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Green Synthesis Of Magnesium Oxide Nanoparticles With Antimicrobial Potential Using Leaf Extract Of Tamarindus Indica

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Abstract
Background: The development of sustainable, eco-friendly protocols for the fabrication of inorganic nanomaterials is a primary objective in modern nanobiotechnology. Magnesium oxide nanoparticles (MgONPs) possess remarkable biocompatibility and therapeutic potential, yet their conventional synthesis often relies on toxic and energy-intensive processes. This study investigates the biogenic synthesis of MgONPs utilizing the aqueous leaf extract of Tamarindus indica as a dual-functional reducing and capping agent.
Methods: The phytosynthesized nanoparticles (Ti-MgONPs) were comprehensively characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy with Energy Dispersive X-ray spectroscopy (SEM-EDX), and High-Resolution Transmission Electron Microscopy (HR-TEM). The biological efficacy of the Ti-MgONPs was evaluated through in vitro antimicrobial assays against Gram-positive and Gram-negative bacteria, DPPH free radical scavenging, and bovine serum albumin (BSA) protein denaturation assays.
Results: UV-Vis spectroscopy confirmed the bioreduction with a characteristic surface plasmon resonance peak at 284 nm. FTIR analysis identified the active participation of plant-derived polyphenols and flavonoids in capping the nanoparticles, alongside the confirmation of the Mg–O lattice at 545 cm^{-1}. XRD and HR-TEM analyses revealed highly pure, face-centered cubic (fcc) crystalline structures with an average size ranging from 18 to 28 nm. The Ti-MgONPs demonstrated potent, dose-dependent broad-spectrum bactericidal activity, exhibiting maximum efficacy against Staphylococcus aureus. Furthermore, the nanoparticles displayed significant in vitro antioxidant activity (74.5% DPPH scavenging at 100 µg/mL) and substantial anti-inflammatory potential (68.4% inhibition of protein denaturation at 100 µg/mL).
Conclusion: The aqueous leaf extract of Tamarindus indica serves as a highly efficient, green, and sustainable medium for synthesizing physically stable and biologically active MgONPs. The multifaceted antimicrobial, antioxidant, and anti-inflammatory properties of these biogenic nanoparticles underscore their substantial promise for integration into advanced biomedical and therapeutic applications.

Introduction

Nanotechnology has emerged as a transformative discipline within modern materials science, providing innovative solutions across the biomedical, environmental, and industrial sectors [1]. The ability to manipulate matter at the nanoscale has opened new frontiers in drug delivery, diagnostics, tissue engineering, and antimicrobial therapy, offering approaches that were previously unattainable using conventional materials. Among the diverse array of nanomaterials, metal oxide nanoparticles have garnered significant attention due to their unique physicochemical properties, high surface-area-to-volume ratio, and remarkable thermodynamic stability, which collectively contribute to their enhanced reactivity and functional versatility [2].
Magnesium oxide nanoparticles, in particular, represent a highly promising class of inorganic nanomaterials with broad applicability in biomedical contexts. Recognized by the United States Food and Drug Administration as safe materials, magnesium oxide nanoparticles are inherently biocompatible and exhibit potent antibacterial, antifungal, and antioxidant characteristics, making them exceptional candidates for therapeutic and broad-spectrum antimicrobial applications [3, 4]. The antimicrobial mechanism of these nanoparticles is primarily attributed to the generation of reactive oxygen species, disruption of bacterial cell membranes, and interaction with cellular components, all of which contribute to effective microbial eradication without the reliance on conventional antibiotics.
Traditionally, the fabrication of magnesium oxide nanoparticles has relied on physical and chemical methodologies, including sol-gel processes, chemical co-precipitation, laser ablation, and hydrothermal techniques [5]. These conventional approaches have been extensively optimized over decades and are capable of producing nanoparticles with defined morphologies, controlled crystallinity, and reproducible properties. However, they frequently involve hazardous precursor chemicals, require high energy consumption, and generate toxic chemical byproducts that pose severe environmental and biological risks [6]. The use of organic solvents, toxic reducing agents, and high-temperature processing conditions in these methods raises concerns regarding their scalability, sustainability, and compatibility with biomedical applications where residual toxicity must be minimized. Consequently, there is a critical global imperative to

develop sustainable, eco-friendly, and cost-effective alternatives for nanomaterial synthesis, a paradigm commonly referred to as green synthesis [7].

Green nanotechnology leverages biological systems, including bacteria, fungi, algae, and plant extracts, as natural reducing and stabilizing agents, thereby eliminating the need for toxic chemicals and reducing energy demands. Among these biological platforms, plant-mediated synthesis is particularly advantageous as it is highly scalable, rapid, and eliminates the need for complex cell culture maintenance and downstream purification steps associated with microbial systems [8]. The rich phytochemical composition of plant tissues, comprising flavonoids, alkaloids, phenols, saponins, and terpenoids, plays a dual functional role in nanoparticle synthesis: actively reducing metal ions into zero-valent nanoparticles and subsequently capping the formed nanoparticles to prevent agglomeration and ensure long-term colloidal stability [9]. These phytochemicals not only facilitate nanoparticle formation but also contribute additional bioactivity to the final product, potentially enhancing its therapeutic efficacy.

Tamarindus indica L., commonly known as tamarind, is a ubiquitous tropical tree belonging to the Fabaceae family and is widely recognized for its extensive pharmacological and medicinal properties across traditional medicine systems. Its leaves are an abundant source of diverse secondary metabolites, notably polyphenols, flavonoids, tannins, and ascorbic acid, which collectively exhibit robust antioxidant and reducing capabilities [10]. These phytochemical constituents have demonstrated efficacy in various biomedical applications, including anti-inflammatory, hepatoprotective, and antimicrobial activities. Despite the well-documented phytochemical profile of T. indica, its specific application as a biogenic reducing agent for the environmentally benign fabrication of alkaline earth metal oxide nanoparticles, specifically magnesium oxide, remains largely underexplored in current literature [11]. The utilization of tamarind leaf extract presents an attractive opportunity for green synthesis due to the widespread availability of the plant, the ease of extract preparation, and the established safety profile of its phytochemical constituents.

Therefore, the present study was designed to investigate the rapid, eco-friendly synthesis of magnesium oxide nanoparticles utilizing the aqueous leaf extract of Tamarindus indica as both a reducing and capping agent. The use of aqueous extract ensures that the synthesis process remains free from organic solvents and other potentially toxic reagents. Furthermore, this research systematically characterizes the structural, morphological, and optical properties of the biosynthesized nanoparticles using complementary analytical techniques to confirm successful synthesis and to elucidate the physicochemical characteristics that underpin their biological activity. Finally, this study rigorously assesses the in vitro antimicrobial efficacy of the synthesized nanoparticles against pathogenic bacterial strains, thereby elucidating their potential as a novel, sustainable nanotherapeutic agent for applications in infection control and biomedical device development.

Materials and Methods

Chemicals and Reagents

Magnesium nitrate hexahydrate with 99% purity, sodium hydroxide, 2,2-diphenyl-1-picrylhydrazyl, bovine serum albumin, ascorbic acid, and diclofenac sodium were procured from Sigma-Aldrich. Mueller-Hinton Agar and standard antibiotic disks containing ciprofloxacin at 5 micrograms per disk were obtained from HiMedia Laboratories. All chemicals and reagents utilized in this study were of analytical grade and were used without further purification. Deionized water was used throughout the experimental procedures to ensure the absence of interfering ions.

Collection and Preparation of Plant Extract

Fresh, mature leaves of Tamarindus indica were collected from the local region. The botanical identification and authentication were confirmed by a plant taxonomist, and a voucher specimen was deposited in the institutional herbarium for reference. The collected leaves were thoroughly washed with running tap water to remove surface dust, dirt, and epiphytes, followed by a final rinse with sterile deionized water to eliminate any residual contaminants. The cleaned leaves were shade-dried at room temperature for 14 days to prevent the degradation of thermolabile phytochemicals that could be compromised by exposure to high temperatures.

The dried leaves were pulverized into a fine powder using a mechanical blender, ensuring uniform particle size for consistent extraction efficiency. For the preparation of the aqueous extract, 10 grams of the leaf powder was suspended in 100 milliliters of deionized water and heated at 60°C for 30 minutes under continuous magnetic stirring. This temperature and duration were selected to optimize the extraction of bioactive phytochemicals while minimizing thermal degradation. The resulting dark brown decoction was cooled to room temperature and filtered sequentially through Whatman No. 1 filter paper to remove particulate matter. The filtrate was stored at 4°C for subsequent nanoparticle synthesis and phytochemical screening.

Green Synthesis of Magnesium Oxide Nanoparticles

The biogenic synthesis of magnesium oxide nanoparticles was initiated by mixing 50 milliliters of 0.1 molar aqueous magnesium nitrate hexahydrate solution with 10 milliliters of the T. indica leaf extract in a 250 milliliter Erlenmeyer flask. The reaction mixture was subjected to continuous magnetic stirring at 80°C to facilitate the interaction between the metal ions and the phytochemical constituents of the extract. To facilitate the precipitation of magnesium hydroxide, 0.1 molar

sodium hydroxide solution was added dropwise to the mixture until the pH reached 10. The formation of a distinctive yellowish-white precipitate indicated the successful reduction and capping process mediated by the phytochemicals present in the leaf extract. The stirring was maintained for an additional 2 hours to ensure completion of the reaction.
The obtained precipitate was separated via centrifugation at 10,000 revolutions per minute for 15 minutes. To remove unreacted precursor ions and excess phytoconstituents that remained adsorbed on the nanoparticle surface, the pellet was washed three times with deionized water and once with absolute ethanol. The purified pellet was dried in a hot air oven at 80°C for 12 hours to remove residual solvents. Finally, the dried precursor was calcined in a muffle furnace at 400°C for 4 hours to yield highly crystalline T. indica-mediated magnesium oxide nanoparticles, which were ground into a fine powder for further characterization and biological assays.

Characterization of Synthesized Magnesium Oxide Nanoparticles
The optical properties of the synthesized T. indica-mediated magnesium oxide nanoparticles were initially monitored using a UV-Vis spectrophotometer in the wavelength range of 200 to 800 nanometers. This analysis allowed for the detection of characteristic surface plasmon resonance bands associated with nanoparticle formation.
Fourier Transform Infrared spectroscopy was employed to identify the functional groups of the phytochemicals responsible for capping and stabilizing the nanoparticles. Spectra were recorded in the range of 4000 to 400 reciprocal centimeters using the potassium bromide pellet technique, which involved mixing the nanoparticle sample with potassium bromide and compressing the mixture into a transparent disc.
The crystalline structure and phase purity were determined by X-Ray Diffraction using a diffractometer equipped with copper K-alpha radiation. The resulting diffraction pattern was compared with standard reference patterns to confirm the crystal structure and to identify any impurities or secondary phases present.
The morphological features and elemental composition were analyzed utilizing Scanning Electron Microscopy coupled with Energy Dispersive X-ray spectroscopy. This technique provided information on the surface morphology, particle aggregation, and the elemental composition of the synthesized nanoparticles.
Furthermore, the particle size, shape, and distribution were precisely examined using High-Resolution Transmission Electron Microscopy, which allowed for direct visualization of individual nanoparticles at high magnification and provided accurate measurements of particle dimensions.

Antimicrobial Activity Evaluation
The antibacterial potential of the biosynthesized T. indica-mediated magnesium oxide nanoparticles was assessed using the agar well diffusion method against two Gram-positive bacterial strains, Staphylococcus aureus and Bacillus subtilis, and two Gram-negative bacterial strains, Escherichia coli and Pseudomonas aeruginosa. These strains were selected to represent clinically relevant pathogens with different cell wall architectures.
Bacterial inoculums were standardized to 0.5 McFarland standard turbidity, corresponding to approximately 1.5×10^8 colony-forming units per milliliter, and uniformly swabbed onto Mueller-Hinton Agar plates using sterile cotton swabs. Wells of 6 millimeters in diameter were punched into the agar using a sterile cork borer. The T. indica-mediated magnesium oxide nanoparticles were ultrasonically dispersed in 5% dimethyl sulfoxide to prepare various concentrations of 25, 50, and 100 micrograms per milliliter. Fifty microliters of each concentration were carefully loaded into the respective wells using a micropipette.
Ciprofloxacin at 5 micrograms served as the positive control, while 5% dimethyl sulfoxide was used as the negative control. The plates were incubated at 37°C for 24 hours to allow for bacterial growth and diffusion of the nanoparticles into the surrounding medium. The antimicrobial efficacy was quantified by measuring the zone of inhibition in millimeters around each well, representing the area where bacterial growth was suppressed. All assays were performed in triplicate to ensure reproducibility and statistical reliability.

In Vitro Antioxidant Assay
The antioxidant capacity of the synthesized T. indica-mediated magnesium oxide nanoparticles was evaluated utilizing the standard 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay. This assay measures the ability of the nanoparticles to neutralize stable free radicals, providing an indication of their potential to mitigate oxidative stress.
Briefly, 1 milliliter of freshly prepared 2,2-diphenyl-1-picrylhydrazyl solution at 0.1 millimolar concentration in methanol was mixed with 1 milliliter of T. indica-mediated magnesium oxide nanoparticles suspension at varying concentrations of 20, 40, 60, 80, and 100 micrograms per milliliter. The reaction mixture was vortexed vigorously to ensure thorough mixing and incubated in the dark at room temperature for 30 minutes to allow for the radical scavenging reaction to proceed. The absorbance was subsequently measured at 517 nanometers using a UV-Vis spectrophotometer. Ascorbic acid was employed as the standard reference antioxidant. The percentage of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was calculated using the following equation:

Scavenging Activity (%) = (Ac - As) / Ac × 100

where Ac represents the absorbance of the control, consisting of 2,2-diphenyl-1-picrylhydrazyl solution without nanoparticles, and As represents the absorbance of the test sample containing the nanoparticles.

In Vitro Anti-inflammatory Assay

The anti-inflammatory activity of the synthesized nanoparticles was investigated using the bovine serum albumin protein denaturation assay. This assay is based on the principle that inflammation is associated with the denaturation of tissue proteins, and agents that inhibit protein denaturation may possess anti-inflammatory properties.

The reaction mixture consisted of 0.5 milliliters of 1% aqueous bovine serum albumin solution and 0.5 milliliters of T. indica-mediated magnesium oxide nanoparticles at varied concentrations ranging from 20 to 100 micrograms per milliliter. The pH of the mixture was adjusted to 6.3 using a small amount of 1 normal hydrochloric acid to simulate physiological conditions. The samples were incubated at 37°C for 20 minutes and subsequently heated in a water bath at 57°C for 15 minutes to induce protein denaturation. After cooling to room temperature, the turbidity, measured as absorbance, was recorded spectrophotometrically at 660 nanometers. Diclofenac sodium was used as the standard anti-inflammatory drug for comparison. The percentage inhibition of protein denaturation was calculated as follows:

Inhibition (%) = (Ac - As) / Ac × 100

where Ac represents the absorbance of the control, consisting of bovine serum albumin without test sample, and As represents the absorbance of the sample containing the nanoparticles. All assays were performed in triplicate to ensure consistency of the results.

Results

UV-Vis Spectroscopy Analysis

The primary confirmation of the bioreduction of the magnesium precursor to T. indica-mediated magnesium oxide nanoparticles by the Tamarindus indica leaf extract was obtained through UV-Vis spectroscopy. This analytical technique detects the interaction of light with the synthesized nanoparticles, providing information about their formation and stability. The localized surface plasmon resonance of the synthesized nanoparticles yielded a distinct, broad absorption band with a maximum peak observed at 284 nanometers. The presence of this characteristic absorption band indicates the successful reduction of magnesium ions to magnesium oxide nanoparticles, as the plasmon resonance arises from the collective oscillation of electrons in response to incident light. The absence of any other significant absorption peaks in the visible region confirms the purity of the synthesized colloidal suspension and indicates the complete reduction of metal ions without the formation of secondary bulk byproducts or unreacted precursor materials.

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared analysis was conducted to identify the dual role of the plant extract's phytoconstituents as both reducing and capping agents in the nanoparticle synthesis process. This technique detects the characteristic vibrational frequencies of functional groups present in the sample, allowing for the identification of chemical bonds and molecular interactions.

The Fourier Transform Infrared spectrum of the T. indica leaf extract exhibited major absorption bands at 3412 reciprocal centimeters, corresponding to O-H stretching vibrations of phenolics and alcohols; 2924 reciprocal centimeters, corresponding to C-H stretching of alkanes; 1635 reciprocal centimeters, corresponding to C=O stretching of amides and flavonoids; and 1058 reciprocal centimeters, corresponding to C-O stretching. These bands are characteristic of the diverse phytochemical constituents present in the extract, including polyphenols, flavonoids, and other secondary metabolites.

In the spectrum of the synthesized T. indica-mediated magnesium oxide nanoparticles, significant band shifts and intensity reductions were observed compared to the pure extract. The O-H band shifted from 3412 to 3390 reciprocal centimeters, and the C=O band shifted from 1635 to 1618 reciprocal centimeters. These shifts suggest the active participation of polyphenols and flavonoids in the bioreduction process, as the functional groups of these compounds interact with the metal ions during nanoparticle formation. Most importantly, a sharp and intense absorption peak emerged at 545 reciprocal centimeters, which corresponds to the characteristic metal-oxygen stretching vibration of the magnesium-oxygen bond. The presence of this peak confirms the successful formation of magnesium oxide nanoparticles and indicates that the calcination process effectively converted the magnesium hydroxide precursor to the oxide form.

X-Ray Diffraction Analysis

The crystalline nature and phase purity of the calcined T. indica-mediated magnesium oxide nanoparticles were evaluated using X-Ray Diffraction analysis. This technique provides information about the crystal structure, crystallite size, and presence of any impurities or secondary phases within the sample.

The diffractogram displayed highly intense and sharp Bragg diffraction peaks at 2-theta values of 36.85 degrees, 42.82 degrees, 62.24 degrees, 74.58 degrees, and 78.51 degrees. These peaks perfectly index to the (111), (200), (220), (311),

and (222) crystallographic planes of the face-centered cubic structure of periclase magnesium oxide, which corresponds to the standard reference pattern for this material. The sharpness and intensity of the diffraction peaks indicate that the synthesized nanoparticles are highly crystalline. The absence of extraneous peaks in the diffractogram indicates the high purity of the synthesized nanomaterial, with no detectable impurities or secondary phases present.
The average crystallite size was calculated using the Debye-Scherrer equation: $D = K\lambda / \beta \cos\theta$, where K represents the shape factor with a value of 0.9, λ represents the X-ray wavelength, β represents the full width at half maximum of the diffraction peak, and θ represents the Bragg angle. The calculated average crystallite size of the T. indica-mediated magnesium oxide nanoparticles was found to be 22.4 nanometers, indicating that the synthesis method produced nanoparticles within the expected nanoscale range.

Morphological and Elemental Analysis
Scanning Electron Microscopy was employed to examine the surface morphology and particle distribution of the synthesized T. indica-mediated magnesium oxide nanoparticles. The micrographs revealed that the synthesized nanoparticles are highly agglomerated due to their high surface energy and the presence of biological capping agents on the particle surfaces. The particles exhibited a quasi-spherical morphology with a relatively uniform surface distribution across the sample.
Energy Dispersive X-ray spectroscopy was performed to confirm the elemental composition of the synthesized nanoparticles. The spectrum displayed strong characteristic signals for magnesium at 1.25 kiloelectron volts and oxygen at 0.52 kiloelectron volts, confirming the presence of these elements as the primary constituents of the nanoparticles. The elemental profile showed 54.2% magnesium, 41.5% oxygen, and 4.3% carbon, with the carbon content attributed to the phytomolecules bound to the nanoparticle surface from the plant extract. This carbon content provides evidence of the capping role of the phytochemicals, which stabilize the nanoparticles and prevent excessive aggregation.
Further microstructural analysis was conducted using High-Resolution Transmission Electron Microscopy to obtain detailed information about particle size, shape, and crystallinity. The micrographs corroborated the Scanning Electron Microscopy findings, showing well-dispersed, roughly spherical nanoparticles with clear lattice fringes indicative of crystalline structure. Particle size distribution analysis from Transmission Electron Microscopy micrographs indicated diameters ranging from 18 to 28 nanometers, aligning perfectly with the crystallite size calculations obtained from X-Ray Diffraction analysis. This consistency between the two techniques confirms the accuracy of the size measurements and indicates that the nanoparticles are predominantly single crystals.

Antimicrobial Activity
The in vitro antimicrobial efficacy of the biosynthesized T. indica-mediated magnesium oxide nanoparticles was evaluated against four pathogenic bacterial strains using the agar well diffusion method. The biosynthesized nanoparticles exhibited robust, dose-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria, with the zone of inhibition increasing proportionally with the concentration of nanoparticles applied.
The maximum zones of inhibition were recorded against Staphylococcus aureus, indicating a higher susceptibility of Gram-positive strains compared to Gram-negative strains. This differential susceptibility may be attributed to the structural differences in the cell walls of these two bacterial groups. Gram-positive bacteria possess a thick peptidoglycan layer that is accessible to the nanoparticles, whereas Gram-negative bacteria have an additional outer membrane that can act as a barrier, potentially reducing nanoparticle penetration. The negative control consisting of 5% dimethyl sulfoxide showed no inhibition, confirming that the observed antimicrobial activity was attributable solely to the synthesized nanoparticles and not to the solvent used for dispersion.
The detailed zone of inhibition measurements are presented in Table 1. The results demonstrate that T. indica-mediated magnesium oxide nanoparticles possess significant antimicrobial potential across a range of clinically relevant bacterial strains.

Table 1. Antimicrobial activity of Ti-MgONPs (Zone of Inhibition in mm)

Bacterial Strain	25_µg/mL	50_µg/mL	100_µg/mL	Ciprofloxacin	DMSO_Control
S._aureus	12.4±0.5	16.8±0.4	21.2±0.6	28.5±0.3	0.0
B._subtilis	10.8±0.3	14.5±0.6	19.4±0.5	26.2±0.4	0.0
E._coli	9.5±0.4	13.2±0.3	17.6±0.4	29.1±0.5	0.0
P._aeruginosa	8.2±0.6	11.5±0.5	15.8±0.7	25.4±0.6	0.0

In Vitro Antioxidant Activity
The antioxidant potential of the synthesized T. indica-mediated magnesium oxide nanoparticles was quantified using the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay. This assay measures the ability of a substance to neutralize stable free radicals, providing an indication of its potential to mitigate oxidative stress, which is implicated in various pathological conditions.

The nanoparticles demonstrated a significant, concentration-dependent ability to neutralize 2,2-diphenyl-1-picrylhydrazyl radicals, manifested by the visible color change of the solution from deep violet to pale yellow as the radical was reduced. The scavenging activity of T. indica-mediated magnesium oxide nanoparticles reached a maximum of 74.5% at the highest tested concentration of 100 micrograms per milliliter. While this activity was slightly lower than that of the standard ascorbic acid, which achieved 94.2% scavenging at the same concentration, the biogenic nanoparticles exhibited substantial antioxidant capacity. This activity is likely synergized by the plant-derived phenolics capping the nanoparticle surface, which themselves possess intrinsic antioxidant properties.
The comparative scavenging percentages are detailed in Table 2, showing the progressive increase in radical neutralization with increasing nanoparticle concentration.

Table 2. DPPH free radical scavenging activity

Concentration_(µg/mL)	Ti-MgONPs_(%)	Ascorbic_Acid_(%)
20	22.3±1.1	35.4±0.8
40	38.6±1.5	52.6±1.2
60	54.2±0.9	71.3±1.0
80	66.8±1.4	85.7±0.6
100	74.5±1.2	94.2±0.5

In Vitro Anti-inflammatory Activity
The ability of the synthesized T. indica-mediated magnesium oxide nanoparticles to inhibit heat-induced bovine serum albumin protein denaturation was assessed to determine their anti-inflammatory potential. This assay is based on the principle that inflammation is associated with the denaturation of tissue proteins, and agents that inhibit this process may possess anti-inflammatory properties.
The nanoparticles exhibited a progressive, dose-dependent inhibition of protein denaturation, with the percentage of protection increasing consistently across the tested concentration range. At a concentration of 100 micrograms per milliliter, the T. indica-mediated magnesium oxide nanoparticles provided 68.4% protection against thermal denaturation, which is comparable to the standard anti-inflammatory drug diclofenac sodium at lower concentrations. This level of activity suggests that the nanoparticles possess significant anti-inflammatory potential, which may be attributed both to the intrinsic properties of magnesium oxide and to the phytochemical capping layer that remains bound to the nanoparticle surface.
The detailed inhibition percentages are provided in Table 3, demonstrating the concentration-dependent nature of the anti-inflammatory activity and confirming the potential utility of the green-synthesized magnesium oxide nanoparticles in managing inflammatory conditions.

Table 3. Inhibition of BSA protein denaturation

Concentration_(µg/mL)	Ti-MgONPs_(%)	Diclofenac_Sodium_(%)
20	18.5±0.8	32.1±1.1
40	34.2±1.2	48.5±0.9
60	49.6±1.5	65.4±1.3
80	61.3±1.0	79.2±0.8
100	68.4±1.4	88.6±0.7

Discussion
The transition toward sustainable, non-toxic methodologies for nanomaterial fabrication represents a critical frontier in nanobiotechnology, driven by the need to minimize environmental impact and to develop biocompatible materials suitable for biomedical applications. In the present study, Tamarindus indica leaf extract was successfully employed as a dual-action biogenic agent for the reduction of magnesium precursors and the subsequent stabilization of magnesium oxide nanoparticles, hereafter referred to as Ti-MgONPs. The use of this widely available plant species offers several advantages, including the abundance of the raw material, the simplicity of extract preparation, and the established safety profile of its phytochemical constituents.
The successful bioreduction was initially monitored by UV-Vis spectroscopy, revealing a prominent absorption peak at 284 nanometers. This surface plasmon resonance band is highly characteristic of magnesium oxide nanoparticles and aligns with previous reports indicating that biogenically synthesized metal oxide nanoparticles exhibit strong absorption in the ultraviolet region due to the excitation of surface plasmon electrons [12]. The absence of additional absorption peaks in the visible region confirmed the purity of the synthesized colloidal suspension and indicated the complete conversion of the magnesium precursor without the formation of secondary byproducts.
The compositional and structural integrity of the synthesized nanoparticles was rigorously validated through complementary analytical techniques. Fourier Transform Infrared spectroscopy elucidated the biomolecular mechanism

of synthesis, confirming that the abundant polyphenols, flavonoids, and carboxylic acids present in T. indica not only facilitate the reduction of magnesium ions but also coordinate on the nanoparticle surface to prevent agglomeration and ensure colloidal stability [13]. The observed shifts in the characteristic bands corresponding to hydroxyl and carbonyl functional groups in the nanoparticle spectrum compared to the pure extract confirmed the active participation of these phytochemicals in the bioreduction and capping processes. The distinct peak at 545 reciprocal centimeters definitively confirmed the formation of the magnesium-oxygen lattice, providing direct evidence of successful nanoparticle synthesis. Furthermore, X-Ray Diffraction analysis corroborated the high phase purity and highly crystalline, face-centered cubic structure of the Ti-MgONPs, with an average crystallite size of 22.4 nanometers. The diffraction peaks indexed perfectly to the standard reference pattern for periclase magnesium oxide, and the absence of extraneous peaks confirmed the absence of impurities or secondary phases. This nanoscale dimension, further confirmed by High-Resolution Transmission Electron Microscopy, which revealed particle diameters ranging from 18 to 28 nanometers, is crucial for the biological activity of the nanoparticles. The surface-area-to-volume ratio increases exponentially as particle size decreases into the nanometer range, fundamentally enhancing the material's surface reactivity, its ability to interact with biological membranes, and its capacity to generate reactive oxygen species [14]. The consistency between the crystallite size calculated from X-Ray Diffraction and the particle size observed through Transmission Electron Microscopy confirms that the nanoparticles are predominantly single crystals with minimal internal defects.

The antimicrobial assays demonstrated that Ti-MgONPs possess potent, broad-spectrum bactericidal efficacy, functioning in a dose-dependent manner across all tested bacterial strains. The zones of inhibition increased progressively with nanoparticle concentration, confirming that the antimicrobial activity is directly related to the amount of nanomaterial present. Notably, the nanoparticles exhibited preferential activity against Gram-positive strains, specifically Staphylococcus aureus and Bacillus subtilis, compared to Gram-negative strains, specifically Escherichia coli and Pseudomonas aeruginosa. This differential susceptibility is fundamentally attributed to variations in bacterial cell wall architecture. Gram-negative bacteria possess a complex outer membrane composed of lipopolysaccharides, which acts as a formidable permeability barrier against reactive molecules and foreign agents. This outer membrane restricts the diffusion of nanoparticles into the cell interior, reducing their effectiveness. Conversely, the thick but porous peptidoglycan layer of Gram-positive bacteria allows for easier penetration of the nanoparticles, making these strains more susceptible to nanoparticle-mediated damage [15].

The biocidal mechanism of magnesium oxide nanoparticles is multifactorial and involves several parallel pathways that collectively lead to bacterial cell death. The primary mechanism involves the generation of reactive oxygen species, such as superoxide radicals and hydroxyl radicals, which induce severe oxidative stress within the bacterial cell. This oxidative stress results in lipid peroxidation of cell membranes, protein oxidation, and DNA damage, ultimately leading to cell death. Additionally, the localized release of magnesium ions from the nanoparticle surface contributes to antimicrobial activity by interfering with cellular ion homeostasis and disrupting essential enzymatic processes. The alkaline microenvironment generated by magnesium oxide nanoparticle hydration on the bacterial surface further compromises membrane integrity and disrupts cellular homeostasis, creating conditions unfavorable for bacterial survival [16].

Beyond antimicrobial applications, the synthesized Ti-MgONPs exhibited significant in vitro antioxidant and anti-inflammatory properties, highlighting their multifaceted therapeutic potential. The robust 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, reaching 74.5% at a concentration of 100 micrograms per milliliter, is an excellent indicator of the nanomaterial's ability to act as a hydrogen or electron donor in neutralizing free radicals. This enhanced antioxidant capacity is likely a synergistic effect arising from two complementary mechanisms: the inherent catalytic defects present on the surface of the magnesium oxide nanoparticles, which can participate in electron transfer reactions, and the potent redox potential of the T. indica phytoconstituents that remain bound to the nanoparticle surface as a capping layer [17]. The presence of these plant-derived molecules not only stabilizes the nanoparticles but also contributes additional antioxidant activity, potentially enhancing the overall efficacy of the material.

Similarly, the substantial inhibition of heat-induced bovine serum albumin denaturation, reaching 68.4% at 100 micrograms per milliliter, underscores the anti-inflammatory promise of these biogenic nanoparticles. Protein denaturation is a well-documented cause of inflammatory diseases, as denatured proteins can act as autoantigens that trigger immune responses and perpetuate inflammation [18]. The ability of Ti-MgONPs to stabilize complex three-dimensional protein structures against thermal degradation suggests that they may interfere with the initiation and propagation of inflammatory cascades. The anti-inflammatory activity observed may be attributed to both the intrinsic properties of the magnesium oxide nanoparticles and the anti-inflammatory phytochemicals derived from the T. indica extract. This dual mechanism of action suggests that the biogenic nanoparticles could offer advantages over conventional anti-inflammatory agents by providing multiple pathways for reducing inflammation.

The ability of Ti-MgONPs to stabilize complex three-dimensional protein structures against thermal degradation suggests their potential utility in formulating novel, nano-based therapeutics for managing acute and chronic inflammatory conditions. Such formulations could potentially bypass the gastrointestinal side effects frequently associated with conventional non-steroidal anti-inflammatory drugs, which are a major limitation of long-term therapy with these agents [19]. By combining antimicrobial, antioxidant, and anti-inflammatory activities within a single nanomaterial, the Ti-MgONPs synthesized in this study represent a versatile platform for further development in biomedical applications,

including wound healing, infection control, and the treatment of inflammatory diseases. The green synthesis approach employed ensures that the material is produced without the use of toxic chemicals, enhancing its biocompatibility and suitability for in vivo applications. Future studies should focus on evaluating the cytotoxicity of these nanoparticles against mammalian cell lines, assessing their safety profile, and exploring their efficacy in in vivo models of infection and inflammation. Additionally, the mechanisms underlying the synergistic effects between the magnesium oxide core and the phytochemical capping layer warrant further investigation to fully elucidate the structure-activity relationships that govern the biological properties of these biogenic nanomaterials.

Conclusion

The present study successfully demonstrates a simple, rapid, cost-effective, and environmentally sustainable approach for the biogenic synthesis of magnesium oxide nanoparticles (Ti-MgONPs) utilizing the aqueous leaf extract of Tamarindus indica. The diverse phytochemicals present in the extract effectively acted as both potent reducing and stabilizing capping agents, entirely eliminating the need for hazardous synthetic chemicals. Comprehensive microstructural and optical characterization unequivocally confirmed the formation of highly pure, quasi-spherical, face-centered cubic MgONPs with a nanoscale average crystallite size of 22.4 nm. The biosynthesized Ti-MgONPs exhibited robust, dose-dependent, and broad-spectrum antimicrobial efficacy against prominent pathogenic bacterial strains, demonstrating notably superior bactericidal activity against Gram-positive bacteria. Furthermore, the nanoparticles displayed substantial in vitro antioxidant and anti-inflammatory capabilities, acting as excellent free radical scavengers and protein denaturation inhibitors. These compelling findings collectively suggest that T. indica-mediated MgONPs possess immense potential as multifunctional therapeutic agents. Future in vivo and comprehensive toxicological studies are highly warranted to fully elucidate their biosafety profile, precise molecular mechanisms of action, and pharmacokinetic behaviors, ultimately paving the way for their seamless integration into advanced biomedical applications, targeted drug delivery systems, and novel antibacterial formulations.

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