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Original Article

Biogenic synthesis and characterization of MgO nanoparticles from banana peel extract with evaluation of their antibacterial and antioxidant activities in cellular models

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Abstract

The current research involved the preparation of Magnesium Oxide Nanoparticles (MgO NPs) by aqueous extraction of banana peels, then characterizing the resulting particles and incorporating them into polymer matrices to provide antimicrobial activity for packaging materials. The resulting particles were characterized by ultraviolet-visible (UV-Vis) and scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), and infrared (FTIR) spectroscopy. The ultraviolet spectrum of Magnesium Oxide nanoparticles showed sharp absorption peaks at 350 and 500 cm^{-1}. Both SEM and TEM revealed MgO NPs as almost spherical granular structures. XRD showed six main peaks of the crystalline mineral elements of the magnesium oxide nanoparticles. FTIR results confirmed that biologically active compounds act as reducing and capping agents for the nanoparticles. The magnesium oxide nanoparticles also showed antibacterial and antioxidant activity and non-toxicity.

Keywords: Magnesium oxide nanoparticles, Antibacterial, Antioxidants, toxicity, Banana peels.

1. Introduction

Food preservation is one of the most important tasks of producers and exporters in the field of food technology. The lack of use of effective food preservation techniques leads to the production of food waste. According to the Food and Agriculture Organization, up to 1.3 billion tons of food are wasted annually, which represents about a third of the total global food production as well as Moreover, preserving food incorrectly can pose a risk to the health of consumers [1-3]. Recent studies have focused on addressing food-related challenges by exploring alternatives such as the use of nanomaterials, including metal-based nanomaterials [4] and subnanomaterials [5]. These nanoparticles also play a significant role in enhancing energy storage and efficiency.

Many plants and microbes have been used for this synthesis [6]. The green synthesis of nanoparticles using living cells or plants is a promising and new tool in the field of bionanotechnology. Biological methods are preferred because they are safe, environmentally friendly, clean, effective, low-cost, easy, high-production and pure, and avoid the use of water to eliminate toxic, harmful and dangerous substances; biosynthesis can be achieved. Through many biological entities, including bacteria, algae, yeast, fungi, and plant extracts [7-8]. Nanomaterials as antimicrobial materials, as well as microbial damage caused by microorganisms, including bacteria and fungi, have a role in reducing the shelf life of foods and increasing the possibility of foodborne diseases and are a major global concern. Foodborne diseases annually cause up to 600 million illnesses and 420,000 deaths worldwide. According to the World Health Organization, producers use different methods to preserve food, such as pasteurization, thermal sterilization, freezing, chemical preservation, etc, to ensure food safety and increase its shelf life all over the world. The most common method is the use of chemical preservatives such as organic acids and their salts, such as citric acid, acetic acid, alcohols, ascorbic acid, potassium sorbate, and others, because of their low prices. For the sake of food and consumer safety, they are not often applied. To reduce microbial risks, antimicrobial packaging has been used, which is one of the applications. Promising

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for active food packaging, antimicrobial packaging interacts with packaged foods in order to reduce, delay and prevent the growth of spoilage and pathogens.[9]. There are several methods for producing antimicrobial packaging, such as placing an antibacterial component in the polymer or fusing the component with the polymer by melting or using solvents. Covalent or ionic incorporation, materials used in antimicrobial packaging include sugars, bacteriocins, vegetable oils, and synthetic antibacterial agents, such as Solutions (benzoic acid, sodium benzoate, sorbic acid, and sodium sorbate) and the solutions provided by nanotechnology [10-11]. Nanomaterials are often used to improve the packaging properties of food materials due to their antimicrobial activity, UV protection, antioxidant potential, etc. Antimicrobial nanomaterials such as Ag, TiO_2, ZnO and magnesium oxide (MgO), due to their high antimicrobial activity, are suitable agents. Very useful for antimicrobial packaging systems, nano TiO_2 materials are non-toxic to the human body and are approved and often applied as food additives or food contact materials in food packaging. More research is needed on the effects on the body after digestion and absorption on the body. To ensure safety in the food industry [12-13]. Nanomaterials as antioxidants can help prevent autoxidation or peroxidation, which are spontaneous reactions of organic molecules with oxygen that lead to the formation of peroxides, aldehydes, ketones, epoxides, and other simpler fragments or insoluble compounds, creating unfavorable conditions for food marketers. By utilizing waste to produce valuable nanoparticles, sustainable solutions can be found that reduce waste. The most important sources of nanoparticles produced by green technologies include iron, zinc, magnesium, and copper. And gold, silver and their oxides. Magnesium oxide nanoparticles act as a safe alternative with highly effective antimicrobial activity. Magnesium oxide nanoparticles have been used as excellent nanocarriers with stable physical, chemical, and biological properties [14-15]. It is also recognized that magnesium oxide nanoparticles have many advantages, including thermal stability, non-genotoxicity, and non-toxicity to humans, which provide notable applications in various agricultural fields. Moreover, these particles help in increasing agricultural yields by promoting seedling growth. Magnesium oxide nanoparticles are also used in plants as food additives, supplements, and for color retention. They possess antimicrobial properties and are effective against foodborne pathogens such as *Escherichia coli* and *Salmonella enterica* [16-17].

The main objective of this study is to biosynthesize magnesium oxide nanoparticles using banana peel extract, characterize their physicochemical properties, and evaluate their antibacterial and antioxidant activities for potential applications in food packaging and safety.

2. Materials and methods

2.1. Preparation and storage of banana peels for nanoparticle synthesis

Ripe banana peels were obtained from local markets in Basrah Governorate. They were thoroughly washed with distilled water to remove impurities and dust and then stored in the refrigerator until use [18].

2.2. Preparation of nanomagnesium particles

Nanomagnesium particles were prepared in two stages. The first stage involved the aqueous extraction of banana peels using the method described by Primožič et al (2021) [19]. The banana peels were washed with distilled water three times to remove any dirt or impurities. They were cut into very small pieces, weighing 100 grams, and placed in a glass beaker with 500 ml of distilled water and boiled at 100°C. At a temperature of 80°C for 10 minutes, the extract was then filtered twice with filter paper to get rid of suspended impurities and large particles. The resulting filtrate was stored at 4°C until use. The second stage: synthesis of magnesium oxide nanoparticles was done by following the method of Salem and Fouda (2021) [20]. To synthesize magnesium oxide nanoparticles with some modifications, 100 ml of banana peel extract and 10 ml of $MgCl_2$ (mM) were mixed using a hot plate with a magnetic stirrer at 600 rpm and heated to 90 °C. When the temperature reaches 90 °C, 100 ml of NaOH is added dropwise using a burette. The solution is then left to react for three hours until $Mg(OH)_2$ forms, resulting in a chalky white precipitate. The precipitate is subsequently centrifuged at 7,500 rpm for 30 minutes at room temperature. The precipitate was washed twice with absolute ethanol (99%) and then dried in an oven to remove any residual water and ethanol. The antibacterial activity of the magnesium oxide nanoparticle solution was evaluated using an established method to measure its inhibitory effectiveness against various bacterial species. Saravanan et al (2021) [21] observed the inhibitory activity against bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

2.3. Detection of nanoparticle toxicity

The toxicity of nanoparticles against red blood cells was evaluated following the methods described [22, 23]. The blood suspension was prepared by adding 1 ml of human blood, which was obtained from the medical unit at University of Basrah, to 20 ml of normal saline, then the concentrations were prepared by dissolving (0.01, 0.03 and 0.05 mg/ml) of Nanomagnesium oxide in distilled water and mixing 100 micrometers of each concentration into 2 ml of blood suspension. Control samples were prepared by adding 100 microliters of distilled water to the blood suspension. The samples were incubated at a temperature of 37°C, and the turbidity was monitored for periods of 3-24 hours.

2.4. Measurement of antioxidant activity

The antioxidant activity of magnesium oxide nanoparticle solution and the synthetic antioxidant Butylated Hydroxy Toluene (BHT) was evaluated according to the method described in Younis et al (2021) [24]. Briefly, 1 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent at a concentration of 0.01 mmol, prepared in 98% methanol, was mixed with 2 ml of nanomagnesium oxide solution at concentrations of 3, 5, 7, and 10 mg/g. The mixture was then incubated in the dark at 25°C (room temperature) for 30 minutes before measurement.

The comparison sample was mixed with 1 DPPH with 2 ml of distilled water, the absorbance was measured at a wavelength of 517 nm, and the antioxidant activity was calculated using the following equation: DPPH1% = (Ao-A1)/Ao]x100 = Ao absorbance of the comparison sample A1=absorbance of nanomagnesium oxide solution, 1 = Inhibition percentage.

2.5. Statistical analysis

Statistical results showed a highly significant effect at the probability level of ($P \leq 0.05$) about our synthesis method in comparison with others and storage duration on the overall acceptability ratings. However, the interaction between the synthesis and storage period had an insignificant effect on the overall acceptability ratings of purity and stability of magnesium oxide nanoparticles, preserved at room temperature and activity. The reason for the use of magnesium nanoparticles may be due to the antibacterial activity and reduced oxidation reactions as well as the negative observation in toxicity test that occur as mentioned in previous section during the storage period, as well as the reduced number of microorganisms that cause contamination, as supported by Khodaei et al. (2023) [25].

3. Results

The banana peel aqueous extract changed in color from honey-yellow to blackish-brown after adding a solution of magnesium dichloride ($MgCl_2$) and sodium hydroxide (NaOH), see Figure (1A&B). This is evidence and confirmation of the synthesis of nanoparticles of magnesium oxide (MgO) with very small sizes. The explanation for this phenomenon is attributed to the presence of ascorbic acid and phenolic compounds, including flavonoids and other compounds such as marins, terpenes, flavonoids, and polymethyl flavonoids in the aqueous extract of banana peels as a reducing agent that reduces magnesium dioxide and as a supporting factor and the solution was kept at laboratory temperature (24°C). SEM test in Figure 2 shows the appearance of MgO as a hexagonal crystal and magnesium oxide grains with a clear structure appeared on the surface. The magnification power was 100 nm, as the image shows the nanoparticles with homogeneous sizes and these nanoparticles appeared in a spherical crystalline form and their diameters ranged between (47.194 - 70.412) nm, due to the effect of the agglomeration of the nanoparticles. TEM data of magnesium oxide nanoparticles in Figure 3 show that the nanoparticles are spherical and indicate that MgO nanoparticles, which were synthesized, revealed three main peaks of crystalline mineral MgO NPs, confirming the presence of Mg and O.

Fig. 1. A: The Banana peel aqueous extract, **B:** color changing.

U.V image in Figure 4 of the MgO spectrum contains several peaks and shows a broad absorption peak between (260-330) nm. The spectrum contains two absorption humps in the range of (320-400) nm, centered at 380 nm and between (500-600) nm, with a peak at 550 nm. FTIR Infrared spectroscopy was used to observe the active aggregates present on the surface. From these aggregates, we can infer the formation of magnesium oxide nanoparticles. Several large absorption peaks appeared in a broad range (3500-500) cm^{-1}, which is specific to the stretching vibration mode of the nano-magnesium oxide. This absorption range indicates that the nano-magnesium oxide powder is crystalline. The infrared spectrum revealed the identification of the active groups of nano magnesium oxide, shown in Figure 5. There are two bands at wavelengths of

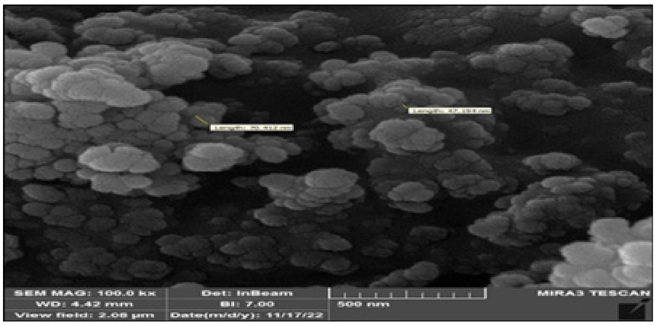

Fig. 2. SEM image of MgO nanoparticles.

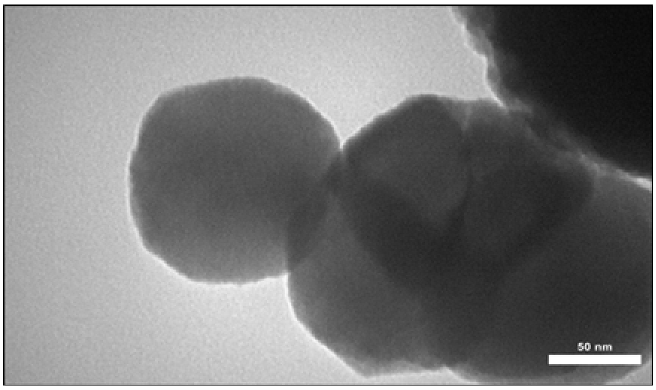

Fig. 3. TEM image of Mgo particles.

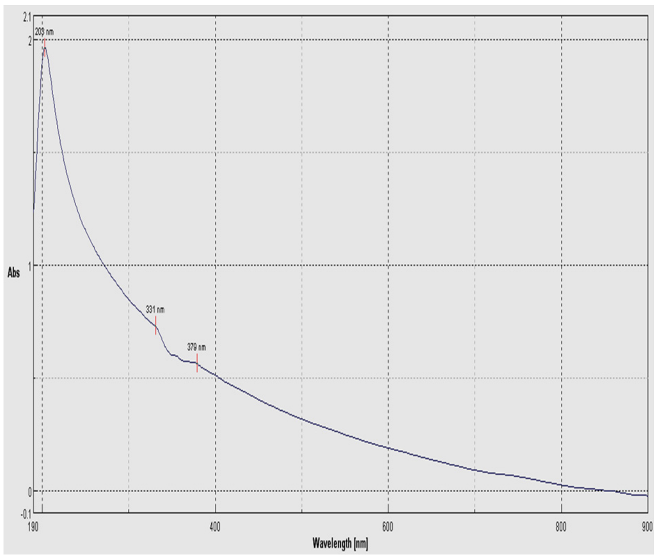

Fig. 4. UV spectrum of nanoparticles of Magnesium Oxide.

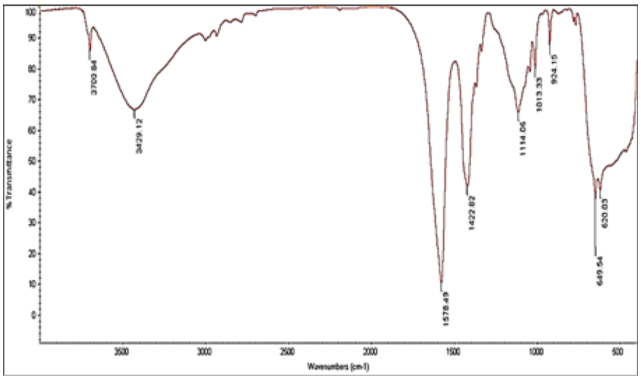

Fig. 5. FTIR of Magnesium Oxide nanoparticles.

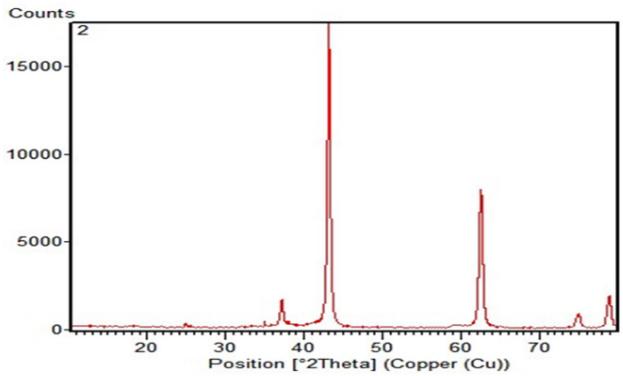

Fig. 6. XRD pattern for MgO nanoparticles.

3700.84 cm^{-1} and 3429.12 cm^{-1}, which are due to the vibration of the N-H and O-H groups of the amine group, in addition to the appearance of two peaks at wavelengths of 1422.82 cm^{-1} and 1578.49 cm^{-1}, which are due to the bending vibration of the C-H groups of the methyl group CH_3. As for the last band between (600-900) cm^{-1}, it is due to the stretching vibration of the C=O group, which indicates the formation of nano magnesium oxide.

The crystalline structure and composition of magnesium oxide nanoparticles were analyzed using XRD. The spectrum in Figure 6 represents magnesium oxide with seven intense peaks in the spectrum over the angle range (20-80°). The spectral results revealed that angles after 20° indicate the formation of magnesium oxide nanoparticles, and the corresponding bands appeared clearly and sharply at 18°, 38°, 51°, 58°, 61°, 68°, and 72°, respectively. The diffraction values provide information about the amount of defects in the crystal, as the diffraction intensity values in these samples were very small, indicating the good crystalline order of the prepared magnesium oxide nanoparticles without any impurities.

The antibacterial activity of magnesium oxide nanoparticles was tested by measuring the inhibition zone against both Gram-positive *Staphylococcus aureus* and Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*. The results shown in Figures (7A, B & C) indicated that magnesium oxide nanoparticles prepared biologically from banana peel extract have antibacterial activity against all tested genera, which shows that the higher the concentration, the greater the inhibitory effect of magnesium oxide nanoparticles.

A toxicity test was conducted by preparing blood suspension and nutrient serum, as well as different concentrations of magnesium oxide nanoparticles. The results showed that they did not cause any changes in human blood cells, such as decomposition or sedimentation after 3, 10 and 24 hours, which indicates that there is no toxic effect of nanoparticles on human blood at all concentrations. Thus, these nanocomposites are safe for human consumption and can be used in various food industries.

The antioxidant activity was estimated by testing the free radical scavenging capacity of magnesium oxide nanoparticle solutions using Diphenyl-1-Picrylhydrazyl (DPPH reagent) compared to the synthetic antioxidant Butylated Hydroxy Toluene (BHT), as shown in Figure 8. It was observed that the antioxidant activity gradually increased with increasing concentration, based on the absorbance-concentration relationship.

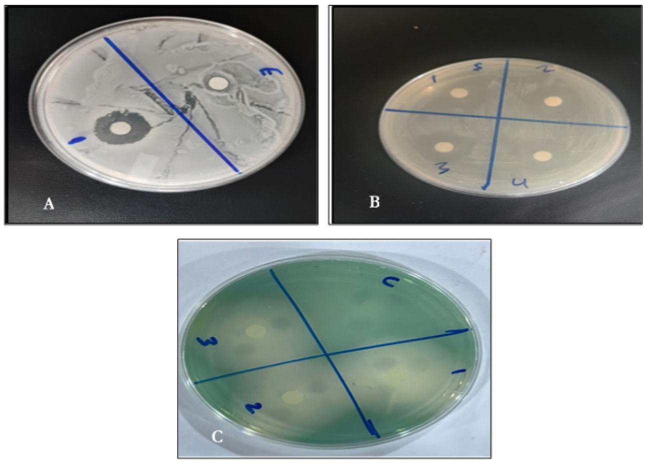

Fig. 7. A: Testing the inhibitory effectiveness of nanomagnesium oxide against *Escherichia coli*, **B:** *Staphylococcus aureus* and **C**-*Pseudomonas aeruginosa* bacteria.

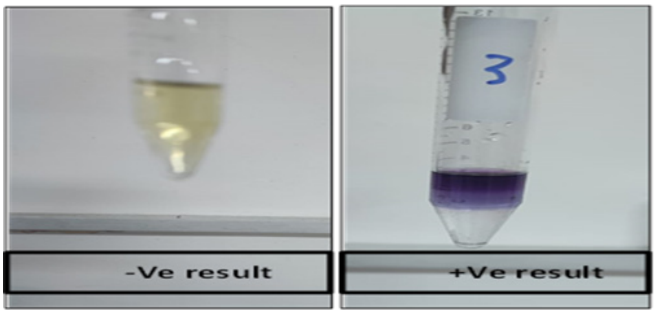

Fig. 8. Antioxidant activity of MgO nanoparticles.

4. Discussion

We used banana (*Musa paradisiaca* Linn.) peel extract to prepare magnesium oxide nanoparticles as a reducing agent by changing the color of the banana peel extract from honey yellow to blackish brown. This change is preliminary evidence of the formation of nanoparticles. This result is consistent with the results obtained by Ogunyemi et al. (2019) [26], who observed the color change of the chamomile flower extract from honey-yellow to blackish-brown, indicating the beginning of the formation of nanoparticles of magnesium oxide (MgO). By scanning electron microscopy (SEM), the morphology and size of MgO nanoparticles synthesized by aqueous extract showed that the particles were spherical, and the percentage of elemental composition of MgO nanoparticles was 47.89% Mg and 52.11% O. SEM revealed that MgO NPs were almost spherical granular structures, and the size was

26.70 nm. The results which were obtained by Abdel-Aziz et al. (2020) [27] are consistent with the current results in this study. TEM images showed that the magnesium oxide particles were spherical, also showed that the TEM pattern revealed three main peaks of crystalline mineral MgO NPs, confirming the presence of Mg and O. TEM examination revealed the MgO NPs as nearly spherical granular structures, which is consistent with the results of Fatiqin et al. (2021) [28]. UV–Vis spectroscopy indicated the formation of MgO nanoparticles due to the presence of an absorption peak at 270 nm in the aloe vera extract, as shown by Almontasser et al. (2019) [29], in his study on the chemical preparation of magnesium oxide nanoparticles from magnesium nitrate hexahydrate $Mg(NO)6H_2O$, ammonia, and distilled water, he pointed out that the UV-vis spectrophotometer was used to record the UV-visible spectra of the absorption of the prepared MgO nanopowder. In the wavelength range between (200-900) nm, the absorption of MgO NPs was determined, and distinct absorption bands of MgO up to 800 nm were obtained. The presence of MgO nanoparticles prepared from the aqueous extract of chamomile was confirmed by the UV-vis spectrophotometer, and the nanoparticles show a strong absorption band at 230 nm.

After that, several tests were conducted to characterize the magnesium oxide nanoparticles. Including the infrared spectroscopy (FTIR), which showed the presence of two bands at wavelengths of 3700.84 cm-1 and 3429.12 cm-1 due to the vibration of the N-H and O-H groups. The amine group has two peaks at wavelengths of 1422.82 cm-1 and 1578.49 cm-1. The bending vibration of the C-H groups of the CH_3 methyl group, while the last band between (600-900) cm-1 belongs to the stretching vibration of the C=O group, which indicates the formation of nano-magnesium oxide and Fatiqin et al., (2021) [28], was shown that the surface-produced MgO nanoparticles do not contain other molecules and these results are consistent with the current study.

Fatiqin et al. (2021) [28] confirmed the formation of MgO nanoparticles in the aqueous extract of Aloe bark using green synthesis and X-ray diffraction (XRD), and the spherical crystalline structure of MgO nanoparticles was confirmed, which is consistent with the current results. The inhibitory zone for Gram-positive *Staphylococcus aureus* was 29 mm at a concentration of 100 µg/ml of magnesium oxide nanoparticles, and it has the highest activity against microorganisms, while for Gram-negative bacteria The inhibitory diameter of *Pseudomonas aeruginosa* was 26 mm, while *Escherichia coli* was less responsive to the inhibitory activity of magnesium nanoparticles than the rest of the tested bacterial species, as it had lower inhibitory diameters at all concentrations, as the inhibitory diameter at the highest concentration of 100 micrograms/ml was 25 mm. These results were consistent with the observation of Safawo et al. (2018) [30]. Patel et al. (2013) [31] indicated that MgO nanoparticles at concentrations less than 200 µM/ml showed no cytotoxic effect, and in the concentration range of (150-250) µM/ml, with a slight toxic effect appearing at higher concentrations (300-350) µM/ml. From the current study, it can be said that MgO nanoparticles can be used at a safe concentration level for normal cells. The antioxidant activity results showed that the synthetic antioxidant BHT was higher than that of the concentrations prepared from magnesium oxide nanoparticle solutions, and the activity increased significantly with increasing concentration**.** MgO nanoparticles showed the highest antioxidant activity, 70.38%, at a concentration of 10 mg/ml compared to the other concentrations, while the lowest antioxidant activity was at a concentration of 3 mg/ml. For nano magnesium oxide particles, it is 49.38%. Shahid et al. (2022) [32] showed that MgO nanoparticles have antioxidant activity when tested by the DPPH method. The scavenging potential of the particles was examined by the degree of color change and comparison with ascorbic acid. This test gave an antioxidant activity for MgO nanoparticles.

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Conflict of interest
The authors have declared that no conflict of interest exists.

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Ethics statement
Not applicable.

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