

Green Synthesis of Magnesium Oxide Nanoparticles Using Alcoholic Extract of *Camellia sinensis* and their Antibacterial and Anti-Biofilm Activity against Multidrug-Resistant Pathogenic Bacteria

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Abstract

Background: Multidrug resistance bacteria is rapidly spreading globally and has created an urgent need for alternative antimicrobial strategies to combat these bacteria. Green nanotechnology is an eco-friendly way of synthesizing Bioactive Nanoparticles with enhanced antimicrobial activities compared to conventional methods.

Objectives: The purpose of this study was to "green" synthesize magnesium oxide nanoparticles (MgO NPs) from an alcohol extract of *Camellia sinensis* (tea plant) and evaluate their antibacterial and anti-biofilm activity against clinical multidrug resistance bacterial isolates.

Methods: Magnesium Oxide nanoparticles were synthesized utilizing an alcohol extract of *Camellia sinensis* as a reducing/stabilizing agent. The synthesized MgO NPs were characterized by UV-Vis Spectroscopy, FTIR, XRD, SEM, AFM, EDX, AAS, and Zeta Potential analyses. The antibacterial activity of the MgO NPs was determined using minimum inhibitory concentration (MIC) determinations while their anti-biofilm activity was assessed by a microtiter plate crystal violet assay against clinical MDR *Enterococcus faecalis* and *Klebsiella pneumoniae* isolates. Data were expressed as mean \pm SD.

Results: According to the study, MgO nanoparticles that were created by biosynthetic means had an average particle size range of 52-104 nm (SEM) and had evidence of nanoscale surface roughness from AFM. They also showed high colloidal stability with zeta potentials of -44.61 mV. MgO nanoparticles exhibited very strong (dose-dependent antibacterial effect on both *E. faecalis* and *K. pneumoniae* with a MIC value of 12.5 μ g/ml, and significantly reduced biofilm formation by 95.45-100% at sub-MIC concentrations.

Conclusion: Green-synthesized magnesium oxide (MgO) nanoparticles using extracts from *Camellia sinensis*, have very good anti-bacterial and anti-biofilm activities against multi-drug resistant (MDR) pathogens, and support the theorized use of green-synthesized MgO nanoparticles as an eco-friendly antimicrobial agent to reduce biofilm-related infections.

Keywords: Antibacterial activity; Biofilm inhibition; *Camellia sinensis*; Green synthesis; Magnesium oxide nanoparticles.

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Introduction

Across the globe, antibiotic-resistant microorganisms represent a serious hazard to public health (1). The increasing growth of multidrug resistance is linked to the lack of new and potent antimicrobials. Therefore, several attempts to develop new and more potent antimicrobial medications, as well as novel and potent medication delivery and targeting methods, were made at the international level.

Multidrug-resistant (MDR) bacteria are worse than ever because many pathogens can form a biofilm, allowing them to be more tolerant of antibiotics and the host immune system than non-biofilm forming pathogens (2). Biofilms are complex communities of micro-organisms within an extracellular polymeric substance (EPS) matrix made up of polysaccharides

that impede antimicrobial penetration into the biofilms and allow for horizontal gene transfer of resistance genes. Biofilm-related infections, such as those caused by *Enterococcus faecalis* and *Klebsiella pneumoniae*, are especially difficult to treat and are associated with longer lengths of hospital stay, increased healthcare costs, and increased mortality rates. Therefore, targeting both planktonic cells and biofilm-associated bacteria has become a key focus of research in developing alternative antimicrobial strategies (3).

Nanobiotechnology is indeed a modern field that focuses on the design, production, and application of nanoparticles (NPs) at the nanoscale. It combines the principles of biology and nanotechnology in an effort to create novel materials and devices for various applications, particularly in the arenas of medicine and healthcare. It is a potential treatment approach

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due to its therapeutic index and excellent antimicrobial effectiveness (4). Besides, it offers a beneficial alternative in the treatment of the majority of bacterial diseases, particularly those including multidrug-resistant organisms. Nanoparticles can also be used alone or together with the antibiotics to deliver strong synergistic results (5).

Green synthesis, an inexpensive, non-toxic, and eco-friendly process, has been widely used to produce metal oxide nanoparticles using a simple methodology (6). The nanoparticles made thus offer a wide variety of applications in the biomedical and pharmaceutical fields, namely, acting as antimicrobial and anti-inflammatory agents, drug delivery, anticancer effects, tumor targeting, bioactivity enhancement, and bioabsorption (7).

Organisms such as bacteria, fungi, algae, and plants frequently act as the raw material sources used in the green synthesis process (8). Plant-derived materials are most often chosen, and the various plant parts, such as roots, stems, leaves, flowers, and seeds, are used in nanoparticle synthesis (6). Leaves are the commonest plant part used, as they can regenerate and are safe and less pathogenic when compared to bacteria or algae (9). Further, leaf extracts show greater efficacy than algae or microbial sources in reducing metal ions (10).

Plant extracts are a veritable reservoir of phytochemicals, which include several flavonoids, alkaloids, and phenolic, apart from other organic compounds such as carbon, hydrogen, and nitrogen (11). The phytochemical bioactive molecules involved in the interaction with metal salts result in the subsequent production of nanoparticles that differ from one another in their physical characteristics of size, shape, and surface attributes (12). Metal ion reduction, which is significantly influenced by phytochemicals, occurs with functional groups like amino groups, which act as catalysts in the conversion of metal oxides (13). Further, the oxygen in the environment from the air or released through phytochemical decay plays a role in metal ion reduction. Metal ions and the stabilizing phytochemicals interacting via electrostatic forces encourage the formation and stabilization of metal oxide nanoparticles, although particle agglomeration may occur. Phenolic compounds that the plant extract contains are very effective in the neutralization of superoxide radicals (one of the main contributors to the Reactive Oxygen Species (ROS)) due to their structural bearing of the carboxyl and hydroxyl groups (14). Also, both the high- and low molecular-weight compounds, like proteins and starches, get involved in the NPC formation process; the proteins that are derived from plants, however, have been found to significantly influence the stabilization of the nanostructures produced (9-13).

Magnesium is abundant in the Earth's crust and is more available than any of the other metals. Magnesium oxide is used as a semiconducting material, a catalyst in organic transformations, and a sorbent for organic and inorganic contaminants from

wastewater. It also possesses strong antibacterial, anticancer, and antioxidant properties (14).

Magnesium oxide nanoparticles have demonstrated a remarkable ability to resist bacteria and fungi through the formation of reactive oxygen species (ROS), as well as their capacity to interact with microbial cell membranes, inducing changes that result in their death (15). These molecules also reveal antioxidant and anticancer activity in some biological studies, which increases their usage in the medical and pharmaceutical fields (16).

Camellia sinensis is an evergreen shrub or plant of the family Theaceae. Tea is made from the leaves and leaf buds (one of the most ancient and famous, globally consumed beverages, with enormous health benefits) (17). Extracts of green tea leaves have been proven to have antimicrobial strength sufficient to kill many types of bacterial strains, such as *Salmonella spp.*, *Enterococcus spp.*, *Escherichia coli*, *Streptococcus mutans*, and *Staphylococcus aureus*. It also exerts an antifungal effect on certain fungi, such as *Candida albicans*, and an antiviral effect on many viruses, such as HIV and Herpes (18).

While the number of studies examining metal oxide nanoparticles as antimicrobial agents has increased, there are still a number of limitations that have not yet been addressed. Much of the literature available focuses on antibacterial activity against standard laboratory strains of bacteria, with little attention to clinically relevant multidrug-resistant isolates or their ability to form biofilms (19, 20). In addition, although green synthesis methods, including the use of plant extracts, have been widely investigated, there is still limited data available regarding the combined antibacterial and anti-biofilm activity of magnesium oxide nanoparticles synthesized from *Camellia sinensis* extracts (21). Additionally, variations in the protocols used to synthesize these materials and the lack of complete physicochemical characterization of the final products have made it difficult to reproduce previous results and compare them meaningfully (22).

To achieve its aims, this research was organized with four primary objectives. First, the research aimed to explore the potential for synthesizing magnesium oxide nanoparticles using an environmentally-friendly approach of synthesizing magnesium oxide nanoparticles from an alcoholic extract of *C. sinensis* using a green method. Second, the chemical, physical, and surface characteristics of the biosynthesized MgO nanoparticles were examined in detail. Third, the antibacterial activity of the synthesized MgO nanoparticles against the multi-drug-resistant organisms of *E. faecalis* and *K. pneumoniae* was evaluated. Finally, the ability of the magnesium oxide nanoparticles to inhibit the growth of biofilms at sub-inhibitory concentrations was also studied.

Materials and Methods

Bacterial Isolates

From Iraqi patients, a total of 150 clinical samples were analyzed and cultured on selective media. The

bacterial isolates were initially identified from culture, morphological and biochemical characteristics. *E. faecalis* and *K. pneumoniae* were selected as clinically relevant and are both multidrug-resistant (MDR). The isolates were confirmed using the Vitek 2 system and antibiotic susceptibility was determined using the Kirby–Bauer disc diffusion method according to standard guidelines (23, 24). The isolates' ability to produce biofilms was evaluated by means of a crystal violet-based 96 well microtiter plate assay (25). Prior to performing the experiments, the isolates were sub-cultured in Mueller–Hinton broth and incubated anaerobically at 37 °C for a period of 18–24 hours.

Ethical approval

Ethical approval for bacterial sample collection was obtained from the Baghdad Hospital Administration, Ministry of Health, Republic of Iraq (Approval No. 2024/12). All samples were collected following informed consent from the patients.

Preparation of *Camellia sinensis* Alcoholic Extract

C. sinensis leaves were purchased from local markets in Baghdad and taxonomically identified by a specialist at the Department of Biology, College of Science for Women. The alcoholic extract was prepared according to Zamil and Al-Hayanni (10). Concisely, dried leaves were powdered and macerated in 70% ethanol at room temperature for 48 h with continuous stirring. The extract was filtered, concentrated under reduced pressure using a rotary evaporator, and stored at 4 °C until further use.

Green Synthesis of Magnesium Oxide Nanoparticles (MgO NPs)

Magnesium oxide nanoparticles (MgO NPs) were synthesized utilizing magnesium sulfate ($MgSO_4$, 99.5% purity; Fisher Scientific) as a precursor and *C. sinensis* alcoholic extract as a reducing and stabilizing agent. Concisely, 50 ml of plant extract was mixed with a 3 mM aqueous $MgSO_4$ solution under continuous stirring. The pH was adjusted to approximately 10 utilizing 1 M NaOH, followed by heating at 100 °C for 30 min. After incubation for 48 h, the formed precipitate was collected, washed with distilled water and ethanol, dried, and calcined at 500 °C for 3 h to obtain MgO nanoparticles (26).

Characterization of Magnesium Oxide Nanoparticles (MgO NPs)

The biosynthesized MgO nanoparticles were characterized for their optical, structural, and morphological properties using UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). Surface morphology and particle size were analyzed by scanning electron microscopy (SEM) and atomic force microscopy (AFM), whereas elemental composition was determined by energy-dispersive X-ray spectroscopy (EDX). Magnesium content was quantified using atomic absorption spectroscopy

(AAS), and colloidal stability was assessed by zeta potential analysis (5, 7, 12, 27, 28).

Antibacterial Activity of Magnesium Oxide Nanoparticles

The minimum inhibitory concentration (MIC) of MgO NPs for bacteria was evaluated according to CSLI guidelines using a resazurin-based microtiter plate assay exactly as described previously (25). This was done by preparing two-fold serial dilutions of MgO NPs using Mueller–Hinton broth in the concentrations of 50 $\mu\text{g/ml}$ –0.78125 $\mu\text{g/ml}$. The bacteria were inoculated into the diluted MgO NPs with a bacterial suspension adjusted to a 0.5 McFarland standard and incubated at 37°C for 18–24 hours before adding the resazurin to each well. The MIC of the MgO NPs was defined as the lowest concentration that showed no color change, which indicates that all bacterial growth has been inhibited.

Anti-Biofilm Activity of Magnesium Oxide Nanoparticles

The investigation into the anti-biofilm effect of MgO nanoparticles was conducted using the crystal violet-based 96-well microtiter plate assay as outlined by Abd Alaameri et al. (5). Bacterial suspension was incubated with sub-MIC concentrations of MgO NPs in Mueller–Hinton broth (with 1% glucose) at 37 °C for 24 h. Following the incubation, unattached cells were washed away, and the biofilm formed (adhered to the plate) was stained with crystal violet. The bound crystal violet was solubilized with ethanol, and the absorbance was measured at 580 nm. Biofilm inhibition was determined by using the following equation:

$$\text{Biofilm inhibition (\%)} = (\text{OD control} - \text{OD sample}) / \text{OD control} \times 100$$

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean \pm standard deviation. Statistical analysis was carried out using SPSS version 25. Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test. A p-value ≤ 0.05 was considered statistically significant (29).

Results

Bacterial Isolates

Out of 150 clinical samples, 8 isolates of bacteria were selected for further experimentation due to their multidrug resistance profiles and biofilm-forming abilities. The selected bacterial isolates included *E. faecalis* and *K. pneumoniae*; both were clinically important pathogens that are associated with persistent and hospital-acquired infections.

Each of the selected isolates demonstrated resistance to at least three classes of antibiotics, which qualifies each of these isolates as multidrug resistant (MDR) by conventional definitions (Table 1). *E. faecalis* isolates exhibited resistance to primarily β -lactams and aminoglycosides, while *K. pneumoniae* isolates

expressed resistance against β -lactams, cephalosporins, and carbapenems.

For all selected bacterial isolates, the results of the biofilm formation assays indicated that they produce biofilms; the OD570 values for the *E. faecalis* isolates ranged from 0.66 to 1.79, and the OD570 values for the *K. pneumoniae* isolates ranged from 0.26 to 0.94, both of which are well above the cut-off for strong biofilm production (Table 4).

Green Synthesis of Magnesium Oxide Nanoparticles

The magnesium oxide nanoparticles were observed visually as they were being created. The $MgSO_4$ solution changed from colorless to green when *C. sinensis* alcoholic extract was added. When sodium hydroxide was added next, the color changed to light brown and then turned dark brown, indicating that the magnesium oxide particles were formed, after incubating for 48 hours (Figure 1).

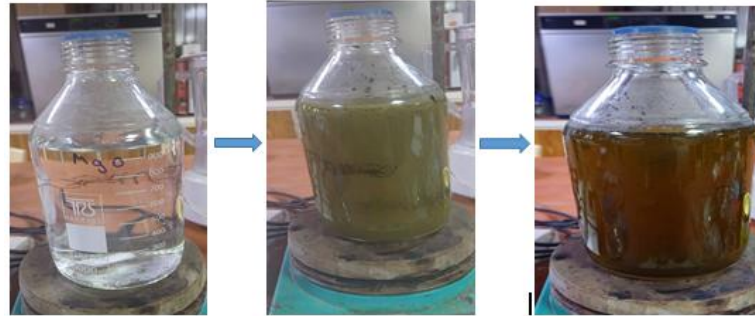


Figure 1: Color changes during the bioreduction of $MgSO_4$ into MgO nanoparticles using *Camellia sinensis* alcoholic extract.

Characterization of Biosynthesized Magnesium Oxide Nanoparticles

By employing a variety of analytical methods to characterize the MgO NPs produced, we confirmed their formation, morphology, functional groups, elemental composition, crystallinity, and surface characteristics. These results confirm how important these methods have proven to be for characterizing MgO NPs properly and scientifically:

UV-Visible Spectrophotometer

The UV-visible spectrum for the biosynthesized MgO NPs was recorded from 200–800 nm. The MgO

NPs had a well-defined absorption band at $\lambda_{max} = 266.5$ nm (Figure 2), while none was present in the alcoholic extract of *C. sinensis* (Figure 3). Furthermore, the intensity of absorbance at this wavelength for the NPs was a significantly greater value than that of the extract (Table 1). Thus, the presence of an absorption band at $\lambda_{max} = 266.5$ nm supports the presence of MgO NPs. MgO NPs do not display a distinct surface plasmon resonance peak, so the UV-Vis absorption band at $\lambda_{max} = 266.5$ nm may be regarded as evidence supporting, but not conclusively establishing, the presence of MgO NPs.

Spectrum Peak Pick Report

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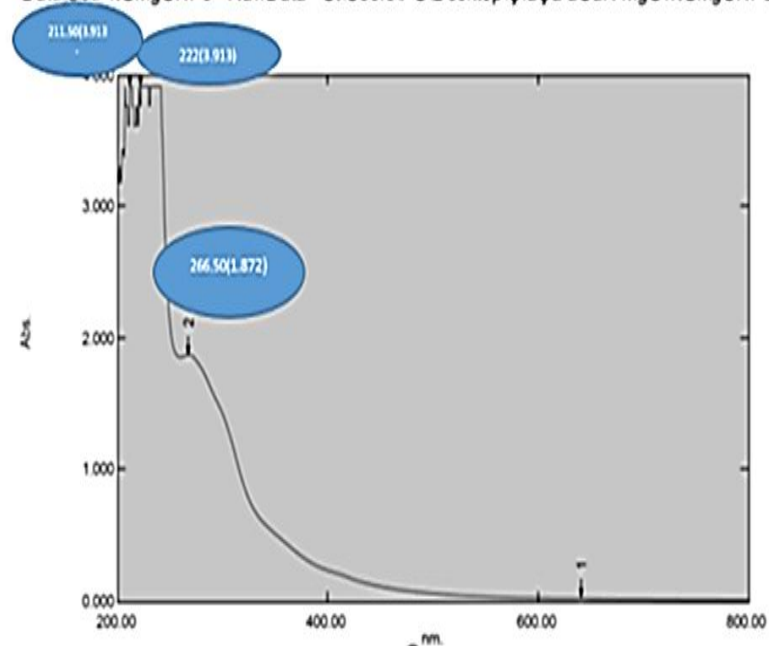


Figure 2: UV-visible spectral analysis of MgO NPs.

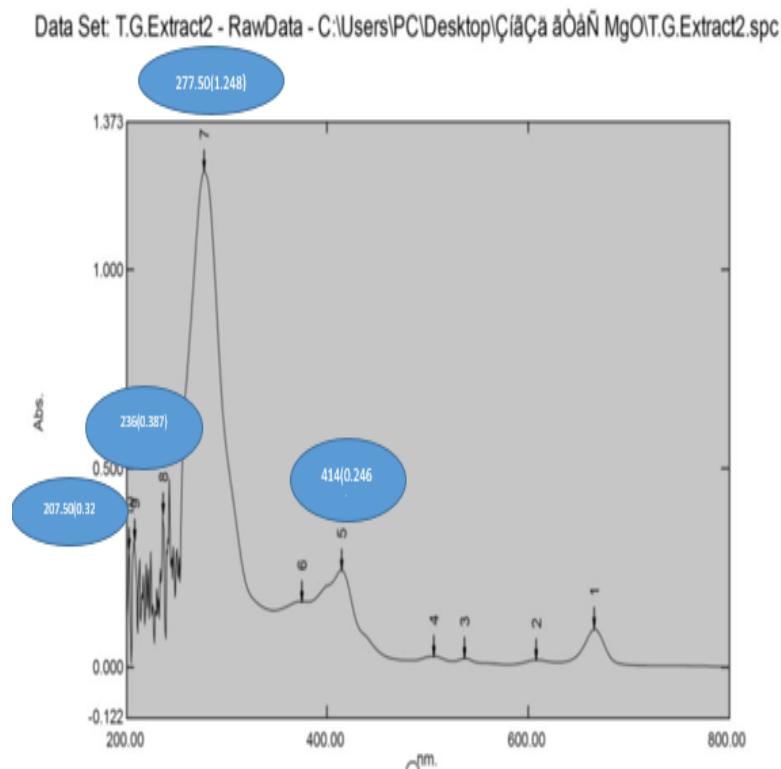


Figure 2: UV-Visible spectral analysis of *Camellia sinensis* alcoholic extract.

Table 1: UV-Visible spectral analysis results of *Camellia sinensis* alcoholic extract and MgO NPs

Peak No.	Wavelength /nm	<i>Camellia sinensis</i> alcoholic extract	MgO NPs
2	266.50	-----	1.872
3	222.00	-----	3.913
4	211.50	-----	3.913
5	414.00	0.246	-----
7	277.50	0.246	-----
8	236.00	0.387	-----
9	207.50	0.320	-----

Fourier Transform Infrared Spectrometer (FTIR)
 Analysis using FTIR was used to demonstrate the stabilization and capping functional groups of MgO nanoparticles (Figures 4 and 5). Through examining the FTIR spectrum of the *C. sinensis* extract its broad band was found at 3383.47 cm⁻¹, representing O-H stretching vibrations of phenol and alcohol. In order to confirm that magnesium oxide nanoparticles have been made, MgO nanoparticle

FTIR spectrum was investigated which found that : at 2926.01 cm⁻¹ had an absorption band (O-H), and at 516-540.07 cm⁻¹ two peaks indicated Mg-O.. Differences in peak positions and intensity from the extract to the magnesium oxide NP demonstrate that plant-derived biomolecules play a role in stabilizing the nanoparticles.

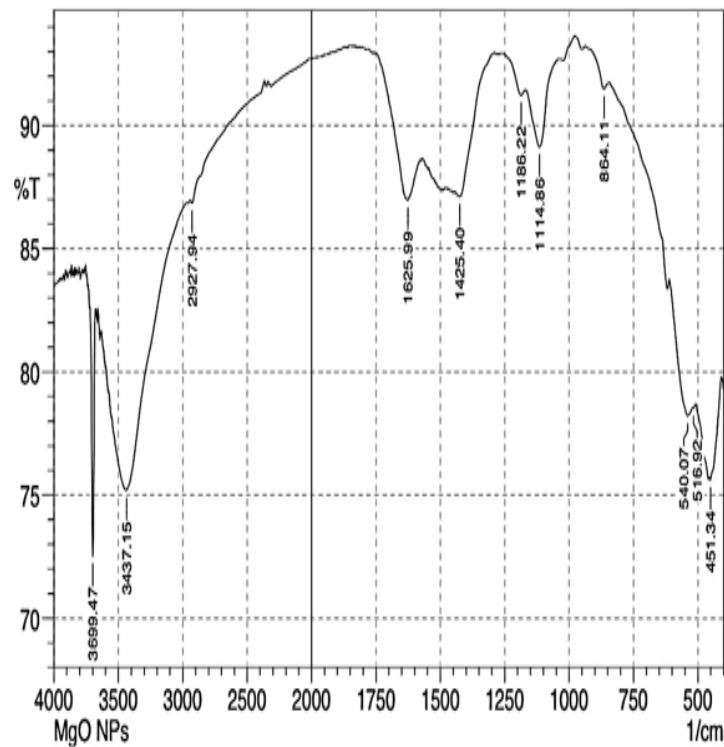


Figure 4: FTIR spectra of the MgO NPs.

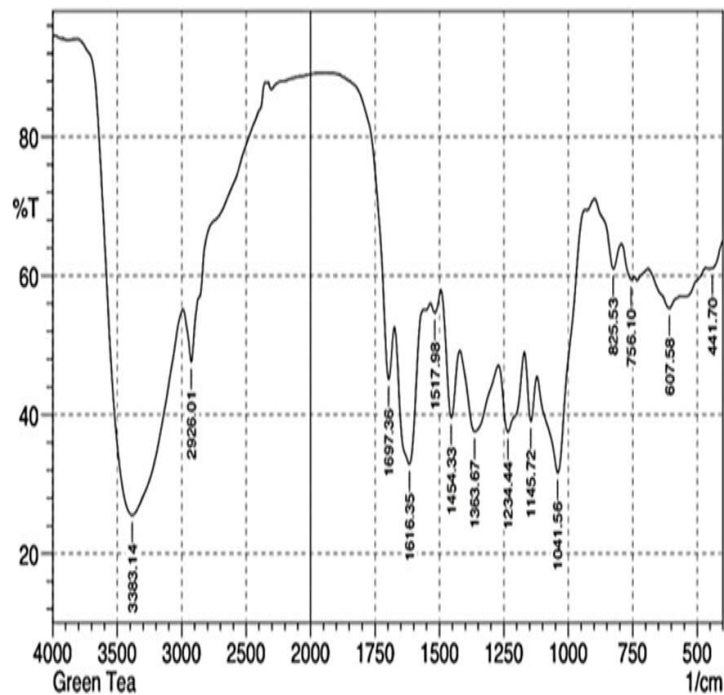


Figure 5. FTIR spectra of the Camellia sinensis alcoholic extract.

X-Ray Diffraction (XRD)

The analysis of the crystalline structure of MgO nanoparticles synthesized biologically was performed via X-Ray Diffraction (XRD) shown in Supplementary [Figures S1 and S2](#). The XRD pattern showed peaks that correlate with the cubic MgO structure at angles 2 Theta = 37.77, 42.9, 36.9, and

58.54 degrees representing the (111) and (220) planes and corresponded to JCPDS Card No. 45-0946 as well.

A very small peak at approximately 18.15 degrees corresponds to residual organic compounds from the plant-based material used to create this material and/or some of the intermediate phases of magnesium

hydroxide resulting from the bio-synthesis process. Based upon the use of the Scherrer Equation from the most intense peak in the XRD data, the average crystallite size of the MgO nanoparticles biosynthesized were approximately 12 nanometers (nm).

Scanning Electron Microscope (SEM)

According to the results of the SEM evaluation, MgO nanoparticles were synthesized using a wide variety of methods; these include both quasi-spherical ("Q-S") and irregularly-shaped ("IS") morphologies however all exhibited very noticeable levels of agglomeration shown in Supplementary (Figures S3). The particle size ranged from 52nm to 104nm. It was observed that the cause behind their observed agglomeration could be due to both the high surface energy of the MgO nanoparticles along with residual phytochemical substances coming from the plant extracts.

Atomic Force Microscopy (AFM)

MgO-nanoparticles' surface topography was studied via AFM shown in Supplementary Figures S4. Based

Table 2: Elemental analysis of MgO NPs via EDX

Element	Wt.%	Wt.% Sigma
O	49.98	0.38
Na	5.11	0.20
Mg	40.23	0.35
S	4.23	0.15
Cl	0.46	0.09
Total:	100.00	

Atomic Absorption Spectrophotometer (AAS)

Using atomic absorption spectroscopy, the magnesium content of synthesized nanoparticles was determined. The concentration of the MgO nanoparticles was measured at 662 ppm, which confirms an efficient incorporation of magnesium during the production process shown in Supplementary Figures S6.

Zeta Potential Analyzer

The magnesium content of the synthesized nanoparticles was quantified via atomic absorption spectroscopy (AAS). MgO nanoparticles have been measured at a concentration of 662 ppm, confirming

on the Ra of 27.5 nm, nanomaterials had a fairly rough surface. Note that the surface roughness does not indicate the actual particle size, but may increase their interactions with biological materials. Variations in particle size between AFM and SEM results could be due to differences in measurement principle and preparation of the sample.

Energy Dispersive X-ray (EDX)

The elemental make-up of the MgO nanoparticles formed by biological synthesis was assessed through EDX analysis. The EDX results are illustrated in Table 2 and Figure S5. The dominant elements in the EDX spectrum were Mg=40.23 wt.% and O=49.98 wt.%, suggesting that MgO nanoparticles were produced. The traces of Na, S, and Cl observed were likely due to the chemical precursors used to create the nanoparticles or pH adjusting agents. Residual plant biomolecules in trace quantities contributed to the additional signal

that magnesium was incorporated efficiently during synthesis shown in Supplementary Figures S7

Antibacterial Activity of Magnesium Oxide Nanoparticles

MgO nanoparticles displayed substantial antibacterial properties towards MDR *E. faecalis* and *K. pneumoniae* (Table 3, Figures S8). The minimum inhibitory concentration (MIC) and 1/2-MIC concentrations were 12.5 µg/ml and 6.25 µg/ml, respectively. Statistical analysis indicated that there was a statistically significant decrease in bacterial growth in comparison to untreated control groups ($P \leq 0.05$).

Table 3: The MIC and sub-MIC results for MgO NPs against MDR *K. pneumoniae* and *E. faecalis* isolates

Bacterial isolate	Concentration of MgO NPs (µg/ml)	
	MIC	sub-MIC
K14	12.5	6.25
K17	12.5	6.25
K19	12.5	6.25
K22	12.5	6.25
E43	12.5	6.25
E96	12.5	6.25
E36	12.5	6.25
E16	12.5	6.25

Anti-biofilm Activity of Magnesium Oxide Nanoparticles

Biofilm formation was significantly reduced in all tested isolates when treated with MgO nanoparticles at sub-MIC levels ($P \leq 0.05$). The percentage of inhibition of biofilm formation by MgO nanoparticles was between 95.45% and 100%, indicating that MgO

nanoparticles possess strong anti-biofilm activity against MDR *K.pneumoniae* and *E. faecalis* (Table 4 and Figures S9).

Table 4: Anti-biofilm activity of MgO NPs against the bacterial isolates

Bacterial isolate	Control biofilm	Biofilm formation inhibition	P-value <i>t</i> -test	The percentage of inhibition \pm S.E
E43	0.73 \pm 0.05	0.00 \pm 0.00	0.001*	100.00 \pm 0.00a
E96	0.66 \pm 0.08	0.03 \pm 0.02	0.002*	95.45 \pm 3.47b
E36	1.79 \pm 0.23	0.00 \pm 0.00	0.002*	100.00 \pm 0.00a
E16	0.89 \pm 0.04	0.00 \pm 0.00	0.001*	100.00 \pm 0.00a
K14	0.26 \pm 0.04	0.00 \pm 0.00	0.002*	100.00 \pm 0.00a
K17	0.27 \pm 0.03	0.00 \pm 0.00	0.001*	100.00 \pm 0.00a
K19	0.94 \pm 0.09	0.00 \pm 0.00	0.001*	100.00 \pm 0.00a
K22	0.49 \pm 0.07	0.00 \pm 0.00	0.002*	100.00 \pm 0.00a
P-Value	0.001*			0.045*

*Significant coefficient differences below $P \leq 0.05$.

Discussion

Nanoparticle biosynthesis using plants has recently become very popular. It is seen as a sustainable, nontoxic, low-cost method of synthesizing nanoparticles that uses biologically active compounds with potential therapeutic applications. Plant-mediated biosynthesis is considered eco-friendly compared with the traditional methodologies (i.e., physical and chemical) used in producing nanoparticles (27).

The biosynthesis of the magnesium oxide (MgO) nanoparticles in this study was indicated by changes in color throughout the synthesis, which reflects the sequential production of magnesium hydroxide (Mg(OH)₂) and its conversion to MgO once it was calcinated. Chemical interactions between the magnesium ions and the polyphenols present in *C. sinensis* extract acted as both stabilizing and capping agents and caused these color changes during synthesis. Reports on color transitions and pathways used to produce MgO nanoparticles using plant-mediated synthesis have been published (26, 30, 31) and support that this environmentally sustainable approach can consistently and reliably yield MgO nanoparticles.

The broad absorption spectrum indicates that MgO nanoparticles were produced through this method, and confirms that nanoparticles exist. The formation of MgO nanoparticles does not display a specific surface plasmon resonance band; rather, the increase in absorbance (or intensity) of the broad absorption band from MgO nanoparticles was due to modifications in size and electronic configuration created by producing the nanoparticles. Additionally, other articles demonstrating the use of plant materials to synthesize MgO nanoparticles report similar UV-Vis spectral characteristics; therefore, the particular phytochemical composition is an important determinant of the optical properties of the MgO nanoparticles (32, 33).

FTIR results supported these conclusions by showing that Mg–O stretching vibrations were present and displaying the presence of hydroxyl and carbonyl functional groups. These functional groups on *C. sinensis* extract contain proteins, flavonoids, and polyphenols that can contribute to particle stability and biological activity due to their ability to effectively functionalize surfaces (34, 35).

Therefore, the XRD analysis confirmed that the material was MgO with a cubic periclase structure

and corroborated with JCPDS reference data. The average crystallite size of MgO (20-30 nm) calculated using the Scherrer equation is within the range of nanocrystalline size range reported being optimal for antimicrobial activity (36). Scanning electron microscopy results demonstrated larger particle sizes of MgO (52-104 nm) due to some level of particle aggregation. The difference between crystallite size and actual particle size is common in biosynthesized nanoparticles and does not affect their biological activity. Other researchers have reported similar size distributions and morphologies observed in MgO nanoparticle synthesized using plant-mediated methods (37, 38).

AFM Data offer supplementary evidence of surface characteristics and nano-topography/roughness which have significant influence on attaching bacteria and nanoparticle-cell interaction (39). The observed height profiles of the nanostructures in the AFM images correlate to structural consistency and surface stability as indicated by the SEM and XRD data. Furthermore, EDX and AAS analyses confirm elemental purity and there were no significant contaminants that would cause interference to the effectiveness of the green synthesis protocol (40, 41). In addition, the high negative zeta potential value observed (–44.61 mV) indicates strong electrostatic repulsion between the nanoparticles and therefore extends the colloidal stability obtained from polyphenol-mediated surface encapsulation, consistent with previous investigations (42).

The antibacterial properties of nanoparticles made of magnesium oxide (MgO) are thought to be a combination of their small size (nanoscale), large surface area, irregular shape, and stability when exposed to air. The majority of studies have reported that the antibacterial properties of MgO nanoparticles are predominantly due to the size of nanoparticles, specifically those between 40 and 60 nm in diameter, as these are known to attach to and disrupt bacterial membranes and ultimately result in the inability to produce ATP, or to undergo normal processes of replication (43).

There are several mechanisms described for how MgO nanoparticles exhibit antibacterial activity;

however, these generally relate to membrane damage, interference with metabolism, and the production of reactive oxygen species (ROS). The addition of ROS causes an oxidative damage to cellular components, including lipids and proteins and the direct effect on bacterial DNA, leading to cell death (44).

The increased antibacterial effect seen in this research could also be due to leftover phytochemicals from *C. sinensis* extract attached to the surface of the nanoparticles. Polyphenols and alkaloids have antimicrobial properties that could produce a synergistic impact on bacteria and contribute to enhancing the inhibition of bacteria more than an MgO nanoparticle by itself. A dose-dependent antibacterial action of green synthesized MgO nanoparticles against resistant strains has been shown previously (42). Differences in antibiotic resistance among isolates highlight the need for a strain specific evaluation when designing antimicrobial agents based on nanoparticles.

MgO nanoparticles displayed substantial inhibitory activity (anti-biofilm) with their penetration of the extracellular polymeric substance (EPS) matrix, disruption of established biofilm architecture, and induction of oxidative stress within the bacterial cells that are embedded in biofilms contributing to their superior efficacy. In agreement, Hashemifard et al. (45) and Huang et al. (46) also reported similar conclusions regarding the greater effectiveness of MgO nanoparticles to prevent rather than eradicate pre-existing biofilms. Moreover, the protective character of the EPS matrix of biofilms is further evidence of the propensity for future uses of MgO nanoparticles as prophylactic agents (e.g., coatings for medical devices). The observation of very high anti-biofilm activity is likely spent with the phenolic and flavonoid constituents from the *C. sinensis* extract and the ability of their extracts to inhibit quorum sensing pathways, suppress the production of EPS (47), and support the use of plant-mediated nanomaterial synthesis for other properties enhance during subsequent processing of the *C. sinensis* extract or formed using bioactive constituents.

This study's results show that disposal of green synthesis, MgO nanoparticles with good antimicrobial activity and also significant antibacterial and anti-biofilm activity against Gram positive and Gram negative MDR pathogens. Green synthesis of MgO nanoparticles is significantly more environmentally friendly and cost-effective, also has better biological properties relative to other metal oxide nanoparticles (48). The findings are consistent with the large amount of evidence that MgO nanoparticles have promise as antimicrobial agents for use in medical and pharmaceutical applications, especially for the treatment of MDR and biofilm-mediated infections (49).

These findings directly support the aims of the present study, which was to evaluate the antibacterial and anti-biofilm potential of green-synthesized MgO nanoparticles against MDR clinical isolates.

Limitations This study was limited to laboratory experiments using a small number of bacterial strains, and the safety, biocompatibility, and in vivo efficacy of the nanoparticles remain to be evaluated., thus limiting a comprehensive evaluation of its therapeutic potential.

Conclusion

The research demonstrated the efficacy of environmentally friendly and cost-effective green synthesis of magnesium oxide (MgO) using an aqueous extract of *C. sinensis* or green tea. Additionally, through characterization using various techniques, MgO was shown to be present as nanosized, crystalline material that is stable with the appropriate surface properties for use as an antimicrobial and anti-biofilm agent against multidrug-resistant (MDR) bacteria such as *E. faecalis* and *K. pneumoniae*. MgO nanoparticles exhibited potent antimicrobial activity against both species of MDR bacteria and were able to inhibit biofilm formation by both microorganisms when tested at sub-inhibitory concentrations with enhancement due to their nanosized nature, rough surface topography, and the presence of phytochemicals that originate from the plant used to produce the nanoparticles. The results of this study demonstrate that green synthesis of MgO nanoparticles provides a viable alternative to conventional antimicrobials and anti-biofilming treatment for use against MDR pathogens within biomedical or pharmaceutical applications.

Author's Declaration

We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript. Author(s) signed on ethical consideration's approval: Ethical approval of isolate collection was obtained from the Baghdad hospital administration affiliated with the Ministry of Health/Republic of Iraq, which numbered (2024/12), as well as after the consent of patients.

Conflicts of Interest: None.

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Authors' Contribution

Study conception & design: (Iman M. Salem, Huda S. A. AL-Hayanni). Literature search: (Iman M. Salem, Huda S. A. AL-Hayanni). Data acquisition: (Iman M. Salem, Huda S. A. AL-Hayanni). Data analysis & interpretation: (Iman M. Salem, Huda S. A. AL-Hayanni). Manuscript preparation: (Iman M. Salem, Huda S. A. AL-Hayanni). Manuscript editing & review: (Iman M. Salem, Huda S. A. AL-Hayanni).

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التخليق الأخضر لجسيمات أكسيد المغنيسيوم النانوية باستخدام المستخلص الكحولي لنبات *Camellia sinensis* ونشاطها المضاد للبكتيريا والمثبط لتكوّن الأغشية الحيوية ضد البكتيريا الممرضة متعددة المقاومة

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الخلاصة

الخلفية: تشهد البكتيريا متعددة المقاومة للمضادات الحيوية (MDR) انتشارًا متسارعًا على المستوى العالمي، مما أوجد حاجة ملحة إلى استراتيجيات علاجية بديلة وفعالة لمكافحة هذه البكتيريا. وتعد تقنية النانو الخضراء نهجًا صديقًا للبيئة في تخليق الجسيمات النانوية ذات الفعالية الحيوية، إذ تمتاز بقدرتها على تعزيز النشاط المضاد للميكروبات مقارنة بالطرق التقليدية.

الأهداف: هدفت هذه الدراسة إلى التخليق الأخضر لجسيمات أكسيد المغنيسيوم النانوية (MgO NPs) باستخدام المستخلص الكحولي لنبات *Camellia sinensis*، وتقييم نشاطها المضاد للبكتيريا والمثبط لتكوّن الأغشية الحيوية ضد عزلات بكتيرية سريرية متعددة المقاومة للمضادات الحيوية.

المواد: تم تخليق جسيمات أكسيد المغنيسيوم النانوية باستخدام المستخلص الكحولي لنبات *Camellia sinensis* بوصفه عامل اختزال وتثبيت. جرى توصيف الجسيمات النانوية المحضّرة باستخدام تقنيات التحليل الطيفي للأشعة فوق البنفسجية-المرئية (UV-Vis)، والتحليل الطيفي بالأشعة تحت الحمراء (FTIR)، وحيود الأشعة السينية (XRD)، والمجهر الإلكتروني الماسح (SEM)، ومجهر القوة الذرية (AFM)، والتحليل الطيفي لتشتت الأشعة السينية (EDX)، والتحليل بالامتصاص الذري (AAS)، وتحليل جهد زيتا. تم تقييم الفعالية المضادة للبكتيريا من خلال تحديد التركيز المثبط الأدنى (MIC)، في حين جرى تقييم النشاط المضاد لتكوّن الأغشية الحيوية باستخدام اختبار الصفيحة الدقيقة بصيغة الكريستال البنفسجي ضد عزلات سريرية متعددة المقاومة من *Enterococcus faecalis* و *Klebsiella pneumoniae*.

النتائج: أظهرت النتائج أن جسيمات MgO النانوية المحضّرة حيويًا تراوح حجمها بين 52–104 نانومتر وفقًا لتحليل SEM، مع وجود خشونة سطحية نانوية مثبتة بواسطة AFM. كما أبدت الجسيمات النانوية استقرارًا غروانيًا عاليًا، حيث بلغت قيمة جهد زيتا -44.61 ملي فولت. وأظهرت جسيمات MgO النانوية نشاطًا قويًا جدًا ومُعتمدًا على الجرعة ضد كل من *E. faecalis* و *K. pneumoniae*، مع قيمة MIC بلغت 12.5 ميكروغرام/مل، إضافة إلى قدرتها على تقليل تكوّن الأغشية الحيوية بشكل معنوي ($p < 0.05$) بنسبة تراوحت بين 95.45–100% عند التراكيز دون المثبطة.

الاستنتاج: تُظهر جسيمات أكسيد المغنيسيوم النانوية المُخلّقة أخضرًا باستخدام مستخلصات نبات *Camellia sinensis* نشاطًا مضادًا للبكتيريا ومثبطًا لتكوّن الأغشية الحيوية بكفاءة عالية ضد الممرضات متعددة المقاومة للمضادات الحيوية. وتدعم هذه النتائج إمكانية توظيف جسيمات MgO النانوية المحضّرة بالطريقة الخضراء بوصفها عوامل مضادة للميكروبات صديقة للبيئة للحد من العدوى المرتبطة بالأغشية الحيوية.

الكلمات المفتاحية: الفعالية المضادة للبكتيريا؛ تثبيط الأغشية الحيوية؛ الشاي الأخضر؛ التخليق الأخضر؛ جسيمات أكسيد المغنيسيوم النانوية.