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Antimicrobial Activity of Zinc Oxide Nanoparticles against bacteria isolated from Goat Wounds

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Abstract

The aims of this study were to determine the main bacteria species that caused wound infections and to investigate the antibacterial effects of ZnO-NPs against them. Samples were taken from 37 goat wounds. Then wound samples were diagnosed using morphological examination, gram stain examination, and biochemical analysis. Results showed that 21 (56.76%) of the cases were positive, and 16 (43.24%) were negative for bacteria isolation. The main species of bacteria isolated most commonly were Staphylococcus aureus (66.67%), Pseudomonas aeruginosa (23.8%), and Escherichia coli (9.5%). ZnO nanoparticles were tested for antibacterial activity using well diffusion assays at different concentrations (16, 8, 4, and 2 mg/ml). In sensitivity testing of isolates to different concentrations of ZnO NPs, the highest area of inhibition was found in S. aureus (19.67±5.50) mm as compared to P. aeruginosa (22.00±5.29) mm and E. coli (20.67±4.61) mm Mean ±SE. In conclusion, in vitro testing of zinc oxide nanoparticles against bacteria showed that when used at different concentrations, they inhibited bacterial growth at different rates.

Keywords: **Wounds, Goats, ZnO NPs, *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli***

Introduction

Wounds are one of the biggest health problems in the world, and infections are one of the most common and serious complications for wound patients with wounds (1). Wound healing complications are common due to infections caused by aerobic and anaerobic microorganisms. These microbes are often resistant to biocides, capable of growing in thick biofilms (2). In addition to complicating wound infections, multi-resistant organisms can spread to the infection site from an infected wound (3). It is considered the most challenging etiology to treat chronic wounds because pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and some enterobacteria form difficult-to-eradicate biofilms in wounds (4).

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The zinc oxide nanoparticles exhibit antibacterial activity against a range of microorganisms, including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis* (5). Next-generation nano antimicrobials using zinc oxide nanoparticles (ZnO NPs) have been developed to treat multi-drug-resistant microorganisms (6). A ZnO nanoparticle (ZnO NP) possesses unique properties, including chemical, physical, optical, and biological. The use of these nanoparticles (NPs) is widely spread across many industries, including the environment, catalysts, optics, agriculture, and biomedicine (7). It has been proposed that ZnO-NPs produce reactive oxygen species, inducing oxidative stress in bacterial cells. ZnO-NPs have also been shown to cause electrostatic breakdown of cell membranes and intracellular material leakage (3,8). Furthermore, soluble Zn2+ is thought to affect the metabolic activities of bacterial cells, inhibiting their growth (8). They are selectively harmful to bacteria while having little effect on human cells (9). This study investigated the antibacterial activity of ZnO-NPs against *Staphylococcus aureus, Pseudomonas spp., and Escherichia coli isolates* from goat wounds in Diyala Province.

Materials and Methods

Collection and isolation of the bacteria from wound samples

Using swabs collected from skin lesions, 37 different samples were collected from goats suffering from wounds. Immediately after the samples were taken, they were transported to the laboratory in the shortest time possible. In order to isolate and examine the cultural characteristics of *Staphylococcus aureus*, *Pseudomonas spp.*, and *Escherichia coli*, enriched cultures were streaked onto various selective and differential culture medium, including Mannitol salt agar, MacConkey's agar, eosin methylene blue agar (EMB), and blood agar (Himedia ; India). Gram staining and biochemical assays were used to identify isolated strains of *Staphylococcus aureus, Pseudomonas spp.*, and *Escherichia coli*.

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Numerous biochemical assays, including the catalase test, the oxidase test, the MR-VP reaction, the indole reaction, and the sugar fermentation test, were carried out (10). Find out which bacteria from this study's isolation of ZnO Np have an antibacterial effect when using NPs with a size 50±10 nm that were acquired from (US Research Nanomaterials in the USA).

Nanoparticle's preparation

Zinc oxide nanoparticles were suspended in colloidal suspension after being resuspended in sterile double-distilled water (11).

Agar well diffusion test of ZnO-NPs

As reported by Geoprincy *et al.*, (2012)(12), both gram-negative and gram-positive bacteria were susceptible to the nanoparticles' antibacterial activity. After the medium solidified, a suspension of each bacteria sample was diluted to 10^{-1}, 10^{-2}, and 10^{-3} (1 ml of 10^{8} cells/ml) and dispersed in Petri plates over solid agar media. We prepared the wells (6 mm) with a sterile cork borer. There were different concentrations of nanomaterial in each well (2-4-8-16 mg/ml). The plates were incubated at 37°C for 24 hours. A transparent ruler was used to measure inhibition zones that formed around the well at the end of incubation.

Results

A total of 37 samples of caprine wounds were collected for this investigation checked the percentages in (Figure 1), 21 (56.76%) of them were positive instances, whereas 16 (43.24%) exhibited no growth. These 21 isolates of various bacteria were obtained, including 14 isolates of Staphylococcus aureus, 5 isolates of P. aeruginosa, and 2 isolates of E. coli, at various rates ranging between 66.67, 23.8, and 9.5% as percentages of the total isolation of the types Staphylococcus aureus, P. aeruginosa, and E. coli, respectively, as shown in (Figure 2).

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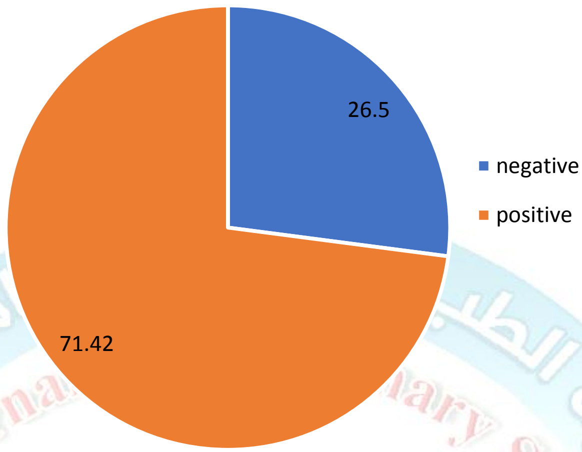

Figure 1: Number of positive and negative wound infection cases out of 37 cases.

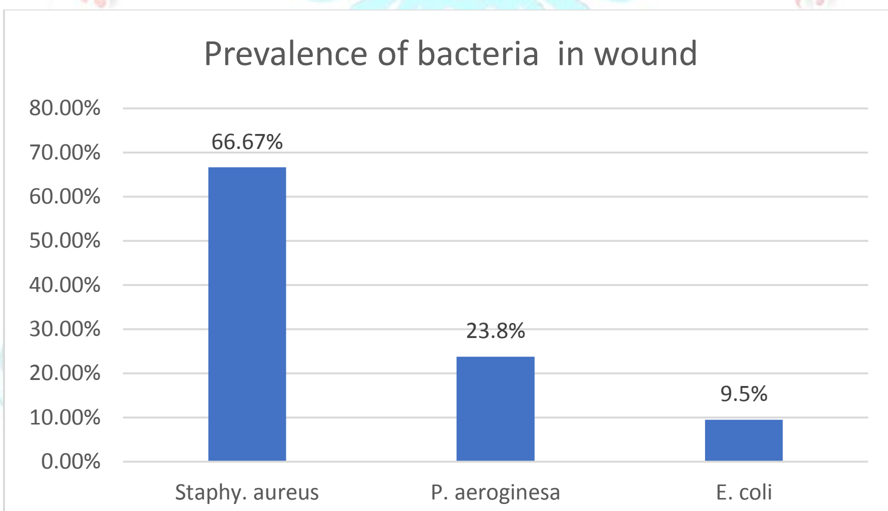

Figure 2: types and ratio of bacteria isolated from wound samples from goats

Identification of isolates

As a result of collection and culture of the samples on different media, all the samples appeared as golden yellow colonies on mannitol salt agar. These colonies were at first identified as S. aureus and confirmed by Gram stain, biochemical tests, and coagulase and catalase activity tests

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(Figure 3). Furthermore, every sample showed a metallic sheen on EMB agar, a characteristic of E. *coli*. A positive result was achieved with indole when IMViC tests (methyl red, Voges-Proskauer, and citrate utilization) were conducted (Figure 4).

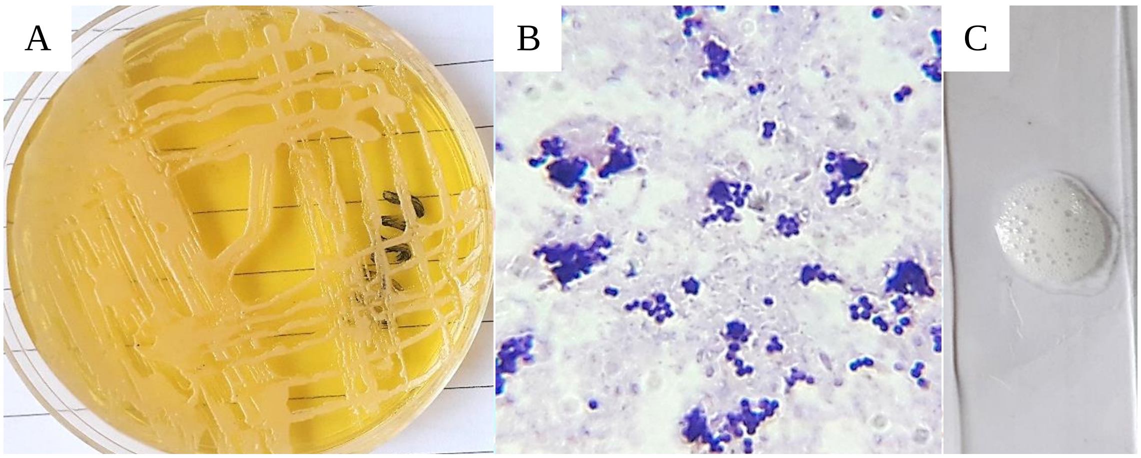

Figure 3: (A) *S. aureus* colonies on mannitol salt agar (golden yellow); (B) S. aureus stained with gram stain (gram-positive); and (C) bacteria that produce bubbles when catalase is present.

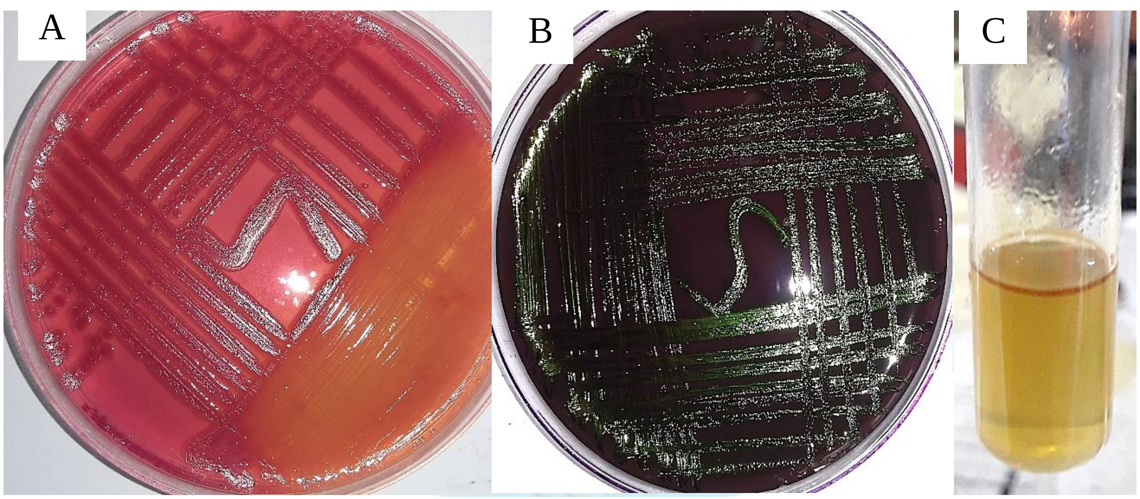

Figure 4: Colonies of E. coli on (A) Macconcy agar, (B) EMB agar, and (C) Indole-positive *E. coli* producing a reddish ring.

Additionally, the remaining samples on nutrient agar display a green color, a musty smell, and have been found to exhibit catalase and oxidase activity, citrate utilization, urea hydrolysis, and sugar fermentation characteristics, all of which are characteristic of Pseudomonas aeruginosa (Figure 5).

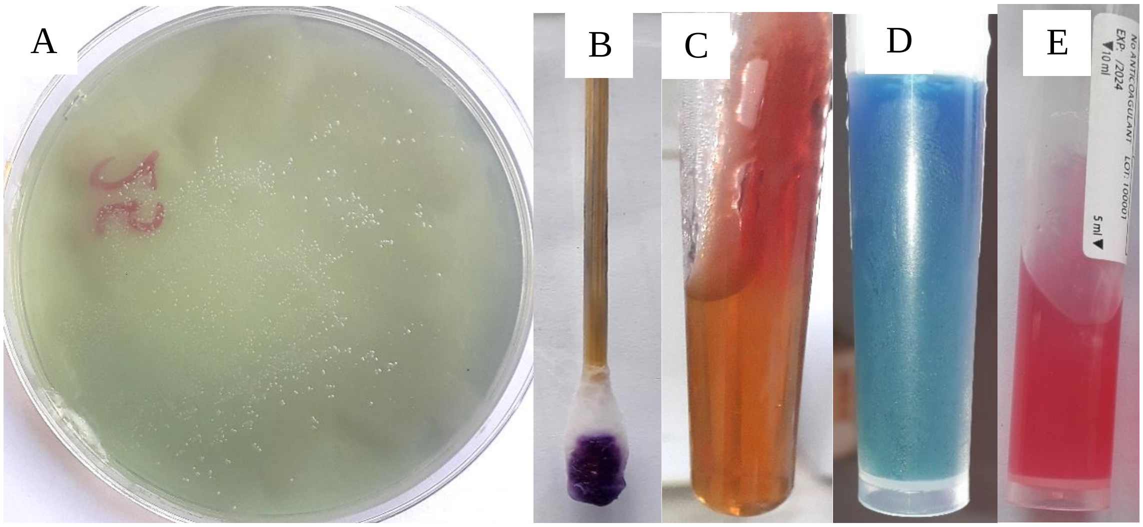

Figure 5: Colonies of *P. aeroginesa* on (A) nutrient agar, (B) oxidase-positive bacteria producing a bluish-purple color, (C) sugar fermentation, (D) simmon citrate-positive, and (E) urease-positive P. aeroginesa Urea hydrolysis changes the color to pink.

Zinc oxide nanoparticles (ZnO-NPs) are sensitive to certain isolates.

According to the results of the sensitivity test, ZnO-NPs exhibited a significant effect, as their sensitivity was high and dose-dependent as observed in (Tables 1,2,3).

Table 1: Antibacterial effect of ZnO NPs against *Staph aureus.*

ZnO-nanoparticles Conc.	*Diameter zone of inhibition(mm)*			*Mean*	*SE.*
16mg/ml	25	14	20	19.67	±5.50
8 mg/ml	20	12	15	16.33	±3.21
4 mg/ml	10	8	12	10	±2
2 mg/ml	0	0	10	3.33	±5.77

Table 2: Antibacterial effect of ZnO NPs against *p. aeroginesa*

ZnO-

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nanoparticles Conc.	*Diameter zone of inhibition(mm)*			*Mean*	*SE.*
16mg/ml	26	24	16	22	±5.29
8 mg/ml	22	22	12	18.67	±5.77
4 mg/ml	20	18	8	15.33	±6.42
2 mg/ml	15	12	0	9	±7.93

Table 3: Antibacterial effect of ZnO NPs against *E. coli.*

ZnO-nanoparticles Conc.	*Diameter zone of inhibition(mm)*			*Mean*	*SE.*
16mg/ml	26	18	18	20.67	±4.61
8 mg/ml	18	14	14	15.33	±2.3
4 mg/ml	16	12	12	13.33	±2.3
2 mg/ml	14	10	0	8	±7.21

During the present study, ZnO-NPS was evaluated for its antimicrobial properties and high antibacterial activity against S. aureus, P. aeroginesa, and E. coli. As a result of 16 mg/ml concentration the maximum inhibited zone reached (19.67 ± 5.50), (22.0 ± 5.29), and (20.67 ± 4.61) mm, respectively. Based on the agar diffusion method and the results of our study, it is obvious that the relationship between the concentration of nano ZnO agent and the inhibition zones of *S. aureus, P. aeroginesa,* and *E. coli* is exponentially proportional, as shown in (Figure 6).

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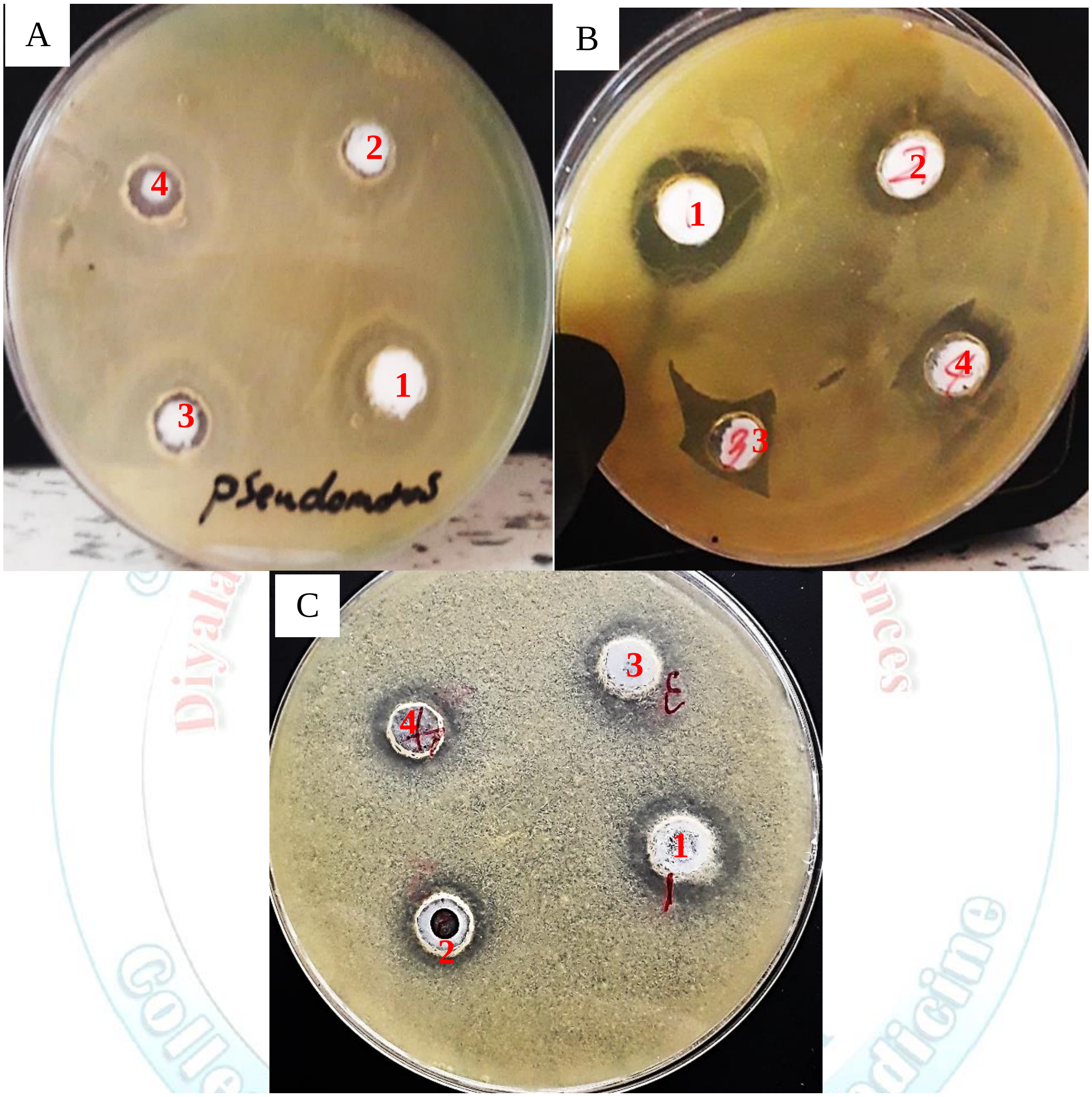

Figure 6: Anti-bacterial effect of ZnO Nps against (A) *Staph. aureus* (B) *P. aeroginesa* (C) *E. coli*; (1) 16 mg of ZnO NPs, (2) 8 mg of ZnO NPs, (3) 4mg of ZnO NPs, (4) 2 mg of ZnO NPs.

Discussion:

Caprine wound infections are a significant concern for animal health and welfare, as well as for the economic sustainability of small ruminant farming. This study investigated the bacterial profile of caprine wound infections and found that more than half of the samples were positive for bacterial growth. The most frequently isolated bacteria were Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, which are known to be common pathogens in both human and animal wound infections.

These findings highlight the diverse bacterial composition of caprine wounds and the potential role of these bacterial species in wound infections. S. aureus is a well-known pathogen commonly associated with skin and soft tissue infections in both humans and animals (12). P. aeruginosa is another opportunistic pathogen that can cause a wide range of infections, particularly in immunocompromised individuals and those with chronic wounds (13). E. coli, although primarily known as a gastrointestinal pathogen, can also cause extraintestinal infections, including skin and soft tissue infections (14).

The use of nanoparticles as antimicrobial agents is an area of active research, and the results of this study suggest that ZnO NPs may have potential as a broad-spectrum antimicrobial agent. Zinc oxide nanoparticles have been shown to have antibacterial properties against a range of Gram-positive and Gram-negative bacteria, including S. aureus, P. aeruginosa, and E. coli (15,16,17).

The observed antimicrobial activity of ZnO NPs against Staphylococcus aureus is consistent with previous studies that have reported the antibacterial properties of ZnO NPs against Gram-positive bacteria (18). The mechanism of action is thought to involve the generation of reactive oxygen species (ROS) and the release of zinc ions, which can disrupt bacterial cell membranes and inhibit cellular processes (19).

Similarly, the inhibitory effect of ZnO NPs on Pseudomonas aeruginosa and Escherichia coli, both Gram-negative bacteria, supports previous findings that ZnO NPs can also be effective against this group of bacteria (20). The difference in the area of inhibition between the bacterial strains may be attributed to variations in cell wall structure and composition, which

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can affect the susceptibility of the bacteria to the antimicrobial action of ZnO NPs (21).

The differences in the sensitivity of these bacterial species to the antimicrobial agent could be attributed to the variations in their cell wall structures and membrane compositions. S. aureus, a Gram-positive bacterium, has a thick peptidoglycan layer that may facilitate the penetration of the antimicrobial agent, leading to a higher area of inhibition (22). In contrast, P. aeruginosa and E. coli, both Gram-negative bacteria, possess an outer membrane that may act as a barrier, reducing their susceptibility to the antimicrobial agent (23).

In addition to their antibacterial properties, ZnO NPs have been shown to have antifungal, antiviral, and antiparasitic effects (17). They have also been used in wound healing and as a coating material for medical devices (24).

In conclusion, this study demonstrates the antimicrobial potential of ZnO NPs against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. These findings contribute to the growing body of evidence supporting the use of ZnO NPs as a promising antimicrobial agent in various applications, such as wound dressings, coatings for medical devices, and water treatment systems. However, further research is needed to optimize the concentration of ZnO NPs and to investigate their potential cytotoxic effects on human cells.

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