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Biosynthesis of fluorescent cadmium sulfide nanoparticles using neem endophytic fungus and evaluation of their anti-proliferative and anti-microbial activities

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ABSTRACT

In the present manuscript, we demonstrate a reliable and eco-conscious approach for the fabrication of technologically important cadmium sulfide (CdS) nanoparticles using neem (*Azadirachta indica*) fungal endophyte, later identified as *Fusarium oxysporum* based on cultural and morphological characteristics. A 10^{-3} M aqueous solution of precursor salt cadmium sulfate ($CdSO_4$) when reacted with endophytic fungus resulted in the bio-construction of copious amounts of well dispersed CdS nanoparticles of 10-40 nm with an average 20 nm size. These bio-constructed CdS NPs were characterized by standard analytical techniques like UV-Visible spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), Fluorescence Spectroscopy (FS), X-ray diffraction (XRD), Transmission Electron Microscopy (TEM), Selected Area Electron Diffraction (SAED) and Energy Dispersive Analysis of X-rays (EDAX). Cytotoxic activity of these nanoparticles was checked against three different cell types viz. human breast cancer (ZR-75-1), Daudi (Human Burkitt's lymphoma) and normal human peripheral blood mononuclear cells (PBMC) where our CdS nanoparticles proved anti-proliferative against cancer cells but safe toward normal cells. Moreover, toxicity assessment toward human RBC revealed less than 0.1 % hemolysis as compared to Triton X-100, thus implying safe nature of our biosynthesized CdS nanoparticles on human cells. Also, our nanoparticles exhibited significant anti-fungal (against *Aspergillus niger*) and anti-bacterial [against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*)] bacteria activity thus suggesting their good potential to be developed as novel therapeutic agents. The present investigation opens up avenues for eco-friendly and reliable fluorescent nanomaterials to be used in a wide variety of application such as in vivo imaging, cell labeling, cell tracking, drug delivery and so on.

Keywords: Anti-microbial, Anti-proliferation, Cytotoxicity, CdS nanoparticles, Endophyte, *Fusarium oxysporum*

INTRODUCTION

Several hundred medicinal compounds are being derived from plants, which is exhausting this valuable resource at a very alarming rate. Attempting to explore a substitute source, researchers discovered that plant-associated endophytic fungi i.e. the ones which exist symbiotically within their host plants, have the capability to mimic the chemistry of their respective hosts and interestingly, can independently generate the same plant-based drugs/ bioactive molecules. This marvelous concept of intergeneric genetic exchange between plants and associated fungi was cemented for the first time when the gibberellin biosynthesis pathway in both the plant associated fungi and higher plants was found to be identical up to GA_{12}. Since then, many endophytic fungi have been employed in synthesis of plant-based bioactive compounds (Gupta and Shukla, 2020; Kumar *et al.,* 2013). As an example, rapid deforestation of *Taxus baccata* tree has halted because the anticancer drug taxol which was previously derived only from this tree is now being synthesized autonomously from the endophytic fungi isolated from the same plant (Sreekanth *et al.,* 2009).

A few years later, our group attempted extending the above concept of solely employing endophytic fungi rather than plants towards the 'green synthesis of nanomaterials' wherein plant parts (Shankar *et al.,* 2004) were and are still largely used. In our previous work, we have demonstrated the synthesis of gold nanoparticles from both the leaves of geranium (*Pelargonium graveolens*) plant and also from the endophytic fungus (*Colletotrichum* sp.) isolated from the same. Although the reduction of metal ions into stable gold nanoparticles was very rapid in both the cases, however, the morphology of the formed nanoparticles was very dissimilar; where the endophyte produced essentially spherical gold NPs while the leaves produced a variety of shapes including rods, flat sheets and triangles (Shankar *et al.,* 2003). Our group is the key contributor to the bio-construction of several nanomaterials using endophytic fungi (Ahmad *et al.,* 2007; Bansal *et al.,* 2006; Bharde *et al.,* 2006; Islam *et al.,* 2021a; Islam *et al.,* 2022; Senapati *et al.,* 2014). This 'biological' method of nanoparticles synthesis outweighs the 'synthetic' chemical and physical protocols as it is non-toxic, occurs at ambient conditions of temperature, pressure and pH, is reliable, rapid and cost-effective. Furthermore, the nanoparticles get capped by natural proteins secreted by the fungus during the process, granting them essential properties like non-agglomeration, water dispersal, high stability and shelf-life, and negating the requirement of any external capping agents which are mostly toxic as observed in chemical and physical synthesis routes.

The past two decades have seen tremendous rise in the studies being conducted up on CdS nanoparticles owing to the very interesting optical and electronic properties exhibited by these semiconductor nanomaterials. The ease with which the optical properties of semiconductor nanoparticles can be tuned during UV-light irradiation simply by altering the nanoparticle size is enticing in various areas of applications like *in vivo* imaging, cell labeling, cell tracking, theranostics, diagnostics and DNA detection (Akshaya *et al.,* 2020; Gu *et*

al., 2007; Hu *et al.,* 2020; Rees *et al.,* 2020; Xiaoke *et al.,* 2020). Thus, CdS nanoparticles are promising candidates for optoelectronic and biological applications as well as luminescent probes and may be developed for fluorescent microscopic techniques with significant advantages over conventional fluorescent dyes. However, semiconductor nanoparticles synthesized by solution-phase synthetic processes tend to easily agglomerate in colloidal solutions are eco-unfriendly, cumbersome and non-uniform in size distribution (Bai *et al.,* 2020; Cargnello *et al.,* 2014; Deng *et al.,* 2012; Feng *et al.,* 2011; Gou and Murphy, 2003; Sonker *et al.,* 2020; Wang *et al.,* 2017; Zhu and Qian, 2009; Zhuang and Peng, 2011). In the first report of its kind (Ahmad *et al.,* 2002), we have already demonstrated the biological extracellular fabrication of CdS nanoparticles of 5-20 nm dimensions by adding wet mycelial mass of *Fusarium oxysporum* to aqueous solution of cadmium sulfate ($CdSO_4$). However, as far as characterization is concerned, we had examined our samples only by UV-Vis, TEM & XRD. Moreover, the nanoparticles weren't checked for any applications whatsoever. Later on, numerous green approaches have been reported for the fabrication of CdS nanoparticles using microbes and mushroom (Prasada *et al.,* 2010; Rajeshkumar *et al.,* 2014; Rao *et al.,* 2017; Sanghi and Verma, 2009; Tudu *et al.,* 2021).

Also, our group has already fabricated gold, silver and core shell nanoparticles using neem (*Azadirachta indica*) leaf extracts (Shankar *et al.,* 2004). Now, in order to shun the several seasonal, geographical and physical barriers which appear while sourcing healthy material for research time and again, we isolated and purified many endophytic fungi from neem leaves gathered from different areas in Pune city, India. A total of 58 endophytic fungi (EA-NEF: 1-58) were isolated and each was screened for the extracellular synthesis of metal sulfide nanoparticles. As the emission of fluorescent nanoparticles is several times more than those of fluorophores, these can be used to replace organic fluorophores for conjugation with biomolecules such as peptides, antibodies and nucleic acids for various applications (Bao *et al.,* 2012). The exploration of newer, more reliable and eco-friendly processes for the synthesis of semiconductor nanoparticles is a crucial step in the emerging field of biomedical nanotechnology; and thus in the present study, we made attempts to fabricate these in a safe and economic manner.

It was found that the culture EA-NEF: 33 when added to aqueous cadmium sulfate ($CdSO_4$) solution, produced profuse amounts of extracellular well dispersed CdS nanoparticles in the size range of 10-40 nm with an average size of 20 nm. The fungus was later identified as *Fusarium oxysporum* based on cultural and morphological characteristics, but appeared texture wise very distinct from our previous encounters which was light-yellow in color and produced a pink pigment in the growth medium. These nanoparticles were completely characterized using standard techniques. Furthermore, we conducted cytotoxicity studies on cancer cells (Human breast cancer: ZR-75-1 and Human lymphoma: Daudi) and normal cells (Human peripheral blood mononuclear cells: PBMC) wherein it was found that our CdS nanoparticles are anti-proliferative against cancer cells and safe towards normal cells. As it is fundamentally established that cancer cells lack DNA repair pathway as compared to normal cells, we are of the opinion that cell repair could be compensated in normal cells in *in-vivo* conditions. Also, evaluation of toxicity toward human RBC revealed less than 0.1 % hemolysis as compared to Triton X-100 implying safe nature of our biosynthesized CdS nanoparticles on human cells.

At present, microbial resistance is a worldwide concern. The efficiency of commercially available antibiotics is on the fall due to the emergence of drug resistant microorganisms. Developing new therapeutic agents with efficacious antimicrobial activity is the crucial need of the hour and thus, the effectiveness of our biosynthesized CdS nanoparticles as antimicrobial agents was scrutinized. Antimicrobial activities of the nanoparticles were carried out against few Gram positive (*Bacillus subtilis* & *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) bacterial strains and as well as fungus (*Aspergillus niger*). Our CdS nanoparticles showed significant antibacterial activity against both Gram positive and Gram negative bacteria, and antifungal activity against *Aspergillus niger*, thus suggesting their potential to be developed as novel therapeutic agents.

MATERIALS AND METHODS

Materials

Cadmium sulfate ($CdSO_4$) was purchased from Sigma-Aldrich and used as received. Stock solution of 10^{-3} M was prepared using distilled water and used for the synthesis of nanoparticles. Components of the media viz. malt extract, yeast extract and peptone belonged to HiMedia (India) while glucose and agar-agar were purchased from Fishers Scientific and used as received.

Biological synthesis of CdS nanoparticles:

The endophytic fungus, EA-NEF-33, was isolated from the leaves of *Azadirachta indica*, maintained on potato-dextrose-agar (PDA) slants at 25°C and was identified to be *Fusarium oxysporum* based on cultural and morphological characteristics (Siddiqui *et al.,* 2016). The fungus was grown in MGYP medium composed of malt extract (0.3%), glucose (1.0%), yeast extract (0.3%), and peptone (0.5%) at 25-28°C, under shaking condition (200 rpm) for 96 h. Mycelia then obtained were washed (by centrifugation at 6000 rpm for 30 min at 15°C) thrice with sterile distilled water under sterile conditions to separate it from culture broth. Harvested mycelia (60 g) were resuspended into 1mM $CdSO_4$ solution in 500 ml Erlenmeyer flasks at pH7 and maintained under shaking condition at 200 rpm. The reaction is carried out for 6 days. The reaction mixture is monitored by visual inspection of changes in the biomass and measurements of UV-visible spectra. After 6th day, the reaction mixture is filtered under sterile conditions using Whatman No.1 filter paper and

lyophilized. The as-synthesized CdS nanoparticles were characterized using TEM, SAED, EDAX, FTIR, XRD, etc. Anti-microbial activity of the CdS NPs against bacteria (both Gram positive and Gram negative) and fungus was assessed alongwith cytotoxicity studies on cancer and normal cells.

Characterization of CdS nanoparticiples

UV-Vis spectroscopy: The bioreduction of $CdSO_4$ in solution was monitored by periodic sampling of aliquots (1 ml) of the aqueous component and measuring the UV-visible spectra of the solution. UV-visible spectra of these aliquots were monitored as function of time of reaction using Perkin Elmer UV-vis-NIR spectrophotometer (Lambda 750) operated at a resolution of 1 nm.

Fluorescence spectroscopy: Fluorescence spectrum of aliquots of reaction mixture was recorded on a Perkin Elmer LS 55 fluorescence spectrometer, with a slit width of 10 nm and excitation at 380 nm.

Transmission electron microscopy (TEM): Samples for TEM analysis were prepared by drop coating biosynthesized CdS NPs solution on carbon-coated copper TEM grids. The film on the TEM grid was allowed to stand for 2 minutes, after which the extra solution was removed and the grid was allowed to dry prior to measurement. TEM measurements were performed on a FEI Technai G2 system operated at an accelerating voltage of 200 kV at room temperature. The Selected Area Electron Diffraction (SAED) analysis and EDAX was carried out on the same grid.

X-ray diffraction (XRD) measurements: To analyze the crystallinity of biosynthesized CdS nanoparticles, thin films of the nanoparticles were drop casted on glass substrates and then subjected to X-ray diffraction analysis and data was recorded on Panalytical 'X' Pert PRO system operating at 40 kV and at a current of 30 mA with CuK_α radiation (=1.5404 Å).

Fourier transform infrared (FTIR) spectroscopy: The biological synthesis of nanoparticles involves protein mediated synthesis in reaction mixture. These secreted proteins cap the nanoparticles and thus make them water dispersible, and to further confirm this finding, FTIR analysis was performed on a Bruker Tensor 27 FTIR-ATR operated in the diffuse reflectance mode at a resolution of 4 cm^{-1}. To obtain good signal to noise ratio, 40 scans of the sample were taken in the range of 650-4000 cm^{-1}.

Antimicrobial activity: The bacterial cultures *Bacillus subtilis* NCIM 2063, *Staphylococcus aureus* NCIM 2079 and *Pseudomonas aeruginosa* NCIM 2200 used for the antimicrobial activity were from our in-house culture collection unit, the National Collection of Industrial Microorganisms (NCIM), Pune, India and the fungal culture *Aspergillus niger* was from our lab itself. Evaluation of antimicrobial activity of CdS NPs was carried out by filter paper bioassay (Islam *et al.,* 2021b). For antibacterial activity, bacterial cultures were inoculated in nutrient broth and incubated at 37°C for 24 h. From the actively growing bacterial culture broth, 100 µL (0.1 ml) of bacterial suspension (with a concentration of 10^5 CFU/ml) was mixed with half strength nutrient broth (0.9 ml) and was immediately overlaid on the surface of sterile nutrient agar plates (90 mm diameter) and incubated at 37°C for some time for initial growth. The same method was followed for fungal cultures where MGYP agar plates were used instead of nutrient agar. Sterile filter papers (Whatman No.3: 1 cm square) were placed on agar plates and then loaded with 100µL suspension of nanoparticles of different concentrations These plates were incubated for 24 h (48 h in case of fungus) and visually monitored for zone of inhibition. Filter paper disc on nutrient agar plate without nanoparticles suspension was used as control. After incubation, the zone of inhibition was measured in millimeters across the filter paper.

Cytotoxicity studies

Cell culture and reagents: Human breast cancer (ZR-75-1) and human lymphoma (Daudi) cells were obtained from NCCS, Pune and were grown in RPMI media, respectively supplemented with 10% fetal bovine serum (cRPMI) and 100 µ/ml Penicillin and Streptomycin (Invitrogen). All cells were grown in humidified atmosphere in 5% CO_2 and 95% air at 37°C. MTT dye was purchased from Himedia, India.

Cell viability assay: ZR-75-1 and Daudi cells (2x104) were seeded in 96 well plates. Cells were treated with biosynthesized CdS NPs for 24h with indicated concentration. Cell survival was determined by the modified MTT method (Mosmann *et al.,* 1983). Treatment was terminated by removing media and MTT [3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide] (0.2 mg/ml) was added. After 4 h, formazan crystals were dissolved in DMSO and absorbance was recorded at 570 nm.

Preparation of peripheral blood mononuclear cells (PBMC): Human PBMC were separated from heparinized whole blood obtained from two healthy volunteers by Ficoll-paque gradient centrifugation (Boyum, 1968). Interface cells were collected and washed three times with PBS (pH 7.2), counted and resuspended in cRPMI medium. The protocol of the study was approved by the Ethics Committee of the University of Pune (Ethics-UoP/2012/19). Written informed consent was obtained from each healthy donor.

Cytotoxicity assay: PBMC suspended in cRPMI (2x104 cells/ml) were seeded in 96-well microtiter plates. After 30 min, cells were treated with CdS NPs for 24h with indicated concentration added to the individual wells, in triplicate, except the wells for control (medium alone). Ethanol was used as a vehicle control. Cell survival was determined by the modified MTT method of Mosmann *et al.* (1983). Briefly, after the treatment with CdS NPs for 24 h, MTT solution (3-4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) was added to each well. Samples were incubated for a further 4 h, followed by the addition of 100 µl of DMSO. Absorbance at 570 nm was measured.

Haemolysis assay: Percent haemolysis of human RBCs (erythrocytes) after treatment of CdS NPs was assessed by haemolysis assay as per US FDA Guidelines (Rockville, 2005). RBCs were obtained from whole heparinized blood of two healthy volunteers, and repeatedly washed with saline (pH 7.4). Thereafter, 1% RBC suspension was prepared and the subsequent erythrocyte suspension was incubated at 37°C for 45 min with different concentrations of the CdS NPs or respective positive controls (0.1% Triton X-100) and vehicle control (50µl/ml Ethanol). Following incubation, the samples were centrifuged at 400 xg for 5 min and the absorbance of the supernatant was read at 540 nm. Percent haemolysis was determined following comparison with 100% lysed erythrocytes.

Statistical analysis: The data reported in cytotoxicity and hemolysis experiments are expressed as mean±S.E. Statistical differences were determined by Student's t test. The p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The fungus EA-NEF-33 was identified based on cultural and morphological features as *Fusarium oxysporum* in the laboratory (Nelson *et al.,* 1994). The fungus when incubated with aqueous solution of 1mM $CdSO_4$, at pH 7 and 25ºC temperature for 7 days under shaking condition on a rotary shaker (200 rpm), resulted in the extracellular production of water dispersible CdS (cadmium sulfide) nanoparticles (**Fig. 1**). The visual change and optical characteristics indicates the formation of CdS nanoparticles in reaction mixture. The fungus reduced and capped the nanoparticles surface but to further probe the extracellular nature of the nanoparticles, we have filtered the aqueous solution from biomass, thereby clearly indicating the extracellular nature of the particles. The biogenic nanocrystals are capped naturally by protein layer, which in turn provides water dispersibility and stability toward aggregation thus maintaining the size of the nanocrystals for long time. **Fig. 2A** shows glass vials containing precursor solution i.e. 1mM $CdSO_4$ (left) and filtrate solution containing CdS nanoparticles (right) under day light (A). The same under UV light are shown in **fig. 2B**. The color change from colorless to yellow (at room temperature) which shows green luminescence when illuminated by UV lamp (365 nm) clearly indicates the generation of CdS nanoparticles.

Bioreduction of Cd^{2+} and SO_4^{2-} ions take place after exposure to fungal biomass. Optical properties of the nanoparticles were monitored by UV-Vis spectroscopy. **Fig. 3A** shows UV-Vis spectrum of the CdS nanoparticles, the spectrum shows CdS band at ~370nm and also provides protein signature (270 nm). These nanoparticles show emission band centered at ~450nm when excited at 380 nm (**Fig. 3B**), thus indicating their remarkable potential to be developed as an imaging and diagnostic agent.

Fig. 1: (A) Fungus EA-NEF-33/*Fusarium oxysporum* (B) Precursor solution (1mM $CdSO_4$), (C) Precursor solution with fungus, (D) Flask showing CdS generation (yellow color)

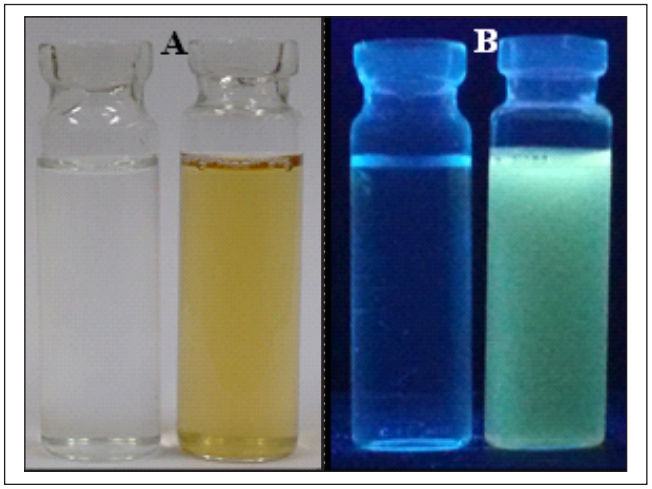

Fig. 2: Glass vials: A) Precursor (left) and biosynthesized CdS nanoparticles (right) under day light. B) The same vials illuminated with UV lamp of 365 nm whereby CdS nanoparticles emit green fluorescence.

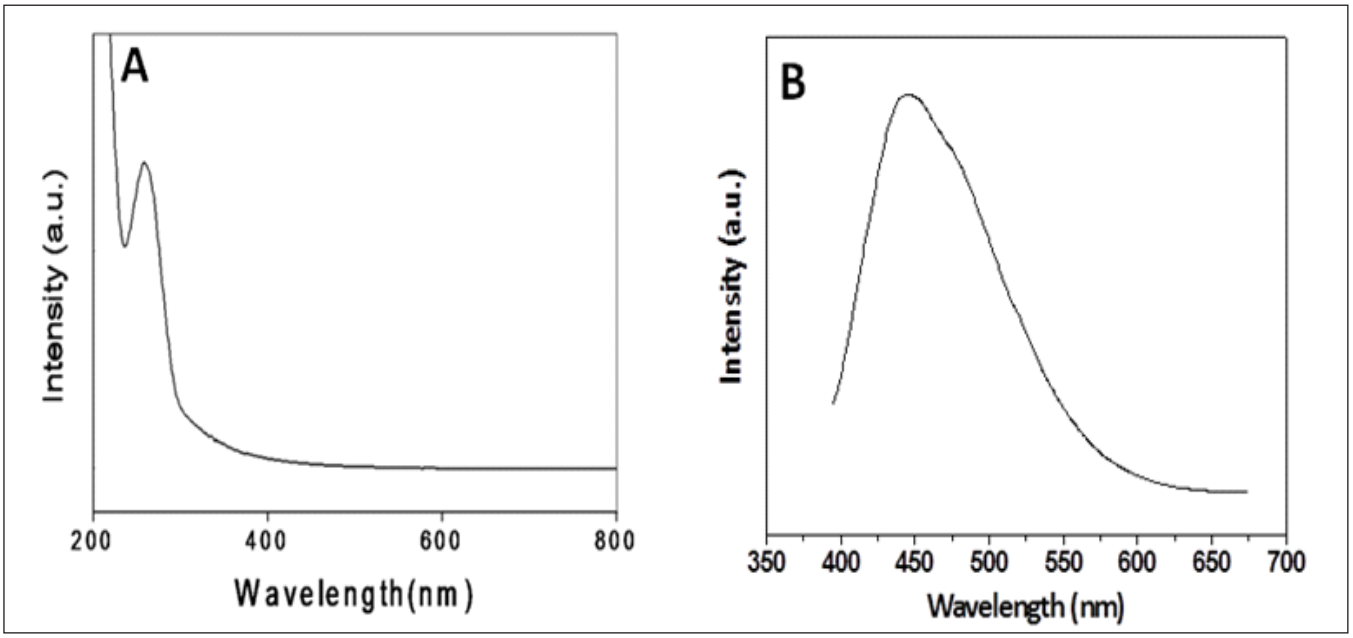

Fig. 3: (A) UV- Visible spectrum and (B) Fluorescence measurement of biosynthesized CdS nanoparticles.

TEM analysis was performed to monitor the size and shape of the CdS nanoparticles (**Fig. 4A**). The biosynthesized nanoparticles exhibit an overall quasi-spherical morphology. Particle size of the nanoparticles (**Fig. 4B**) was measured from the TEM images. The graph shows that nanoparticles are in the size range of 10-40 nm and average size is 20 nm. The distinct, individual particles formed in the as-prepared reaction mixture may have given way to a more aggregated structure in which the individual particles are barely discerned. These nanoparticles are capped by secreted protein in reaction mixture and are very well dispersed as evident from the TEM micrograph. The SAED (**Fig. 4C**) pattern shows crystalline nature of the nanoparticles and EDAX (**Fig. 4D**) analysis was utilized to determine the sample composition. The spectrum shows signals from C,N,O,Cd and S. The signals C,N, and O are originating from the biomolecules that caps the nanoparticle surface.

The crystal structure of the biosynthesized CdS nanoparticles was evaluated by XRD analysis (**Fig. 5A**). The biosynthesized CdS nanoparticles obtained was amorphous in nature due to the presence of organic entities in the powder sample. Hence to get the Bragg's reflection, the powder sample containing CdS nanoparticles was calcined at 350^{o}C for 4h. After calcination Bragg's reflection were obtained which corresponded to planes (100), (101), (102) and (110) which were indexed with the JCPDS card number (80-0006). The Bragg's reflections in the XRD pattern show that the CdS nanoparticles are crystalline in nature. The peak broadening shows the nanoscale size dimension of these nanoparticles. Fungus when subjected to aqueous $CdSO_4$ secreted the biomolecules (mostly proteins) which reduced and stabilized the precursor salt solution. To further confirm the presence of the protein layer, we conducted FTIR analysis of the CdS nanoparticles; the results show the presence of Amide I and Amide II bands (**Fig. 5B**) centered at 1646 and 1549 cm^{-1} (Gole *et al.,* 2000).

Antimicrobial activity of the CdS nanoparticles

Antibacterial and antifungal activity of the CdS nanoparticles synthesized using EA-NEF-33 was carried out against Gram positive (*Staphyllococcus aureus* and *Bacillus subtilis*) and Gram negative (*Pseudomonas aeruginosa*) bacterial and fungal strain (*Aspergillus niger*). Varying concentrations of the nanoparticles were used in the assessment. The filter paper bioassay for antimicrobial activity depicted a clear zone of inhibition which developed around the filter paper disc against the bacterial growth of both Gram-positive and Gram-negative bacteria. Zone of inhibition was even obtained in case of the fungus *Aspergillus niger* (**Fig. 6**). As our CdS nanoparticles have both antibacterial and antifungal activity, thus it can be stated that they have a broad spectrum inhibitory action.

Cytotoxicity studies

Cytotoxicity studies indicated enhanced sensitivity of Daudi cells to CdS nanoparticles as compared to ZR-75-1 cells. CdS nanoparticles exhibited significant toxicity in Daudi cells from concentration of 300-500 µg/ml and induced about 50% cell death at 500 µg/ml concentration. CdS nanoparticles had relatively less effect on ZR-75-1 cells at a lower concentration of 5µg/ml. These studies suggest that CdS nanoparticles show significant and cell specific anti-proliferative activity. ZR-75-1 and Daudi cells were treated with CdS nanoparticles at

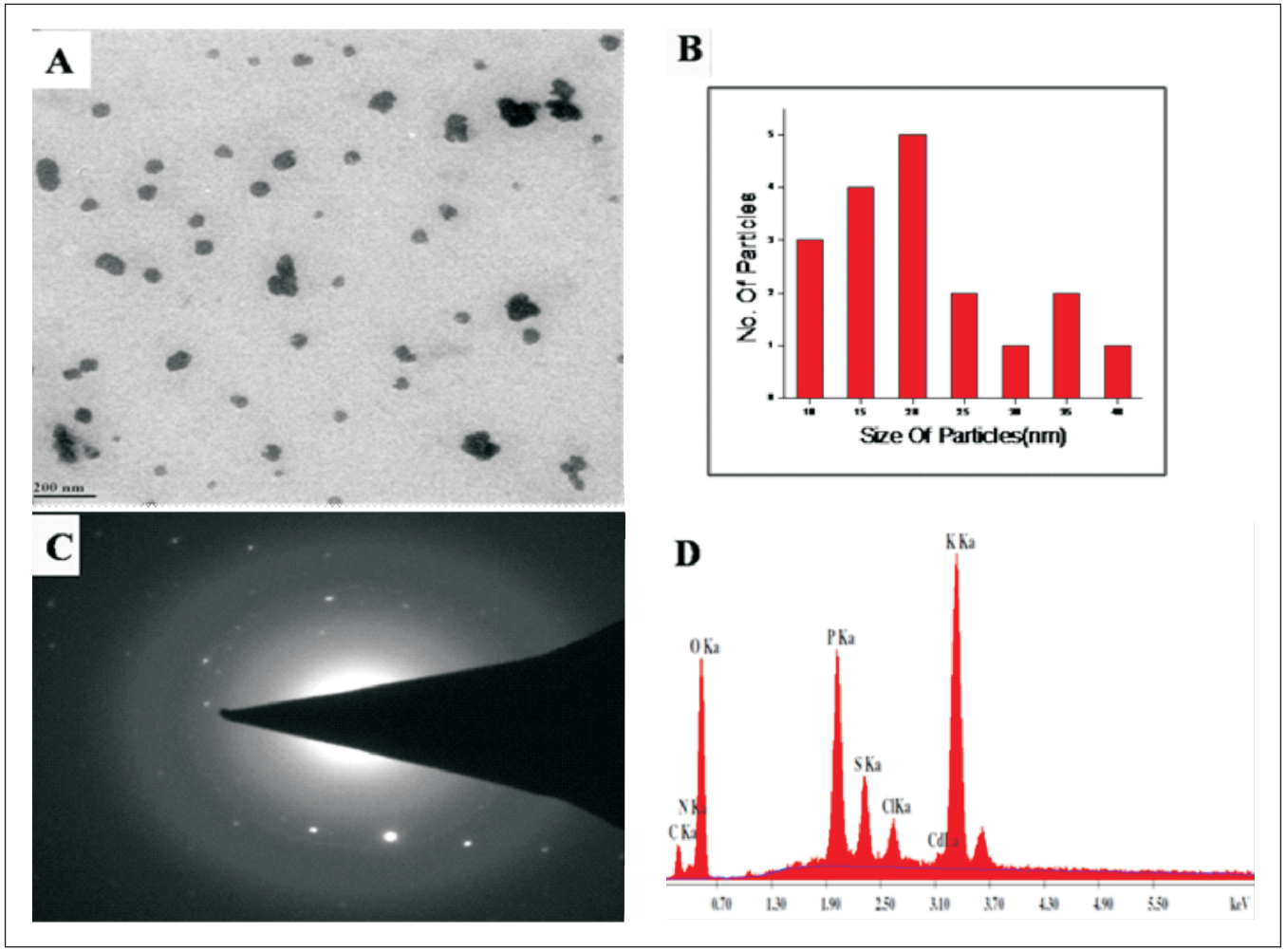

Fig. 4: (A) TEM image, (B) Particle size histogram (C) SAED image and (D) EDAX image of so-formed CdS nanoparticles

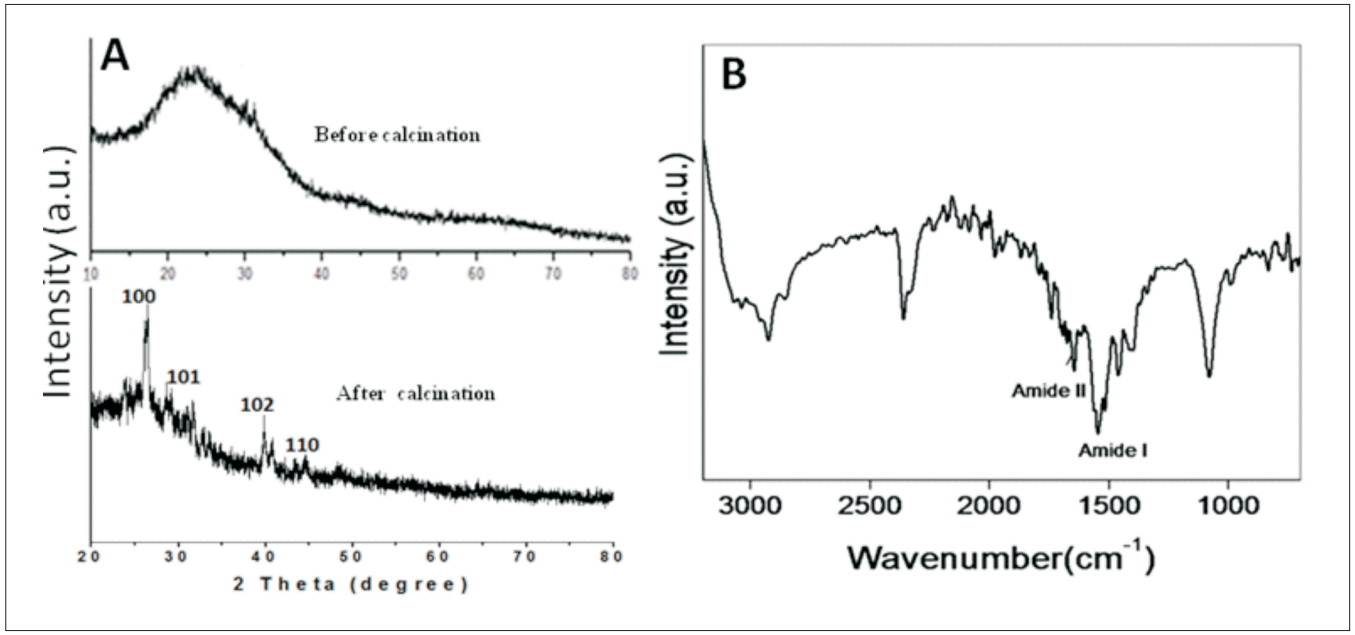

Fig. 5: (A) XRD pattern and (B) FTIR spectrum of biosynthesized CdS nanoparticles.

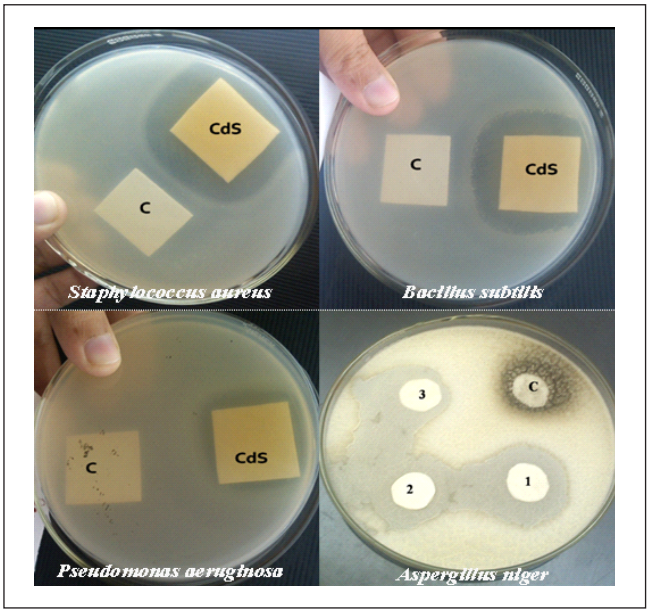

Fig. 6: Filter paper bioassay: Antimicrobial activity of CdS nanoparticles against Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Pseudomonas aeruginosa*) bacteria; and antifungal activity against fungus *Aspergillus niger.*

varying concentrations from 5-500 µg for 24 h and cell viability was determined by MTT assay wherein it was observed that these biosynthesized nanoparticles were anti-proliferative at varying concentrations. At higher concentrations of CdS nanoparticles, the cell death observed was around 50-60% (**Fig. 7A**)

Percent cytotoxicity and hemolytic activity

Peripheral blood mononuclear cells (PBMC) showed more than 90% viability when treated with 5 to 400 µg concentration of CdS nanoparticles. There was a dose dependent decrease in viability at 5, 50, 100, 200, 300, 400 and 500 µg concentrations. However, the percent viability was steady from 85% to 88% in the above mentioned concentrations. The graph (**Fig. 7B**) concludes that up to 50 µg, our biosynthesized CdS nanoparticles were not toxic to the PBMC. Therefore these nanoparticles were deemed safe for normal cells at 50 µg. RBC was treated with CdS nanoparticles at varying concentrations from 5-500 µg for 24 h and hemolysis was measured at 540nm. No significant toxicity was observed up to 50 µg and therefore our biosynthesized CdS nanoparticles were considered safe (i.e. no hemolytic activity was observed at 50 µg).

CONCLUSIONS

The aim of the present study was to synthesize well dispersed cadmium sulfide (CdS) nanoparticles, to be used in cell labeling, cell tracking, targeted drug delivery, diagnostic and therapeutic applications; with properties such as bio-compatibility, high stability, non-toxicity, etc. The CdS nanoparticles which we have synthesized using the endophytic fungus *Fusarium oxysporum* isolated from neem

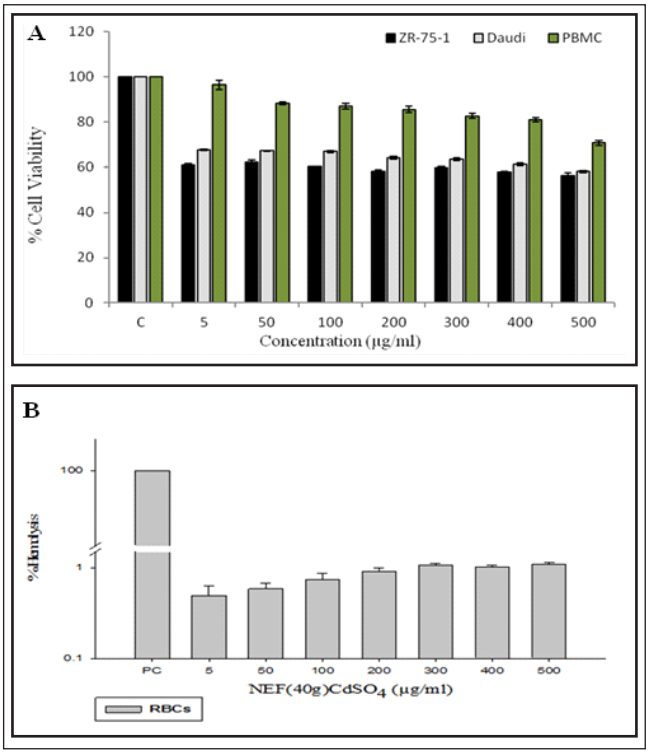

Fig. 7: A. Percentage cell viability of cancer cells (ZR-75-1 and Daudi) and normal human PBMC, and B. Percent Hemolysis of CdS nanoparticles

(*Azadirachta indica*) leaves are water dispersible, natural protein capped and highly stable at room temperature. As these inorganic nanoparticles are in the size range of 10-40 nm, these may find various applications in targeted drug delivery systems without chances of toxicity as owing to their small size, and these may easily pass through the kidneys and be excreted through urine. Cytotoxicity studies suggested that our CdS nanoparticles are anti-proliferative against cancer cells and safe towards normal cells. Moreover, these nanoparticles exhibited broad spectrum antibacterial as well as antifungal activity. Therefore, our CdS nanoparticles can serve as a new antimicrobial agent at a time when drug resistance is increasing at an alarming rate. Since our biosynthesized CdS nanoparticles are fluorescent and non-toxic in nature, these may be applied for *in-vitro* and *in-vivo* imaging and can no doubt completely replace the toxic organic dyes which are currently being used in imaging and diagnostics. Thus an efficient, eco-friendly and non-toxic method for the synthesis of extracellular CdS nanoparticles was established which can have wide-ranging applications in various fields such as therapeutics, theranostics, electronics, catalysis, advanced materials, energy and related sectors.

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