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RESEARCH PAPER

Exploration of Some Biological Activities of Biogenic Magnesium Oxide Nanoparticles Synthesized via *Heyndrickxia coagulans*

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ABSTRACT

Infections of the diabetic foot, often known as DFIs, are frequently linked to the overuse of prescribed antibiotics. This study aimed to use nanomaterials as an alternative or auxiliary approach to antibiotic therapy against *P. aeruginosa* isolates from DFI patients. Fifty-five bacterial isolates were identified as *P. aeruginosa* (27 isolates, 49%) from DFI. Bacterial isolates from the soil synthesized MgO nanoparticles, the isolate Z11, which was identified as *Heyndrickxia coagulans* was chosen for its efficiency in producing white precipitate. Characterization of MgO nanoparticles was conducted using Field Emission Scanning Electron Microscopy (FESEM), X-ray Diffraction (XRD), and Atomic Force Microscopy (AFM). The antibacterial activity of the synthesized nanoparticles against *P. aeruginosae* shows that increasing MgO nanoparticles enhanced the inhibitory activity. Additionally, MgO nanoparticles demonstrated antioxidant activity by scavenging DPPH free radicals at concentrations of 1000, 500, 250, and 125 µg/mL. Importantly, no hemolytic activity was observed for MgO nanoparticles tested with whole blood samples.

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INTRODUCTION

Diabetic foot infections (DFIs) are increasingly common complications of diabetes mellitus, posing significant clinical and economic challenges to healthcare systems. Over 3 million adults experience DFIs yearly, with associated annual healthcare costs exceeding $13 billion [1-3]. DFIs are also the leading cause of diabetes-related hospital admissions, with infection-related readmission rates reported as high as 40% [4]. Managing DFIs effectively is complicated by several factors, including discrepancies in optimal antibiotic regimens, treatment durations, wound care intensity, and infectious disease consultations' impact. A broad spectrum of pathogens has been implicated in DFIs, with many infections being polymicrobial in nature. This diversity makes selecting appropriate antibiotic therapy particularly challenging, often leading to overprescribing broad-spectrum antibiotics [5-8]. The rise of multidrug-resistant organisms (MDROs), including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* species, and extended-spectrum β-lactamase-producing gram-negative bacilli, further complicates DFI management [9,10]. The most pressing issue in public health is that certain bacterial strains have developed resistance

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to antibiotics commonly used in human medicine. This has severely curbed the range of treatments available and jeopardized affected individuals' lives [11]. Globally, antimicrobial-resistant infections claim over 700,000 lives annually, with projections estimating up to 10 million deaths per year by 2050 due to antimicrobial resistance (AMR) [12].

Nanotechnology is the engineering of materials at the nanoscale (less than 100 nm), enabling advanced applications in drug delivery and antimicrobial agents [13-19], where exceptional chemical, physical, and biological properties develop, enabling advanced applications across numerous majors [20-29]. Their applications include drug delivery, antimicrobial agents, and diagnostic tools, which are crucial in modern technological developments. Previous studies demonstrated the antifungal and antimicrobial potential of nanoparticles, such as silver nanoparticles (AgNPs), combined with microbial agents like *Pseudomonas fluorescens* and *Bacillus circulans*, which effectively inhibited the growth of *Aspergillus niger* [30] Such interacted methods could additionally advance the effectiveness of nanoparticles such as Ag NPs, Fe_3O_4, and MgO in fighting infections caused by multidrug-resistant pathogens and different-associated microorganisms.

Magnesium oxide (MgO) nanoparticles offer promising antimicrobial properties due to their biocompatibility, biodegradability, and cost-effectiveness. Their efficacy increases with higher concentrations and smaller sizes, making them a potential alternative or complement to antibiotics. Previous studies have demonstrated that the bactericidal efficacy of MgO NPs increases with decreasing size and concentration [31-33]. New progressions in the nanotechnology field have enabled the alteration of surface characteristics of nanomaterials, such as stiffness, wettability, stability, bioavailability, and topography, through regulatory ion beam parameters. These alterations are crucial in improving the biocompatibility and functionality of nanomaterials, particularly in managing infections caused by multidrug-resistant pathogens and in drug delivery processes [34]. The use of altered nanoparticles such as iron oxide or MgO as carriers for drugs and vaccines enhances and minimizes potential side effects, contributing to more efficient and sustainable strategies [35,36]. The integration of nanoparticles in drug delivery systems demonstrates the transformative potential of nanotechnology in tackling global health challenges and combating infectious diseases like malaria [37] and cancer, particularly in studying tumor microenvironments, drug screening, and personalized medicine, which are crucial for cancer research offering new possibilities for therapeutic development [38]. For medicinal applications of MgO's antibacterial capabilities, it is required to ascertain the MIC, MBC, and MFC of MgO concerning common infectious yeasts and bacteria [39,40]. This study explores the synthesis and characterization of biogenic MgO nanoparticles using *Heyndrickxia coagulans*, focusing on their potential application in managing infections caused by multidrug-resistant pathogens. Precisely, the investigation of the antibacterial efficacy of MgO nanoparticles against multidrug-resistant pathogens, their anti-biofilm activity, and their antioxidant properties. By addressing the encounters modelled by antibiotic resistance and oxidative stress, this study pursues to examine MgO nanoparticles as a practical substitute or complement to conventional antibiotics. The findings are expected to contribute to the development of innovative, eco-friendly nanotechnological solutions for managing complex infections, such as those associated with diabetic foot infections (DFIs).

MATERIALS AND METHODS

Isolation and Identification of Bacteria from DFI Infections

One hundred samples of diabetic foot patients were collected from patients attending the Diabetic Centre at AL-Manathara Hospital and AL-Sadr Hospital in Al-Najaf Province, Iraq. Fifty-five bacterial isolates were isolated from diabetic foot infections (DFIs). These isolates were. The bacterial isolates were identified [41] based on morphology, microscopic examination, biochemical tests, and the VITEK2 compact system.

Selection of the Efficient Isolate for MgO NP Synthesizing

A total of Thirty soil samples were collected to detect present bacterial isolates that are efficient for producing MgO, the isolates were cultured in brain heart infusion broth and incubated at 37°C for 24 hours. After incubation, magnesium nitrate ($Mg(NO_3)_2$) 0.2M was added to each culture, and the samples were additionally incubated at 37°C for 18 hours in a shaking incubator (200 rpm).

The colloidal suspensions were then centrifuged at 15,000 rpm for 25 minutes to separate the precipitate [42]. All isolates (Z1-Z30) were screened against *Pseudomonas aeruginosa* using the Mueller-Hinton agar well diffusion assay. Following an 18-hour incubation at 37°C, isolate Z11 was carefully chosen as the most efficient MgO nanoparticle producer based on its ability to form a white precipitate and exhibit antimicrobial activity. The isolate Z11 demonstrated identified as *Heyndrickxia coagulans* based on its morphology, biochemical characteristics, and molecular sequencing by using universal primers 16S rRNA [43].

Characterization of Biosynthesized Magnesium Oxide Nanoparticles

The physical Characteristics of biosynthesis nanoparticles were characterized using different techniques [44]. Field Emission Scanning Electron Microscopy (FESEM) analysis was employed to test the magnesium oxide nanoparticles, the examination was conducted at the University of Baghdad, Electron Microscopy Unite, Iraq. Atomic Force Microscope (AFM) was used to estimate the size of MgO NPs at the University of Baghdad. The X-ray Diffraction (X-ray diffraction) was used for the characterization of Mg NPs at Tehran University, Iran.

Antibacterial Activity of Mgo NPs

The antibacterial activities of MgO NPs were tested with different concentrations against Multi Drug-Resistant (MDR) bacteria: Gram-negative (*P. aeruginosa)* isolated from DFI, bacteria using the diffusion method. With 100µl of bacterial suspension, the agar plate was incubated, and using a sterile cork Porer, 7 mm diameter pores were created and filled with Mgo NPs (100ul). then incubated at 37°C for 18 hours antibacterial activities were evaluated and the values were provided a means of triplicate by measuring the growth inhibition zone diameter in millimeters [45].

Antioxidant activity of MgO nanoparticles

The DPPH solution (0.006 % w/v) was prepared in 95 percent methanol. Freshly prepared DPPH solution was placed in test tubes, and MgO NPs (0.12, 0.25, 0.5, 1 mg/ml) were applied to each test tube until the final volume was 2ml, and discoloration was calculated at 517 nm after 30 minutes in the dark incubation. Measurements were implemented at least in triplicate. DPPH solution was used as a control which contained the same volume (without chitosan NPs) and 95% methanol was used as the blank. The percentage of DPPH free radical scavenging was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Ao} - \text{A1}}{\text{Ao}} \times 100$$

where A_o represents the absorbance of the control and A_1 represents the absorbance in the presence of MgO nanoparticles. The real absorption decrease caused by test compounds was compared with the positive controls [46,47].

RESULTS AND DISCUSSION

A total of One Hundred samples were collected from DFIs infections. The patients attending (AL-Manathara Hospital and Al-Sadr Hospital in Al-Najaf Province. Among the 55 isolates, *Pseudomonas aeruginosa* was the most prevalent accounting for 27 isolates (49%). To prepare the biogenic MgO, thirty bacterial isolates from soil named Z1 to Z30 of a Probiotic strain Heyndrickxia *coagulans* were tested for their ability to synthesize magnesium oxide (MgO) nanoparticles. The most effective isolate Z11 was selected based on its ability to form a white precipitate, which was then tested against the previously isolated *Pseudomonas aeruginosa* as a significant antimicrobial activity. *Heyndrickxia coagulans* is well recognized as a safe and edible option because of their remarkable effectiveness in a range of biological processes [48], Due to each microbe having a unique metabolic process and enzyme activity, not all bacteria can manufacture nanoparticles. The current study method was selected because of the benefits of external synthesizing over intracellular biosynthesis. Extracellular biosynthesis has several advantages, including a more straightforward, less artifact-prone approach [49,50]. Nano biosynthesis is not possible in all organisms. The exact mechanisms that lead to the creation of biogenic MgO NPs have yet to be determined.

FESEM was used to validate the morphology and size of biogenic MgO nanoparticles. The obtained images reflected well-distributed and spherical-shaped MgO nanoparticles generated by *Heyndrickxia coagulans* with a measured size of (24.23-79.2 nm), the size average was

51.72 nm as shown in Fig. 1. Atomic Force Microscopy (AFM) image of MgO nanoparticle synthesis by *Heyndrickxia coagulan,* although the lateral dimensions are influenced by the shape of the probe, the morphology of MgO NPs was reported. The height measurements be able to provide the elevation of nanoparticles with a high point of precision and accuracy. the average diameter of MgO NPs biosynthesis from *Heyndrickxia coagulan* was 9.83nm as illustrated in Fig. 2, which showed three-dimensional images, and granularity accumulation distribution charts of MgO NPs. Atomic Force Microscopy's extraordinary resolution allows for precise three-dimensional visualization of molecular structures, as well as atomic-scale strategies. The procedure for preparing samples for AFM is straightforward. Because samples can be viewed under near-physiological conditions, AFM can record the critical procedures of molecules, organelles, and other structures in living cells in real time [51]. The cubic crystal system of the synthesized MgO was confirmed by the XRD. The 2θ peak positions were well identified with the JCPDS NO: 01-076-1363.

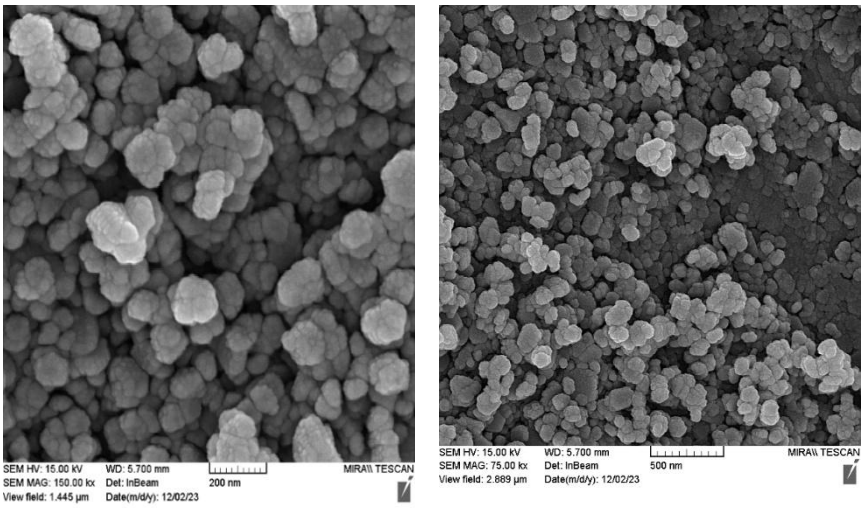

Fig. 1. FESEM Micrograph of biogenic Mgo nanoparticle synthesized by *Heyndrickxia coagulan*. At scale 100nm (right) and 200nm (left).

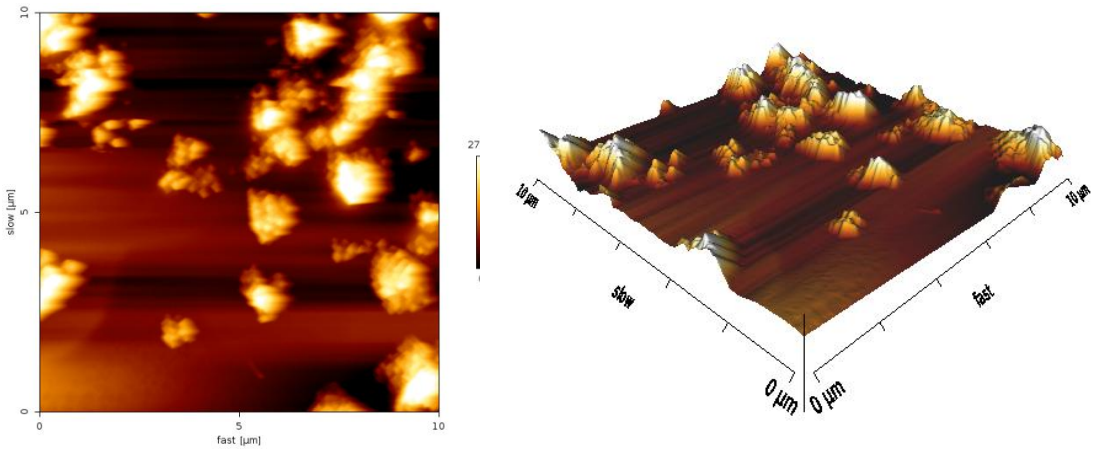

Fig. 2. AFM analysis of biogenic Mgo synthesis by *Heyndrickxia coagulan.*

Also, the crystalline size was evaluated by the following Debye Scherrer's formula D = 0.94λ/β cosθ as presented in Fig. 3 [52]. The strong peak suggested the inclusion of bioorganic coupons/

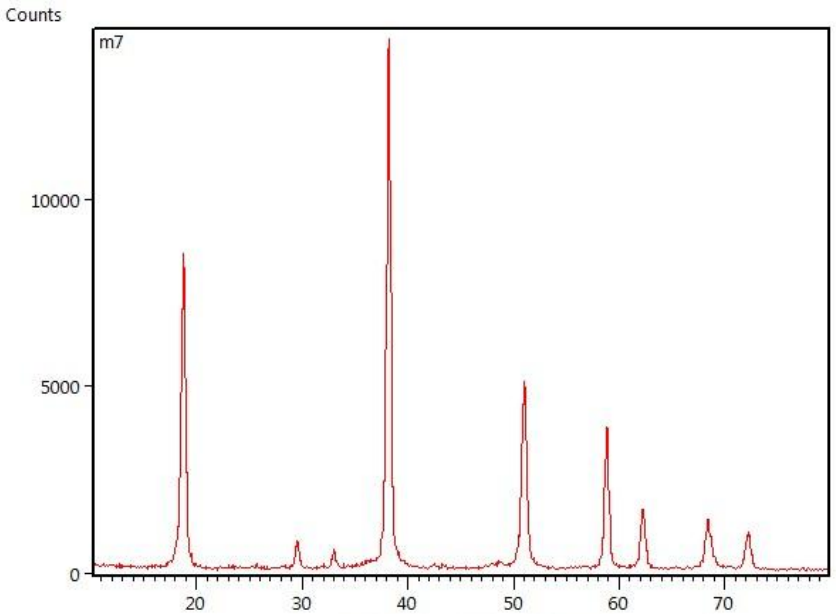

Fig. 3. XRD analysis of MgO nanoparticles synthesized by *Heyndrickxia coagulan.*

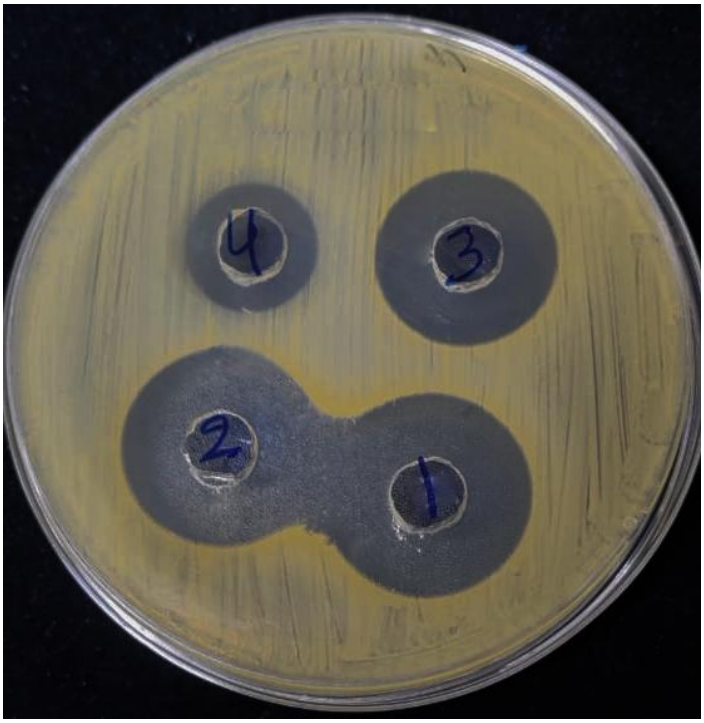

Fig. 4. Antibacterial activity of MgO NPs against *P. aeruginosa.*

proteins in the nanoparticles throughout the manufacturing process.

Antibacterial Activity of MgO nanoparticles

The antibacterial activity of MgO nanoparticles synthesized using *Heyndrickxia coagulans* was tested against multidrug-resistant (MDR) *Pseudomonas aeruginosa* using the agar well diffusion method [53]. The nanoparticles exhibited significant antibacterial effects as aligned with [54] when examined at different concentrations of 125, 25, 500, and 1000 µg/mL, with the largest inhibition zone (26 mm) as illustrated in Fig. 4 observed at a concentration of 1000 µg/mL (Table 1). The results showed that increasing concentrations of MgO nanoparticles enhanced their inhibitory activity. The inhibition zone assay confirmed a concentration-dependent response, with higher concentrations of MgO nanoparticles exhibiting superior antibacterial activity. As shown in Fig. 4, the inhibition zone diameter increased with the nanoparticle concentration, ranging from 14 mm at 125 µg/ml to 26 mm at 1000 µg/ml. The representative plate in Fig. 4 confirms these findings, showing distinct and clear zones of inhibition around the wells containing MgO nanoparticles. These results highlight the potential of MgO nanoparticles as effective antibacterial agents, particularly against multidrug-resistant pathogens like *Pseudomonas aeruginosa*. These findings align with previous studies demonstrating that if the quantities of MgO NPs were raised, the inhibitory impact became stronger. The MgO nanoparticles were effective against both Gram-negative and Gram-positive bacteria, with Gram-negative bacteria showing higher sensitivity. It was comparable to earlier studies that found that high concentrations of Mgo NPs inhibited bacterial growth [55].

The scientist has made their aim to create a successful alternative to replace current antibiotics that have resistance features in some regions, as well as an effective new entry drug complement to antibiotics, because of the mounting problem of Extensive Drug Resistance (XDR). Nanoparticles are now being welcomed

Table 1. Inhibition zone using the biogenic MgO NPs against *P. aeruginosa.*

Conc. No.	MgO NPs CONC. µg/ml	Inhibition Zone/ mm
1	1000	26 mm
2	500	24 mm
3	250	21 mm
4	125	14 mm

Table 2. Antioxidant effect of MgO NPs synthesized by *Heyndrickxia coagulan* in DPPH test.

No.	Conc. Mg/ ml	Antioxidant effect %
1	0.12	60
2	0.25	63
3	0.5	66
4	1	70

Table 3. Effect of MgO NPs on blood hemolysis.

Concentration mg/ml	Test result	Hemolysis %
1	0.6942	0
0.5	0.6891	0
0.25	0.6831	0
0.12	0.5193	0
positive	2.3639	100
Negative	0.3665	0

as a possible alternative to antibiotics, with the potential to solve the bacterial XDR problem [56]. In Gram-negative bacteria like *P. aeruginosa* and Gram-positive bacteria like *S. aureus*, MgO NPs are reported to have antibacterial activities [57,58]. MgO NPs were shown to be efficient antimicrobial medicines against a wide range of clinically relevant infections. It has been shown that the fundamental mechanism of its antibacterial action is attacking an outer membrane protein called lipopolysaccharide (LPS), which damages cell membranes and causes bacteria to die at neutral PH [59]. Joining a microfluidic system can simulate the chemotactic environments where bacteria can reproduce, allowing real-time observation of nanoparticle-bacteria interactions. Such technology would permit accurate characterization of MgO nanoparticles' effects on microbial motility [60].

Antioxidant Activity of MgO Nanoparticles

The antioxidant activity of MgO nanoparticles was evaluated using the DPPH free radical scavenging assay [61]. The antioxidant effects increased with the increase of the concentration of nanoparticles, as presented in Table 2. This concentration-dependent effect demonstrates the potential of MgO nanoparticles as effective antioxidants that neutralize free radicals and mitigate oxidative stress. The DPPH scavenging effect of nanoparticles increased as their concentration increased, indicating that higher concentrations of MgO NPs result in a greater proportion of DPPH inhibition. This suggests that DPPH was inhibited more effectively due to increased electron donation at higher nanoparticle concentrations [62,63]. Antioxidants work by searching for free radicals and inhibiting their formation. On oxidative stress, free radicals have a strong bactericidal action. Not only has the membrane been destroyed, but biological macromolecules such as proteins, lipids, DNA enzymes, and RNA that trigger cell death have also been harmed [64]. The findings also highlight the advantages of biosynthesized nanoparticles, such as abridged environmental impact and cost-effectiveness. This aligns with the comprehensive mechanisms of antioxidant action described in the previous research [65], which highlights the role of antioxidants in scavenging free radicals, chelating metals, and preventing oxidative damage to lipids, proteins, and DNA. However, the exact mechanisms underlying the antibacterial and antioxidant effects of MgO nanoparticles remain to be fully elucidated. Additional studies exploring these mechanisms and in vivo testing and clinical trials are necessary to validate their therapeutic potential. The incorporation of Artificial Intelligence (AI) into nanotechnology development represents a transformative progression. AI performances, enable scientists to enhance nanoparticle fabrication methods, estimate the different biological activities, and minimize resource waste. The employing of AI in recent nanoparticle research could assist in solidifying their role in addressing antimicrobial resistance and oxidative stress-related conditions [66].

Hemolytic Activity of MgO Nanoparticles

The hemolytic activity of MgO nanoparticles was tested using whole blood samples to assess their biocompatibility and safety for biomedical applications. Across all tested concentrations, the nanoparticles showed no hemolytic effects as presented in Table 3, indicating their biocompatibility with red blood cells (RBCs), a critical factor when considering nanoparticles for therapeutic or drug delivery applications and safety for potential therapeutic applications. These findings are consistent with earlier studies such as those reported in [67], which reported the non-hemolytic nature of nanoparticles synthesized via biogenic methods. The absence of hemolysis suggests that the MgO nanoparticles do not disrupt the integrity of RBC membranes, a property likely attributed to their surface properties and biogenic synthesis. Biogenic synthesis often results in nanoparticles with a natural biocompatible coating, reducing the risk of cytotoxicity and enhancing their potential for in vivo applications [68]. Future studies could expand on this by exploring the interaction of MgO nanoparticles with other blood components, such as platelets and plasma proteins, to ensure comprehensive biocompatibility. Additionally, understanding the long-term effects of these nanoparticles in vivo will further substantiate their safety profile and potential in clinical applications.

CONCLUSION

This study successfully demonstrated the biosynthesis of magnesium oxide (MgO) nanoparticles using *Heyndrickxia coagulan* as

an efficient and eco-friendly approach. The synthesized MgO nanoparticles exhibited significant antibacterial activity, particularly against multidrug-resistant *Pseudomonas aeruginosa*, a major pathogen implicated in diabetic foot infections (DFIs). In addition to their antimicrobial properties, MgO nanoparticles showed remarkable antioxidant activity, effectively scavenging free radicals in a dose-dependent manner. Importantly, the nanoparticles were non-hemolytic, underscoring their biocompatibility and safety for potential therapeutic applications. The results of this study highlight the potential of biogenic MgO nanoparticles as a promising alternative or complementary solution to conventional antibiotics in managing infections caused by resistant pathogens. Future research should focus on elucidating the detailed mechanisms of action of MgO nanoparticles and conducting in vivo studies and clinical trials to assess their efficacy and safety in real-world applications. This could assist in their integration into clinical practice, addressing the growing threat of antimicrobial resistance and improving outcomes for patients with DFIs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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