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Investigation of the Antimicrobial Effects of Magnesium Oxide Nanoparticles Used in the Food Industry on Gram Positive and Gram Negative Bacteria

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Abstract

Background and Objective: Previous studies have demonstrated that certain nanoparticles possess antimicrobial properties. In recent years, new applications of these nanoparticles have been developed to enhance public health in everyday life. The aim of the present study is to evaluate and compare the antimicrobial activity of magnesium oxide nanoparticles against Gram positive and Gram negative bacteria.

Materials and Methods: The antimicrobial activity of magnesium oxide nanoparticles was evaluated against two Gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and one Gram positive bacterium, *Staphylococcus aureus*. Suspensions of magnesium oxide nanoparticles were prepared at different concentrations, and their antimicrobial activity was assessed using the agar diffusion method. In addition, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the nanoparticles were determined. Statistical analysis was performed using ANOVA.

Results: Magnesium oxide nanoparticles exhibited significant antimicrobial activity against both Gram positive and Gram negative bacteria. The minimum inhibitory concentration (MIC) of the nanoparticles was 0.78 mg/mL, while the minimum bactericidal concentration (MBC) was 1.56 mg/mL.

Conclusion: Considering the effective antimicrobial activity of magnesium oxide nanoparticles against Gram positive and Gram negative bacteria, these nanoparticles have the potential to be utilized in the development of antimicrobial materials that can compete with other similar compounds.

Keywords Bacteria; Antimicrobial activity; Magnesium oxide nanoparticles; MIC; MBC.

1. Introduction

With the rapid development of human life, controlling the harmful effects of microorganisms has become inevitable. Researchers have reported that nanoparticles based on metal ions can exhibit broad-spectrum cytotoxic activ-

ity and show effective antimicrobial action against bacteria, fungi, and viruses (1). Studies conducted on Gram positive and Gram negative bacteria have demonstrated the antimicrobial properties of zinc oxide nanoparticles. Additionally, investigations on the antimicrobial charac-



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teristics of iron oxide, silver, calcium carbonate, titanium dioxide, chromium oxide, and other nanoparticles have been carried out, each showing specific effects against various bacteria. Therefore, scientists believe that such nanoparticles may serve as suitable alternatives to conventional antibiotics (2).

The discovery of new antibiotics has not kept pace with the rapid increase in bacterial resistance; consequently, innovative approaches are required to combat bacterial infections (3–6). Formulating compounds in nanoparticle structures, compared with their conventional forms, may facilitate the development of new antibacterial drugs in the future. The permeability of microbial cell membranes changes depending on nanoparticle concentration, eventually leading to disruption of the cell wall and bacterial death (7–8). Considering the characteristics of metallic nanoparticles and their bactericidal ability against both Gram positive and Gram negative bacteria, the present study aims to evaluate the antibacterial activity of magnesium oxide nanoparticles against three important infection causing bacteria.

The antibacterial activity of copper oxide nanoparticles against both Gram negative and Gram positive bacteria has been demonstrated. However, the potential toxicity and other characteristics of these nanoparticles have limited their widespread application. Antibacterial tests have shown that coated copper oxide exhibits antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*. It should be noted that the antibacterial activity of silver is lower than that of the two types of coated copper oxide nanoparticles. Differences in resistance levels among these materials against bacteria may be related to variations in the major functional groups present on the bacterial surface (2).

In 2009, Robert P. Allaker investigated the identification of metal oxide nanoparticles with antibacterial properties (9). In 2012, Mohammad Hassan Shirazi and colleagues studied the antimicrobial properties of metallic nanoparticles. Their findings indicated that ZnO nanoparticles possess antibacterial activity against both Gram positive and Gram negative bacteria. The results also showed that fabrics containing zinc nanoparticles represent a promising method for eliminating bacteria from textile materials (10). In 2017, Habibi and colleagues examined the antifungal activity of magnesium oxide and copper oxide nanoparticles against different species of *Aspergillus*. Their results demonstrated that the application of nanotechnology, particularly metal oxides, can reduce fungal contamination in food products (11).

A study by Espitia et al. in 2013 demonstrated that Fe₂O₃, ZnO, and MgO nanoparticles exhibit antibacterial activity against Gram positive and Gram negative bacteria,

as well as antifungal activity against *Aspergillus niger*, *Saccharomyces*, and other fungi (12). Although relatively few studies have investigated the antibacterial effects of magnesium oxide nanoparticles, their low synthesis cost and lower toxicity compared with silver and copper nanoparticles make them a more economical option. Magnesium oxide has been reported to possess bactericidal activity against pathogenic *Escherichia coli* (13). Metallic nanoparticles are considered important antibacterial agents, and among them, MgO nanoparticles exhibit lower toxicity toward mammalian cells compared with many other nanoparticles. Therefore, the aim of the present study is to investigate and compare the inhibitory effects of magnesium oxide nanoparticles on the growth of three bacteria: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The antimicrobial activity of magnesium oxide nanoparticles against these bacteria was evaluated using the agar well diffusion method, determination of the minimum inhibitory concentration (MIC), and determination of the minimum bactericidal concentration (MBC).

Materials and Methods

Preparation of Bacterial Suspensions

To prepare the bacterial suspensions, the obtained bacterial strains were first inoculated into Tryptic Soy Broth (TSB). The cultures were then streaked onto nutrient agar plates, which were incubated at 37°C for 18–24 hours to obtain fresh microbial cultures in the logarithmic growth phase. Subsequently, microbial suspensions were prepared from the bacteria grown on nutrient agar. For preparation of the bacterial suspension used in the experiments, bacterial cells were collected from the agar surface using a sterile loop and transferred into Ringer's solution. After shaking, a uniform suspension with a defined turbidity was obtained. To ensure the presence of approximately 1.5×10^8 CFU/mL in the suspension, the turbidity was compared with that of a 0.5 McFarland standard, and the absorbance was measured using a UV–visible spectrophotometer at 620 nm. Based on the instrument calibration, absorbance was adjusted to the range of 0.5–0.6. A surface culture was then prepared from the microbial suspension on agar plates (6, 14).

Assessment of Antimicrobial Activity Using the Well Diffusion Method

To evaluate bacterial susceptibility to the nanoparticles via the well diffusion method, a surface culture was first prepared on nutrient agar using a bacterial suspension containing 1.5×10^8 CFU/mL. Wells of uniform diameter were then created on the plates. Magnesium oxide nanoparticle suspensions were

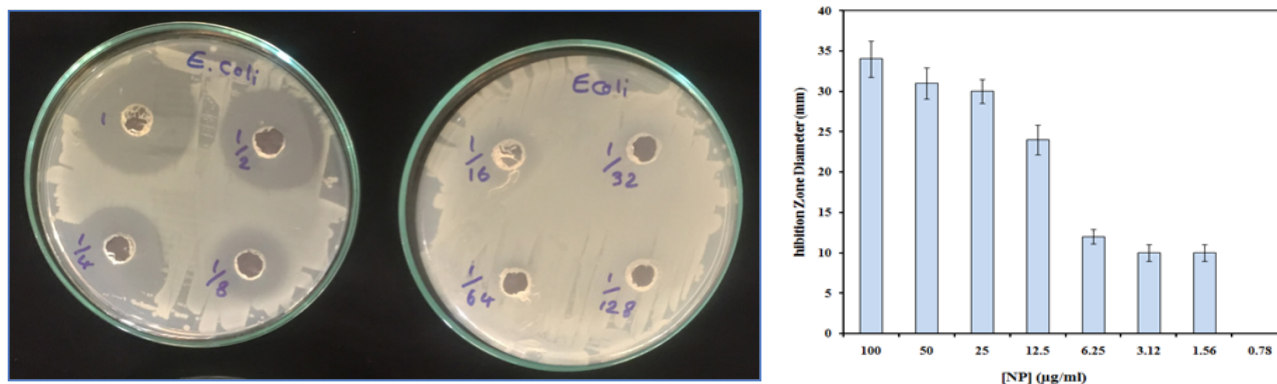


Fig 1. Diameter of the inhibition zones of *Escherichia coli* in the presence of various concentrations (0.78–100 µg/mL) of magnesium oxide nanoparticles.

prepared using serial dilutions at concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/mL. From each nanoparticle dilution, 100 µL was added into each well. The plates were incubated at 37°C for 18–24 hours, after which the diameter of the inhibition zones around the wells containing the nanoparticles was measured (15). All procedures were performed in triplicate, and the mean values were reported.

For statistical comparison among treatments, one-way analysis of variance (One-way ANOVA) was used. Post-hoc comparisons among means were performed with the Scheffé test at a significance level of $P < 0.05$. Statistical analysis was conducted using SPSS version 22, and graphs were generated in Microsoft Excel 2013.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the antibacterial compound capable of inhibiting visible bacterial growth under laboratory conditions. The minimum bactericidal concentration (MBC) was defined as the lowest concentration capable of killing the bacteria. Nanoparticle suspensions were prepared at serial dilutions of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/mL. From each dilution, 500 µL was added to tubes containing 0.5 mL of nutrient broth. Then, 1 mL of the microbial suspension was added to each tube, and the tubes were incubated at 37°C for 18–24 hours. After incubation, microbial growth was visually examined, and the first dilution showing no visible turbidity was recorded as the MIC (16, 17).

To determine the MBC, streak cultures were prepared on nutrient agar from all tubes showing no turbidity. The plates were incubated at 37°C for 18–24 hours. Bacterial growth on the plates was then assessed, and the MBC was determined as the lowest nanoparticle concentration at which 99.99% of the bacteria were killed.

Results

Antimicrobial Activity of Nanoparticles Assessed by the Well Diffusion Method

The antibacterial effects of the nanoparticles were evaluated separately for each bacterial strain using the well diffusion method. The results of the assay for the three bacteria are presented in Figures 1 to 4.

The results obtained from the well diffusion method showed that with increasing concentrations of magnesium nanoparticles, the diameter of the inhibition zones for all three bacterial strains increased. At a concentration of 100 µg/mL of magnesium nanoparticles, the inhibition zone diameters were 34.28 ± 0.92 mm for *Escherichia coli*, 36.27 ± 1.19 mm for *Staphylococcus aureus*, and 32.38 ± 1.11 mm for *Pseudomonas aeruginosa*.

In contrast, with the gradual decrease in the concentration of magnesium nanoparticles, a reduction in the inhibition zone diameter was observed. At a concentration of 0.78 µg/mL of magnesium nanoparticles, the inhibition zone diameter for *Staphylococcus aureus* was 15 ± 0.9 mm, while no inhibition zone was observed for *Escherichia coli* and *Pseudomonas aeruginosa*. These findings indicate that the antibacterial activity of magnesium nanoparticles decreases at lower concentrations.

By comparing the diameters of the inhibition zones for the three bacterial species cultured on nutrient agar and based on the statistical analysis performed, it was determined that all three bacteria were sensitive to magnesium nanoparticles. Among the tested bacteria, the largest inhibition zone diameter was observed for *Staphylococcus aureus* (36.27 ± 1.19 mm), which showed a statistically significant difference compared with the inhibition zone diameter of *Pseudomonas aeruginosa* ($P < 0.05$), indicating greater sensitivity. However, no statistically significant difference was observed between the inhibition zone diameters of *Staphylococcus aureus* and *Escherichia coli* ($P > 0.05$). The results of the statistical analysis are presented in Tables 1 and 2.

Results of Antimicrobial Activity Assessment Using MIC and MBC Methods

The antibacterial effects of magnesium nanoparticles against the tested bacterial strains, as determined by the MIC and MBC methods, are presented in Table 3. Culture tubes containing various concentrations of the nanoparticles along with the three bacterial species are shown in Figures 5–7.

The minimum inhibitory concentration (MIC) of magnesium nanoparticles for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was determined to be 0.78, 0.78, and 1.56 µg/mL, respectively. Figure 8 shows the culture plates of the bacteria at these MIC concentrations.

The minimum bactericidal concentration (MBC) of magnesium nanoparticles for *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was determined to be 1.56, 1.56, and 3.125 µg/mL, respectively. All three bacterial strains were found to be sensitive to magnesium nanoparticles.

Table 1. One way analysis of variance (ANOVA) for comparison of inhibition zone diameters among the three bacterial groups: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Group	Mean inhibition zone	Standard deviation	F	P
Pseudomonas aeruginosa	32.38	1.11	9.61	P =0.013
Escherichia coli	34.28	0.92		
Staphylococcus aureus	36.27	1.19		

Table 2. Results of Scheffé post hoc analysis.

Group	Mean inhibition zone	Standard deviation	F	P
Pseudomonas aeruginosa	32.38	1.11	9.61	P =0.013
Escherichia coli	34.28	0.92		
Staphylococcus aureus	36.27	1.19		

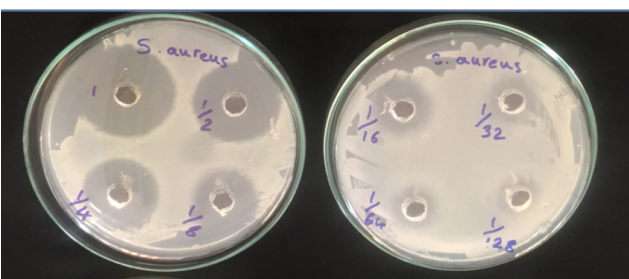


Fig 2. Diameter of the inhibition zones of *Staphylococcus aureus* in the presence of different concentrations (0.78–100 µg/mL) of magnesium oxide nanoparticles.

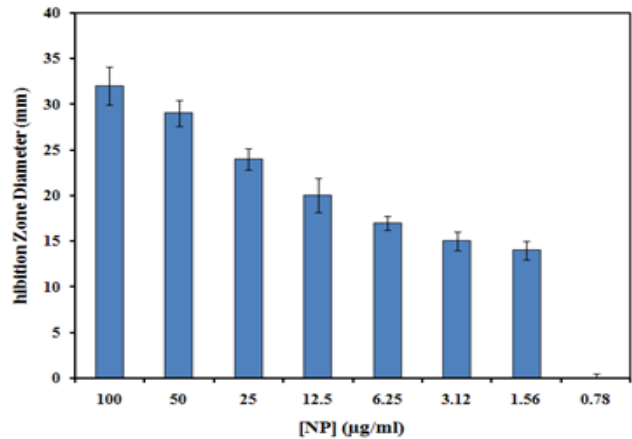
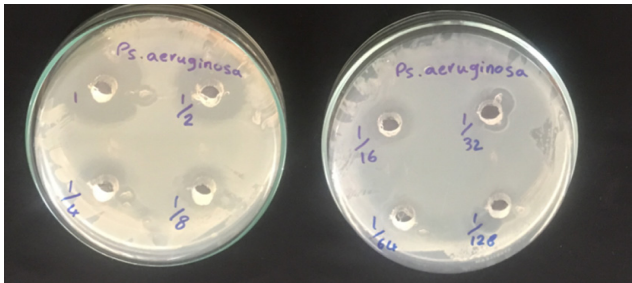


Fig 3. Diameter of the inhibition zones of *Pseudomonas aeruginosa* in the presence of various concentrations (0.78–100 µg/mL) of magnesium oxide nanoparticles.

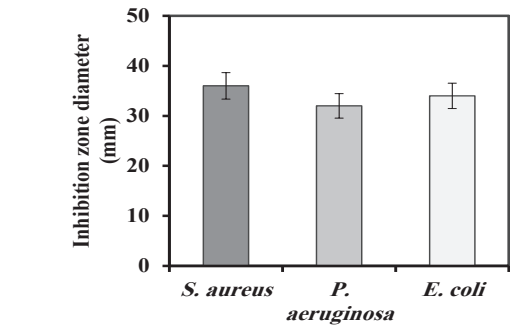


Fig 4. Comparison of inhibition zone diameters of the three bacterial strains in the presence of magnesium nanoparticles at a concentration of 100 µg/mL.

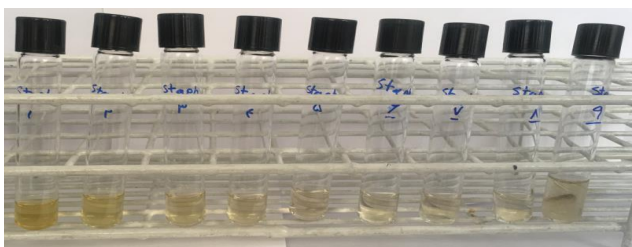


Fig 5. Serial dilutions of magnesium nanoparticle samples at concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/mL prepared in tubes containing 0.5 mL of nutrient broth for determination of the MIC of *Staphylococcus aureus*.

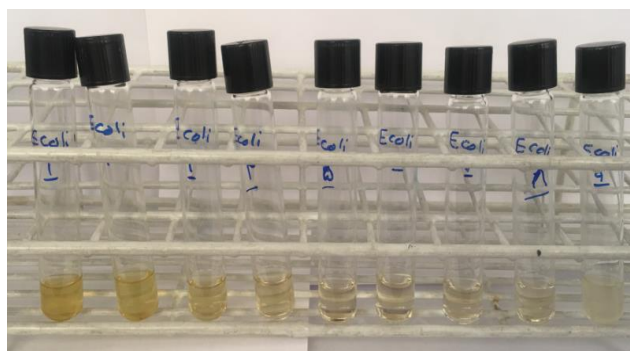


Fig 6. Serial dilutions of magnesium nanoparticle samples at concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 $\mu\text{g/mL}$ prepared in tubes containing 0.5 mL of nutrient broth for determination of the MIC of *Escherichia coli*.

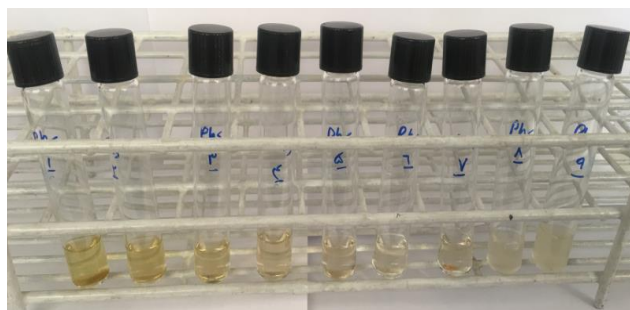


Fig 7. Serial dilutions of magnesium nanoparticle samples at concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 $\mu\text{g/mL}$ prepared in tubes containing 0.5 mL of nutrient broth for determination of the MIC of *Pseudomonas aeruginosa*.

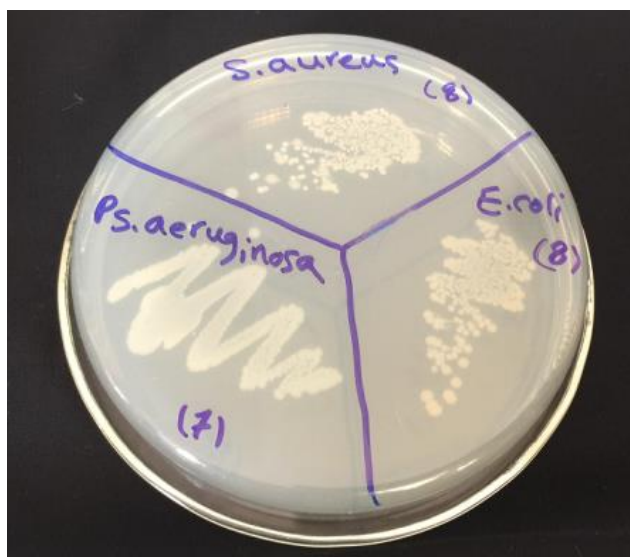


Fig 8. Streak culture from the MIC tubes on nutrient agar medium. The MIC values were 0.78 $\mu\text{g/mL}$ for *Staphylococcus aureus*, 1.56 $\mu\text{g/mL}$ for *Pseudomonas aeruginosa*, and 0.78 $\mu\text{g/mL}$ for *Escherichia coli*.

Discussion

With the rapid advancement of human life, controlling the harmful effects of microorganisms has become unavoidable, as their rapid and uncontrolled growth can lead to serious problems. Over the past decade, the development of nanotechnology has created valuable opportunities for exploring the antibacterial effects of metallic nanoparticles. Since the introduction and widespread use of antibiotics in medicine, bacteria have continuously attempted—under the principle of natural selection—to develop resistance to these agents. The excessive and indiscriminate use of antibiotics in recent years has caused numerous problems, including toxic side effects and, more importantly, the emergence of resistant strains. Metallic nanoparticles, in addition to exerting particle specific inhibitory effects, possess strong antibacterial activity due to their small size and large surface to volume ratio, which increases their contact with the external environment (1).

Chemical disinfectants are widely used in hospitals, clinical centers, and medical offices to prevent the spread of pathogenic microorganisms. Fortunately, various studies have revealed that certain nanoparticles exhibit antibacterial properties that, in comparison with other available antimicrobial agents, are highly effective. Research indicates that magnesium oxide nanoparticles (MgO NPs) can be used alone or in combination with other antimicrobial agents as a potent potential antibacterial compound to enhance food safety. In the medical field, magnesium oxide is used for relieving heartburn and gastric discomfort, as an antacid, detoxifying agent, and for bone regeneration. Recently, MgO nanoparticles have also been applied in cancer therapy, nanoscale cryotherapy, and hyperthermia. Thus, there is substantial potential for investigating the antimicrobial effects of these nanoparticles against various bacterial species (18–20).

In this study, the antimicrobial properties of magnesium nanoparticles were evaluated against important Gram positive and Gram negative bacterial pathogens by determining inhibition zone diameters as well as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

At a concentration of 100 $\mu\text{g/mL}$ of magnesium nanoparticles, the inhibition zone diameters were 34.28 ± 0.92 mm for *Escherichia coli*, 36.27 ± 1.19 mm for *Staphylococcus aureus*, and 32.38 ± 1.11 mm for *Pseudomonas aeruginosa*. These results demonstrate that magnesium oxide nanoparticles are effective against the Gram positive bacterium *Staphylococcus aureus* and the Gram negative bacteria *E. coli* and *P. aeruginosa*. The greatest antibacterial effect was observed against *S. aureus*, while the lowest effect was seen against *P. aeruginosa*. No sig-

nificant differences were observed between the two Gram negative bacteria (*E. coli* and *P. aeruginosa*), nor between *S. aureus* and *E. coli*.

The MBC values for *S. aureus*, *E. coli*, and *P. aeruginosa* were 1.56, 1.56, and 3.125 µg/mL, respectively, and the MIC values were 0.78, 0.78, and 1.56 µg/mL, respectively. Consistent with the findings of this research, other studies have also reported the antibacterial effectiveness of MgO nanoparticles, generally at much higher concentrations than those used in the present study. He et al. (2016) investigated the synthesis and antibacterial activity of magnesium nanoparticles against *Campylobacter jejuni*, *Escherichia coli*, and *Salmonella enteritidis*. Their results showed MIC values of 0.5, 1, and 1.5 mg/mL, respectively—much higher than the concentrations applied in the current study. They concluded that MgO nanoparticles exert strong antimicrobial activity, suggesting that interactions between the nanoparticles and bacterial cells lead to membrane leakage, induction of oxidative stress, and eventual cell death (15).

Similarly, Cai et al. (2018) assessed the antibacterial activity of magnesium oxide nanoparticles against *Ralstonia solanacearum* and reported concentration dependent antimicrobial effects. The MIC and MBC values were estimated at 200 and 250 µg/mL, respectively. TEM and SEM analyses demonstrated that MgO nanoparticles bind to the bacterial surface and exert antimicrobial effects through this interaction. They concluded that MgO nanoparticles possess favorable antimicrobial properties and have potential for future antimicrobial applications (16).

Espitia et al. (2013) reported that magnesium nanoparticles exhibit antibacterial activity against various Gram positive and Gram negative bacteria, including *S. aureus* and *E. coli*, as well as against *Aspergillus niger*, *Saccharomyces*, and other fungi. Their study also showed that Gram positive bacteria were more susceptible to MgO nanoparticles compared to Gram negative bacteria (12). They emphasized that due to the low production cost of MgO nanoparticles and their lower toxicity compared to silver and copper nanoparticles, their use may be more economically favorable in the future.

Samadi et al. (2016) investigated the antimicrobial effects of MgO nanoparticles against *E. coli* and *Listeria monocytogenes*, reporting that at a concentration of 4.1 mg/mL, MgO nanoparticles inhibited the growth of both bacteria (4). In comparison, the concentration used in this study was much lower.

Additional investigations have evaluated the effects of MgO nanoparticles on the bacterial inactivation kinetics of both Gram positive and Gram negative species. The results showed that at concentrations equal to one or two times the MIC, *E. coli* populations declined more rap-

idly over time than other species, suggesting that MgO nanoparticles could serve as effective disinfectants for controlling Gram negative bacteria such as *E. coli* (6). Further comparative studies have examined the antibacterial activity of silver and zinc nanoparticles against pathogenic bacteria such as *P. aeruginosa* and *S. aureus*. The results revealed that at a concentration of 0.5%, both nanoparticle types exhibited bactericidal activity, while 0.01% showed bacteriostatic effects. Additionally, the antibacterial efficacy was positively correlated with nanoparticle concentration (11). Similar trends were observed in the present study, as higher concentrations of MgO nanoparticles resulted in greater bactericidal and bacteriostatic effects, with the highest impact occurring at 100 µg/mL.

Other researchers have incorporated MgO nanoparticles into dental filler materials and reported MIC and MBC values of 500 and 1000 µg/mL, respectively—substantially higher than those observed in this study (21). Studies on biogenic zinc oxide nanoparticles (ZnO NPs) have reported MIC values similar to those of the present study, suggesting that nanoparticle properties such as size, synthesis method, and surface chemistry can significantly influence antimicrobial activity (3).

Regarding the mechanisms of antibacterial activity, several hypotheses have been proposed, including electrostatic interactions between nanoparticles and bacterial surfaces leading to membrane disruption, alterations in membrane permeability, and penetration of metal ions due to changes in proton motive force, as well as reduced activity of specific periplasmic enzymes (1, 15, 16).

Combined antimicrobial effects of MgO nanoparticles with other antimicrobial agents have also been studied. Samadi et al. demonstrated that MgO nanoparticles and the natural antimicrobial poly L lysine exhibited partial synergistic effects against *E. coli* and *L. monocytogenes* (5). Other studies have investigated metallic nanoparticle combinations, reporting that silver–copper oxide nanocomposites exhibited greater antibacterial activity than individual particles, although still weaker than the activity of MgO nanoparticles observed in the present study (4).

Research also shows that MgO, ZnO, and Fe₂O₃ nanoparticles display antibacterial activity against a wide range of Gram positive and Gram negative bacteria as well as fungi, with Gram positive bacteria generally being more susceptible (12, 13). Furthermore, the combined use of MgO and Fe₂O₃ nanoparticles in fruit juices demonstrated greater sensitivity in *S. aureus* than in *E. coli* (22).

Conclusion

The results of the present study demonstrated that magnesium nanoparticles possess antimicrobial potential. In-

creasing the concentration of magnesium nanoparticles led to an increase in the diameter of the inhibition zones in all three Gram positive and Gram negative bacterial strains examined. At a concentration of 100 µg/mL of magnesium nanoparticles, the inhibition zone diameters were 34.28 ± 0.92 mm for *Escherichia coli*, 36.27 ± 1.19 mm for *Staphylococcus aureus*, and 32.38 ± 1.11 mm for *Pseudomonas aeruginosa*. In contrast, a gradual decrease in nanoparticle concentration resulted in a reduction in inhibition zone size. At 0.78 µg/mL, the inhibition zone diameter for *Staphylococcus aureus* was 15 ± 0 mm, while no inhibition zones were observed for *E. coli* or *P. aeruginosa*, indicating that the antibacterial activity of magnesium nanoparticles declines at lower concentrations.

Comparison of inhibition zone diameters for all three bacteria grown on nutrient agar showed that they exhibited relatively similar levels of sensitivity to magnesium nanoparticles. However, among the tested bacteria, *Staphylococcus aureus* demonstrated the greatest susceptibility, as reflected by its largest inhibition zone diameter (36.27 ± 1.19 mm), indicating lower resistance to the nanoparticle treatment.

Overall, considering the strong antimicrobial effect of magnesium oxide nanoparticles observed in this study against both Gram positive and Gram negative bacteria—compared with previously reported studies—it is recommended that future investigations evaluate the combined use of MgO nanoparticles with other nanoparticle types.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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