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Green-fabricated MgO nanoparticles: A potent antimicrobial and anticancer agent

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ABSTRACT

MgO nanoparticles are gaining popularity because of their potential applications in several different industries, such as bioengineering, medicine, and environmental protection. The fabrication of MgO nanoparticles with enhanced biological properties remains difficult, despite the growing interest in this area. In this paper, we describe a method that minimizes environmental impact when manufacturing MgO nanoparticles using garlic (Allium sativum L.) extract. For ages, one of the most vital functions in human nutrition and medicine has been played by garlic. Garlic also contains phytochemicals that are effective against cancer and microbes. Our goal was to increase the medicinal relevance of MgO nanoparticles while decreasing our reliance on harmful chemicals. Various characterization techniques were employed to confirm the synthesis of MgO nanoparticles, including SEM, TEM, EDX, and XRD. The study findings indicate that the synthesis of MgO nanoparticles yielded a polycrystalline cubic configuration with a crystal size of 55-60 nm. The results of the EDX analysis suggest that only Mg and O elements are present, without any detectable impurities. The efficacy of MgO nanoparticles against grampositive (Staphylococcus epidermidis), gram-negative bacteria (Escherichia coli) and a fungal pathogen (Candida albicans) was investigated. The results indicated that MgO nanoparticles exhibited high effectiveness against all three microorganisms. The study revealed that MgO nanoparticles exhibit potent anticancer properties against human liver (HepG2) and lung (A549) cancer cells. Reactive oxygen species production in cancer cells by MgO nanoparticles suggests oxidative stress pathwav-mediated anticancer action. The present research highlights the significance of utilizing medicinal plants in the synthesis of nanoparticles for enhanced anticancer and microcidal properties. © 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access

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1. Introduction

Due to their distinct physicochemical properties like low toxicity, cationic capacity, corrosion resistance, dielectric resistance, optical transparency, high stability, and redox properties, MgO nanoparticles have recently emerged as potential materials for widespread industrial and commercial applications (Karthik et al., 2017; Ramezani Farani et al., 2023). The distinctive characteristics of MgO nanoparticles make it useful in numerous contexts, such as those of catalysts, sensors, photonic devices,

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additives in refractories, superconducting products, paint, and a pivotal material in bioremediation (Srivastava et al., 2015; Verma et al., 2020). In addition to its promising applications in the treatment of heart burns and bone regeneration, MgO nanoparticles have also been found to be effective anticancer and antibacterial agents (Karthik et al., 2017; Krishnamoorthy et al., 2012a).

The synthesis of nanoscale materials can be achieved through various methods, including physical, chemical, and biological approaches (Ahamed et al., 2023). Physical methods involve vapor decomposition, plasma irradiation, and ultrasonication, while chemical methods include sol–gel, co-precipitation, chemical reduction, and hydrothermal techniques (Pal et al., 2019). Biological methods, on the other hand, utilize plant and microbial sources (Jadoun et al., 2021). However, the physical method necessitates a high capital expenditure for operating equipment and uses a lot of heating or electric energy (Behzad et al., 2021). The utilization of toxic chemicals in the chemical method's protocols leads to negative environmental consequences. Hence, the attainment of a crit-





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ical green strategy through physical and chemical methods may pose significant challenges (Ahamed et al., 2022a).

The effectiveness, tunability, and eco-friendliness of the biological method make it the most suitable approach for the green synthesis of MgO nanoparticles (Verma et al., 2020). Biomolecules derived from biological sources (e.g., plants) have been found to be highly effective reducing, capping, and stabilizing agents, resulting in significant improvements in MgO nanoparticles application (Rotti et al., 2023). Biological substrates have the potential to serve as a safe and cost-effective alternative to highly expensive and toxic chemicals or energy-consuming physical instruments (Ramezani Farani et al., 2023). The biogenic synthesis of MgO nanoparticles is considered a green synthesis due to its ability to reduce potential risks from chemical and physical methods, avoid the production of hazardous intermediates, and prevent secondary pollution.

The biogenic synthesis of MgO nanoparticles is a relatively new field, with only a handful of studies reporting on it so far. In this research, we used an extract from garlic cloves (Allium sativum L.) to create MgO nanoparticles. The extract of garlic contains a number of active phytochemicals that were used to create MgO nanoparticles by acting as stabilizing and reducing agents (Ansary et al., 2020). Inflammation, high blood pressure, infections, and even cancer are just some of the many conditions for which garlic has long been used as a curative food-based medication (Mondal et al., 2022). There are a number of bioactive compounds in garlic, including organosulfur, that have broad-spectrum antibacterial and anticancer effects (Rouf et al., 2020). The therapeutic activity of garlic nanoformulations was found to be higher than that of garlic phytochemicals in recent research (Mondal et al., 2022). SEM, TEM, EDX, and XRD characterization confirmed that MgO nanoparticles were successfully fabricated with the help of garlic extract. Antimicrobial activity of synthesized samples was determined in gram-positive bacteria (Staphylococcus epidermidis), gram-negative bacteria (Escherichia coli), and a fungal pathogen (Candida albicans). MgO nanoparticles anticancer efficacy was evaluated in human liver (HepG2) and lung cancer (A459) cells. It was determined that MgO nanoparticles likely exert their anticancer impact by generating reactive oxygen species (ROS).

2. Materials and methods

2.1. Green synthesis of MgO nanoparticles by garlic extract

Garlic cloves acquired locally were peeled, washed in deionized water, chopped, and air dried. A grinder pulverizes whole cloves into a powder. The maceration procedure was used for the extraction. Garlic powder (15 g) was steeped in 300 ml of deionized water for 24 h with continuous stirring. Finally, filter paper with a pore size of 0.2 m was used to remove any remaining solids from the extract before it was kept at 4 °C for later use.

A solution was produced by adding 1 g of magnesium nitrate $(Mg(NO_3)_2 \cdot 6H_2O)$ (Sigma-Aldrich, St. Louis, MO, USA) with 20 ml of garlic extract, followed by stirring for 30 min at room temperature. Following this, a 5 ml of sodium hydroxide solution (0.2 M) was slowly added to the amalgam until a visible precipitate was formed. The precipitate was then subjected to multiple washes with deionized water and subsequently dried at a temperature of 60 °C for overnight. Following this, the sample was subjected to a calcination procedure at a temperature of 500 °C for a period of 2 h to get fine powder of MgO nanoparticles. Fig. 1 illustrates the green process of synthesizing MgO nanoparticles using garlic extract.

2.2. Characterization

The structural properties of the as-prepared MgO nanoparticles were analyzed by X-ray diffraction (XRD) (PanAlytical X'pert Pro, Malvern Instruments) employing CuK radiation (λ = 1.5406). Field emission scanning electron microscopy (FESEM) (JSM-7600F, JEOL, Tokyo, Japan), transmission electron microscopy (FETEM) (JEM-2100, JEOL), and energy dispersive X-ray spectroscopy (EDX) were used to further investigate the structural characteristics, particle size, and elemental composition of MgO nanoparticles.

2.3. Antimicrobial assay

The antibacterial activity of as-synthesized nanoparticles was evaluated using Staphylococcus epidermidis (ATCC-14990, grampositive bacteria), Escherichia coli (ATCC-BAA-2471, gramnegative bacteria), and Candida albicans (ATCC-10231, fungal pathogen). Slants of nutritional agar broth were inoculated with 0.5 McFarland (1 \times 10⁸ CFU/ml) of bacteria and incubated for 18 h at 37 °C (Zheng et al., 2020). Sabouraud dextrose broth slants were used to cultivate the fungi for 24 h at 30 °C with an inoculum of 2×10^6 PFU/mL. The disc-diffusion assay was used to measure the antimicrobial activity (Azam et al., 2012). Each well was filled with a 50 µl (50 µg) solution of MgO nanoparticles. Streptomycin was used to treat bacteria, while nystatin was used to treat fungus as a positive control (PC). The minimum inhibitory concentrations (MICs) of as-prepared MgO nanoparticles against bacterial and fungal pathogens were determined using the micro-broth dilution technique.

2.4. Cell lines and anticancer assay

The HepG2 and A549 cell lines of human hepatocellular carcinoma and lung carcinoma, respectively, were bought from American Type Cell Culture Collection (ATCC, Manassas, Virginia, USA). Both types of cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. The cell culture was sustained within an incubation chamber at a temperature of 37 °C, with a 5% concentration of CO₂. Cells were treated to MgO nanoparticles for 24 h at concentrations ranging from 1 to 200 µg/ml. The MTT test, with appropriate changes, was used to investigate anticancer activity (Ahamed et al., 2021). The 2' -7'-dichlorodihydrofluorescein diacetate (H2DCFDA) was used to measure the intracellular concentration of ROS (Ahamed et al., 2021).

2.5. Statistical analysis

The data was analyzed with one-way analysis of variance (ANOVA) and then Dennett's multiple comparison tests. The significance level was set at p < 0.05. Three separate experiments (n = 3) provided the quantitative results displayed as mean \pm SD.

3. Results and discussion

3.1. Electron microscopy study

Fig. 2 shows the FESEM characterization of environmentalfriendly produced MgO nanoparticles. The typical SEM micrographs of MgO nanoparticles are shown in Fig. 2A and B at varying magnifications. These SEM images reveal that the MgO nanoparticles are about 60 nm in size. The elemental composition was determined using SEM-EDX analysis, and the results showed a stoichiometric amount of Mg (52.36 wt%) and O (47.64 wt%)



Fig. 1. Illustration of green process MgO nanoparticles synthesis using garlic extract.



Fig. 2. FESEM micrographs of MgO nanoparticles of different magnifications (A and B). SEM-EDX spectra (C) and elemental composition (D) of MgO nanoparticles.



Fig. 3. Low-resolution (A) and high-resolution (B) FETEM micrographs of MgO nanoparticles.

(Fig. 2C and D). The present research's FESEM characterization data aligns with previously published work (Su et al., 2023; Yousefi et al., 2017).

Green-manufactured MgO nanoparticles were further characterized by FETEM and shown in Fig. 3. The findings presented in Fig. 3A indicate that the MgO nanoparticles exhibited an irregular shape and possessed an average diameter of 58 nm, which is consistent with the results obtained from the SEM analysis. The appearance of lattice fringes in the high-resolution TEM image confirmed that MgO had a cubic structure (Chen et al., 2020). As can be seen in Fig. 3B, the MgO cubic structure has a (200) diffraction plane with a corresponding lattice inter-planar distance of 0.235 nm. TEM-EDX analysis of MgO nanoparticles' elemental composition confirmed the pure presence of the Mg and O. The utilization of a carbon-coated copper grid in TEM resulted in the production of peaks corresponding to carbon and copper, as depicted in Fig. 4.

3.2. XRD study

The XRD technique was employed, utilizing CuK α radiation, to investigate the phase-purity and crystallinity of the synthesized MgO nanoparticles. Fig. 5 displays the X-ray diffraction spectra of MgO nanoparticles. The observed peaks at angles 37.23°, 43.13°, 62.50°, 74.94°, and 78.86° correspond to the (111), (200), (220), (311), and (222) planes (JCPDS No. 87–0653), indicating the presence of a polycrystalline cubic structure of MgO. The absence of any additional impurity phase in the XRD pattern corroborates the findings of the EDX data. The XRD spectra indicate a high degree of crystallinity in the MgO nanoparticles synthesized through the green method, as evidenced by the presence of intense peaks. The determination of crystal size in the synthesized samples was carried out by utilizing Scherrer's formula (Ahamed et al.,



Fig. 5. XRD pattern of MgO nanoparticles.

2017) and applying the most prominent peak (200). Results reveals that the crystal dimensions of MgO nanoparticles were estimated to be approximately 55 nm, which is consistent with the size determined through the utilization of TEM and SEM.

3.3. Antimicrobial study

Green-manufactured MgO nanoparticles have been the subject of increasing research into their antibacterial properties (Abinaya and Kavitha, 2023; Rotti et al., 2023). In this research, MgO nanoparticles created using garlic extract were highly effective against both bacterial and fungal pathogens. *Staphylococcus epidermidis* (29.6 µg/ml), *Escherichia coli* (26.8 µg/ml), and the fungal



Fig. 4. TEM-EDX spectra of MgO nanoparticles.



Fig. 6. Antimicrobial effects of MgO nanoparticles. Zone of inhibition (A) and minimum inhibitory concentration. SE: Staphylococcus epidermidis, EC: Escherichia coli, CA: Candida albicans, PC: positive control.

pathogen *Candida albicans* (17.7 μ g/ml) all showed zone of inhibition formation in Fig. 6A. Fig. 6B shows the minimal inhibitory concentration (MIC) of MgO nanoparticles against specific microorganisms. The *S. epidermidis, E. coli*, and *C. albicans* all had MICs of 19.3 μ g/ml, 22.7 μ g/ml, and 27.4 μ g/ml, respectively. These findings imply that the MgO nanoparticles produced in a sustainable manner have potent antimicrobial and bacteriostatic properties. Rotti et al. demonstrated similar antibacterial activity in plant extract mediated manufactured MgO nanoparticles against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, supporting the current findings (Rotti et al., 2023). The bactericidal and fungicidal activities of MgO nanoparticles against a variety of common pathogenic bacteria and yeasts were also reported by Nguyen and co-workers (Nguyen et al., 2018).

3.4. Anticancer study

Research on the effectiveness of MgO nanoparticles as an anticancer agent is gaining interest (Akhtar et al., 2018;



Fig. 7. Anticancer effects of MgO nanoparticles in human liver (HepG2) and lung (A549) cancer cells. *p < 0.05 vs. control.



Fig. 8. ROS generation of MgO nanoparticles in human liver (HepG2) and lung (A549) cancer cells. *p < 0.05 vs. control.

Krishnamoorthy et al., 2012b). Human liver (HeG2) and lung (A549) cancer cells were used to test the anticancer effect of MgO nanoparticles generated in an eco-friendly manner. Anticancer activity was measured using the MTT test after cancer cells were exposed to MgO nanoparticles at concentrations ranging from 1 to 100 μ g/ml for 24 h. In both HepG2 and A549 cancer cells, MgO nanoparticles exhibited dose-dependent cytotoxicity, as shown in Fig. 7. MgO nanoparticles had IC₅₀ values of 18.6 g/ml and 20.3 g/ml against HepG2 and A549 cancer cells, respectively. Potent anticancer activity of MgO nanoparticles may have been facilitated by garlic phytochemicals during the synthesis. Previous research has shown that the phytochemicals in garlic have strong anticancer effects on several different types of cancer, including those of the lung, liver, breast, and skin (Mondal et al., 2022). Nanocomposite ZnO/RGO produced from garlic extract showed anticancer action in human colon cancer HC116 and breast cancer MCF7 cells, as we reported in our prior study (Ahamed et al., 2022b). The ability of MgO nanoparticles to fight cancer has been reported in earlier research. For example, human breast cancer (MCF-7) cells were found to be sensitive to MgO nanoparticles exposure (Amina et al., 2020). Ali et al. recently observed the anticancer effects of Abrus precatorius bark extract manufactured MgO nanoparticles in human melanoma cancer (A375) (Su et al., 2023).

It has been claimed that oxidative stress may play a part in the treatment of cancer. Due to the use of numerous medications, ROSmediated oxidative stress is a frequent occurrence in cancer treatment. The ROS-generating ability of MgO nanoparticles has been also observed in earlier research. Previous studies have shown that the depletion of antioxidants can lead to the damage of tumor cells, especially when used in conjunction with treatments that induce the generation of ROS. This study aimed to investigate the impact of MgO nanoparticles on the oxidative stress response in cancer cells (HepG2 and A549 cells). The cells were exposed to varying concentrations (1-100 µg/ml) of MgO nanoparticles for 24 h. The oxidative stress response (ROS level) was then measured. The results of Fig. 8 indicate that there is a positive correlation between the concentration of MgO nanoparticles and the level of ROS in cancer cells. A recent study conducted by Pugazhendhi et al. also revealed that the induction of apoptosis in A549 cells was observed upon exposure to MgO nanoparticles, which was attributed to the accumulation of intracellular ROS (Pugazhendhi et al., 2019).

4. Conclusion

In this study, a novel method was developed for the synthesis of MgO nanoparticles utilizing galic (*Allium sativum* L.) extract as a green and cost-effective reducing agent. Green manufactured MgO nanoparticles was characterized by SEM, TEM, EDX, and XRD. Characterization data showed the preparation of highly pure,

polycrystalline cubic MgO nanoparticles. We found that MgO nanoparticles have outstanding antibacterial activities against a variety of pathogens, including gram-positive bacteria (*S. epider-midis*), gram-negative bacteria (*E. coli*), and a fungal strain (*C. albicans*). The study further observed that MgO nanoparticles displayed remarkable anticancer properties when tested on human cancer cells (HepG2 and A549). ROS generation in cancer cells following MgO nanoparticles exposure indicated the role of oxidative stress in the anticancer effect of prepared nanoparticles. The results of this study provide valuable insights into the potential use of green-fabricated MgO nanoparticles as a novel therapeutic agent for the treatment of microbial infections and cancer.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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