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Exploration of Antibacterial Activity of Plant-Mediated Green Synthesis of Cadmium Sulfide Nanoparticles

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Abstract: Today, cadmium sulfide nanoparticles are extensively used in various fields, including medical sciences, engineering, drug delivery, photovoltaic cells, bio-imaging techniques, molecular pathology, and bio-sensing. This study uses a green, eco-friendly approach to explore biosynthesized CdS NPs characterization and their antibacterial activity. A uniform color change from light yellow to greenish yellow indicated the formation of cadmium sulfide nanoparticles, further confirmed by a blue shift at 450 nm in optical absorption spectra. The XRD (X-ray diffraction) analysis confirmed the crystallinity of elements present in nanoparticles, while the coating of *Coronopus didymus* phytochemicals around the nanoparticle surface was confirmed by Fourier transform infrared (FT-IR) analysis. Next, SEM analysis was performed to investigate the morphology and size of the CdS nanoparticles. The antibacterial activity of the synthesized CdS nanoparticles was evaluated against gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*) and gram-positive bacteria (*Staphylococcus aureus*); their result shows the highest zone of inhibition at a medium concentration of 40 mg/ml in *Klebsiella pneumoniae* (27.5±0.70), *Escherichia coli* (21.5±0.70), and *Staphylococcus aureus* (12.15±0.25). Our approach presents a successful, green, and cost-efficient method for synthesizing CdS nanoparticles and exploring their antibacterial activity.

Keywords: extraction; *Coronopus didymus*; cadmium sulfide (CdS) nanoparticles, Antibacterial activity

1. Introduction

Nanotechnology is a rapidly emerging field in the 21st century, leading to numerous scientific and technological developments. It involves working with materials at the nanometer scale, ranging from 1 to 100 nanometers in diameter [1]. Nano-science and technology have immense applications in the fields of biotechnology, health care, pharmaceuticals, agriculture, cosmetics, food technology, bio-medicines, biosensors, catalysis, solar cells, water treatment, electrochemistry, semiconductors [2-10], green packaging, and generation of renewable energy[11]. For this purpose, different traditional methods have been employed to synthesize nanoparticles. Still, the green method gains importance because green nanotechnology is environment-friendly, less expensive, simpler to characterize, and has fewer failure risks. Nanoparticles like Ag, Au, MgO, CuO, Al, TiO2, CdS, etc., have an effective role against viruses, bacteria, fungi, and microbes [12] as well as extensive applications in the medical fields due to their distinctive chemical and physical properties[13].

Cadmium sulfide nanoparticles (CdS NPs) have been reported as an excellent material with many applications in medical sciences and engineering. It is used in many areas, such as nano-medicines, drug delivery, photovoltaic cells, bio-imaging techniques, molecular pathology, and bio-sensing [14-16]. It is effective against AGS and MCF-7 cancerous human cells [17] and has therapeutic applications in lung cancer cells [18,19]. The study reported that green synthesized CdS NPs showed marvelous activity against microbes including Bacillus cereus, *Escherichia coli*, *Salmonella typhi*starims, *Staphylococcus aureus*, Basillus subtilis, Vibrio, Bacillus licheniformis, Serratiamarcescens, Streptococcus pyogens, Pseudomonas aeruginosa, Klebsiellapneumoniae Serratia nematodiphila, and K.planticola [19-25], and fungi including Aspergillus flavus fungus, Penicillium expansum, and Aspergillus niger Fusarium oxysporum [23,24]. However, we investigate the synthesis and antibacterial activity of CdS nanoparticles for the first time using an extract of *Coronopus didymus*.

*Coronopus didymus*is is one of the most important medicinal plants belonging to the Brassicaceae or Cruciferae/mustard family, commonly known as the mustard family [26]. The Brassicaceae are the dicot family of the plant kingdom with a total of 338–360 genera and 3,709 species spread worldwide [27]. Its genus is Lepidium, which is generally written as*Coronopus didymus*(L). They are commonly known as wart-cress [28], lesser swine cress [29], hogs cress, and twin cress [30].*Coronopus didymus*is is a fast-growing and easily adaptable species that grows during the winter (October to February) and is available in gardens, vacant plots, pastures, and along roadsides [29]. It has been reported that *Coronopus didymus* contains numerous compounds including flavones, e.g., chrysoeriol, chrysoeriol-4′-β-D-glucoside [20], benzyl cyanide [31], flavonoids (quercetin), phenolics [32],1,8- dihydroxy anthraquinone at a concentration of 0.003% [33], glucotrapaeolin [34], and Flavonoids (15.55%) [35]. *Coronopus didymus* is a traditional medicinal plant that has the potential to cure different diseases and is widely used by the population as a primary source of health care in respiratory diseases e.g. asthma, emphysema, flu, bronchitis, allergic rhinitis, cough, chronic obstructive pulmonary disease, malaria [36-38], allergies, ulcers, arthrosis, wounds, gastric illness, muscular pain, bruises, inflammations [39]and for blood purification [33]. For instance, the aqueous extract of *Coronopus didymus* has been shown to exhibit antifungal activity against A. alternate [40], while the ethyl acetate fraction of root extract of *Coronopus didymus* is effective against *S. rolfsii* because of antifungal constituents [41]. Additionally, it also showed anti-inflammatory activity through the inhibition of pro-inflammatory enzymes (myeloperoxidase, adenosine deaminase) [32,42] and was also involved in anticancer and anti-malarial properties [33]. It was concluded that *Coronopus didymus* species contains various natural products like flavonoids, saponins, and tannins, which are responsible for the biological activities, e.g., hypoglycemic, antiallergic, antipyretic, and hepatoprotective [43]. Among all other species reported, the *Coronopus didymus* species possesses a significant and greatest amount of antimicrobial activity [44], i.e., 98 % of all other species [45].

Thus, the current study aims to produce CdS nanoparticles from *Coronopus didymus* using it as a green course and test their antibacterial activity against clinical pathogens.

2. Materials and Methods

2.1. Chemicals.

Ethanol (99.8% purity), Methanol (99.8% purity), acetone (99.5% purity), n-hexane (98.5% purity), and DMSO (98% purity) are the necessary chemicals were procured from BDH

laboratory supplies Poole, BH 15 1TD, England. Distilled water was utilized to prepare all the required solutions.

2.2.Identification of plant.

Coronopus didymus, an important medicinal plant that belongs to the family of Cruciferae or Brassicaceae, was recognized and approved by the botanist and studied through several literature surveys.

2.3.Collection of plant.

It was collected from the small villages (Gujrat, Katlang, and Toru) located in the city of Mardan, Khyber Pakhtunkhwa province, Pakistan.

2.4.Drying and grinding of plant

The fresh green *Coronopus didymus* plant was collected from the fields and washed with distilled water thrice to remove dust particles. The clean plant was then placed on a wooden table for drying under the shade. The plant was kept under thin clothes for about 15-20 days to protect it from light exposure and environmental pollution. The dried plant was then ground using a grinding machine to convert it into powdered form to increase its surface area for better extraction. After grinding, the plant was made ready for the process of extraction.

2.5.Soxhlet extraction.

The finely ground, uniformed size 25 gm powder plant samples are placed in a thimble, and a thimble is then placed into the Soxhlet's thimble chamber using a porous bag made of a cellulose strong filter which is prepared by hand. For the extraction, the distillation flask of the Soxhlet had 250 ml of ethanol. The solvent was heated at a moderate temperature, roughly 45°C using a siphon mechanism, and the solvent vaporized and traveled to the sample thimble chamber, condensed, and fell back when the liquid extract reached the siphon arm and was repeatedly emptied into a distillation flask of a Soxhlet. The upper section was built with a condenser that allowed water inflow and outflow. The operation was repeated for 40-48 hours until the solvent drop could not evaporate without leaving residues.

2.6. Synthesis of nanoparticles.

Cadmium sulfide nanoparticles (CdS NPs) were prepared using a green method using plant extract. Plants are rich in organic compounds containing different functional groups that act as a source of natural products. These compounds get activated by metal atoms in a basic medium to give desired NPs [46-49]. To prepare CdS NPs, a 45ml of stock solution was prepared by adding 1g of plant extract in 45ml of distilled water. Next, 1.33g of cadmium acetate was dissolved in 35 ml of the stock solution, while 0.4g of sodium sulfide was dissolved in the remaining 10 ml of the stock solution. After it the process of titration began, add a dropwise homogenous solution of sodium sulfide from the burette to a 10ml solution of cadmium acetate taken in the flask. A greenish or bright yellow color appeared on continued stirring for about half an hour, indicating the formation of CdS NPs. Further, the CdS NPs formation was confirmed by taking UV-visible spectra.

2.7. Washing and drying.

The CdS NPs were processed for separation by centrifuge at 7000 rpm for about 20 minutes. The resulting paste of CdS NPs was washed thrice with distilled water, ethanol, and n-hexane to remove contaminants. The purified nanoparticles of cadmium sulfide were then dried in an oven and stored in a clean bottle for further characterization and activity assessments.

2.8. Characterization of synthesized nanoparticles.

2.8.1.UV-Visible Spectrophotometry (UV-Vis).

The synthesized CdS NPs were further monitored by using a UV-visible spectrophotometer (Shimadzu Corporation made in Japan). The sample was dissolved in distilled water to make a dilute solution and then analyzed at the UV range from 200nm to 700nm.

2.8.2. X-ray diffraction (XRD).

X-ray Diffraction is an analytical technique used to measure the compound crystal structure, geometry, and crystal lattices and to identify an unknown compound. Here, the crystalline structure of the CdS nanoparticles was characterized by Power X-ray Diffractometer model No. JDX-3532 (JEOL, Japan) operated at room temperature with voltage 20-40Kv, current 2.5-30mA, with radiations CuKα at λ=1.5418 Å, angle range 0 to 160 degrees. The sample was scanned with 2θ range from 20 to 100 degrees.

2.8.3. Fourier Transform Infrared Spectroscopy (FTIR).

Fourier transform infrared is another tool to analyze the capping of biomolecules over the nanoparticles. The FTIR spectrum of CdS NPs was recorded in the wavelength range from 4000 to 500 cm^{-1} by using a Perkin spectrum (100 FTIR spectrometer) with a resolution of 4 cm^{-1} after 32 scans.

2.8.4. Scanning Electron Microscopy (SEM).

Utilizing the scanning electron microscopy technique is significant in visualizing the microstructure and morphology of a sample material. The sample was scrutinized under the Jeol 6480LV JSM microscope with a voltage range of 10-20 kV and a vacuum level of around 10-5 torr to conduct the observation. Before examination, the synthesized solution of cadmium sulfide nanoparticles was sonicated with distilled water to ensure a homogenous mixture. A small amount of the sample was carefully deposited onto a glass slide and left to dry. This method ensures that the sample is well-prepared for observation and helps to achieve accurate and reliable results.

2.9. Antibacterial assay.

The standard disc diffusion method evaluated the synthesized CdS nanoparticles' antibacterial properties against various bacterial strains [46,50]. The powdered CdS NPs were dissolved in DMSO (Dimethyl sulfoxide) to make a 30mg/ml solution. To ensure sterility of all the materials used for the antibacterial assay, they were subjected to autoclaving at 121°C

for 15 minutes. The Mueller-Hinton agar culture of 100µl was spread evenly all over the plates as a medium for the growth of bacteria using an L-shaped spreader. The bacterial strains gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*), and gram-positive (*Staphylococcus aureus*) were obtained from Bacha Khan Medical College Mardan, Pakistan, and grown in the fresh culture medium of agar solution. The bacterial suspensions of *E. coli*, *S. aureus,* and K. pneumonia were swabbed on separate nutrient agar plates using L-rod. Wells of 6 mm diameter were made on all the Petri plates using Whatman filter paper. Then, the three wells were filled with 40 μl of nanoparticles synthesized solution of low, medium, and high concentrations. Two Antibiotic discs, Gentamicin and Ciprofloxacin 30µg (Oxoid Ltd, UK), were taken as a positive control. All the Petri plates were sealed with parafilm tape to avoid contamination and incubated at 37°C for 24 h for bacterial culture. After 24 h, the diameter of zones of inhibition formed around the well was calculated using a meter ruler.

3. Results and Discussion

The result of biosynthesized Cadmium Sulfide Nanoparticles (CdS NPs),its antibacterial activity, and its characterizations are further discussed.

3.1. UV-Visible analysis.

The formation of CdS nanoparticles was preliminarily done by simple visual observation. As shown in Figure 1, a uniform color change was observed from light yellow to greenish-yellow, confirming the formation of CdS NPs. Furthermore, the formation of CdS NPs was confirmed by UV-visible absorption spectra. The UV-visible spectrum of the synthesized nanoparticles showed broad absorption peaks between 380 nm and 450 nm, as shown in Figure 2. A blue shift in the absorption peak at 450 nm arises due to the excitation of high binding energy of CdS[51,52]. The broad peaks in the optical absorption indicate stable CdS nanoparticles' formation.

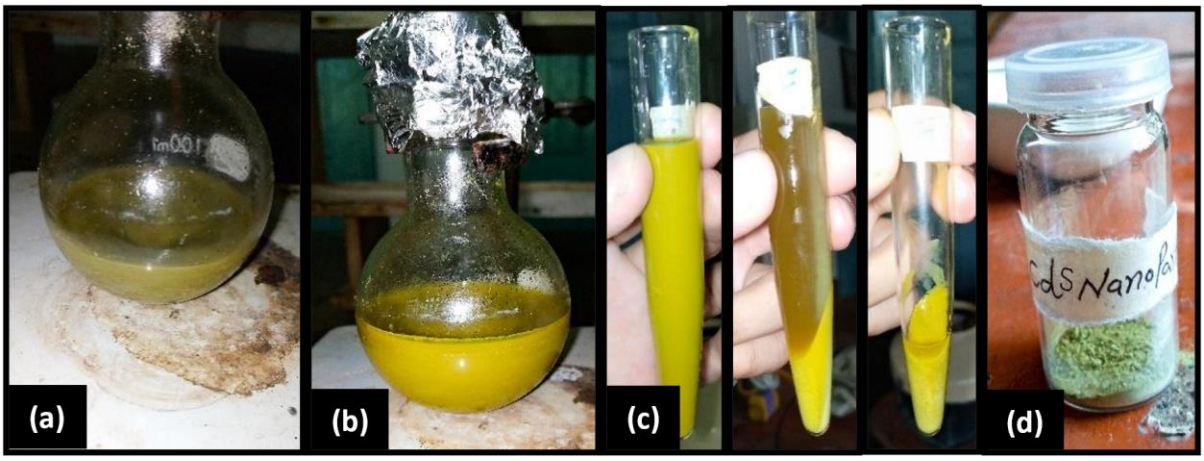


Figure 1. A stepwise formation of CdS NPs:**(a)** CdS+Extract, **(b)** CdS+Extract, after 30 minutes, **(c)** Paste after centrifugation, **(d)** Paste of CdS NPs washed then dried and kept in a clean bottle.

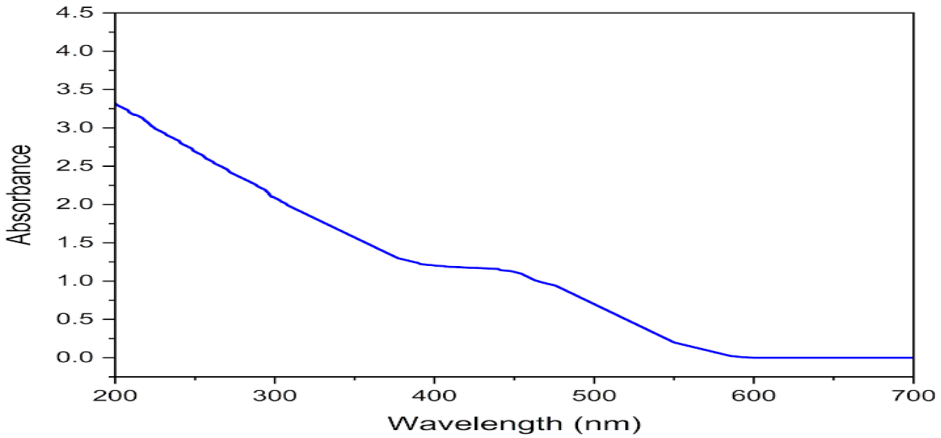


Figure 2. UV- visible spectrum of CdS nanoparticles.

3.2. Fourier Transform Infrared Spectroscopy (FTIR) analysis.

The FTIR analysis was used to determine the functional groups present in the synthesized compound to specify the molecule's structure. The number of peaks and position of bands depends on the chemical composition, morphology, and crystalline structure of the synthesized compound, film width, and deposition time [41]. The FTIR spectrum of CdS nanoparticles is shown in Figure 3. Various types of biomolecules were observed in the plant [53,54]. The high-range absorption band corresponding to 1392 cm^{-1} represents the O-C=O asymmetric stretching vibrations. The shortest band at 723 cm^{-1} corresponds to the Cd–S bond, which confirmed the formation of CdS nanoparticles. The peak at 860 cm^{-1} shows a minute impurity present in the compound in the form of SO^{-4} [55].

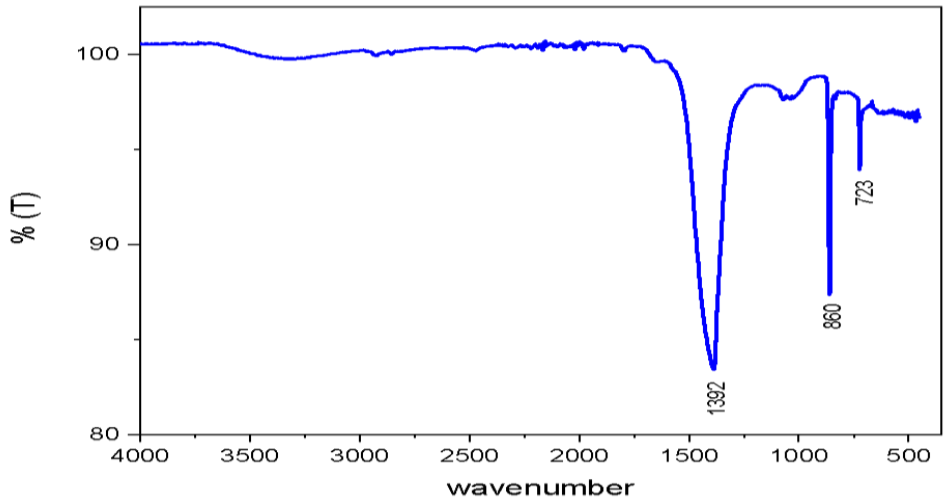


Figure 3. FTIR spectrum of synthesized CdS nanoparticles.

3.3. XRD analysis.

As shown in Figure 4, the diffraction peaks of CdS nanoparticles were obtained at 23.54°, 30.32°, 36.44°, and 49.96° corresponds to (111), (200), (220), and (311) planes of face-centered crystalline CdS respectively. The vast peaks show that the particles were formed as fine crystalline sizes, and clearly, no impurity peak was seen. Our result is matching with the peaks of pure CdS crystals reported by the Joint Committee for Powder Diffraction Standards (JCPDS (ICDD) file number 10- 454 for CdS) [22]. The average crystallite size of the CdS

nanoparticles was found to be 26 nm, which is calculated by using the Debye–Scherrer's equation:

$$D = K\lambda/\beta Cos\theta$$

where λ represents the wavelength of X-ray radiation, and D shows the average particle size. β represents full width at half maximum (FWHM) in radians, K represents the shape factor, and θ is the Bragg diffraction angle.

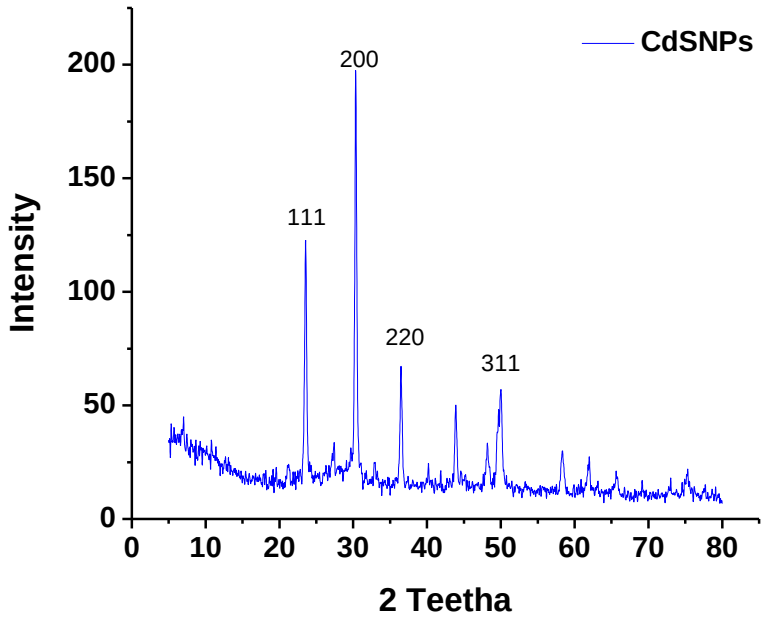
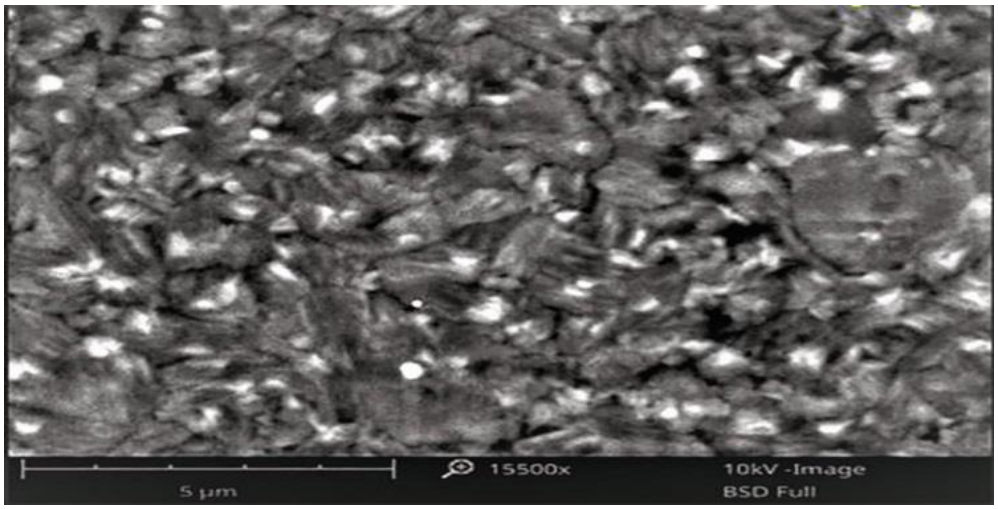


Figure 4. XRD pattern of synthesized CdS nanoparticles.

3.4. SEM analysis.

The SEM analysis was used to investigate the morphology and size of the CdS nanoparticles, as shown in Figure 5. The nanoparticles that were produced were primarily triangular in shape and had sizes ranging from 20 to 40 nm, as observed on a scale bar of 5 μm. Although some aggregates were visible in the nanoparticles, this indicates that proteins are critical in serving as capping agents for the nanoparticles, preventing them from clumping together and providing stability and a specific structure to the nanoparticles [22].



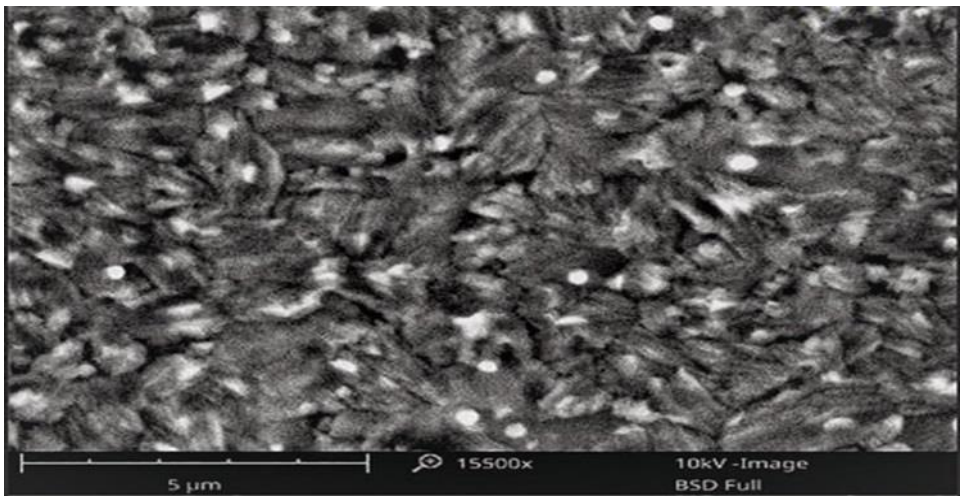


Figure 5. SEM morphological characterization of CdS nanoparticles.

3.5. Antibacterial activity of Cadmium sulfide (CdS) nanoparticles.

The synthesized cadmium sulfide nanoparticles' bacterial susceptibility action was investigated against 3 different bacterial strains, including gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) and gram-positive (*Staphylococcus aureus*). During the antibacterial susceptibility test followed by the Disc diffusion method, the zone of inhibition for each bacterial strain was expressed as mean ± SD by taking four replicates. A medium concentration of 40 mg/ml CdS NPs showed the highest inhibition against all bacterial strains, as shown in Figure 5. The diameter of the zone of inhibition of all bacteria pathogens is presented in Table 1. Strong antibacterial potential was seen in the order of *Klebsiella pneumoniae* (27.5±0.70) Figure 5(a) followed by *Escherichia coli* (21.5±0.70) Figure 5(c) and *Staphylococcus aureus* (12.15±0.25) Figure 5(b). The effectiveness of CdS nanoparticles in eradicating bacteria is dependent on their size. Particles with smaller dimensions can effectively infiltrate the bacterial cell wall due to their increased number of surface atoms, which results in greater surface area for interaction with the bacterial cell. Moreover, smaller nanoparticles contain a higher proportion of atoms located at corners, edges, and steps, which are low coordination and high energy sites. This characteristic makes them more active compared to larger particles [49]. When the positively charged CdS nanoparticles come into contact with the negatively charged proteins on the surface of bacterial cells, electrostatic interactions occur, releasing ions. These ions interact with the thiol groups of the proteins found in the cell wall, causing the production of reactive oxygen species. This, in turn, leads to the destruction of cells [56-58]. Due to the strong binding of the nanoparticles to proteins, the active transport, dehydrogenase, and enzymatic activity in the periplasmic region are inhibited, ultimately resulting in the suppression of DNA, RNA, and protein synthesis. This process eventually leads to cell lysis [21,59]. Standard antibiotics were tested against the same bacterial pathogens to compare the antibacterial action with that of the CdS nanoparticles. Salmonella showed a greater zone of inhibition (36.75±0.35) against Ciprofloxacin, as shown in Figure 6(a). E. coli and S. aureus showed maximum zone of inhibition (34.5±0.70) and (34.75±0.28) against Gentamicin, shown in Figure 6(b) and (d), respectively. The result clearly shows an enhanced antibacterial potential of CdS nanoparticles against human pathogens used in this experiment.

Table 1. Antibacterial activity of CdS nanoparticles against different bacteria.

Bacteria	Concentration of Cds NPs (mg/ml) of low, medium, and high	Volume of CdS NPs (µl)	CdS NPs Zone of inhibition in (mm) of different concentrations
Escherichia coli	30, 40, 50	40	(18.5±0.70) (23.5±0.70) (22.5±0.70)
Klebsiella pneumoniae	30, 40, 50	40	(18.5±0.70) (27.5±0.70) (22.5±0.70)
Staphylococcus aureus	30, 40, 50	40	(5.5±0.70) (7.5±0.70) (5.5±0.70)

± Standard deviation.

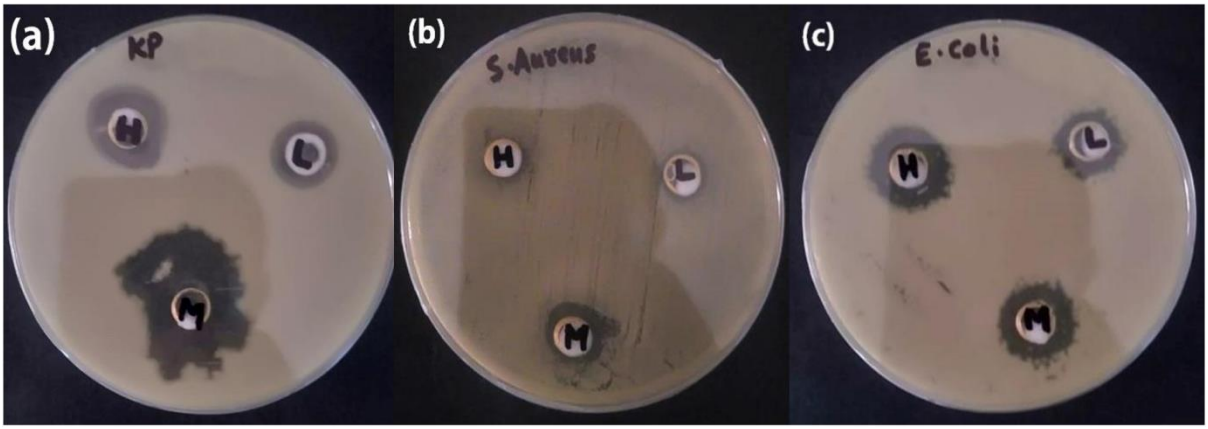


Figure 6. Antibacterial activity of CdS nanoparticles against three bacterial strains:(**a**) *Klebsiella pneumoniae*, (**b**) *Staphylococcus aureus*, (**c**) *Escherichia coli* at different concentrations. For further details, refer to Table 1 above.

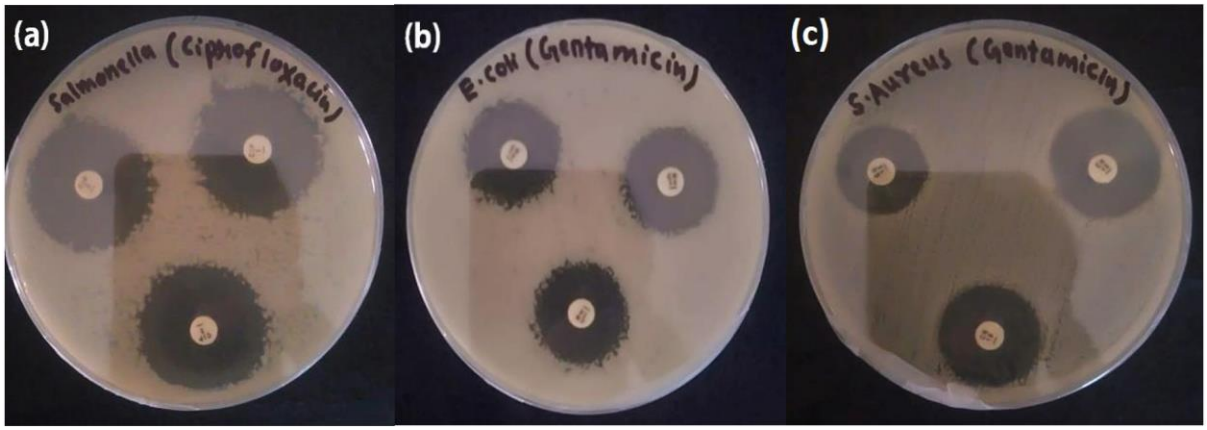


Figure 7. Antibacterial activity of standard antibiotics against three bacterial strains:**(a)** *Salmonella typhi*, **(b)** *Escherichia coli*, **(c)** *Staphylococcus aureus*. For further details, refer to Table 2 below.

Table 2. Antibacterial activity of standard antibiotics against different bacteria.

Bacteria	Zone of inhibition (mm)	
	Gentamicin	Ciprofloxacin
Escherichia coli	(34.5±0.70)	-
Staphylococcus aureus	(34.75±0.28)	-
Salmonella typhi	-	(36.75±0.35)

4. Conclusions

Green nanotechnology gets attention due to its ability to be environment friendly, less expensive, has fewer failure risks, is safe, sustainable, nontoxic nano-products production, and is simpler to characterize. In light of these benefits, our current study investigated antibacterial activity from the extract of *Coronopus didymus* plant-mediated synthesis of CdS nanoparticles. Various techniques, including UV, XRD, FT-IR, and SEM characterized the synthesized CdS

nanoparticles. Furthermore, the nanoparticles were tested against various bacterial strains. In our study, the synthesized CdS NPs exhibited strong antibacterial potential against gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*)and gram-positive (*Staphylococcus aureus*) bacterium. We hope that the chemically synthesized cadmium sulfide nanoparticles using the plant species *Coronopus didymus* and their applications as antibacterial potential can open a new door for treating and curing human pathogens in food, packaging, and biofilm industries.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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