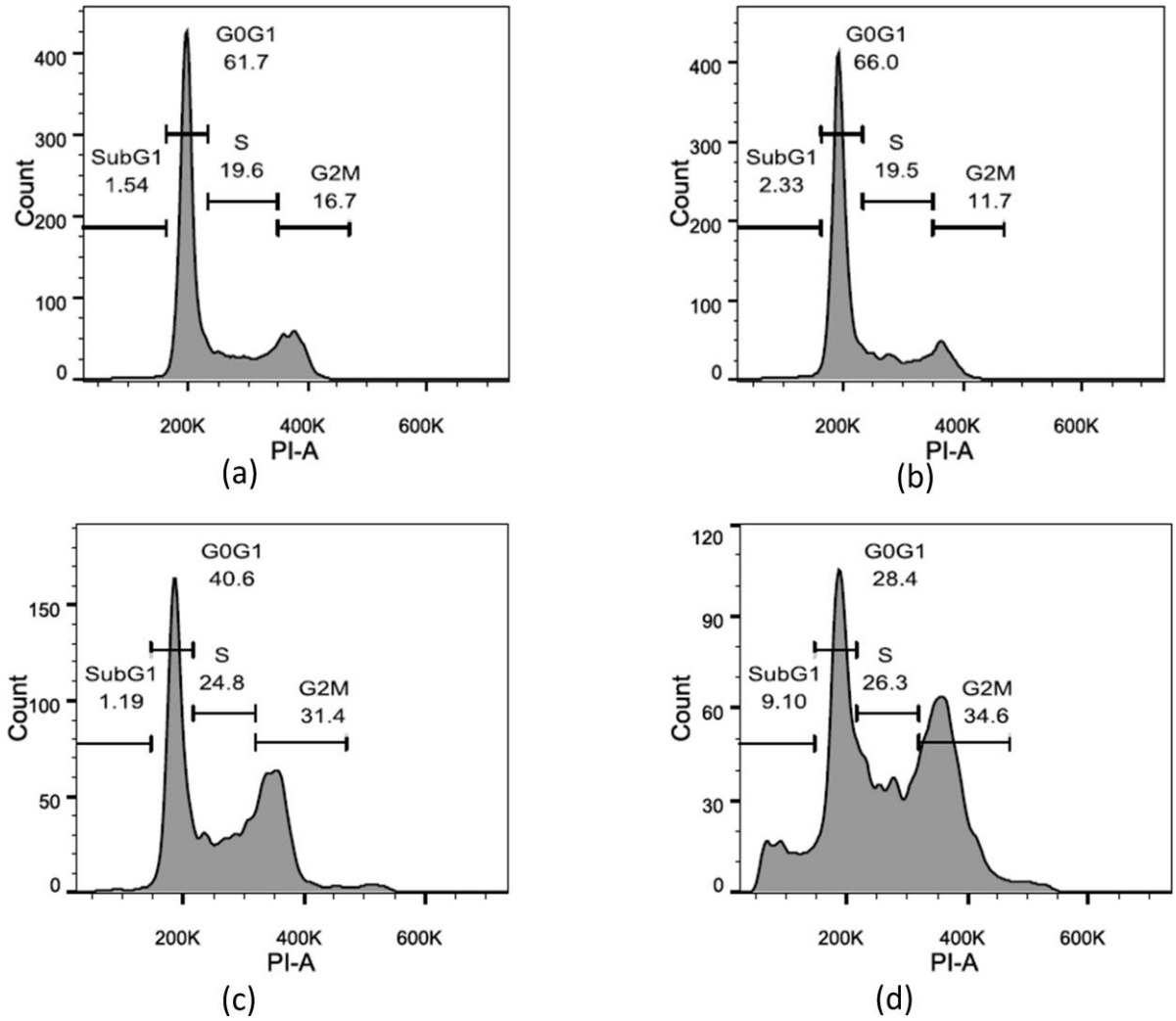

Figure. 5.7. The image illustrates the cell cycle arrest analysis using flow cytometry. (a) represents the control (untreated) MDAMB-231 cells, while (b) depicts the MDAMB-231 cells treated with DH-CdSNP. Similarly, (c) represents the untreated PA1 cells, and (d) represents the CdS nanoparticle-treated PA1 cells.

5.5. Conclusion –

In this study, biosynthesis of DH-CdSNP was performed using the methanolic extract of Daruharidra (Berberis aristata). Extensive efforts and resources are being dedicated to discovering a potent and effective drug to combat cancer and mitigate its threat to humanity.

However, many conventional cancer drugs are associated with numerous severe side effects. Therefore, the development of more targeted herbal medicines holds great promise for the prevention and treatment of cancer, as they have the potential to offer greater specificity and fewer adverse effects. As seen, the cadmium sulfide nanoparticles show significant results about the anticancer effect of the same. FACS analysis before and after treatment showed DNA damage, possibly due to intercalation of the nanoparticles, which resulted in cell cycle arrest and apoptosis.

Nevertheless, this requires additional research with the combined efforts of material scientists and natural product chemists. Further evidence that the biosynthesized DH-CdSNP possess bactericidal capabilities comes from testing the antibacterial activity of the resulting DH-CdSNP against pathogenic microorganisms. Since CdS NPs make extensive contact with the thiol groups in crucial bacterial respiratory enzymes, they impede bacterial cell growth. As CdS NPs pass through the membrane, their positive charge interacts electrostatically with the negatively charged proteins on the surface of bacterial cells, changing the internal structure of the bacterial cells. The combination of plant extract and DH-CdSNP has the potential to revolutionize the approach to cancer therapy, representing an unconventional strategy. This promising approach calls for further research in the field, with the aim of providing substantial evidence supporting the utilization of berberis plant extract in the field of oncology.