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Antibacterial Activity of the Chemically Synthesized Zinc Oxide Nanoparticles against Gram-Negative and Gram-Positive Bacteria

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Abstract

Zinc oxide nanoparticles (ZnO NPs) are highly important due to their high photocatalytic and antibacterial activities. ZnO NPs are used as antibacterial agents because they have significant antibacterial properties, are inexpensive, easy to synthesize, have a high surface area-to-volume ratio, and are non-toxic to human cells, making them effective and safe for medicinal applications. This study also concentrates on the characterization and chemical synthesis of ZnO NPs, as well as an evaluation of their antibacterial properties. The ZnO NPs were created utilising a simple chemical method that included aqueous solutions of sodium hydroxide and zinc chloride, conducted in an alcoholic methanol media. The obtained product was examined using ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The mean crystallite size of ZnO nanocrystals was 32.64 nm, as calculated from main peaks of X-ray diffraction through Scherrer equation. Functional groups containing carbonyl and carboxyl were found on the surface of ZnO nanoparticles using FTIR analysis, which enhance interactions with bacterial cell membranes and contribute to their antibacterial activity. ZnO nanoparticles with a prominent and broad absorption peak at a maximum wavelength (λmax) of 375 nm demonstrate highly effective antibacterial properties. The synthesized ZnO nanoparticles displayed antibacterial activity against pathogenic strains of *Staphylococcus aureus* (gram-positive). as well as *Klebsiella pneumoniae and Escherichia coli (gram-negative)*. The findings revealed effective antibacterial properties of the chemically synthesised ZnO nanoparticles.

Keywords Chemical reduction, zinc oxide nanoparticles, antibacterial activity

1.0 INTRODUCTION

ZnO NPs are considered to be an attractive inorganic material having great importance in various specialized areas of nanotechnology, such as communications, cosmetics, electronics, sensors, biological, medicinal, and environmental protection [1]. The significance of these materials stems from their features and activities, such as their high chemical stability, conductivity, catalytic, optoelectronic, photonic, UV filtering, and antimicrobial properties. [2]. They can be produced by various methods such as chemical precipitation reaction, hydrothermal, sol-gel, and sonochemical [3] etc, with different morphologies such as nanocombs, nanoflowers, nanorods, nanosheets and nanowires [4]. However, challenges exist in the production areas on a large scale for their demanding users in various fields with focused morphology, size, and surface area.

In the above-stated applications, ZnO NPs as antimicrobial agents are frequently applied against microbes such as viruses, fungi and bacteria as antivirals, antifungals and antibacterials [5], which are creating great challenges for health issues. Although, there are a lot of commercially available antimicrobial agents which are increasingly used, they have now

been located to be unsuccessful against microbes due to high drug resistance in them. This drug resistance in microorganisms is also considered to be a serious challenge on a global scale [6] that could be solved by nanotechnology-based nanoparticles. Recent advances in nanotechnology, especially, the formation of nanomaterials of various sizes and shapes, led to the development of novel biocidal agents, which are considered to be wonders in modern medicine. The killing power of such nanomaterials for different disease-causing microorganisms is approximately 100 times greater than antibiotics [7].

It is well known that microbes severely contaminate food industries, hospitals and medical settings, which produce a serious health problem [8]. To cope with such problems, the development of antimicrobial agents has gained significant interest from scientists in recent years. Where, amongst the organic and inorganic antimicrobial agents, the second one has the advantages of greater stability at high temperature and pressure, robustness, long shelf life, and the ability to be used in harsh processes. These advantages attracted scientists to focus on replacement of conventional organic compounds by developing various inorganic metal oxides such as calcium oxide (CaO), copper/copper (II) oxide (Cu/CuO_2), Zinc oxide (ZnO) [9] and etc., which are not only considered to be highly stable under various circumstances, but also to be as protected materials for animals and humans [10].

ZnO NPs have long been recognised as antimicrobial agents that are considered to be highly efficient against a variety of multidrug-resistant human microorganisms. For example, antibacterial property of ZnO nanoparticles against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), and their good biocompatibility to human cell have already been reported [11]. Different mechanisms for ZnO's antibacterial action have been postulated. One of the proposed mechanisms is the production of hydrogen peroxide, which inhibits bacterial growth; the second mechanism is the detection of active oxygen sites induced by metal oxide entities; and the third mechanism is based on the removal of Zn^{+2} ions, which causes the cell membrane to break and interact with intracellular components [12].

A lot of research has been done to synthesise zinc oxide nanoparticles, as an important antimicrobial drug against numerous microbes. Wherein, Zakharova and Kolesnikov [13] studied the effect of physicochemical properties of ZnO nanoparticles in particle size ranges of 20–100 nm and 50–300 nm and concentrations of 0.001–1000 mg/L in normal saline and distilled water for their toxicity against *E. coli*. Wherein the ZnO NPs' antibacterial effects were found to be influenced by particle sizes and their aggregates in colloidal solutions, suspension storage time, and the chemical composition of the media. Dobrucka and Dlugaszewska [14] reported the preparation of ZnO NPs utilizing Trifoliumpratenseflower extract and found them with high antimicrobial activity against clinical and standard strains of *E. coli*, *Pseudomonas aeruginosa* (P. aeruginosa) and *S. aureus*. Azam et al., [15] studied the synthesis of nanoparticles like ZnO, Fe_2O_3 and CuO using sol-gel combustion technique and examined their antimicrobial activity against both gram-positive (*Bacillus subtilis* (*B. subtilis*) and *S. aureu*) and gram-negative bacteria (*P. aeruginosa*) and *E. coli*). The antibacterial activities were obtained in the order of ZnO>CuO> Fe_2O_3. Happy et al., [16] performed the green synthesis of ZnO NPs to get spherical shaped particles within average size range of 60-80 nm and found them with good antibacterial activity against *E. coli*. Reddy et al., [17] also prepared ZnO NPs using precipitation method and investigated their antibacterial activity and mode of action against *K. pneumonia*. The materials were found to be very effective in breaking the membrane of the bacterial cell wall and tightly sticking to the intracellular mass. Similarly, Mohamed et al., [18] investigated the sol-gel technique to synthesise ZnO NPs with various calcination temperatures for influence on their molecular structure, morphological, optical, and antibacterial evaluation against various gram-negative bacteria (*K. pneumonia* and *P. aeruginosa*) and gram-positive bacteria (*S. aureus* and *B. subtilis*). The higher antibacterial effects of ZnO NPs on the gram-negative and gram-positive pathogenic bacteria were found at a calcination temperature of 300 ^oC.

In this paper, an attempt has been made to prepare ZnO NPs through precipitation method utilizing $ZnCl_2$ and NaOH as precursors. The aim of this work is also focused on optimising zinc oxide nanoparticle synthesis process parameters and their antibacterial activity assessment against *B. subtilis* (gram-positive), *E. coli*, and *K. pneumonia* (gram-negative) bacteria. A detailed investigation of the ZnO NPs is also performed using XRD, FTIR, UV, and SEM.

2.0 EXPERIMENTAL

2.1 Materials

The chemicals used in this study, including zinc chloride ($ZnCl_2$), sodium hydroxide (NaOH), and methanol (CH_3OH), were purchased from Sigma-Aldrich and were of analytical grade.

2.2 Synthesis of ZnO NPs

ZnO NPs were synthesized through a chemical method employing $ZnCl_2$ and NaOH as precursor reagents. The chemical reaction was followed according to the chemical formula equation, given as in equation (1). For this purpose, 0.1 M an aqueous 50 % methanol solution of $ZnCl_2$ (0.436 g in 16.6 ml) was taken in a glass beaker of 250 ml and stirred to dissolve

completely the ZnCl2 powder. Similarly, an aqueous 50 % methanol solution of 0.2 M NaOH (0.2568 g in 16.6 ml) was also prepared in the same way. After formation of both the precursor solutions, the 0.2 M NaOH solution was added carefully drop wise into 0.1M $ZnCl_2$ solution for 25 mins. After complete addition of NaOH solution, the resulted colourless solution was further subjected to constant magnet stirring (2 h) and then sealed up for 12 h. After that the $Zn(OH)_2$ was found to be settled at the bottom and the extra liquid on top which was thoroughly removed from there by using a syringe. The remaining contents of the solution were centrifuged for 10 minutes, and the precipitation was collected after washing repeatedly with water (deionized) and methanol to get rid of byproducts, which were cohered with $Zn(OH)_2$ and then allowed to dry at 100 ^oC in an oven for 3 h. Following the drying process, the zinc hydroxide precursor was converted into zinc oxide nanoparticles (ZnO NPs), as demonstrated by the reaction shown in Equations 2. The schematic reaction mechanism for the formation of ZnO NPs is also provided in Figure 6.

Following the drying process, the zinc hydroxide precursor was converted into ZnO nanoparticles, as illustrated in Equations 1 and 2. Figure 6 shows a schematic chemical pathway for the creation of ZnO nanoparticles.

$$2NaOH + ZnCl_2 \rightarrow 2NaCl + Zn(OH)_2 \quad (1)$$
$$Zn(OH)_2 \rightarrow H_2O + ZnO \quad (2)$$

2.3 Characterization

An X-ray diffractometer equipped with X-rays of CuKα (λ = 1.5418 Å) (model JDX-3532, Japan) was utilized for identification of the crystal structure and phase transformation of ZnO NPs. For the ZnO nanoparticle's size determination, the Scherrer equation was employed, given as below:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where K for Scherrer constant (0.9), β for FWHM (full width at half maximum) in radians, λ for wave length, θ for Bragg angle and similarly d for diameter (size) of the particle in nanometers. Functional groups in ZnO NPs powder sample were identified using Fourier transform infrared spectrometry (Eco-ATR-Spectrometer, Alpha-Bruker). UV absorption analysis of nanoparticles was performed through a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The morphology of the particles was detected using the SEM technique (Model: JSEM5910, JEOL, Japan).

2.4 Antibacterial assessment of ZnO NPs

The testing for antibacterial activity of the synthesized ZnO NPs against various bacterial strains, including *K. pneumoniae*, *E. coli* (gram-negative) and *B. subtilis* (gram-positive), was carried out by agar well diffusion method [19]. Nutrient agar plates were prepared, and a bacterial lawn was created by inoculating the molten agar with 24-hour-old bacterial cultures. The wells (6 mm) were created in each Petri plate using a sterile borer. ZnO NPs at various concentrations (100, 150, and 200 µg/mL) were ultrasonically dispersed in deionized water and added to wells. Ciprofloxacin, a positive control antibiotic, was dissolved in deionized water (5 mg/mL) and 40 µL was added to separate wells. The plates were incubated at 37°C for 24 hours, after which the diameter of the resulting zones of inhibition (in mm) was measured for each treatment.

3.0 RESULTS AND DISCUSSION

3.1 X-ray diffraction

The X-ray diffraction profile of ZnO NPs is given in Figure 1. The profile contains numerous reflection bands at 31.8, 34.5, 36.3, 47.6, 56.6, 62.8, 66.4, 67.9, 69.1, 72.5 and 76.9 correspond to planes to planes (100), (101), (002), (102), (110), (103), (200), (112), (201), (202) and (004), respectively, exhibiting characteristic hexagonal structure of crystalline ZnO NPs (JCPDS reference code: 01-075-0576) having an average crystallite size of 32.64 nm, as previously discussed [18, 20, 21]. XRD examination indicates that the ZnO NPs have smaller particle sizes and higher crystallinity, which increases their antibacterial activity.

3.2 Infrared Spectroscopy

The FTIR spectrum of the synthesized ZnO NPs is given in Figure 2. Numerous bands are present in the wave number rang 4000-500 cm^{-1}. The band at 524 cm^{-1} is ascribed to the Zn-O (stretching vibrations), which shows the Zn-O bonds in ZnO nanoparticles. Peak at 898 cm^{-1} is attributed to bending vibrations of Zn-OH, which correspond to the nanoparticle's surface OH (hydroxyl) groups. Peak at 1423 cm^{-1} is assigned to the C-O (stretching vibrations), suggesting the presence of carbonate

(CO_3) species, which could arise from atmospheric carbon dioxide absorption. Band at 1462 cm^{-1} corresponds to C-H (bending vibrations), indicating organic contaminants or residual carbon-based materials on the surface of the ZnO nanoparticles. Peak at 1514 cm^{-1} is related to C=C (stretching vibrations), which shows unsaturated carbon bonds, possibly from organic impurities. The band at 1646, 1691, and 1743 cm^{-1} may be ascribed to the stretching vibration of carbonyl (C=O) carboxyl (COOH) groups, suggesting the presence of organic compounds containing carbonyl and carboxyl functional groups on the surface of the ZnO nanoparticles. The band at 2318 cm^{-1} corresponds to the stretching vibration of atmospheric carbon dioxide (CO2), suggesting the presence of molecular CO2 absorption or adsorption on ZnO nanoparticle surfaces. Similarly, band at 3619 cm^{-1} may be associated with the stretching vibration of the hydroxyl (OH) groups, indicating the presence of hydroxyl functional groups on the surface of the ZnO nanoparticles, as reported earlier [21-23]. The FTIR revealed the presence of functional groups on the surface of ZnO nanoparticles, which contribute to antibacterial activity by enhancing interactions with bacterial cell membranes.

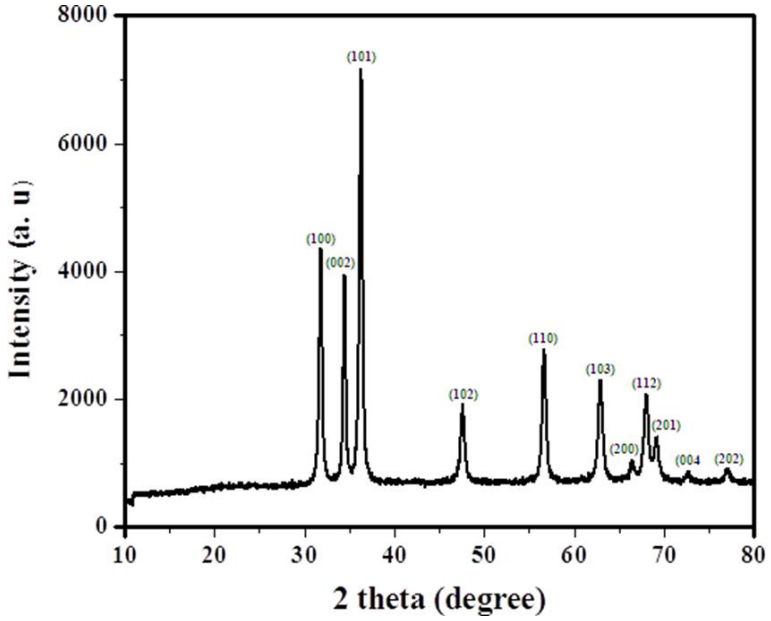

Figure 1 X-ray diffractogram of ZnO nanoparticles.

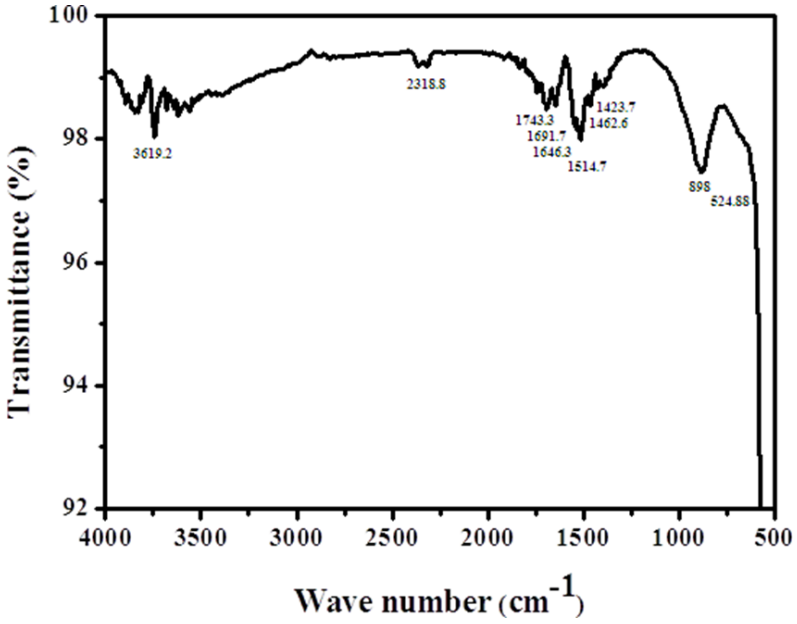

Figure 2 ATR spectrum of ZnO nanoparticles.

3.3 UV-Vis Spectroscopy

UV absorption spectrum of the synthesized ZnO NPs is given in Figure 3. Wherein, the ZnO NPs displayed strong and broad peak with λ_{max} of 375 nm, which is considered to be a characteristic band for the pure ZnO NPs and also exhibited a blue light (fluorescence) by means of Handheld UV Lamp of 365 nm (UVGL-25, Compact UV Lamp). The blue shift found in the ZnO structure is produced by the size effect, rather than bulk ZnO, as previously reported in the literatures [23, 24]. Therefore, ZnO nanoparticles that possess enhanced UV absorption capabilities are expected to demonstrate high antibacterial activity through the reactive oxygen species (ROS) created during exposure to UV light. Thus the generated ROS then induces oxidative stress and leads to the damage of bacterial cells.

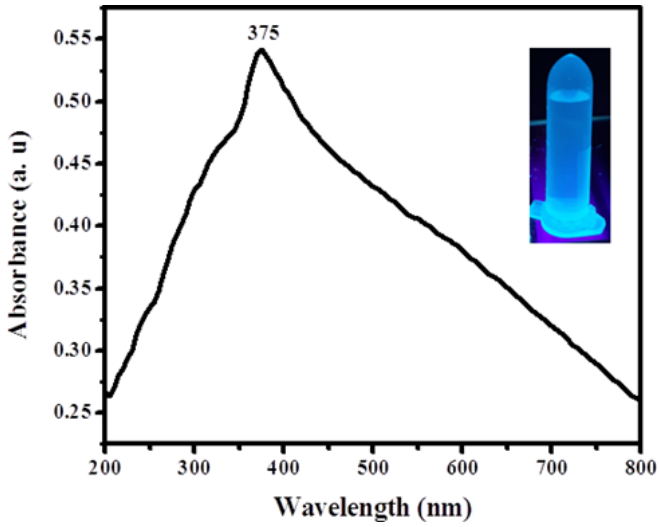

Figure 3 UV spectrum of ZnO NPs and its view of fluorescence image.

3.1.4 Scanning Electron Microscopy

SEM microphotograph of ZnO NPs is given in Figure 4, which confirms spherical shape of the synthesized nanoparticles with an average particle size of about 32.64 nm as determined by applying the Scherrer equation. The particles are observed as an aggregated form which can be considered due to the polar nature of ZnO NPs and their electrostatic attraction as reported in the literatures [20, 22].

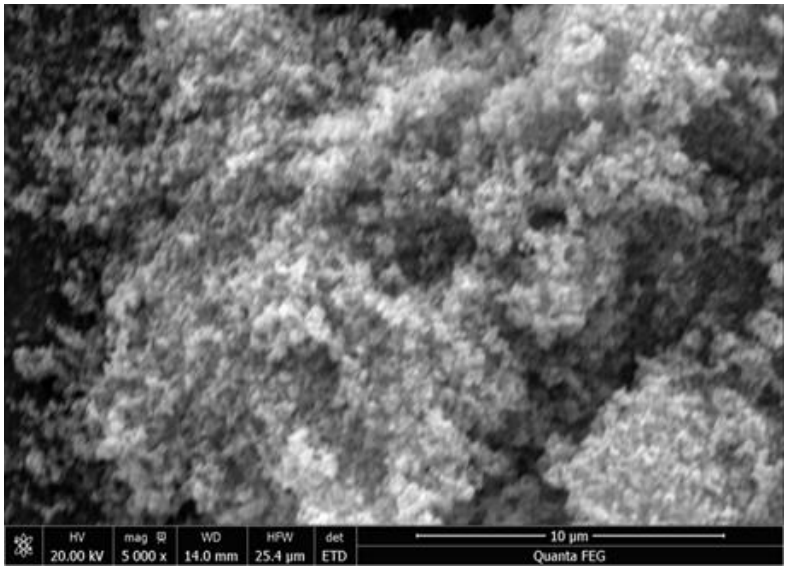

Figure 4 SEM micrograph of ZnO NPs.

3.2 Antibacterial activity of ZnO NPs

The antibacterial activities of ZnO NPs against the tested bacterial strains are tabulated in Table 1. Higher antibacterial activity was recorded at 200 µl of ZnO NPs. Interestingly; gram-negative bacteria like *E. coli* and *S. aureus* were more susceptible to ZnO NPs. The images for the inhibition zone of ZnO NPs antibacterial activity against *E. coli*, *S. aureus* and *K. pneumonia* at various concentrations of ZnO NPs together with the control drug Ciprofloxacin are shown in Figure 5 (a-c). The ZnO nanoparticles kill bacteria by (a) causing the generation of reactive oxygen species, (b) downregulating transcription of oxidative stress-resistance genes in bacteria, (c) rupturing bacterial cell membranes and (e) interrupting biofilm development by bacterial pathogens [22, 25]. Figure 6 depicts the proposed mode of action of ZnO nanoparticles against bacteria.

Treatments		Zone of inhibition (mm)		
		E. coli	*K. pneumonia*	*S. aureus*
Ciprofloxacin	(40 uL/mL)	26	21	25
ZnO NPs	(1). 100 µg/mL	13	14	12
	(2). 150 µg/mL	15	15	15
	(3). 200 µg/mL	24	15	18

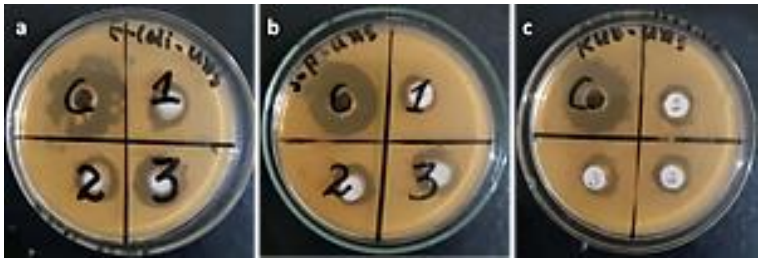

Figure 5 Zone of inhibition of ZnO NPs on (a) *E. coli*, (b) *S. aureus* and (c) *K. pneumonia* at different ZnO NPs concentrations and control drug Ciprofloxacin.

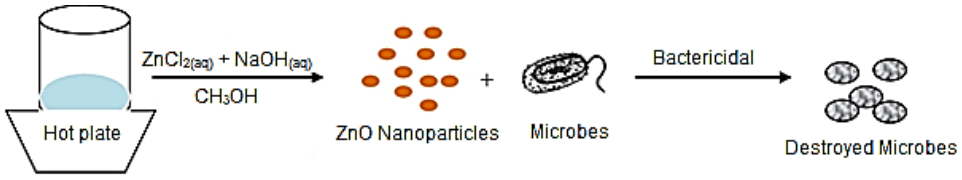

Figure 6 Schematic diagram of the synthesis and mechanism of action of ZnO NPs against bacteria.

4.0 CONCLUSION

The present study established the preparation and characterization of ZnO NPs via a simple and cost-efficient reduction process, as well as their antibacterial activities against both gram-negative and positive bacteria. An XRD investigation verified that ZnO NPs have a typical hexagonal nanostructure with an average crystallite size of 32.62 nm. Smaller particle sizes increase surface area, enhancing interactions with bacterial cells and potentially boosting antibacterial activity. Carbonyl and carboxyl functional groups were discovered on the surface of ZnO nanoparticles using FTIR analysis, increasing interactions with bacterial cell membranes and contributing to antibacterial activity. UV analysis identified a broad and intense absorption band at 375 nm, indicating the maximum absorption of ZnO nanoparticles. This absorption characteristic can potentially influence their antibacterial activity against bacteria. The resulting ZnO NPs were found to be antibacterial against the harmful bacterial strains tested. The observed antibacterial activity of synthesized ZnO NPs can be attributed to their small size, which enables them to potentially disrupt the bacterial cell membrane and interact with intracellular molecules. Based on the findings, it can be concluded that the simple and cost-effective production process described here is suitable for large-scale manufacturing of ZnO nanoparticles. Furthermore, these nanoparticles exhibit significant potential as effective antibacterial agents for various biomedical applications.

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