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Study on Biological Synthesis of Cadmium Sulfide Nanoparticles by *Bacillus licheniformis* and Its Antimicrobial Properties against Food Borne Pathogens

Aishwarya Shivashankarappa, K R Sanjay*

Department of Biotechnology, Sri Jayachamarajendra College of Engineering, JSS Technical Institutions Campus, Mysuru
*Corresponding author: sanjayrajesh@gmail.com

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Abstract The green synthesis of cadmium sulfide (CdS) nanoparticles has been regarded as the most promising technique for their prospective applications in biological system. The bacterial strain *Bacillus licheniformis* has shown to be efficient in synthesizing cadmium sulfide nanoparticles. We report the effect on nanocrystal formation by varying the ratios of cadmium chloride and sodium sulfide ranging from 1:1, 2:1, 3:1 and 4:1 respectively. The resultant CdS nanoparticles was tested for antimicrobial activity against a range of food borne bacteria *E coli*, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* and fungi *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium expansum.* The results showed that the CdS nanoparticles were crystalline in nature with size varying from 20-40 nm. The stability of nanoparticles was due to protein interaction which may have played an important role as capping agents. The antimicrobial activity showed that the CdS nanoparticles of ratio 4:1 of cadmium chloride and sodium sulfide at a concentration of 40 mg/ml showed highest zone of inhibition in *Psedomonas aeruginosa* (26.5±0.70) and A*spergillus flavus* (27.8±0.28). The present study explains a simple, cost effective way of nanoparticle synthesis suitable for large scale production. The green synthesis approach extends the horizon of applications to biological systems as an effective medicinal agent.

Keywords: *Cadmium sulfide (CdS) nanoparticles, Semiconductors, Antimicrobial activity, Antifungal activity*

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1. Introduction

The synthesis and application of semiconductor nanoparticles like cadmium sulfide (CdS) have gained tremendous interest due to their wide range of applications as biosensors, photo catalysts, solar cells, diodes [4,14,32] and quantum dots for targeted drug delivery and therapy [40]. The size and composition of the nanoparticles play a critical role in determining the wide range of applications. As a result there has been extensive work on developing new techniques in a cost effective manner. Various methods involving chemical synthesis [20,24] micro emulsion method, ultrasonic irradiation [6,39] sol-gel and photo-itching methods [22,37] have been adapted to synthesize size specific nanoparticles. However these methods are not cost effective and require huge investments. As a result, biological means of producing nanoparticles have gained much momentum in the recent years. Many microorganisms and plant sources have been used for the synthesis of various metal and semiconductor nanoparticles [3,11,14,35]. The micro organisms have the ability to produce nanoparticles either extracellular or intracellular depending on the type of organism used. Although the extracellular produced nanoparticles have wide range of commercial application their polydispersive nature requires optimization of growth parameters to obtain monodispersive nanoparticles. On the other hand, the intracellular nanoparticles produced are mostly monodispersive in nature which makes them suitable for many in-vivo applications [19,21]. The biosynthesis mechanism of semiconductor nanoparticles involves the reduction of inorganic metals in the solution which is facilitated by the enzyme sulphate reductase present in most of the bacterial species. In the intracellular production of nanoparticles the transport of ions takes place into the cell which utilizes the intracellular enzymes for the production, whereas in extra cellular production of nanoparticles the metal ions and enzymes are trapped on the cell surface to produce nanoparticles [21,36]. Metals like gold, silver, titanium, copper have been long known for their bactericidal agents. They have been used as biofilms in many packed food and in toothpastes to prevent the deposition of plaque in teeth [7,8,16]. It has been showed that the release of Cd^{2+} ions from the particles play an important role in the cytotoxic activity of cadmium nanoparticles. And hence, the decrease in the size of nanoparticle provides more surface to volume ratio which increase the chance of Cd^{2+} exposure to the bacterial cells [10,15].

In the present work, we report the biological synthesis and characterization of cadmium sulfide nanoparticles by *Bacillus licheniformis.* The effect of variation in the ratio of cadmium chloride and sodium sulfide in the formation of nanoparticles has been addressed and the antibacterial and antifungal activity of the cadmium sulfide nanoparticles has been tested on various food borne bacteria and fungi.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals used for experiments were of high purity and analytical grade. Cadmium chloride was purchased from SRL. Sodium sulfide, nutrient agar, nutrient broth, Muller Hinton agar, potato dextrose agar and maltose extract agar were purchased from Himedia, India.

2.2. Microbial Strains

All microbial and fungal strains were got from Microbial Type Culture Collection (MTCC), Chandigarh, India. *E coli* (10312), *Bacillus licheniformis* (2465), *Staphylococcus aureus* (7443), *Bacillus cereus* (430), *Aspergillus flavus* (9606), *Fusarium oxysporum* (8608) and *Penicillium expansum* (8241). The *Pseudomonas aeruginosa* was got from American Type Culture Collection (ATCC), US.

2.3. Bacterial Strain and Growth Conditions

Bacillus licheniformis strain MTCC 2465 was grown on nutrient agar slants under aerobic conditions at 37°C and stored at 6°C until use.

2.4. Preparations of Supernatants

Seed culture was prepared by transferring one loop of *Bacillus licheniformis* into 3 ml nutrient broth and grown for 24 hrs at 37°C at 150 rpm on rotary shaker. The seed culture was further enriched by transferring 1 ml of culture into 50 ml nutrient broth and grown for 24 hrs at 37°C at 150 rpm. The culture broth was centrifuged at 8000 rpm for 20 minutes and the supernatant was collected for further studies.

2.5. Synthesis of CdS Nanoparticles

The synthesis of cadmium sulfide nanoparticles involves the reaction between cadmium chloride and sodium sulfide under the influence of bacterial supernatant.

$$CdCl_2 + Na_2S \rightarrow CdS + 2NaCl$$

0.25 M concentration of cadmium chloride and sodium sulfide was used for the reaction to synthesize CdS. Four different ratios of cadmium chloride and sodium sulfide ranging 1:1, 2:1, 3:1 and 4:1 respectively was taken to check the effect of cadmium chloride on nanoparticle formation. A volume of 5, 6.6, 7.5 and 8 ml of cadmium chloride and 5, 3.3, 2.5 and 2 ml of sodium sulfide corresponding to ratio 1:1, 2:1, 3:1 and 4:1 were added in different screw cap tubes and allowed to react. This reaction produced an orange-yellow colour of cadmium sulfide suspension to which equal volume of supernatant was added to each of the tubes and mixed thoroughly. The mixture was kept in water bath at 60°C for about 10-20 minutes until there was fluffy orange yellow deposition seen at the bottom, indicating the formation of nanoparticles [26]. The suspension was left to cool and incubated at room temperature over night. Following day, the solution was observed for coalescent orange yellow clusters deposited at the bottom of the tube.

2.6. Purification of Nanoparticle

The sodium chloride formed from the reaction of cadmium chloride and sodium sulfide was removed without disturbing the CdS nanoparticle precipitate. The precipitate was washed with acetone and water to remove if any contaminants present and dried in hot air oven at 45° - 50°C.

2.7. Characterization of CdS Nanoparticles

The formation of cadmium sulfide nanoparticles was characterized by Shimadzu UV-2.42 UV-visible spectrophotometer. The spectrum was recorded from 300 nm to 650 nm. The size and morphology of the CdS nanoparticles were analyzed by coating the air dried nanoparticles on the copper grid and observed under Scanning Electron Microscope (ZEISS). The elemental analysis was performed by Energy Dispersive X-ray spectroscopy (EDX). The functional groups of nanoparticles were analyzed using the Fourier Transform Infrared Spectroscopy (FTIR) and scanned in the infrared region of 400 to 4000 cm^{-1}. The crystalline nature of the CdS nanoparticles was assessed by powder X-Ray Diffractormeter – Rigaku SmartLab. The intensities were recorded from 10° to 80° at 2θ angles.

2.8. Antimicrobial Activity of Cadmium Sulfide Nanoparticles

The antimicrobial activity of cadmium sulfide nanoparticles were assessed by well diffusion method against a set of food borne pathogens *E coli, Bacillus licheniformis, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus cereus* which were obtained from MTCC, India. The Muller Hinton agar plates were prepared and 100 µl of each culture were added to individual plates and spread uniformly with an L-shape spreader. Well of 6 mm diameter was made using a sterile cork borer and 20 µl of 40 mg/ml of CdS nanoparticles was used for all strains except *Pseudomonas aeruginosa* which was treated with 30 µl of 40 mg/ml CdS nanoparticles. All four ratios 1:1, 2:1, 3:1 and 4:1 were added into individual wells and incubated at 37°C for 24 hrs and the Zone of Inhibition (ZOI) formed surrounding the well was noted. The zone of inhibition was compared against a set of standard antibiotics Ampicillin (A^{25}), Norfloxin (NX^{10}), Trimethoprim (COT^{25}), Ceftazimidime (CA^{30}) and Cephatoxime (CTX^{30}) treated for all strains.

2.9. Antifungal Activity of Cadmium Sulfide Nanoparticles

The antifungal activity of CdS nanoparticles was tested against fungal strains *Fusarium oxysporum, Aspergillus flavus,* and *Penicillium expansum.* Using a sterile loop, the

fungal spores were dispersed in 1 ml distilled water and mixed thoroughly. Petri plates containing Potato dextrose agar (PDA) for *Fusarium oxysporum* and *Aspergillus flavus* and Maltose extract agar for *Penicillium expansum* were prepared and 100 µl of fungal spore suspension was added to each plate and spread evenly with an L-shape spreader. 6 mm diameter wells were made with a sterile cork borer and 20 µl CdS nanoparticles of 26 mg/ml for *Aspergillus flavus*, 40 mg/ml for *Fusarium oxysporum* and 50µl of 66 mg/ml for *Penicillium expansum* of four different ratios of 1:1, 2:1, 3:1 and 4:1 was added to individual wells and kept for incubation at 28^0C for 72 hrs. An initial concentration of 26 mg/ml of CdS nanoparticles was used against all the fungal strains. But however, the fungal strain *Fusarium oxysporum* and *Pseudomonas aeruginosa* proved to be more resistant at this concentration and hence a higher concentration of 40 mg/ml and 66 mg/ml respectively was used for inhibition studies. The zone of inhibition formed was measured after 72 hrs of incubation.

3. Results and Discussions

3.1. Visual Observation

In the present study, the synthesis of cadmium sulfide nanoparticles was achieved by using the bacteria *Bacillus licheniformis.* The reaction between cadmium chloride and sodium sulfide was reduced to cadmium sulfide nanoparticles under the influence of enzyme sulfate reductase. The formation of coalescent orange-yellow clusters at the bottom of the tube indicated the formation of nanoparticles (Figure 1). The precipitation was highest in the ratio of 1:1 and was found to be least in the ratio of 4:1 of cadmium chloride and sodium sulfide. The formation of CdS precipitate is said to be inversely proportional to the amount nanocrystal formation and the maximum synthesis of nanoparticles been reported to form at stationary phase of cell cycle [2,21]. The results reported by Sweeney et al. [35] also showed that the cells obtained at stationary phase showed little precipitation when compared with cells of late logarithmic phase which had bulk CdS precipitation. Our results correlate with the above findings showing highest nanoparticles formation and least precipitation at the ratio of 4:1.

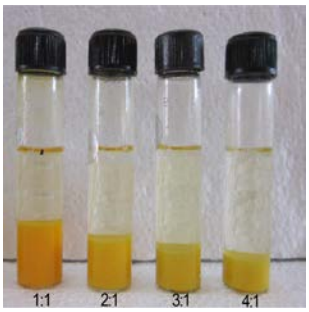

Figure 1. Biosynthesized CdS nanoparticles by *Bacillus licheniformis* with different ratios of cadmium chloride and sodium sulfide 1:1, 2:1, 3:1 and 4:1 respectively

3.2. UV-Visible Spectroscopy

The CdS nanoparticles were analyzed using UV-Visible spectrophotometer (Figure 2). The absorbance spectra for the nanoparticles was measured from 300 nm to 650 nm which showed strong absorption peaks from 420 nm - 440 nm. The broad peaks formed correspond to the surface plasmon resonance indicating the presence of stable nanoparticles. The nanoparticles of 4:1 ratio showed broad peak which may be due to the variation in the size of the nanoparticles. The increasing intensity of the peak can be attributed to the increase in the number of nanoparticles in the solution [17].

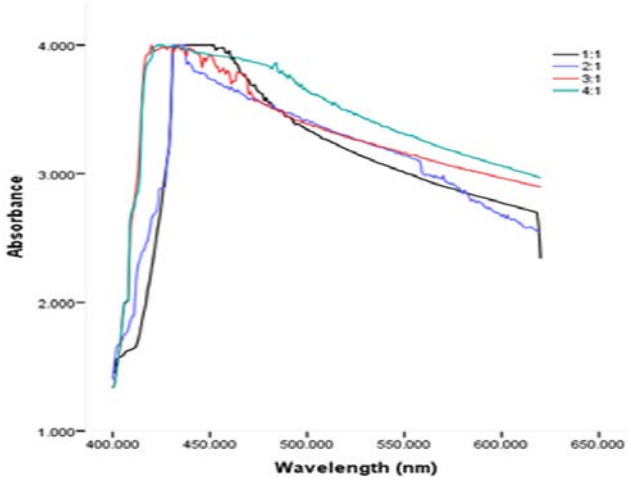

Figure 2. UV- visible absorption spectrum of CdS nanoparticles of all four ratios 1:1, 2:1, 3:1 and 4:1

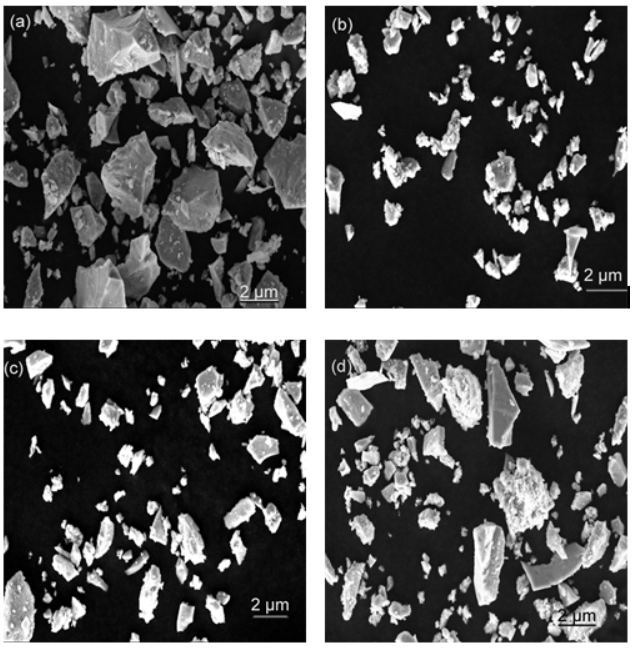

Figure 3. SEM micrograph of CdS nanoparticles taken at a scale bar of 2 µm. Images of different ratios of cadmium chloride and sodium sulfide 1:1 (a), 2:1 (b), 3:1 (c) and 4:1 (d)

3.3. Scanning Electron Microscopy (SEM)

The size and morphology of CdS nanoparticles were examined by SEM analysis (Figure 3). The images were analyzed for all four ratios 1:1 Figure 3(a), 2:1 Figure 3(b), 3:1 Figure 3(c) and 4:1 Figure 3(d) of cadmium chloride and sodium sulfide which showed the presence of nanoparticles. The synthesized nanoparticles were mostly triangle in shape with size ranging from 20 - 40 nm taken in a scale bar of 2 µm. There were few aggregates seen in

the nanoparticles suggesting that the proteins play an important role as capping agents for nanoparticles thus preventing internal agglomeration and providing stability and specific structure to the nanoparticles [27].

3.4. Energy Dispersive X-Ray Spectroscopy (EDX)

The elements present in the sample were analyzed by EDX (Figure 4). The results showed strong signals of Cd and S indicating the nanoparticles were made of Cadmium sulfide metals. The spectrum plotted between energy (KeV) and X-ray counts for all different ratios 1:1 Figure 4(a), 2:1 Figure 4(b), 3:1 Figure 4(c) and 4:1 Figure 4(d) showed optical absorption band peak from 3 - 4 KeV which is the typical absorption peak for metallic CdS nanocrystals obtained due to surface plasmon resonance. Studies conducted by Pandian et al. [23] and Rajeshkumar et al. [27] showed similar results of CdS nanoparticles. The result showed that the wt% of the Cd gradually increased and showed highest concentration at the ratio of 4:1.

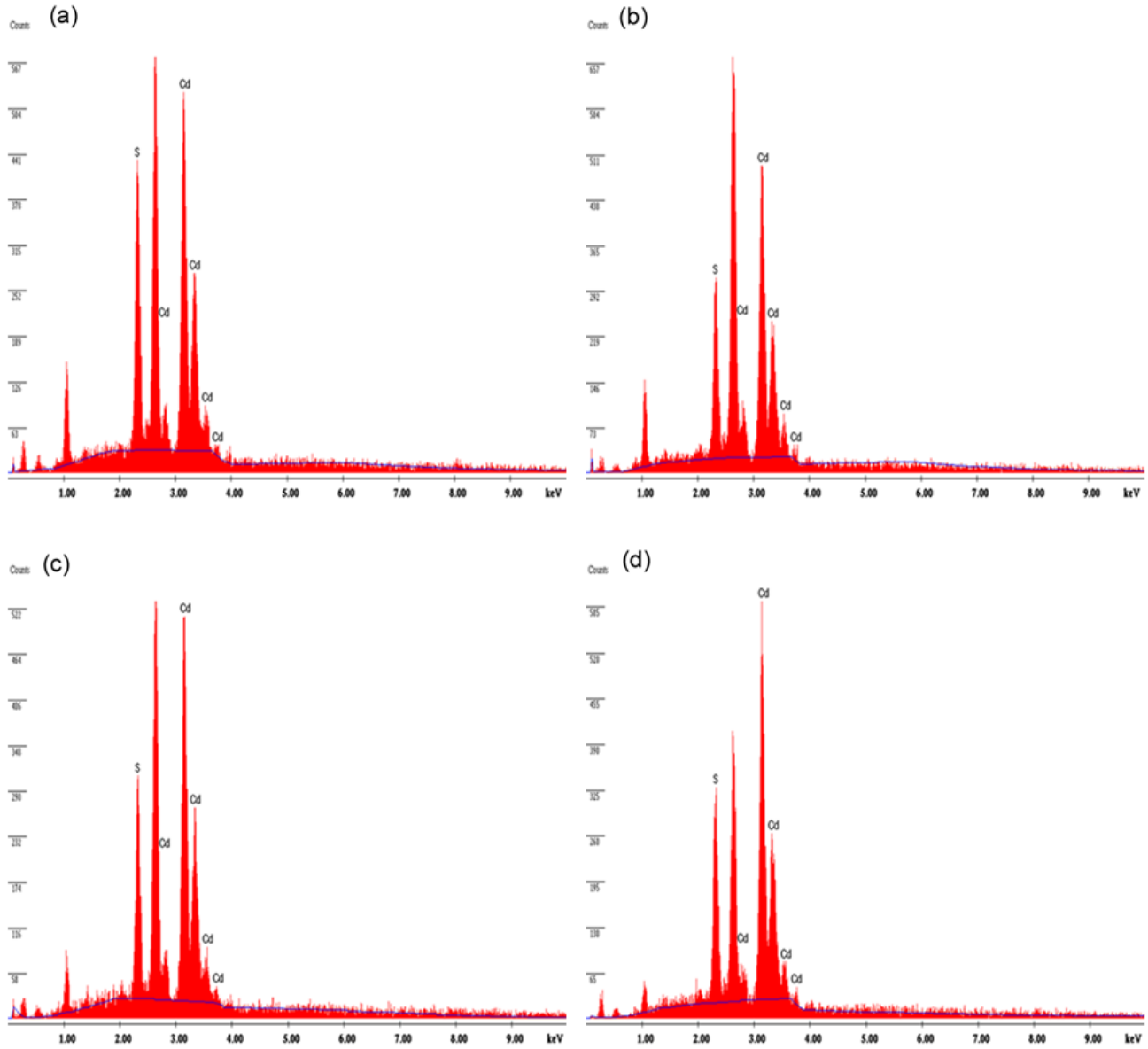

Figure 4. EDX analysis of biosynthesized CdS nanoparticles. Graphs plotted for different ratios 1:1 (a), 2:1 (b), 3:1 (c) and 4:1 (d) shows the presence of Cd and S metals

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR) was carried on CdS nanoparticles to identify the functional groups and the biomolecules responsible for the stability of the reduced sulfide nanoparticles (Figure 5). The FTIR results showed a number of absorption bands in the region 4000 - 400 cm^{-1}. The Figure 5(a) (1:1) shows absorption peaks at 3335.28, 2928.38, 2861.84, 1645.95 and 1405.85. Figure 5(b) (2:1) shows absorption peaks at 3322.75, 2928.38, 2860.88, 1645.95 and 1406.82, Figure 5(c) (3:1) shows peaks at 3324.68, 2928.38, 2856.06, 1647.88 and 1406.82 and Figure 5(d) (4:1) shows peaks at 3307.32, 2923.56, 2855.1, 1655.59, 1536.02, 1406.82 and 1006.66. The peaks occurring from 3335 – 3300 cm^{-1} corresponds to O-H stretching vibrations of the carboxyl group along with N-H stretching vibrations [5]. The N-H stretching vibration is due to the primary and secondary amine linkages of proteins and amino acid residues respectively. The stability of the CdS nanoparticles is brought by the

binding of the proteins to nanoparticles either by free amino groups or through cysteine residues [19,33]. The proteins act as surface coating agents and thus prevent the internal agglomeration of nanoparticles. Three peaks observed at 2928 cm^{-1}, 2861 cm^{-1} and 2855 cm^{-1} corresponds to C-H stretching vibrations of alkanes. The strong peak at 1645 cm^{-1} – 1655 cm^{-1} can be assigned to N-H bending vibrations of amide I and amide II proteins [42]. The CdS nanoparticles of ratio 4:1 Figure 5(d) shows a strong peak at 1536.02 cm^{-1} and 1006 cm^{-1} which corresponds to the C=C bending vibrations of aromatics and C-O stretching of ethers respectively. The peak at 1406 cm^{-1} corresponds to the presence of aromatic groups [18]. The results confirmed that nanoparticles synthesized with the ratio 4:1 showed maximum functional groups compared to other ratios.

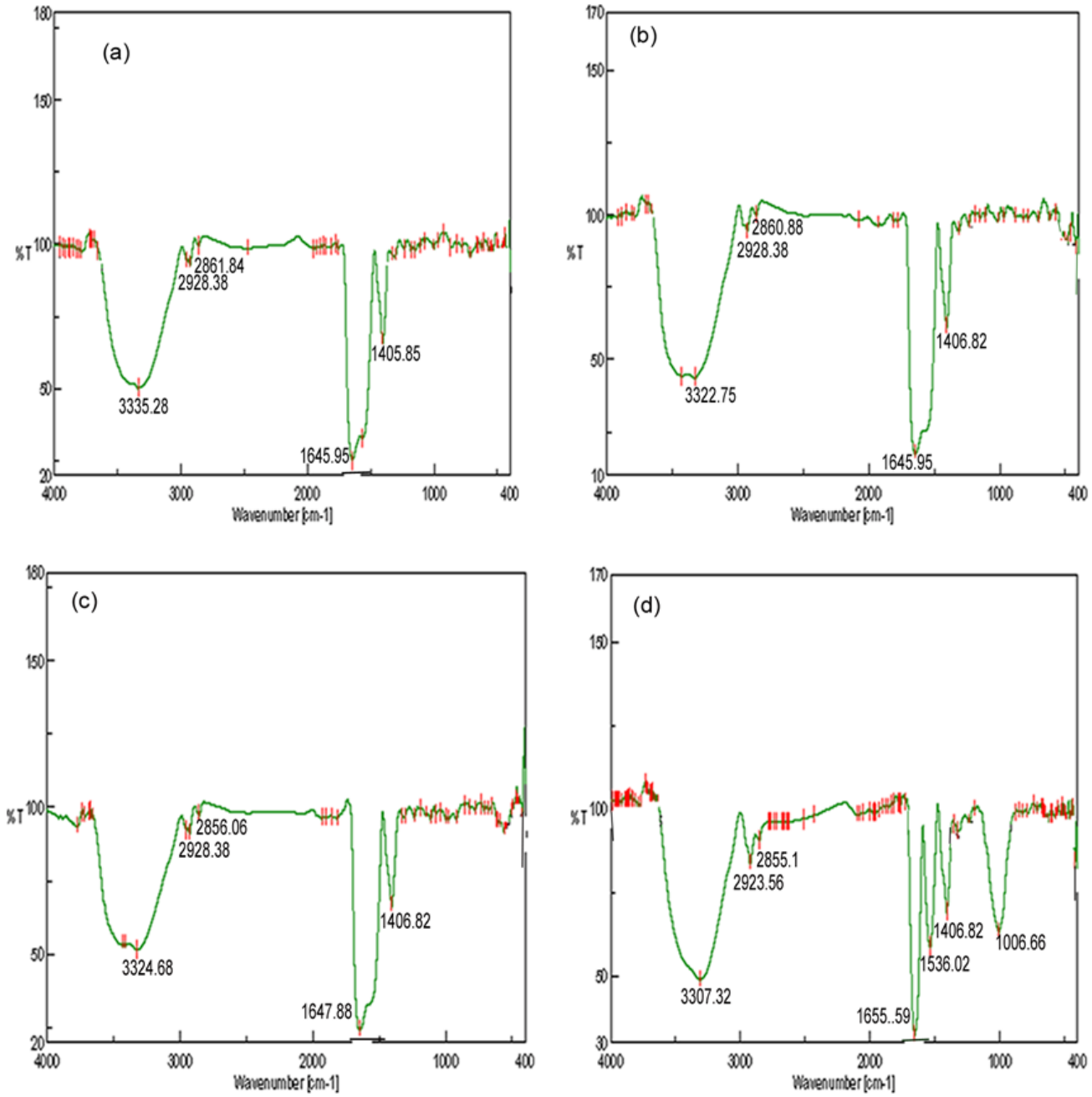

Figure 5. FTIR spectra recorded for CdS nanoparticles of all ratios 1:1 (a), 2:1 (b), 3:1 (c) and 4:1 (d)

3.6. X-Ray Diffraction Analysis (XRD)

The crystalline structure of the cadmium sulfide nanoparticles was characterized by Rigaku, Smart Lab X-Ray Diffractormeter (Figure 6). The diffraction pattern for all four ratios 1:1 Figure 6(a), 2:1 Figure 6(b), 3:1 Figure 6(c) and 4:1 Figure 6(d) was measured at 2θ values with a range varying from (10° ≤2θ ≤ 80°). Strong diffraction peaks were seen at 26.3°, 31.7°, 43.5°, and 51.5° which can be indexed to the (111), (200), (220) and (311) planes of face centered crystalline CdS respectively. These results matched with the peaks of pure CdS crystals published by Joint Committee for Powder Diffraction Standards (JCPDS (ICDD) file number 10- 454 for CdS). The average crystallite size of the nanoparticles was found to be 35 nm which was calculated by full width half maximum (FWHM) of the strongest peak in the plane (200) using the Scherrer's equation:

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

Where λ is the wavelength of X - ray radiation, β is the full width at half maximum (FWHM) in radians, θ is the Bragg angle of diffraction and K is the shape factor. The particles size correlates with the results obtained by SEM analyses. The lattice plane of the CdS nanoparticles showed a spacing (d) of 3.3 nm corresponding to the strongest peak in the plane (200) of the face centered crystalline nanoparticles which correlates to the results obtained by Rodriguez-Fragoso et al. [31] and Malarkodi et al. [18]. The particle size also depends on the reaction time, as the reaction time increases the particle size also increases. But however, the agglomerations of the neighbouring crystals help restrict the particle size to nanoscale range. As a result, the XRD peak intensity decreases and the width of the peak increases with decreasing crystallite size [28]. The effect of changing cadmium chloride ratio did not have an impact on the crystal structure of the nanoparticles.

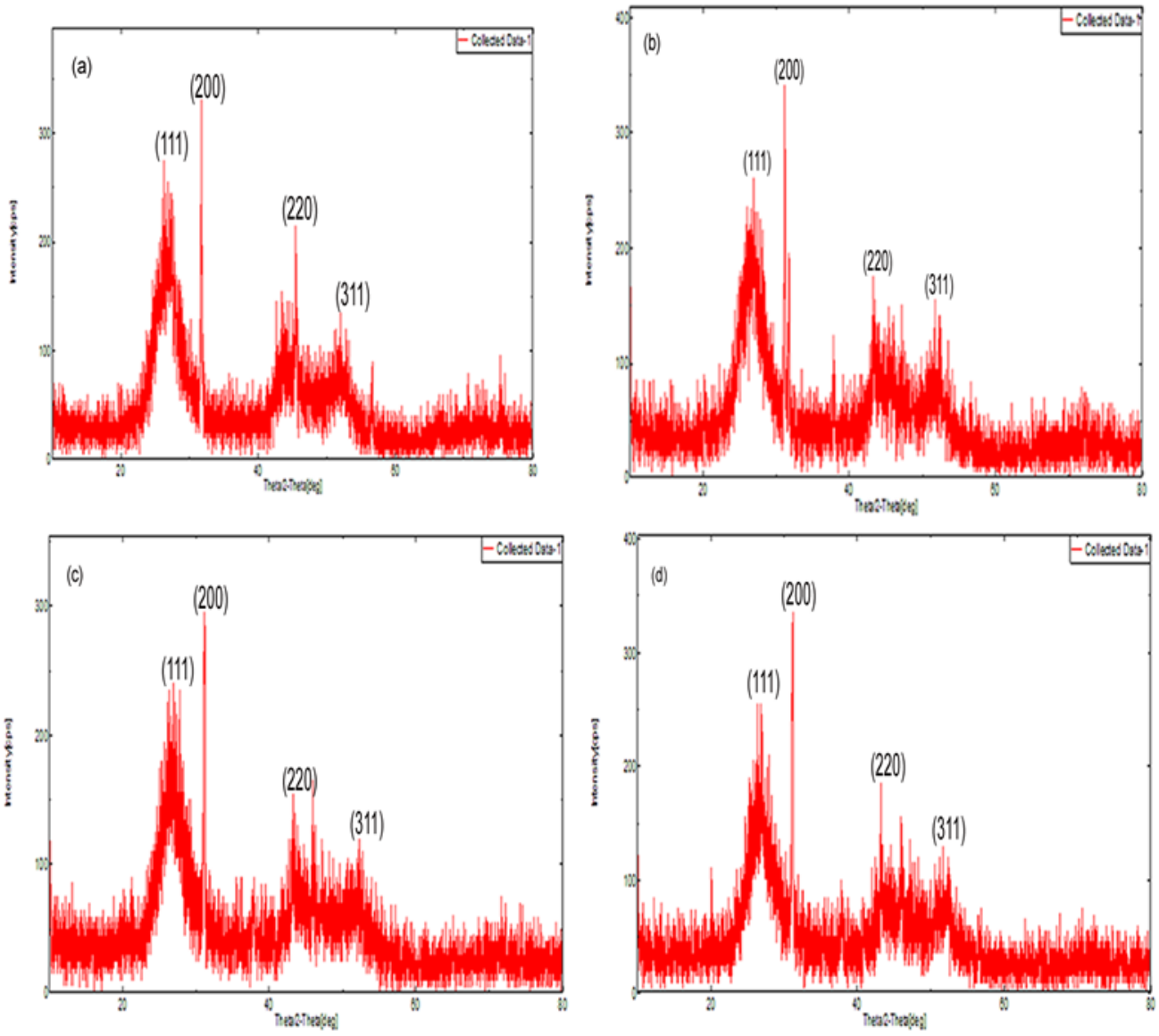

Figure 6. X-Ray Diffraction pattern of powder CdS nanoparticles of ratios 1:1 (a), 2:1 (b), 3:1 (c) and 4:1 (d) showing peaks at 111, 200, 220 and 311 of the lattices planes

3.7. Antimicrobial Activity of CdS Nanoparticles

The antimicrobial activity of the CdS nanoparticles were investigated against food borne pathogens *E coli, Bacillus licheniformis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus*. (Figure 7). A concentration of 40 mg/ml CdS nanoparticles showed highest inhibition on all strains. Table 1 shows the diameter of the zone of inhibition (mm) of all food pathogens. The highest antimicrobial activity was seen in the order of *Pseudomonas aeruginosa* (26.5±0.70) Figure 7(a) followed by *Bacillus licheniformis* (23.5±0.70) Figure 7(b), *Bacillus cereus* (22±0.01) Figure 7(c), *E coli* (19.1±0.14) Figure 7(d) and *Staphylococcus aureus* (18.25±0.35) Figure 7(e). Among the four different ratios 1:1, 2:1, 3:1 and 4:1 of cadmium chloride and sodium sulfide, the highest inhibition was obtained in the ratio 4:1 in all strains. The antimicrobial activity of CdS nanoparticles is due to the fact that the nanoparticles can easily impregnate into the cell wall of the bacteria. The positive charge of the CdS nanoparticles interacts electrostatically with the negative charge of proteins on the surface of the bacterial

cell. The CdS nanoparticles releases ions which interacts with the thiol groups of the proteins in the cell wall which results in the formation of reactive oxygen species resulting in the disruption of cells [29,34,38]. The strong binding of nanoparticles to the proteins causes inhibition of the active transport, dehydrogenase and the enzymatic activity in the periplasmic region and thus inhibits the synthesis of DNA, RNA and proteins which leads to cell lysis [25,30]. In the present study, the highest inhibition was seen in gram negative bacteria. Since gram negative bacteria possess a slender peptidoglycan layer, it facilitates the easy penetration of the nanoparticles into the cell when compared to gram positive bacteria which have a thick peptidoglycan layer. The efficiency of antimicrobial activity also depends on the size of the nanoparticles. Smaller nanoparticles have more surface atoms which gives them a larger surface area for interaction with the bacterial cell. It has also been shown that smaller nanoparticles have larger fractions of atoms on low coordination and high energy sites like corners, edges and steps which makes them more active than larger particles [41]. The antimicrobial activity of CdS nanoparticles were compared against a set of standard antibiotics (Figure 8) (Table 2) Ampicillin (A^{25}), Norfloxin (NX^{10}), Trimethoprim (COT^{25}), Ceftazimidime (CA^{30}) and Cephatoxime (CTX^{30}). *Pseudomonas aeruginosa* Figure 8(a) showed maximum zone of inhibition (34.75±0.35) and (23.5±0.70) to Norfloxin and Ceftazimidime respectively. *Bacillus licheniformis* Figure 8(b) showed highest zone of inhibition to Norfloxin (30.5±0.70), moderate inhibition to Ampicillin (20±2.8) and Trimethoprim (18.5±0.70) and low zone of inhibition to Ceftazimidime (13.5±0.71). *Bacillus cereus* Figure 8(c) showed moderate zone of inhibition to Norfloxin (22±2.12) and low zone of inhibition to Ampicillin (8.5±0.70). *E coli* Figure 8(d) showed highest zone of inhibition to Norfloxin (26.5±2.12) and low zone of inhibition for Ampicillin (12.75±0.35) and Ceftazimidime (8.25±0.35). *Staphylococcus aureus* Figure 8(e) showed highest zone of inhibition in Ampicillin (28.5±2.12) followed by Norfloxin (25.5±0.70), Trimethoprim (23.75±0.35), Ceftazimidime (12.5±0.70) and Cephatoxime (7.75±0.35). It was clear from the study that CdS nanoparticles had better antimicrobial activity on food borne pathogens compared to Ampicillin, Trimethoprim and Cephatoxime which had no effect on *Pseudomonas aeruginosa*, *Bacillus cereus* and *E coli* used in the experiment.

Table 1. Antimicrobial activity of CdS nanoparticles against food borne pathogens

Bacteria	Concentration of Cadmium sulfide nanoparticles (mg/ml)	Volume of the Cadmium sulfide nanoparticles (µl)	CdS nanoparticles in four different ratios Zone of inhibition (mm in diameter)			
			1:1	2:1	3:1	4:1
Pseudomonas aeruginosa	40	30	18.5±0.70	23.75±1.0	24.5±0.7	26.5±0.70
Bacillus licheniformis	40	20	11.5±0.71	19.75±1.0	22±1.4	23.5±0.70
Bacillus cereus	40	20	14.75±0.56	19.65±0.49	20.11±0.14	22±0.01
E coli	40	20	14.5±0.70	18.55±0.70	18.15±0.21	19.1±0.14
Staphylococcus aureus	40	20	14.5±0.71	17.1±0.14	18.5±0.70	18.25±0.35

± Standard deviation.

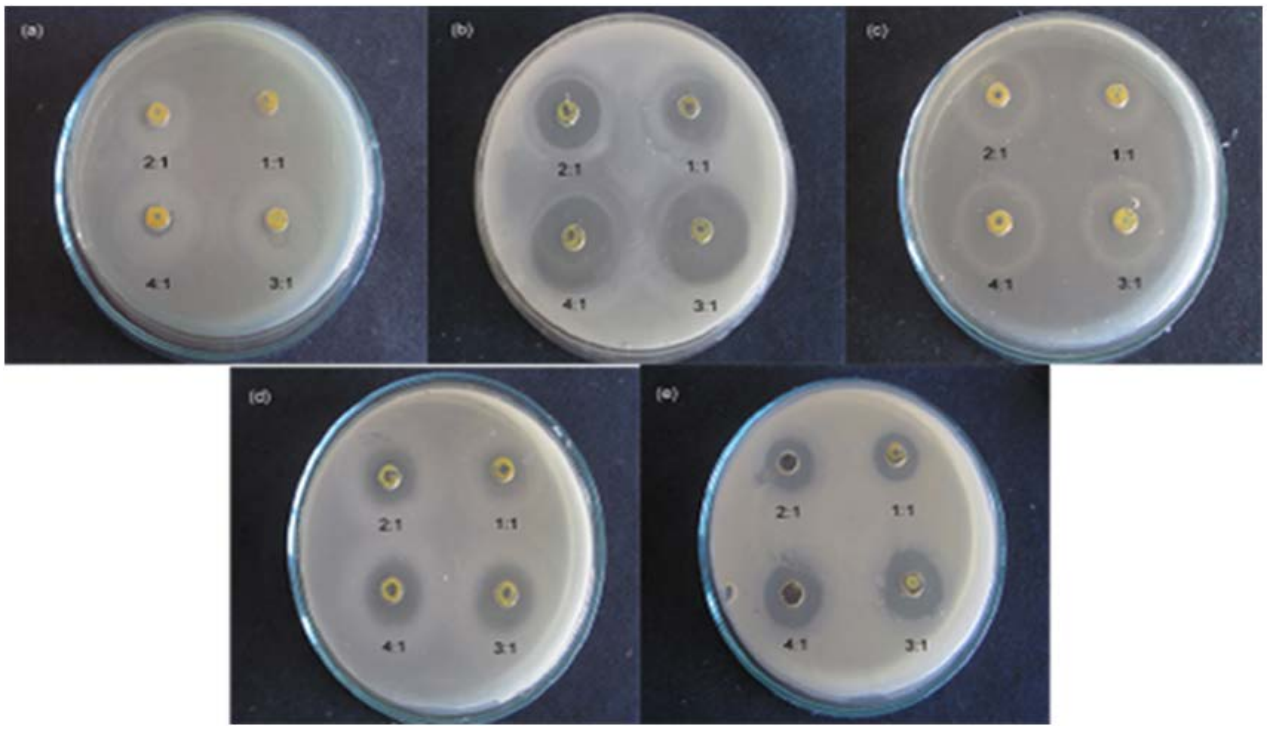

Figure 7. Antimicrobial activity of CdS nanoparticles against five food borne pathogens. (a) *Pseudomonas aeruginosa*, (b) *Bacillus licheniformis*, (c) *Bacillus cereus*, (d) *E coli* and (e) *Staphylococcus aureus*

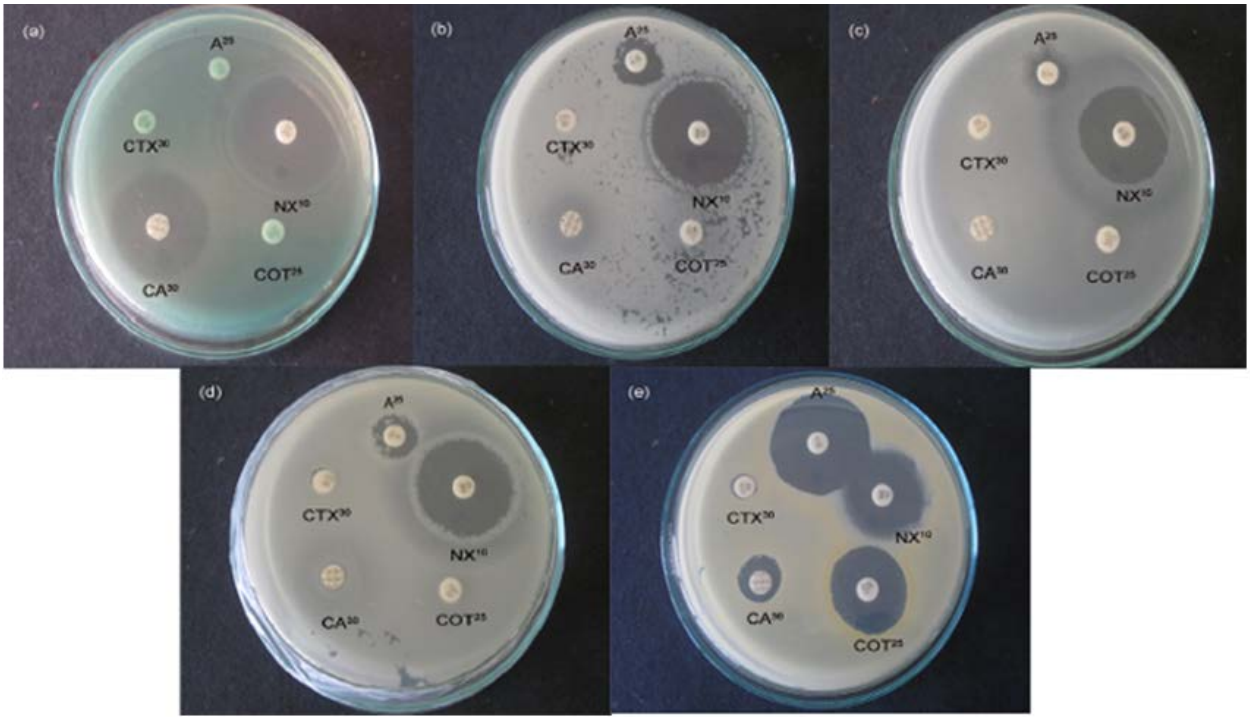

Figure 8. Antimicrobial activity of standard antibiotics against five food borne pathogens. (a) *Pseudomonas aeruginosa*, (b) *Bacillus licheniformis*, (c) *Bacillus cereus*, (d) *E coli* and (e) *Staphylococcus aureus*

Table 2. Antimicrobial activity of standard antibiotics against food borne pathogens

Bacteria	Zone of inhibition (mm in diameter)				
	Ampicillin (A^{25})	Norfloxin (NX^{10})	Timethoprim (COT^{25})	Ceftazimidime (CA^{30})	Cephatoxime (CTX^{30})
Pseudomonas aeruginosa	-	34.75±0.35	-	23.5±0.70	-
Bacillus licheniformis	20±2.8	30.5±0.70	18.5±0.70	13.5±0.71	-
Bacillus cereus	8.5±0.70	22±2.12	-	-	-
E coli	12.75±0.35	26.5±2.12		8.25±0.35	
Staphylococcus aureus	28.5±2.12	25.5±0.70	23.75±0.35	12.5±0.70	7.75±0.35

± Standard deviation.

3.8. Antifungal Activity of CdS Nanoparticles

The antifungal activity of CdS nanoparticles was performed against three fungal strains *Fusarium oxysporum, Aspergillus flavus* and *Penicillium expansum* (Figure 9) (Table 3). A concentration of 40 mg/ml of CdS nanoparticles showed high inhibition on growth of *Aspergillus flavus* (27.8±0.28) Figure 9(a) and *Fusarium oxysporum* (18.5±0.70) Figure 9(b) but however the *Penicillium expansum* with the same concentration did not show inhibition and proved to be more resistant. An increased concentration of 66 mg/ml of CdS nanoparticles showed a zone of inhibition of (15.5±0.70) Figure 9(c) for *Penicillium expansum.* It was observed that the ratio 4:1 of cadmium chloride and sodium sulfide showed highest inhibition in all strains. This may be due to the presence of more CdS nanoparticles in 4:1 ratio when compared with the other three ratios. A study previously conducted by He et al. [9] has shown that the antifungal activity varies depending on the growth morphology of the fungi. The fungi that grow more densely on the surface of the agar medium tend to show more inhibition as they are exposed more to the nanoparticles. The antifungal activity of nanoparticles is believed to affect the cell functions which cause an increase in the nucleic acid content due to the stress response of fungal hyphae. It has also been suggested that there is an increase in the carbohydrate content in the cell which may be due to the self defence mechanism of the fungi against the nanoparticles [1]. Significant exposure to nanoparticles results in the formation of 'pits' on the surface of the cells which ultimately leads to the formation of pores and cell death. Flow cytometric assay conducted to study the physiological changes in the fungal cells showed the nanoparticles inhibited the cellular process involved in budding through destruction of membrane integrity [12,13].

Table 3. Antifungal activity of CdS nanoparticles against food borne fungus

Fungal Strain	Concentration of cadmium sulfide nanoparticle (mg/ml)	Volume of the Cadmium sulfide nanoparticles (µl)	CdS nanoparticles in four different ratios Zone of inhibition (mm in diameter)			
			1:1	2:1	3:1	4:1
Aspergillus flavus	26	20	19.75±0.3	24.5±0.70	25.75±1.0	27.8±0.28
Fusarium oxysporum	40	20	13.8±0.28	15±0.01	17±1.41	18.5±0.70
Penicillium expansum	66	50	4.65±0.49	11.75±1.0	13.5±0.70	15.5±0.70

± Standard deviation.

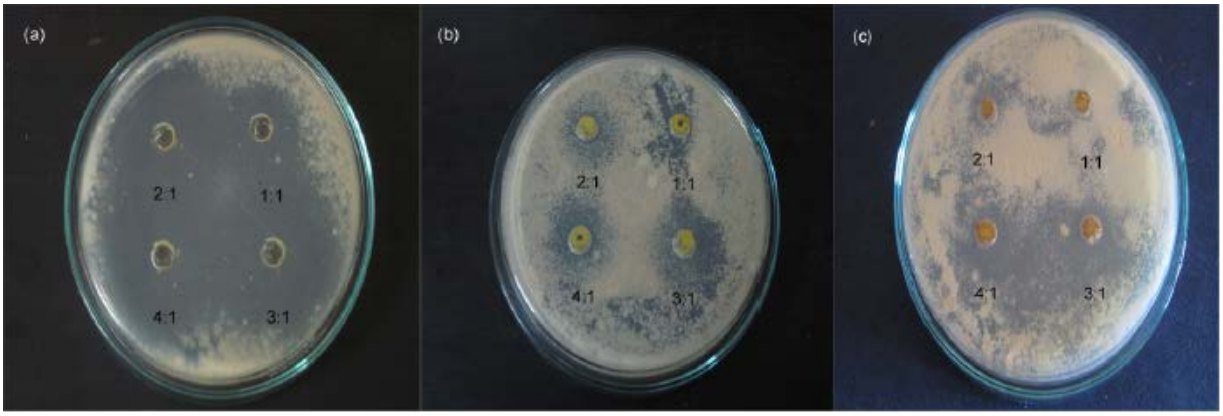

Figure 9. Antifungal activity of CdS nanoparticles against three food borne fungi. (a) *Aspergillus flavus*, (b) *Fusarious oxysporum* and (c) *Penicillium expansum*

4. Conclusions

The present study demonstrates the green synthesis of cadmium sulfide nanoparticles using the bacteria *Bacillus licheniformis* and the effect of varying ratios of cadmium chloride on nanoparticle formation. The synthesized nanoparticles proved to be stable for more than three months in water. The morphological and structural studies showed the nanoparticles were face centered crystals with a lattice plane spacing (d) of 3.3 nm. The FTIR results showed that the proteins might have played an important role as capping agents in stabilizing the CdS nanoparticles. Using varied ratios of cadmium chloride and sodium sulfide showed changes in the formation of functional groups of the nanoparticles which can act beneficial for CdS capping. The CdS nanoparticles possess significant antimicrobial activity against different food borne bacteria and fungi which was successfully demonstrated by well diffusion method. Among the pathogens, the bacteria *Pseudomonas aeruginosa* and fungi *Aspergillus flavus* showed greater inhibition to the nanoparticles compared to other strains used in the experiment. It was noted that highest inhibitory activity in both bacteria and fungi was seen in the ratio 4:1 which had the highest concentration of cadmium chloride. When compared with standard antibiotics, the CdS nanoparticles had higher inhibitory effect on the pathogens. This eco-friendly method of CdS nanoparticle synthesis and their application as bactericidal and antifungal agents makes them potential candidates for food safety applications, biofilms in food packaging and biomedical markers.

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Statement of Competing Interest

The authors declare that there is no competing interests

Ethical Standards

The manuscripts does not contain clinical studies or patient data.

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