RESEARCH ARTICLE

Biosynthesis of Platinum Nanoparticles using Azadirachta indica for the Antibacterial Activity

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ABSTRACT

This research explores the antibacterial effects of platinum nanoparticles (PlNPs) created with an aqueous leaf extract from Azadirachta indica. Biosynthesized PlNPs has shown excellent antibacterial action against both gram +ve and gram -ve bacteria., indicating their potential for use in biomedical fields. The efficacy of PlNPs was assessed by measuring the inhibition zone (ZOI) and conducting killing kinetics studies. Furthermore, the biocompatibility of the biosynthesized PlNPs was evaluated against chemically synthesized PlNPs using the MTT assay on L929 cell lines.

Key Words: Nanoparticles, Azadirachta Indica, Platinum, Antibacterial Activity.

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INTRODUCTION

The advent of nanotechnology has enabled the creation and refinement of nanosized particles, which exhibit enhanced interactions with biological targets due to their larger surface area. Platinum nanoparticles (PlNPs) have been found to possess remarkable antibacterial properties, often outperforming bulk platinum. Despite the established antibacterial efficacy of platinum in various forms, the effects of elemental platinum in nanoparticle form on a range of microorganisms are still not well understood. This research aims to explore the antibacterial potential of PlNPs synthesized using Azadirachta indica leaf extract. [1-4]

EXPERIMENTAL

Synthesis and Purification of Platinum Nanoparticles (PlNPs): [5-8]

PlNPs were synthesized using a leaf extract from Azadirachta indica. For comparative purposes, PlNPs were also synthesized chemically by reducing sodium platinate salt with L-cysteine.

MTT Assay and Cytocompatibility Analysis: [9-12]

Cytotoxicity Evaluation

Assessment of Cytotoxicity: The cytotoxicity of both biosynthesized and chemically synthesized platinum nanoparticles (PlNPs) was evaluated using the MTT assay on L929 cell lines. The MTT assay measured the cytotoxic effects of these nanoparticles on L929 cells, providing valuable insights into their potential biocompatibility and safety profiles.[13]

Cell Culture Maintenance: L929 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with kanamycin, fetal bovine serum, penicillin G, and sodium bicarbonate. This culture maintenance regimen ensured optimal growth conditions for the L929 cell lines, facilitating their proliferation and viability in vitro.[14]

Bacterial Strain Maintenance and Antibacterial Activity Evaluation: The antibacterial properties of biosynthesized PlNPs were assessed against both gram-negative (Proteus vulgaris and Pseudomonas aeruginosa) and gram-positive (Staphylococcus aureus and Bacillus cereus) bacteria. These evaluations aimed to determine the potential of PlNPs as antibacterial agents against a range of bacterial strains.[15]

Methodology for Antibacterial Activity: The effectiveness of PlNPs as antibacterial agents was determined by measuring the zone of inhibition (ZOI) using the well diffusion method. This technique allowed for the quantitative assessment of the antibacterial activity of PlNPs by observing the extent of bacterial growth inhibition surrounding the nanoparticle-containing wells.[16]

Killing Kinetics Studies: Studies on the killing kinetics of PlNPs against bacterial strains were conducted. These investigations provided insights into the dynamics of PlNP-induced bacterial cell death over time, offering a deeper understanding of their antibacterial mechanisms and efficacy against different bacterial species.[17]

RESULTS AND DISCUSSION

Antibacterial Activity of Biosynthesized PlNPs: Platinum nanoparticles synthesized using Azadirachta indica leaf extract exhibited concentration-dependent antibacterial effects against all tested bacteria, as depicted in Figure 1 and summarized in Table 1 and Figure 2 summarizes The kinetics of PlNPs. This observation underscores the potential of these biosynthesized nanoparticles as effective antibacterial agents.[18]

Comparison with Ampicillin: While the antibacterial activity of the biosynthesized PlNPs was slightly lower than

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Table. 1: PlNPs Zone of Inhibition.

Bacterial Strain	(ZOI)[a] (mm)			
	P[b]	PlNPs		Ampicillin
		20 µg/mL	40 µg/m L	(15 µg/mL)
S. aureus	*	12	13	16
B. cereus	*	08	10	11
P.s aeruginosa	*	11	16	19
P. vulgaris	*	13	14	17

that of ampicillin, it remained consistent regardless of nanoparticle size. This comparison highlights the relative efficacy of the PlNPs and emphasizes their potential as alternative antibacterial agents.[19]

Effectiveness of Different PlNP Sizes: Interestingly, PlNPs of approximately 153 nm (synthesized after 5 minutes) and 278 nm (synthesized after 10 minutes) demonstrated comparable antibacterial effectiveness. This finding suggests that the antibacterial activity of the PlNPs is not significantly influenced by their size within this range.[20]

Significant Activity Against Pseudomonas aeruginosa: The highest antibacterial activity was observed against Pseudomonas aeruginosa, with notable zone of inhibition (ZOI) values recorded at 20 and 40 µg/mL concentrations. This highlights the potential of the biosynthesized PlNPs as effective agents against this bacterial strain.[21]

Lowest Activity Against Bacillus cereus: In contrast, the lowest antibacterial activity was noted against Bacillus cereus, with comparatively smaller ZOI values observed at similar concentrations. This observation indicates variations in the susceptibility of different bacterial strains to the antibacterial effects of the PlNPs.[22]

ZOI for Other Bacterial Strains: ZOI measurements ranged within specific parameters at different PlNP concentrations for the remaining bacterial strains. This suggests varying degrees of susceptibility among different bacterial species to the antibacterial effects of the biosynthesized PlNPs.[23]

Bacteria Killing Kinetics: The study demonstrated that the antibacterial activity of the PlNPs was dependent on both concentration and interaction duration with bacterial cells. This highlights the dynamic nature of the antibacterial effects exerted by the PlNPs over time.[24]

Survival Percentage: Notably, the survival percentage of bacterial strains exhibited a significant decrease within the first 40 minutes of interaction with PlNPs at both 20 and 40 µg/mL concentrations. This rapid decline underscores the potent antibacterial activity of the biosynthesized PlNPs.

Interaction Time and Bacterial Survival: Furthermore, prolonged interaction with PlNPs resulted in a further decrease in bacterial survival, albeit at a reduced pace. This emphasizes the cumulative effect of interaction time on the antibacterial efficacy of the PlNPs.

Effectiveness at 80 Minutes: Importantly, a substantial proportion of bacterial cells were killed within 80 minutes of interaction with the PlNPs, further highlighting their rapid and potent antibacterial action.[25]

Control Experiment: The absence of antibacterial activity in the aqueous leaf extract of Azadirachta indica at a specific concentration confirms that the observed antibacterial effects were solely attributed to the biosynthesized PlNPs.

Confirmation of Antibacterial Activity: Overall, these findings provide robust confirmation that the observed antibacterial activity was exclusively due to the biosynthesized PlNPs, highlighting their potential as effective antibacterial agents with promising applications in various fields.[26]

The cytocompatibility of both biosynthesized and chemically synthesized platinum nanoparticles (PlNPs) was investigated using the L929 cell line via the MTT assay (Figure 3). Biosynthesized PlNPs, with sizes approximately 153 nm and 278 nm, were tested after 24 and 48 hours exposure in liquid media. Concentrations ranging from 0.2

Table 2. Cytocompatibility Results.

PlNPs (mg/mL)	% L929 Cell viability							
	PlNPs Synthesized using *A. indica*				Chemically synthesized PlNPs			
	153 nm		278 nm		150 nm		278 nm	
	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
Control	93.48	93.48	93.48	93.48	93.48	Control	93.48	93.48
0.2	86.64	81.13	88.16	80.94	82.84	0.2	86.64	81.13
0.4	85.12	79.89	84.08	78.95	80.18	0.4	85.12	79.89
0.6	84.46	78.66	82.94	77.24	78.19	0.6	84.46	78.66
0.8	83.03	76.29	81.70	75.43	77.05	0.8	83.03	76.29
1	76.95	75.43	78.09	72.49	71.44	1	76.95	75.43

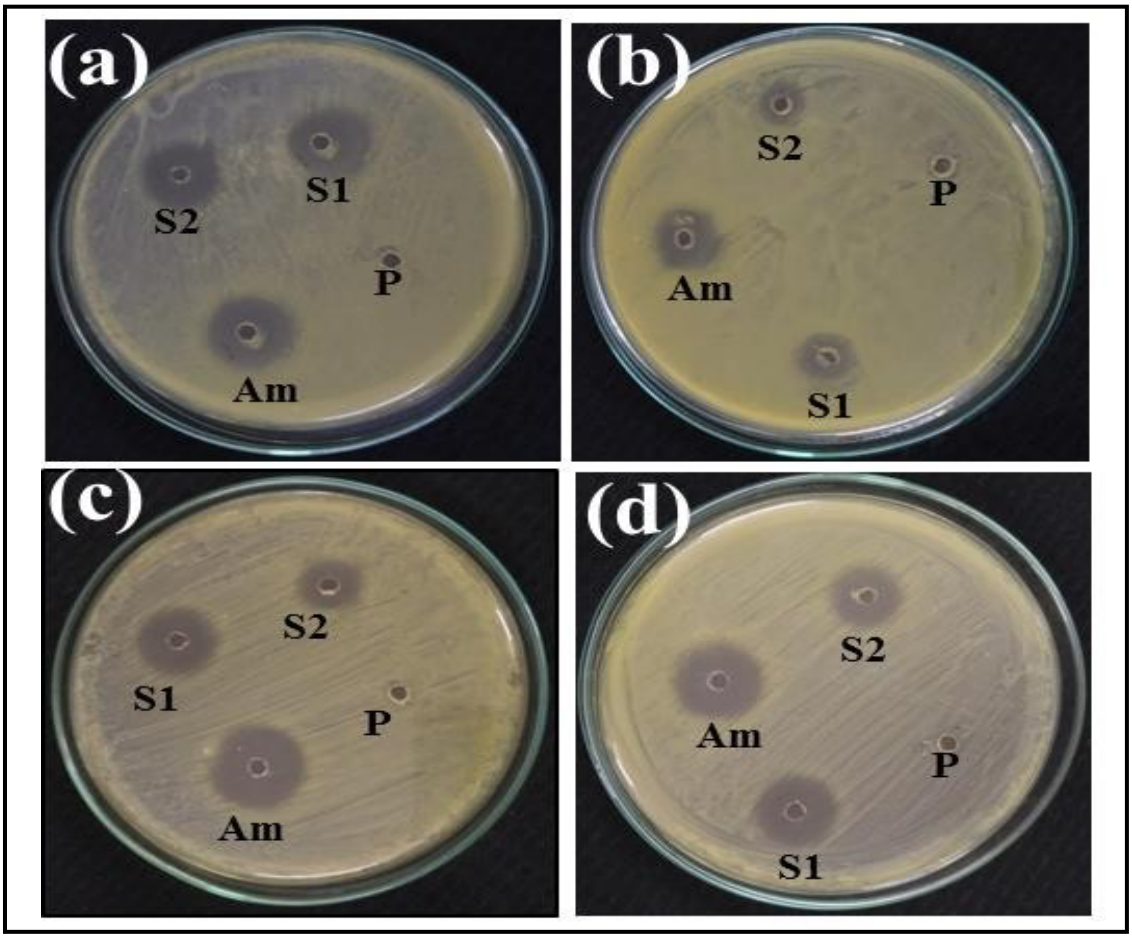

Figure. 1. The ZOI of PlNPs (a) Proteus vulgaris, (b) *Pseudomonas aeruginosa*, (c) Staphylococcus aureus, (d) Bacillus cereus.

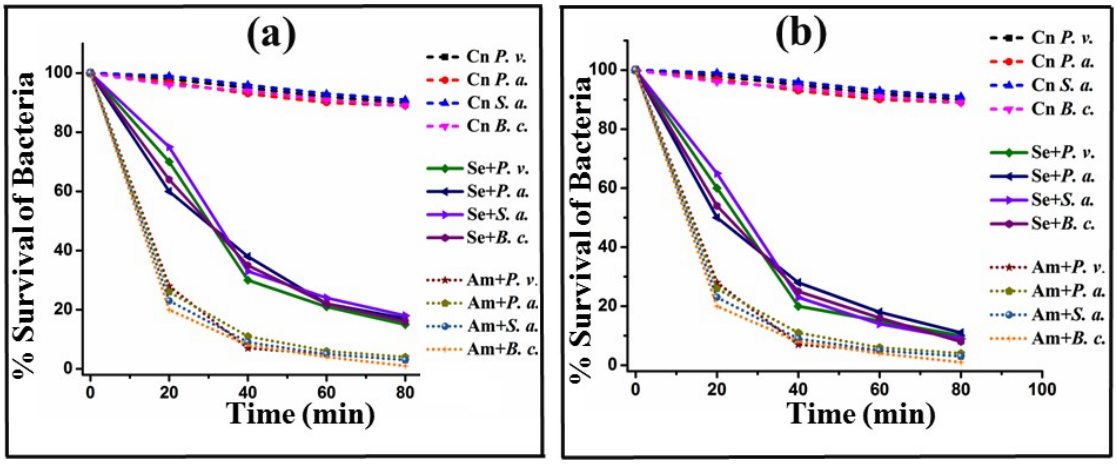

Figure. 2. The kinetics of PlNPs , (a)20µg/mL, (b) 40µg/mL

to 1 mg/mL of PlNPs were assessed for their impact on the L929 cell line, with untreated cells serving as the control group. Concurrently, chemically synthesized PlNPs, approximately 150 nm and 280 nm in size, produced using L-cysteine as a reducing agent, underwent cytocompatibility testing on the L929 cell line using the MTT assay. This comprehensive evaluation aimed to discern the potential cytotoxic effects of both biosynthesized and chemically synthesized PlNPs on cellular viability, providing valuable insights into their biocompatibility and suitability for various applications. [26] For instance, at a concentration of 1 mg/mL and 48 hours of incubation, the minimum cell survival percentage was 76.3% for biosynthesized PlNPs (both ~153 nm and ~287 nm), whereas for chemically synthesized PlNPs under the same conditions, the cell survival percentage was 65.2%. These findings underscore the greater cytocompatibility of biosynthesized PlNPs coated with phytochemicals from Azadirachta indica when compared to their chemically synthesized counterparts..

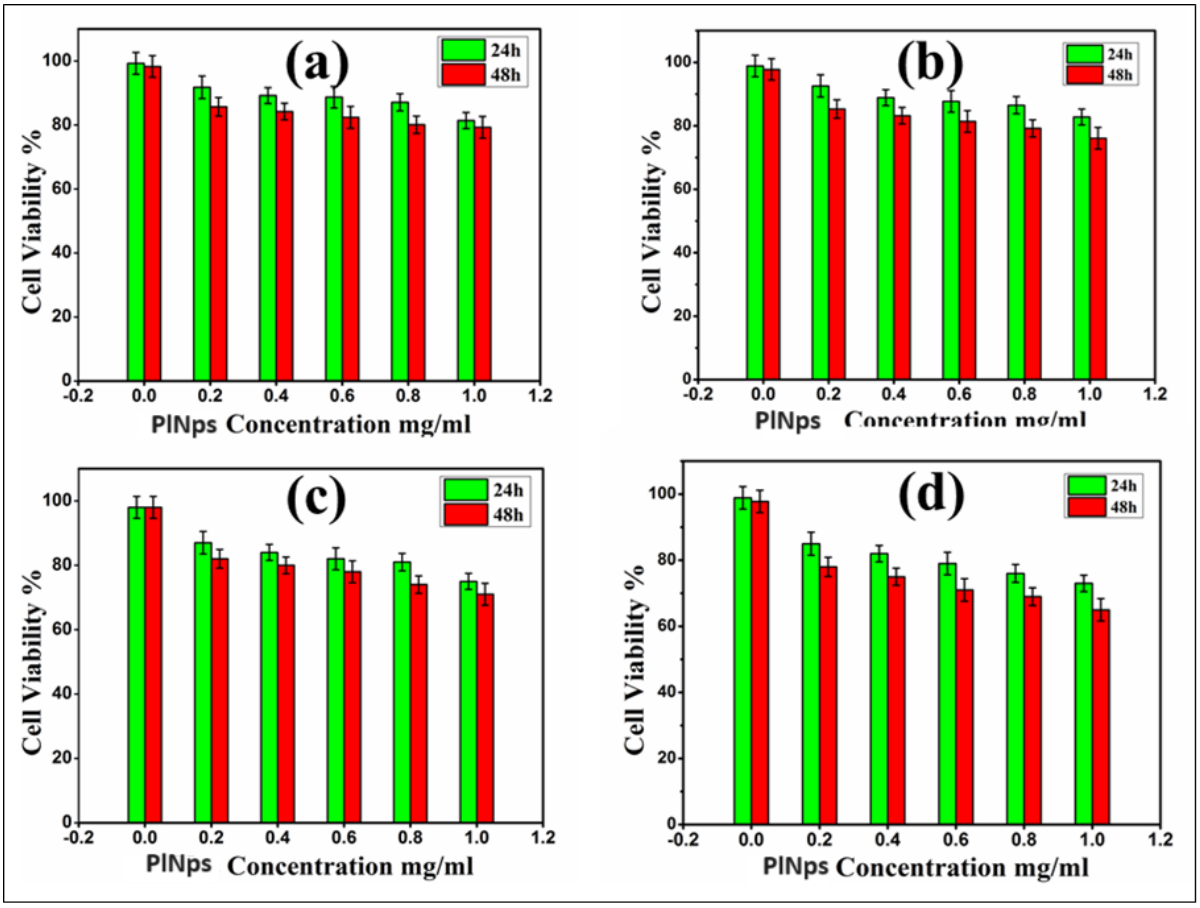

Figure.3. Cytocompatibility evaluation of PlNPs (*In-vitro*), (a) ~153nm, (b) 278 nm, (c) ~150 nm (d) ~ 280nm.

Cytocompatibility: The MTT assay results showed ((Table 2) that biosynthesized PlNPs were cytocompatible with L929 cell lines at various concentrations and exposure times. Both biosynthesized and chemically synthesized PlNPs demonstrated similar levels of cytocompatibility, indicating their potential for safe biomedical applications.

CONCLUSION

Platinum nanoparticles synthesized using Azadirachta indica leaf extract demonstrate significant antibacterial efficacy against both gram-positive and gram-negative bacteria, with their antibacterial properties remaining independent of nanoparticle size. This study underscores the size-independent antibacterial activity of the biosynthesized nanoparticles, showcasing their ability to combat a broad spectrum of bacterial strains effectively. Moreover, the cytocompatibility of these biosynthesized PlNPs suggests their safety for use in biological environments, indicating no harm to mammalian cells and rendering them suitable for various biomedical applications. Considering their antibacterial efficacy and cytocompatible nature, these nanoparticles hold promise as antimicrobial coatings for medical devices, offering potential solutions to prevent bacterial infections in clinical settings. Future research endeavors should focus on exploring the clinical applications of these biosynthesized PlNPs, delving into their efficacy in real-world medical scenarios, which could pave the way for innovative advancements in antimicrobial treatments and medical device coatings.

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