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Murraya Paniculata Mediated Synthesis Of CdS Nanoparticles For Potent Biomedical Applications

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Abstract

The leaf extract of Murraya paniculata (MPL) is used to create cadmium sulphide nanoparticles, or CdS NPs and the reducing and stabilizing agent is plant extract. A crucial capping agent in nano production is played by phytochemicals. Analytical methods such as XRD and FTIR are used to characterize CdS NPs. The biomedical applications of prepared CdS NPs were examined, including their anticancer, antifungal, and antibacterial properties. The antibacterial performance was carried out with S.flexneri, C.perfringens, S.typhimurium, and E.faecalis which were all susceptible to the antibacterial action of CdS NPs. At 150µg/ml, S.flexneri and E.faecalis showed a maximum zone of inhibition is 20mm. In addition, A.niger and C.albicans were used to test the antifungal activities, results shows the concentration of 400 µg/ml CdS NPs inhibited the growth of A.flavus (16mm). By using the NRU assay, it was found that the biosynthesized CdS NPs exhibited cytotoxic action against the MCF-7 cell line. Analysis using the NRU assay revealed that treating cell lines with increasing concentrations of NPs had lethal effects. The 24-hour treatment's IC_{50} was found to be 153.2 µg/ml.

Keywords: Murraya paniculate, Cds nanoparticles, biomedical applications, Antibacterial, Antifungal, Cytotoxic activity

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1. Introduction

One technology that is gaining popularity around the globe and has a big impact on pharmacology and medicine is nanotechnology [1]. Nanotechnology produces extremely small-scale particles called nanoparticles (NPs) that exhibit many characteristics in the fields of drug delivery, diagnostics, nanomedicine, biomarkers, cell labeling, and antibacterial and anticancer agents [2–5]. Researchers discovered that the properties of the NPs, which are generally classified as organic, inorganic, and carbon-based particles, are improved in comparison to bigger sizes of the pertinent materials [6, 7].

There are many different kinds of metallic nanoparticles (NPs): gold, silver, alloys, magnetic, etc. [8, 9]. Cadmium (Cd) is a metal with special qualities, including high malleability, high electrical conductivity, and resistance to corrosion [10, 11]. It has been established that Cd is toxic to humans and that it has no biological purpose [12]. The antioxidant defense system may be compromised by reactive oxygen species (ROS) generated as a result of oxidative stress caused by Cd bioaccumulation in human tissues. This can then result in a number of health issues [13]. Furthermore, because of its superior optical and electrical qualities, photocatalytic activity, and reduced toxicity compared to Cd, cadmium sulfide (CdS) is a fluorescent substance that is utilized in medicine [14–16].

Different kinds of CdS NPs are produced by physical, chemical, and biological processes. The properties of the synthesized NPs, such as size, shape, and surface charge, which depend on their synthesis method, determine the application of CdS NPs [11]. Biosynthesised CdS NPs are more frequently employed in the medical sciences due to their superior compatibility with biological systems and decreased toxicity [17]. CdS NPs are used as medications and diagnostic tools in vivo and in vitro models due to their non-toxicity as well as their antioxidant, antimicrobial, anticancer, imaging probe, and drug delivery capabilities [18–21].

This study focuses on the characterization and environmentally friendly synthesis of CdS NPs using Murraya paniculata plant extract. We have also concentrated on a few particular biomedical uses of CdS NPs, including their antimicrobial, antifungal, and anticancer properties.

2. Experimental

2.1 Chemicals and Reagents

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

Murraya paniculata (MPL) plants were collected, and their dried leaves were repeatedly cleaned with distilled water to get rid of any dust and debris. They were then ground into a fine powder, dried under shade, and kept in an airtight glass container. As previously mentioned, the Aegle marmelos bark (AMB) and Citrus nobilis peel (CNP) were also gathered, powdered, and stored.

One gram of powder was added to a 250 mL Erlen Meyer flask along with 100 mL of Milli-Q water, and the mixture was heated to 60° for 25 minutes to create 1% aqueous leaf, bark, or peel extract. Next, whatman paper was used to filter the extract after it had cooled.

The filtrate was used in a subsequent procedure after being refrigerated at 4°C.

2.2 Preparation of cadmium sulphide nanoparticles

In 50 mL of cadmium chloride (0.1 M) solution, 5 mL of leaf (or) bark (or) peel extract was added. Under magnetic stirring, sodium sulfide (50 mL, 0.1 M) dissolved in de-ionized water was added dropwise to the cadmium chloride solution. Later, the contents were placed on a rotatory orbital shaker set to 200 rpm and 30℃ for 12 hours in dark solution. The particle formation was monitored by sampling an aliquot (3 mL) of the mixture after 12 hours and measuring the UV-Vis spectra with a spectrophotometer. The optical density of the content is monitored from wavelength 250-700 nm to determine the absorption maximum.

2.3. Characterization techniques

The crystallite sizes and phase structures of the prepared materials were investigated using the RIGAKU powder X-ray diffractometer with Cu-Kα radiation and applied current, accelerating voltage, and wavelength maintained at 30 kV, 40 mA, and 1.54, respectively.

Fourier-Transformation Infrared Spectrometer (Bruker ALPHA-E) with a resolution of 4 cm1 was used to investigate the functional groups present in prepared materials. 2.4 Antimicrobial assay Nutrient agar medium (High-Media) was dissolved in water in a 100 mL conical flask and autoclaved at 121°C, 15lbs for 15 minutes before being poured into sterilized petri plates. As a positive control for antibacterial activity, chloramphenicol was used. Agar well diffusion was used to test the antibacterial activity of plant extract [22]. With a sterile glass spreader, inocula were spread across the surface of agar plates. Using a sterile cork borer, four wells were made at equal distances. The plant extract was made to a final concentration of 100mg/mL to test its antibacterial activity. Aliquots of the compound (80µg/mL, 100µg/mL, and 150µg/mL) were poured into each well, and the plates were incubated for 24 hours at 37°C in an incubator. The diameter (mm) of the clear inhibitory zone that formed around the well was also measured.

2.6 Cytotoxic Effects

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

3. Results and Discussion

3.1 XRD analysis

X-ray diffraction (XRD) analysis was adapted to study the crystal structure and phase of the synthesized CdS NPs. The diffraction peaks for CdS NPs are obtained from the XRD micrograph. The diffraction peaks were noticed as 26.6^0, 44^0, and 51.8^0, for CdS at 2θ values, therefore revealing a hexagonal wurtzite structure while being identical to the (JCPDS No.: 01-089-0511). The XRD pattern of CdS NPs, indicates high crystalline which can be seen from the diffraction peaks.

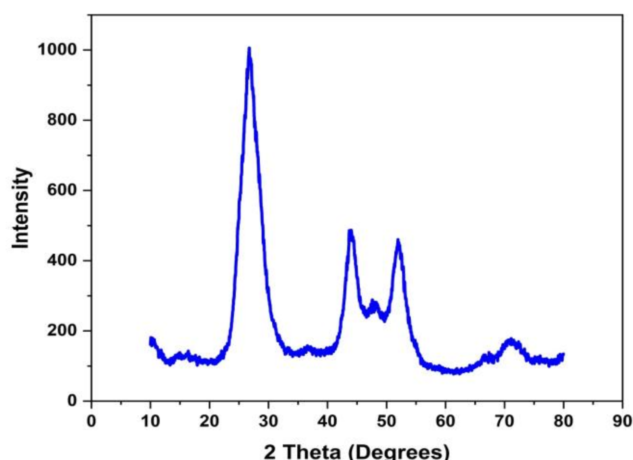

Fig.1: X-ray diffraction analysis of CdS-NPs

3.2: FTIR

For studying the behaviour of the chemical bonds present in the synthesized samples, the FTIR spectrum of CdS NPs was captured from 400 to 4000 cm^{-1}. The sharp peak was noticed at 3802 cm^{-1} which is due to the O-H stretching bond. For CdS at 1028 cm^{-1} and 1392 cm^{-1}, the stretching vibrations of Cd-S and bending vibrations of O-H occurs respectively. These findings validate the presence of CdS NPs and support the XRD data.

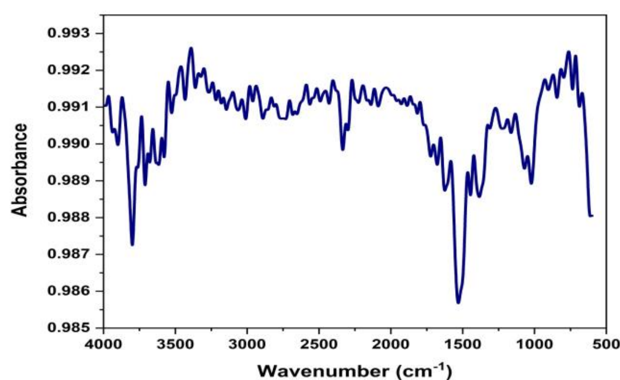

Fig.2: FTIR spectrum of CdS-NPs

3.3 Antimicrobial activity

The anti-bacterial activity of CdS-NPs against various harmful microorganisms was investigated. The diameter of the inhibition zones grew for the entire test pathogens in comparison to the control (Table 1). CdS-NPs made from MPL could prevent the growth of four common harmful bacteria, including Salmonella typhimurium, Shigella flexneri, Clostridium perfringens, and Enterococcus faecalis. As a result, CdS-NPs may be regarded as superior broad-spectrum antibacterial agents. Furthermore, CdS-NPs demonstrated potent antibacterial activity against a variety of infections. At 150µg/ml, S.flexneri and E.faecalis showed a maximum zone of inhibition is 20mm (Fig.3). Because the biosynthesized CdS-NPs demonstrated significant antibacterial activity, they have the potential to be widely used in clinical applications. Recent works revealed that the green synthesized CdS-NPs showed promising activity independently and also in combination with antibiotics [23] against E.coli, S.marcescens, S.aureus, and B.substills. Similar type of work was also presented by [24] where they showed the excellent antibacterial activity of CdS-NPs against E.coli and S.aureus.

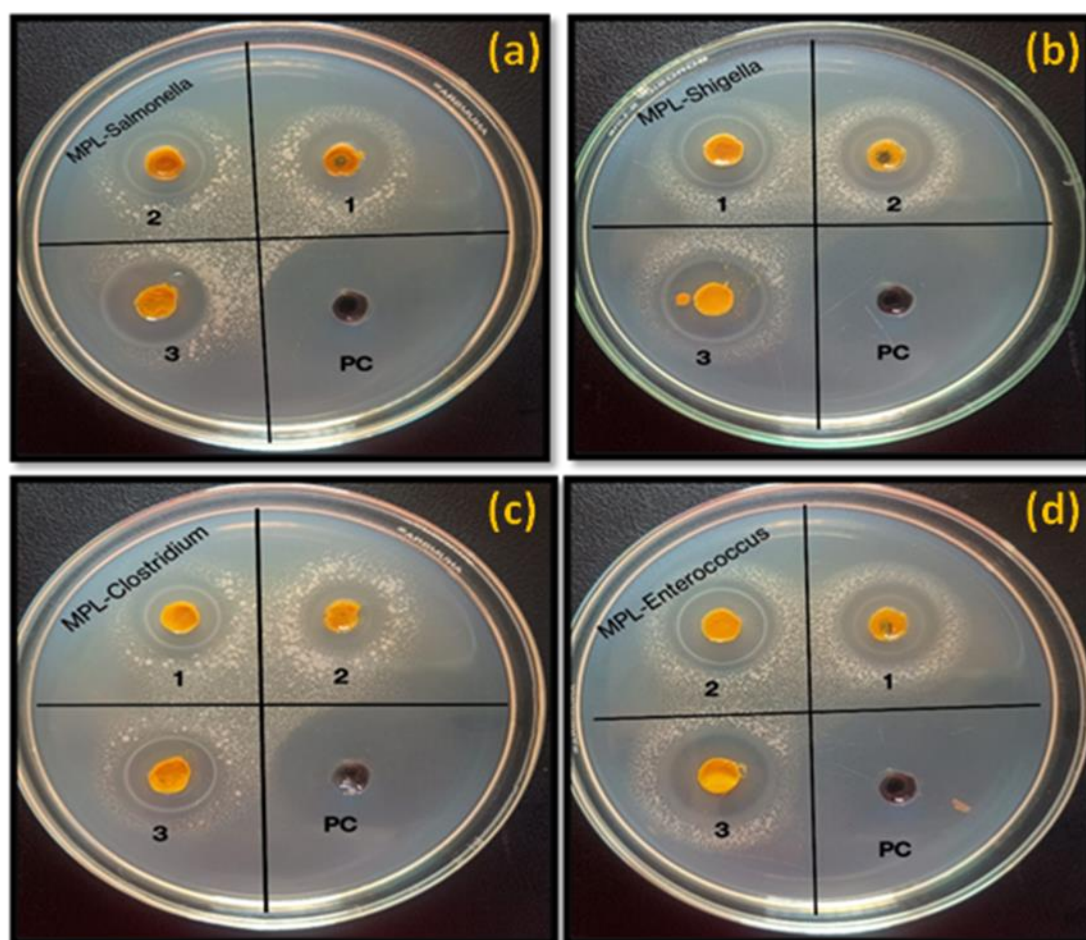

Fig.3: Antibacterial activity of MPL extract over (a) S.typhimurium (b) S.flexneri , (c) C.perfringens and (d) E.faecalis

Table 1: Antibacterial activity of CdS-NPs

Test Organism		Zone of inhibition (mm)		
		MPL extract (µg/mL)		
	80	100	150	Standard (Chloramphenicol) 30µg/mL
S.typhimurium	15	17	19	22
S.flexneri	16	18	20	23
C.perfringens	15	16	18	21
E.faecalis	17	18	20	24

3.4 Antifungal activity

CdS NPs were tested for antifungal activity against two fungal strains, A.flavus and C.albicans (Fig.4). Table 2 shows the concentration of 400 µg/ml CdS NPs inhibited the growth of A.flavus (16mm) but not C.albicans (13mm). The antifungal activity varies depending on the fungi's growth morphology, reported earlier [25]. Fungi that grow more densely on the surface of the agar medium exhibit more inhibition because they are more exposed to the nanoparticles. The antifungal activity of nanoparticles is thought to affect cell functions, causing an increase in nucleic acid content as a result of fungal hyphae's stress response. It has also been proposed that there is an increase in carbohydrate content in the cell, which could be due to the fungi's self-defense mechanism against nanoparticles [26]. Significant nanoparticle exposure causes the formation of 'pits' on the surface of the cells, which eventually leads to the formation of pores and cell death. A flow cytometric assay used to investigate the physiological changes in fungal cells revealed that nanoparticles inhibited the cellular process involved in budding by destroying membrane integrity [27, 28].

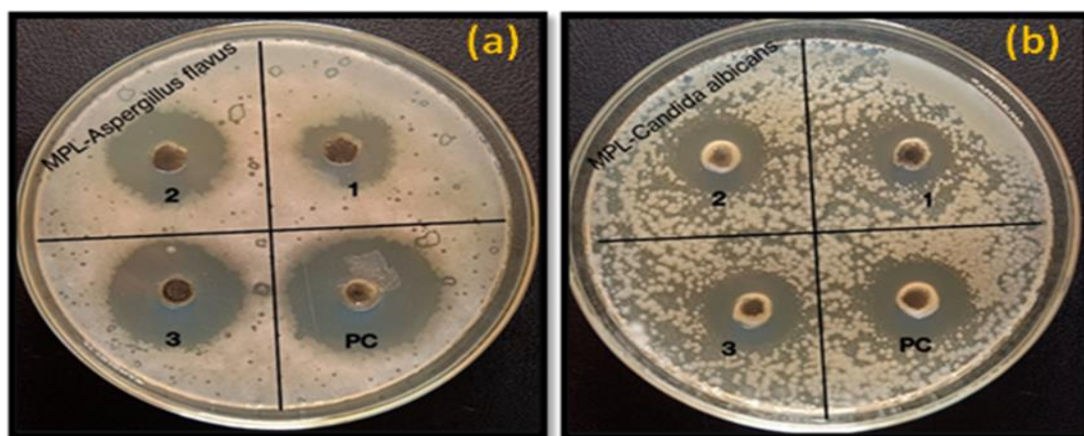

Fig.4: Antifungal activity of MPL extract against (a) A.Flavus and (b) C.albicans

Table 2: Antifungal activity of CdS-NPs

Test Organism	Zone of inhibition (mm)			
	MPL (µg/mL)			
	200	300	400	Standard (Nystatin) 50µg/mL
A. flavus	12	14	16	20
C.albicans	10	12	13	14

3.5 Cytotoxic Activity

MCF-7 human breast cancer cells were treated with the resulting CdS nanoparticles. The NRU Assay was used to test different NP concentrations on cell lines. Cell viability was observed and calculated over a 24-hour period. In Fig.5, the IC_{50} for CdS NP was determined to be 153.2µg/mL for MCF-7. Green CdS particles most likely interacted with the phosphorus moieties in DNA. This could have resulted in the inactivation of DNA replication, further inhibiting enzyme functions and resulting in viability loss [23].

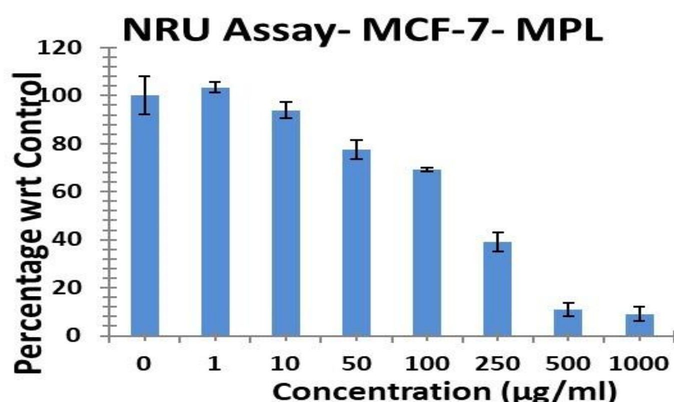

Fig.5: Cytotoxic effect of CdS-NPs on MCF-7 cell line

Conclusion

In this work, we prepared CdS nanoparticles (NPs) via green synthesis route of Murraya paniculata leaf extract by using cadmium chloride as precursor. The prepared CdS NPs were characterized by XRD and FTIR instruments. The CdS NPs were applied to the biomedical applications like antimicrobial activity and anticancer activities. The concentration of 400
μg/ml CdS NPs inhibited the growth of A.flavus (16mm) but not C.albicans (13mm). The IC_{50} for CdS NP was determined to be 153.2μg/mL for MCF-7. The authors conclude that the prepared CdS NPs may be used as potent antimicrobial agent for biomedical applications.

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