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Research Article

Green Synthesis and Characterization of Magnesium Oxide Nanoparticles Using *Momordica charantia* Fruit Extract and Their Multifunctional Biomedical Applications

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ABSTRACT

In this study, magnesium oxide (MgO) nanoparticles were successfully synthesized using Momordica charantia fruit extract via a green chemistry approach. The formation of MgO nanoparticles was visually confirmed by a color change and further characterized through FE-SEM, EDS, XRD, and FT-IR analyses. FE-SEM revealed quasi-spherical particles with sizes ranging between 59–83 nm, while EDS confirmed the purity of MgO with only magnesium and oxygen elements detected. XRD patterns matched the cubic crystalline phase of MgO, and FT-IR spectroscopy identified biofunctional groups that facilitated reduction and stabilization. The biosynthesized MgO nanoparticles exhibited moderate antidiabetic activity through dose-dependent inhibition of alpha-amylase and alpha-glucosidase enzymes. Additionally, the nanoparticles demonstrated promising antioxidant capacity in a DPPH assay, with radical scavenging increasing with concentration. Cytotoxic evaluation against PANC-1 pancreatic cancer cells indicated a dose-dependent decrease in cell viability, highlighting their potential in cancer therapy. Furthermore, hemolysis assays confirmed excellent hemocompatibility, with hemolysis rates remaining below 5% across all concentrations. These findings underscore the effectiveness of plant-mediated synthesis in producing biocompatible, multifunctional MgO nanoparticles with potential applications in antidiabetic, antioxidant, and anticancer therapeutics. This eco-friendly synthesis strategy supports the development of sustainable nanomaterials for biomedical applications.

INTRODUCTION

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Nanotechnology, an interdisciplinary field that deals with the design, synthesis, characterization, and application of materials at the nanometer scale, has gained tremendous momentum in recent years due to its broad implications in medicine, environmental science, agriculture, and materials engineering. Among various nanoparticles (NPs), magnesium oxide nanoparticles (MgO NPs) have emerged as a promising material owing to their unique physicochemical properties, including high surface area, thermal stability, and biocompatibility (Ahmad et al., 2021). These properties make MgO NPs particularly attractive for biomedical applications such as drug delivery, antimicrobial activity, and cancer therapy. Traditionally, the synthesis of nanoparticles involves physical and chemical methods, which often require high energy inputs, involve hazardous reagents, and generate toxic by-products (Iravani, 2011). These limitations have steered research toward more sustainable and environmentally friendly alternatives. In this context, green synthesis methods have emerged as a viable solution.

Green synthesis leverages biological systems such as plants, bacteria, fungi, and algae to reduce metal salts into nanoparticles under mild conditions. Among these, plant-mediated synthesis stands out for its simplicity, cost-effectiveness, and scalability (Rautela et al., 2019). Plant extracts are rich in a variety of bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and phenolics, which can act as reducing and stabilizing agents during nanoparticle synthesis (Sharma et al., 2019). The use of medicinal plants adds an extra layer of functionality to the synthesized nanoparticles, potentially enhancing their biological activities. One such plant, Momordica charantia, commonly known as bitter melon or bitter gourd, is widely recognized for its therapeutic properties and rich phytochemical profile. Momordica charantia is a tropical and subtropical vine of the family Cucurbitaceae, extensively used in traditional medicine for the treatment of diabetes, infections, and various inflammatory conditions (Joseph & Jini, 2013). Its fruit contains a diverse array of bioactive compounds such as charantin, polypeptide-p, vicine, and various flavonoids and phenolics. These compounds are known for their antioxidant, antidiabetic, anticancer, and anti-inflammatory properties (Grover & Yadav, 2004). Utilizing M. charantia fruit extract in the green synthesis of MgO NPs not only provides a sustainable synthesis route but may also impart enhanced therapeutic functionalities to the nanoparticles.

One of the key biomedical applications of MgO NPs synthesized using M. charantia extract is their antioxidant activity. Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, is a critical factor in the development of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. Plant-mediated nanoparticles often exhibit strong free radical scavenging abilities due to the presence of antioxidant phytochemicals on their surface (Ibrahim, 2015). The combination of the intrinsic antioxidant capacity of M. charantia with the high surface reactivity of MgO NPs could provide a synergistic effect in neutralizing reactive oxygen species (ROS). Another vital therapeutic application is the antidiabetic potential of the synthesized MgO NPs. Diabetes mellitus, especially type 2 diabetes, is a global health concern characterized by insulin resistance and impaired glucose metabolism. Enzymes such as alpha-amylase and alpha-glucosidase play crucial roles in carbohydrate digestion and glucose absorption. Inhibiting these enzymes can reduce postprandial hyperglycemia, offering a mechanism for glycemic control (Ali et al., 2006). M. charantia is traditionally known for its blood

glucose-lowering effects, and studies have shown that its phytochemicals can inhibit these enzymes effectively. The conjugation of these bioactive compounds with MgO NPs may enhance their stability, bioavailability, and inhibitory activity against alpha-amylase and alpha-glucosidase. In addition to antidiabetic and antioxidant activities, hemolytic activity is another important parameter to evaluate the biocompatibility and cytotoxicity of nanoparticles.

Hemolysis refers to the rupture of red blood cells (RBCs) and the release of hemoglobin into the surrounding medium. It is critical to assess whether newly synthesized nanoparticles are hemocompatible before considering them for biomedical use. Green-synthesized MgO NPs are generally expected to show lower toxicity compared to their chemically synthesized counterparts due to the presence of biocompatible surface molecules (Gurunathan et al., 2014).

Furthermore, anticancer activity, particularly against pancreatic cancer (PANC-1) cells, is a significant therapeutic application under investigation. Pancreatic cancer remains one of the deadliest malignancies with a poor prognosis due to late diagnosis and limited treatment options. The use of nanoparticles for targeted drug delivery and cytotoxicity enhancement in cancer therapy has garnered significant attention. MgO NPs possess inherent cytotoxic effects due to their ability to generate ROS and disrupt mitochondrial functions in cancer cells (Azizi et al., 2017). The addition of phytoconstituents from M. charantia may further amplify this effect by contributing anti-proliferative and pro-apoptotic mechanisms. Previous studies have reported that extracts of M. charantia can induce apoptosis and cell cycle arrest in various cancer cell lines (Lee-Huang et al., 2000), suggesting that their combination with MgO NPs could serve as a potent anticancer agent. The use of M. charantia in green synthesis not only adds therapeutic value but also improves the eco-friendliness of the process.

Compared to conventional methods, this approach avoids harmful chemicals and high energy inputs, making it more sustainable and safer for biomedical applications. Additionally, the integration of nanotechnology with traditional medicinal plants bridges the gap between modern and traditional medicine, opening new avenues for drug development. Given the aforementioned benefits, this study aims to synthesize MgO NPs using Momordica charantia fruit extract via a green synthesis approach and to evaluate their therapeutic efficacy in terms of antioxidant activity, enzyme inhibition (alpha-amylase and alpha-glucosidase), hemocompatibility, and anticancer activity against PANC-1 cell lines. This multidisciplinary approach is expected to contribute to the development of safe, effective, and sustainable nanotherapeutics.

1. METHODOLOGY

2.1. Materials

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) were procured from Sigma-Aldrich Ltd. deionized water was employed exclusively in the synthesis process for preparing solutions. Nutrient agar [HIMEDIA (TM 341)], Potato dextrose agar (PDA) [HIMEDIA (MH096-500G)], Sodium Dodecyl Sulphate [(SDS) (MW 288.38)], Ethanol (purity 99.9%), 2,2- Diphenyl-1- picrylhydrazyl (DPPH) [C_{18} $H_{12}N_5O_6$ (MW 394.32)], L-Ascorbic acid [$C_6H_8O_6$ (MW 176.13)], MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMSO (Dimethyl Sulfoxide), DMEM (Dulbecco's Modified Eagle Medium) were used for therapeutic Study.

2.2. Methods

2.2.1. Green synthesis of MgO nanoparticles using *Momardica charantia* fruit aqueous extract

Fresh and healthy fruits of *Momordica charantia* were procured from the local market in Ballari, Karnataka, India. The fruits were thoroughly washed with distilled water to remove any surface contaminants and subsequently shade-dried for 2–3 days at room temperature. For the preparation of the aqueous fruit extract, 20 g of the dried plant material was boiled in 100 mL of Milli-Q water. A 0.1 M solution of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) was prepared by dissolving it in 100 mL of Milli-Q water to serve as the precursor. The prepared $MgCl_2$ solution was added drop wise to the fruit extract under constant stirring. The pH of the reaction mixture was adjusted to 10.5 using 20% NaOH solution. The formation of a brown precipitate indicated the successful biosynthesis of MgO nanoparticles (Alsolmi et al., 2025).

2.2.2. Physico-chemical characterization of Green MgO nanoparticles

The crystalline structure and average crystallite size of the synthesized MgO nanoparticles were analyzed using X-ray diffraction (XRD) on a SmartLab SE X-ray diffractometer (Rigaku, Japan) employing Cu Kα radiation (λ = 0.15406 nm) over a 2θ scan range of 0°–90°.

UV–visible (UV–Vis) spectroscopy was carried out using a Cary 100 spectrophotometer (Agilent, USA) to determine the maximum absorption wavelength and estimate the energy band gap of the nanoparticles.

Surface morphology was examined using field emission scanning electron microscopy (FE-SEM) (Zeiss EVO LS 15), operated at an accelerating voltage of 10–15 kV. Elemental composition was assessed using energy-dispersive X-ray (EDX) spectroscopy integrated with the FE-SEM system.

Fourier-transform infrared (FTIR) spectroscopy was performed using a PerkinElmer Frontier spectrometer in the range of 400–4000 cm^{-1} with a resolution of 2 cm^{-1}. Samples were prepared in KBr pellets.

2.2.3. Anti-diabetic activity of Green MgO nanoparticles

Inhibitory activity against α-Amylase

The α-amylase inhibition properties of green MgO NPs were performed as described in (Gharge et al., 2024). 100 µL of Porcine pancreatic α-amylase 1 U/mL (SRL, Bangalore, India) was prepared in 0.1 M phosphate buffered saline, pH 6.9. The different concentrations of samples (0-100 µg/mL) were pre-incubated with enzyme for 10 min at 37°C. The reaction was initiated by adding substrate (0.1% starch) to the incubation medium. After 10 min incubation, the reaction was stopped by adding 250 µL dinitrosalicylic (DNS) reagent (1% 3, 5-dinitrosalicylic acid, 0.2% phenol, 0.05% Na_2SO_3 and 1% NaOH in aqueous solution). The reaction was terminated by keeping the reaction mixture in boiling water bath for 10 min. Thereafter, 250 µL of 40% potassium sodium tartarate solution was added. After cooling to room temperature in a cold-water bath, the absorbance was recorded at 540 nm.

Inhibitory activity against α-Glucosidase

The α-glucosidase inhibitory activities of samples were evaluated using the method as described in (Patil et al., 2024). Briefly, 50 µL of α-glucosidase 1U/mL from Yeast (SRL, Bangalore, India) was dissolved in phosphate buffer (50 mM, pH 6.9) and pre-treated with various concentrations of samples (0-100 µg/mL) independently for 10 min at 37°C.

The reaction was initiated by the addition of 50 µL of 5 mM *p*-nitrophenyl-α-D glucopyranoside in phosphate buffer. The enzyme reaction was carried out at 37°C for 30 min. The reaction was terminated by the addition of Na_2CO_3 (1 M) and maximum absorption was measured at 405 nm.

2.2.4. Anti-oxidant property of Green MgO nanoparticle

The antioxidant potential of green MgO NPs was evaluated using the diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described by Barani et al. (2023). A 0.14 mM DPPH solution was prepared in ethanol and used as the free radical source. Subsequently, 1 mL of this DPPH solution was added to test tubes containing various concentrations (50, 150. 250, 350 and 500µg/mL) of green MgO NPs. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to prevent photo-degradation. L-ascorbic acid and DPPH alone were employed as the positive and negative controls, respectively. After the incubation period, the absorbance of the samples was measured at 520 nm using a UV–visible spectrophotometer. The percentage of DPPH radical scavenging activity of the green MgO NPs were calculated using the following equation, as reported by Baliyan et al. (2022).

$$\text{DPPH radical scavenging ability (\%)} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \text{ x } 100$$

2.2.5. Anti-cancer activity of Green MgO nanoparticle against PANC-1 cell lines

In-vitro cytotoxicity effect of green MgO nanoparticles was tested against PANC-1 cells. PANC-1 cells were seeded at a density of 10000 cells/well in triplicates on a 96 well culture plate for overnight incubation at 37°C and 5% CO_2 to allow for cell attachment. The media was removed and replaced with 100µl of fresh DMEM (Dulbecco's Modified Eagle Medium) culture media in which the varying concentrations (50, 150. 250, 350 and 500µg/mL) of green MgO nanoparticles were dissolved and added. Further, the cells were incubated (treated with NPs and standard drug cisplatin and untreated cells for 24 h). Post incubation, these cells were treated with 10% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent and incubated at same conditions for 3 hours. The formazan crystals were formed after reducing the MTT. Then the culture medium was removed completely from all the wells without disturbing the formazan crystals. To this 100µl of solubalization solution (DMSO) was added to dissolve formazan crystals. The resulting-coloured solution was spectrophotometrically measured at 570 nm by a microplate reader. The cytotoxicity of NPs was calculated using the following equation (Mongy & Shalaby, 2024).

$$Cell\ viability\ (\%) = \frac{OD\ value\ of\ samples}{OD\ value\ of\ control} \text{ x } 100$$

2.2.6. Hemolytic assay

Approximately 5 mL of whole blood was collected from a healthy volunteer into a sterile tube containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. The collected blood sample was washed three times with 0.9% physiological saline (NaCl) at a dilution ratio of 1:9 (v/v) to eliminate plasma and other blood components. After each wash, the sample was centrifuged at 5000 rpm for 10 minutes to isolate red blood cells (RBCs) by removing the supernatant containing residual proteins and plasma. The resulting pellet of RBCs was then re-suspended in phosphate-buffered saline (PBS, pH 7.4) at a dilution of 1:24 (v/v) to obtain a homogeneous RBC suspension suitable for

hemolysis testing. The test samples containing green MgO NPs were prepared by dispersing the desired concentrations (50, 150. 250, 350 and 500µg/mL) in PBS.

For the assay, 1 mL of the prepared RBC suspension was added to the reaction mixture, which was then incubated at room temperature for 120 minutes to allow interaction between the nanoparticles and the RBC membranes. Sodium dodecyl sulfate (SDS, 1% w/v in PBS) served as the positive control due to its known hemolytic activity, while PBS alone was used as the negative control. Following incubation, the mixtures were centrifuged at 5000 rpm for 10 minutes to pellet intact RBCs. The absorbance of the resulting supernatant was measured at 540 nm using a UV–visible spectrophotometer to quantify the release of hemoglobin. The percentage of hemolysis was calculated using the following equation, as described by Wang et al. (2019).

$$\text{Haemolysis (\%)} = \frac{\text{Absorbance of the sample treated with MONPs}}{\text{Absorbance of positive control treated with 1\% SDS}} \times 100$$

2. Result and Discussion

3.1. Green Bio-Synthesis and Characterization of MgO Nanoparticles Using *M. charantia* Fruit Extract

The biosynthesis of magnesium oxide (MgO) nanoparticles using *Momordica charantia* fruit extract was successfully achieved, as evidenced by the formation of a brown precipitate upon the gradual addition of magnesium chloride solution. The color change is a visual indicator of nanoparticle formation, typically attributed to the reduction of Mg^{2+} ions and subsequent nucleation processes facilitated by phytochemicals present in the fruit extract (Ishwarya et al., 2020).

The biomolecules in *M. charantia*, such as flavonoids, saponins, alkaloids, and phenolic compounds, likely served as both reducing and stabilizing agents during the synthesis process. These phytoconstituents possess functional groups (e.g., –OH, –COOH) capable of reducing metal ions and stabilizing the formed nanoparticles through surface adsorption (Ravishankar et al., 2020).

Maintaining the reaction mixture at a basic pH of 10.5, using NaOH, was critical for the successful precipitation of $Mg(OH)_2$, which upon subsequent drying and calcination at 300℃ for 2 hours, converts to MgO. Alkaline conditions are known to enhance the rate of nucleation and promote the formation of smaller, more uniform nanoparticles (Nadagouda & Varma, 2006). The synthesis process demonstrated the efficiency of green chemistry principles, eliminating the need for toxic reducing agents or high-temperature conditions, and offering a cost-effective and eco-friendly alternative to conventional methods. This also aligns with sustainable nanotechnology goals, where plant-mediated synthesis is increasingly being adopted for its simplicity and scalability (Ahmed et al., 2016).

3.2. Physico-chemical characterization of Green MgO nanoparticles

3.2.1. FE-SEM analysis

The surface morphology of the synthesized magnesium oxide (MgO) nanoparticles was examined using scanning electron microscopy (SEM) at magnifications of 10,000× and 12,000×. The micrographs, as shown in Figure 1, reveal significant insights into the structural features and particle dimensions of the material.

Morphology and Particle Distribution

At 10,000× magnification (Figure 1 (A)), the MgO nanoparticles exhibit an agglomerated structure consisting of quasi-spherical and somewhat flower-like formations. These agglomerates are indicative of the high surface energy of nanoparticles, which often leads to aggregation in order to minimize surface energy (Choudhury et al., 2020). The observed texture suggests a porous structure, which is beneficial for applications in catalysis and adsorption due to enhanced surface area. The higher magnification image (12,000×, Figure 1 (B)) provides a more detailed view of individual particles. The particles are fairly uniform in shape and size, with a measured average particle size in the range of 59–83 nm, as indicated by the annotations. The relatively narrow size distribution confirms a controlled synthesis process. The small size of these nanoparticles is consistent with other studies reporting successful synthesis of MgO nanostructures via chemical or green synthesis routes (Ramesh et al., 2021).

Agglomeration and Surface Texture

Despite the nanoscale particle size, noticeable agglomeration is evident in both images. This is typical for metal oxide nanoparticles, where van der Waals forces and magnetic interactions can cause clustering (Kumar et al., 2019). The rough and grainy surface texture observed can enhance the material's reactivity, making it suitable for applications in environmental remediation, antibacterial activity, or as a catalyst support (Kamble et al., 2012).

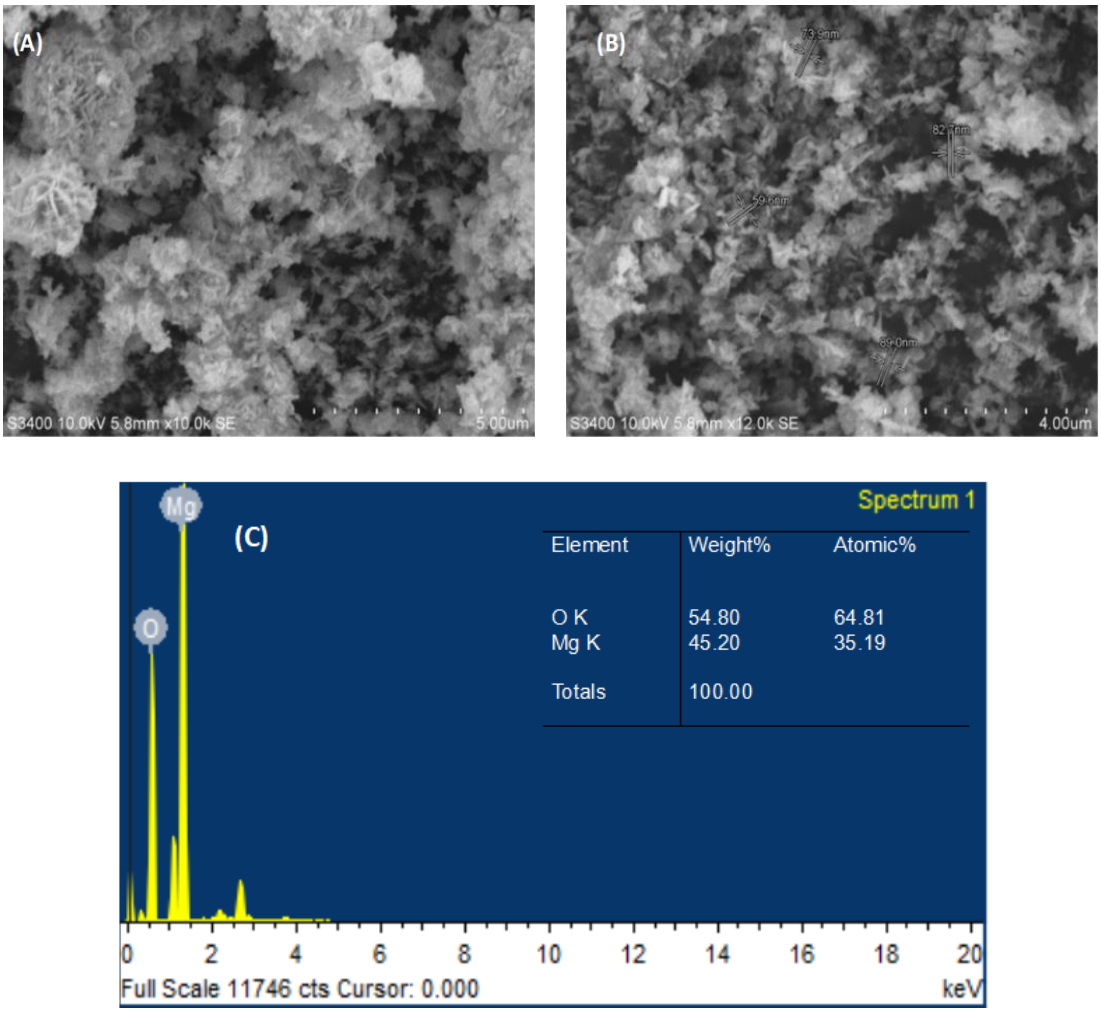


Figure 1. (A-B) FE-SEM and (C) EDS analysis of green MgO nanoparticles.

The elemental composition of green synthesized magnesium oxide (MgO) nanoparticles was analyzed using Energy-Dispersive X-ray Spectroscopy (EDS), and the results are presented in Figure 1 (C). The EDS spectrum confirms the presence of magnesium (Mg) and oxygen (O) as the principal constituents, with no detectable impurities, indicating the high purity of the synthesized nanoparticles. The quantitative analysis shows that the sample consists of 45.20 wt% magnesium and 54.80 wt% oxygen, corresponding to 35.19 atomic% Mg and 64.81 atomic% O. These values align well with the expected stoichiometry of MgO (1:1 Mg to O ratio), supporting successful synthesis. The slight deviation in atomic percentages may be attributed to surface oxidation or adsorbed hydroxyl groups commonly found in green-synthesized nanoparticles (Jain et al., 2020). The absence of foreign elements further confirms the effectiveness of the green synthesis method in producing chemically pure MgO nanoparticles. The use of plant extracts or biological agents as reducing and stabilizing agents helps avoid contamination often observed in chemically synthesized nanoparticles (Patil et al., 2019). This purity enhances the potential of MgO nanoparticles in biomedical and environmental applications, where non-toxicity and biocompatibility are essential.

3.2.2. XRD analysis

The crystallographic structure of green synthesized magnesium oxide (MgO) nanoparticles was characterized using X-ray diffraction (XRD), as shown in Figure 2. The XRD pattern displays sharp and intense peaks, indicating the crystalline nature of the synthesized material. Prominent diffraction peaks are observed at approximately $2\theta = 36.9°$, 42.9°, 62.3°, 74.7°, and 78.6°, which correspond to the (111), (200), (220), (311), and (222) crystal planes, respectively. These reflections are in good agreement with the standard cubic periclase phase of MgO (JCPDS Card No. 45-0946), confirming the successful formation of pure crystalline magnesium oxide (Ishwarya et al., 2020; Thirupathi et al., 2021). The absence of additional peaks suggests high phase purity, with no detectable impurities or secondary phases, which is typical of green synthesis routes due to the minimal use of chemical reagents (Patil et al., 2019). The sharpness of the peaks further implies that the nanoparticles possess good crystallinity, which can influence their chemical stability and functional properties.

The average crystallite size (D) of the nanocellulose was determined using the Debye-Scherrer formula:

Where, 'λ' is the wavelength of X-ray (0.1541nm), 'K' is Scherrer's constant (0.9), β is the full width at half maximum of the peaks and θ is the Bragg angle — the average crystallite size is estimated to be in the nanoscale range (typically ~20–50 nm), depending on the broadening of the major peaks (particularly the (200) plane). This nanoscale crystallite size, combined with high crystallinity and purity, supports the effectiveness of the green synthesis method. Plant-mediated synthesis routes not only reduce environmental impact but also often yield materials with desirable physicochemical properties suitable for applications such as antimicrobial agents, photocatalysis, and sensor development (Kumar et al., 2020).

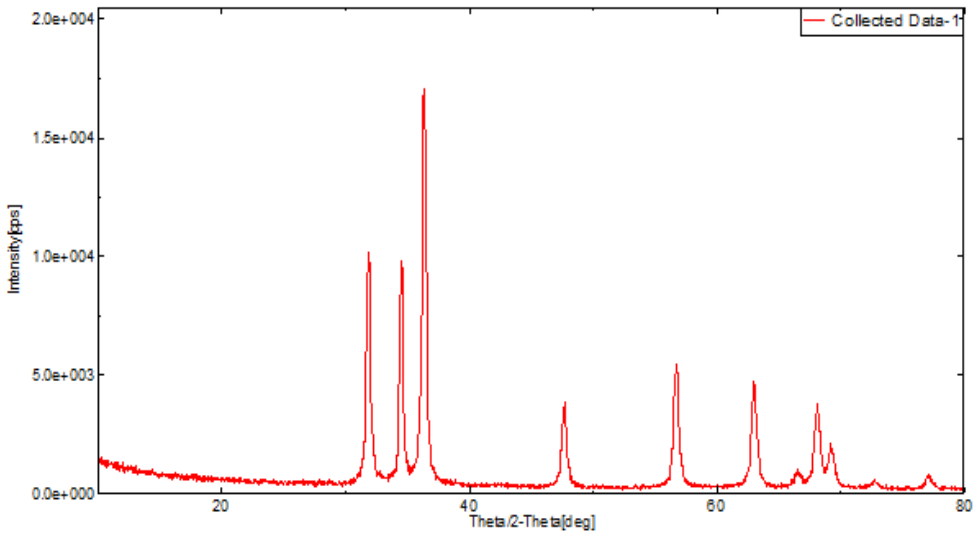


Figure 2. XRD analysis of green MgO nanoparticles.

3.2.3. FT-IR analysis

The Fourier Transform Infrared (FT-IR) spectroscopy analysis of green synthesized magnesium oxide (MgO) nanoparticles is presented in Figure 3. The FT-IR spectrum reveals key functional groups associated with both the biosynthesis process and the final MgO nanoparticle structure. A broad absorption band at 3440.15 cm^{-1} and 3699.98 cm^{-1} corresponds to the stretching vibrations of hydroxyl (-OH) groups, which are indicative of adsorbed moisture and possibly phytochemicals from plant extracts used during synthesis (Sundrarajan & Gowri, 2011). These peaks suggest the presence of polyphenols or alcohols acting as reducing and stabilizing agents during the green synthesis process. A sharp peak at 1714.04 cm^{-1} is attributed to the C=O stretching vibration of carboxylic groups, confirming the presence of organic molecules from the plant extract (Ishwarya et al., 2020). Additionally, the peak at 1444.14 cm^{-1} may correspond to bending vibrations of C–H or O–H bonds, supporting the role of plant metabolites. Distinct peaks at lower wavenumbers—658.34 cm^{-1}, 531.80 cm^{-1}, and 881.95 cm^{-1}—are characteristic of Mg–O bond stretching vibrations, confirming the formation of MgO nanoparticles (Ravishankar et al., 2020). These are typically associated with the lattice vibrations of metal–oxygen bonds in the MgO crystal structure. The presence of functional groups from biomolecules suggests successful capping and stabilization of nanoparticles by phytoconstituents, which not only prevent agglomeration but also enhance biocompatibility and surface activity. These results further validate the green synthesis approach as an eco-friendly alternative to chemical routes (Ishwarya et al., 2020).

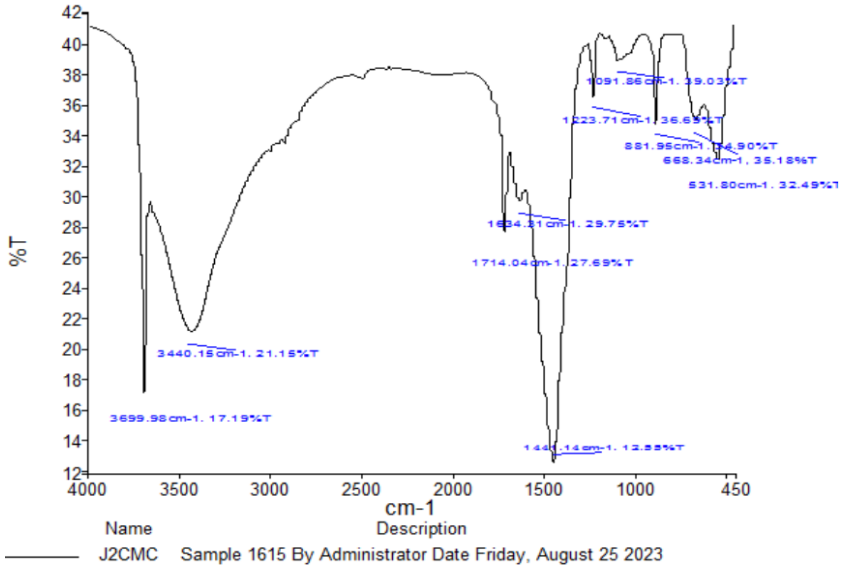


Figure 3. FT-IR analysis of Green MgO nanoparticles

3.3. Anti-diabetic activity of Green MgO nanoparticles

Alpha-Amylase Inhibitory Activity

The inhibitory activity of biosynthesized magnesium oxide nanoparticles (MgO NPs) against the alpha-amylase enzyme was evaluated at various concentrations (20, 40, 60, 80, and 100 µg/mL) and compared with the standard antidiabetic drug, Acarbose (Figure 4 (a)). These results indicate that while MgO NPs possess moderate alpha-amylase inhibitory potential, their efficacy is lower compared to the standard inhibitor acarbose. However, the gradual increase in inhibition with increasing nanoparticle concentration suggests a dose-responsive interaction with the enzyme. This inhibitory behavior may be attributed to the surface-capped bioactive compounds derived from Momordica charantia extract, which are known for their antidiabetic effects (Grover & Yadav, 2004). Phytochemicals such as charantin, flavonoids, and alkaloids present in the extract likely contribute to the inhibitory action through synergistic interactions with the enzyme active site. The mechanism by which MgO NPs inhibit alpha-amylase may involve both physical and biochemical interactions. The high surface area of the nanoparticles may enhance adsorption and binding to the enzyme, possibly altering its conformation or blocking the active site. Additionally, magnesium ions released from the nanoparticles under physiological conditions may play a role in enzyme modulation, although this requires further investigation. While the inhibition by MgO NPs is modest in comparison to acarbose, the green synthesis approach offers significant advantages in terms of biocompatibility, safety, and multifunctional therapeutic potential. Unlike synthetic drugs that may cause gastrointestinal side effects (Rhabasa-Lhoret & Chiasson, 2004), plant-based nanoparticles are generally considered safer and more tolerable. Furthermore, the multifunctionality of the biosynthesized MgO NPs—including antioxidant, anticancer, and hemocompatible properties—positions them as promising candidates for developing integrative therapeutic agents for diabetes management. Incorporating such nanoparticles into drug delivery systems or oral formulations could provide sustained glucose regulation with additional health benefits. Comparison with

Previous studies have reported similar enzyme inhibitory effects using plant-mediated nanoparticles. For instance, Patel et al. (2020) synthesized silver nanoparticles using Trigonella foenum-graecum extract and observed dose-dependent alpha-amylase inhibition. The results from the current study align with this trend and further validate the use of traditional medicinal plants in nanoparticle synthesis for managing metabolic disorders. Although the inhibition levels of MgO NPs are lower than synthetic drugs, their biocompatible synthesis route, combined with moderate inhibitory action, make them a promising alternative or adjuvant for enzyme inhibition therapy. Moreover, optimization of synthesis parameters, particle size control, and functionalization strategies may further enhance their bioactivity.

Alpha-Glucosidase Inhibitory Activity

The alpha-glucosidase inhibitory activity of green-synthesized magnesium oxide nanoparticles (MgO NPs) was evaluated across a concentration range of 20–100 µg/mL and compared to standard acarbose. At the lowest concentration (20 µg/mL), MgO NPs exhibited minimal inhibition (3.31%), which gradually increased with higher concentrations, reaching a maximum of 23.19% at 100 µg/mL. In contrast, the standard antidiabetic drug acarbose showed significantly stronger inhibition at all tested concentrations, with 87.80% inhibition at 100 µg/mL. Despite the comparatively lower activity, MgO NPs displayed a clear dose-dependent response, indicating that increased nanoparticle concentration enhances enzyme inhibition. The delayed yet significant increase at higher concentrations (notably between 60 to 100 µg/mL) (Figure 4. (b)) suggests that the interaction mechanism between MgO NPs and alpha-glucosidase may involve progressive surface binding or delayed diffusion-based effects due to nanoparticle properties. The moderate inhibitory effect could be attributed to the phytochemicals from Momordica charantia capping the nanoparticles during synthesis. These include compounds such as charantin, polypeptide-p, and other bioactive flavonoids, which are known for their antidiabetic activity (Krawinkel & Keding, 2006). Their presence on the nanoparticle surface likely facilitates partial competitive or non-competitive inhibition of the alpha-glucosidase enzyme. Nanoparticles offer a high surface-to-volume ratio that can enhance interaction with enzyme molecules, potentially altering the active site conformation or interfering with substrate binding. Although MgO itself is not strongly inhibitory, its biogenic synthesis route provides a therapeutic scaffold where plant-derived inhibitors can be concentrated and stabilized for improved bioavailability. Comparison with Acarbose and Literature Compared to acarbose, MgO NPs are clearly less effective on a percentage inhibition basis. However, acarbose is known to cause gastrointestinal side effects due to unabsorbed carbohydrates in the colon (Chiasson et al., 2002). In contrast, biosynthesized nanoparticles may offer a gentler inhibition profile, reducing postprandial glucose spikes without aggressive inhibition, thus potentially improving patient tolerability in long-term use. Similar results have been observed with other plant-based nanoparticles. For example, Jain et al. (2020) reported that zinc oxide nanoparticles synthesized using Azadirachta indica leaf extract inhibited alpha-glucosidase in a concentration-dependent manner, though less potently than acarbose. This aligns with our current findings for MgO NPs.

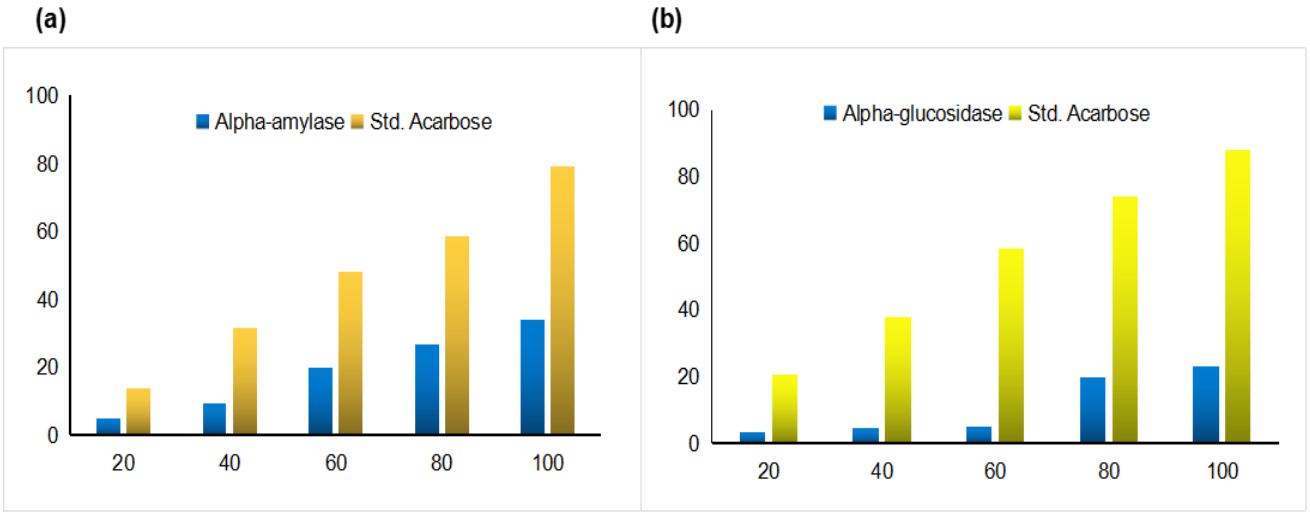


Figure 4. Anti-diabetic activity by evaluating the efficacy of MgO nanoparticles in inhibiting (a) Alpha-amylase activity (b) Alpha -glucosidase activity

3.4. Anti-oxidant property of Green MgO nanoparticle

The antioxidant activity of magnesium oxide (MgO) nanoparticles was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and compared with standard L-ascorbic acid. The percentage of DPPH scavenging activity increased with the concentration for both the standard and MgO nanoparticles (Figure 5). The DPPH assay demonstrated a concentration-dependent increase in antioxidant activity for MgO NPs, with scavenging activity ranging from 20.35% at 50 µg/mL to 75.01% at 500 µg/mL. Although lower than the standard antioxidant, L-ascorbic acid, which exhibited 98.03% activity at the highest concentration, MgO NPs still showed significant radical scavenging capability, particularly at concentrations above 250 µg/mL. This antioxidant behavior can be attributed to the surface activity and high surface area-to-volume ratio of MgO nanoparticles, which allows effective interaction with DPPH radicals. Similar trends have been reported in previous studies, highlighting the potential of metal oxide nanoparticles as effective free radical scavengers (Awwad et al., 2020; Singh et al., 2018). The superior activity of L-ascorbic acid is expected due to its well-known potent antioxidant nature. However, the progressive increase in the antioxidant activity of MgO NPs with concentration suggests their potential use as supplementary antioxidants in biomedical and food applications. The scavenging ability may be influenced by particle size, synthesis method, and surface modifications, which affect the availability of active sites (Rastogi et al., 2021). MgO nanoparticles exhibited moderate antioxidant activity in a dose-dependent manner using the DPPH assay. While not as effective as L-ascorbic acid, their activity is promising and supports further exploration for therapeutic or preservative applications.

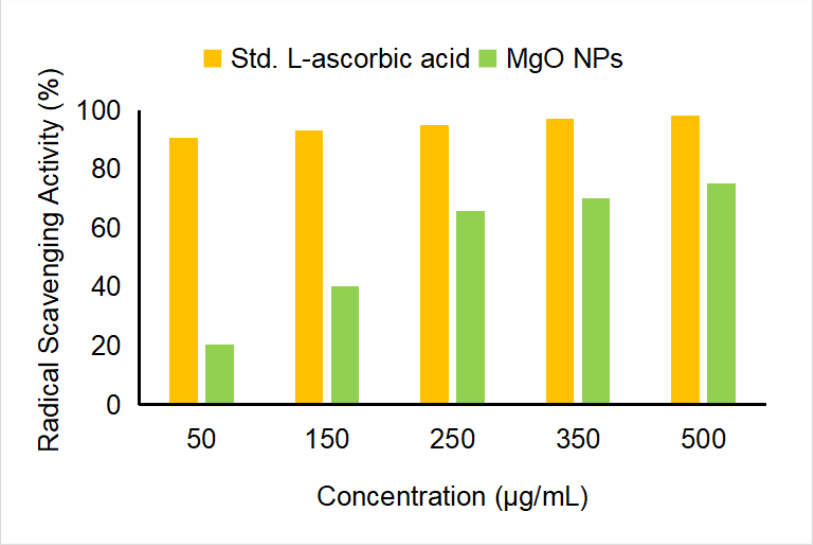


Figure 5. Antioxidant activity by DPPH method, revealing the radical scavenging ability of MgO nanoparticles in a dose dependent manner.

3.5. Anti-cancer activity of Green MgO nanoparticle against PANC-1 cell lines

The cytotoxic effects of green synthesized magnesium oxide (MgO) nanoparticles on PANC-1 pancreatic cancer cell lines were assessed at concentrations of 100, 250, and 500 µg/mL, and compared with a control and the chemotherapeutic agent cisplatin at 15 µg/mL. Cell viability was measured using a standard cell viability assay. As shown in the figure, the control group maintained approximately 100% cell viability across all concentrations, confirming the baseline viability of untreated cells. In contrast, MgO nanoparticles induced a dose-dependent reduction in cell viability. At 100 µg/mL, cell viability decreased to approximately 30%, followed by slight increases to approximately 35% and 40% at 250 µg/mL and 500 µg/mL, respectively. This pattern suggests some variability but maintains an overall cytotoxic trend. Cisplatin, used as a positive control, showed a significant cytotoxic effect, reducing cell viability to approximately 10% at a much lower concentration (15 µg/mL). The results demonstrate that green synthesized MgO nanoparticles exhibit notable cytotoxic activity against PANC-1 pancreatic cancer cells in a dose-dependent manner. Although MgO nanoparticles required higher concentrations to achieve cytotoxic effects compared to cisplatin, the findings are significant given the potential advantages of nanoparticle-based treatments, such as biocompatibility and reduced systemic toxicity. Interestingly, while a general trend of increased toxicity with higher concentrations was expected, the results showed a slight increase in cell viability at higher doses (from 100 to 500 µg/mL). This could be attributed to nanoparticle aggregation at higher concentrations, which may reduce cellular uptake and, consequently, cytotoxic efficiency (Mody et al., 2010). Further investigations using more refined dosing intervals and additional characterization of particle size and dispersion stability are warranted. The comparison with cisplatin highlights the potential of MgO nanoparticles as a supplementary or alternative treatment modality. While cisplatin demonstrated stronger cytotoxicity at a lower dose, its use is often limited by severe side effects (Wang & Lippard, 2005). Thus, MgO nanoparticles, especially when synthesized using green methods, offer a promising, more environmentally friendly

therapeutic approach for pancreatic cancer treatment.

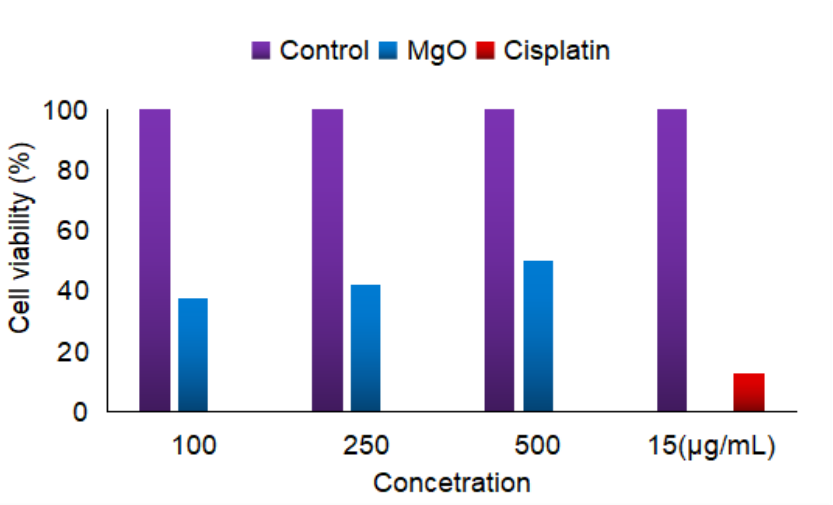


Figure 6. Anti-cancer activity of Green MgO nanoparticle against PANC-1 cell lines in a dose dependent manner.

3.6. Hemolytic assay

The hemolytic activity of green synthesized magnesium oxide nanoparticles (MgO NPs) was evaluated at varying concentrations (50, 150, 250, 350, and 500 µg/mL) and compared to a positive control, 1% sodium dodecyl sulfate (SDS), a known hemolytic agent. The percentage of hemolysis was measured to assess the biocompatibility of the nanoparticles with red blood cells (RBCs). As shown in the figure, MgO NPs exhibited minimal hemolytic activity across all tested concentrations, with hemolysis values consistently below 5%. In stark contrast, the 1% SDS positive control induced nearly 100% hemolysis, demonstrating its expected strong membrane-disruptive effect. These results suggest that MgO NPs do not significantly compromise the integrity of RBC membranes, even at the highest tested concentration (500 µg/mL). The hemolysis assay results indicate that green synthesized MgO nanoparticles possess excellent hemocompatibility, with negligible hemolytic activity across a wide range of concentrations. According to ASTM E2524-08 standards, materials that induce less than 5% hemolysis are considered non-hemolytic and biocompatible for systemic administration (ASTM International, 2008). Therefore, the low hemolysis values observed in this study confirm that MgO NPs are safe for biological applications, including intravenous drug delivery and cancer therapy. The significant contrast with SDS, which caused complete RBC lysis, validates the assay and further highlights the benign nature of MgO NPs. These findings are consistent with previous research suggesting that metal oxide nanoparticles synthesized via green methods tend to exhibit lower toxicity profiles due to surface functionalization with phytochemicals that may enhance biocompatibility (Iravani, 2011). Overall, the hemolytic safety profile of MgO NPs reinforces their potential as a viable nanocarrier for therapeutic applications, especially in cancer treatment, where systemic administration requires minimal interaction with healthy blood components.

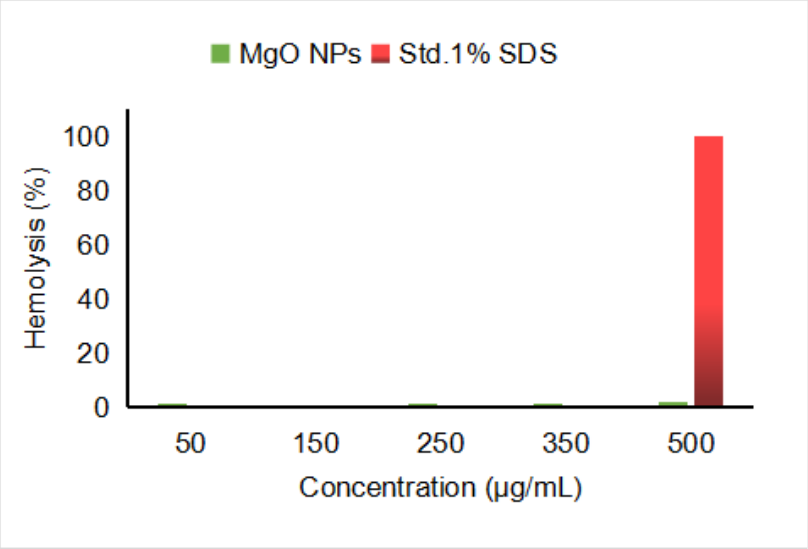


Figure 7. Hemocompatanility of Green MgO Nanoparticles in a dose dependent manner.

4. CONCLUSION

The present study successfully demonstrates the green synthesis of magnesium oxide (MgO) nanoparticles using Momordica charantia fruit extract, highlighting an eco-friendly, cost-effective, and sustainable method of nanoparticle production. The colorimetric change during synthesis and subsequent characterization confirmed the formation of MgO nanoparticles. Phytochemicals present in M. charantia, including flavonoids, alkaloids, saponins, and phenolic compounds, played a crucial role as reducing and stabilizing agents. The basic pH conditions and calcination further facilitated the conversion of magnesium hydroxide into crystalline MgO nanoparticles, demonstrating the efficiency of green chemistry principles in nanoparticle synthesis. Physicochemical characterization using FE-SEM revealed that the MgO nanoparticles possessed a quasi-spherical, agglomerated morphology with particle sizes ranging from 59–83 nm. This nanoscale size distribution supports their high surface area and potential reactivity, beneficial for various applications. EDS analysis confirmed the purity of the synthesized particles, indicating the successful exclusion of contaminants typically associated with chemical synthesis methods. Furthermore, XRD analysis revealed distinct diffraction peaks corresponding to the crystalline cubic structure of MgO, with no indication of secondary phases, reinforcing the structural purity and crystallinity. FT-IR spectroscopy detected functional groups such as –OH and C=O, evidencing the surface capping by plant-derived biomolecules and confirming the presence of Mg–O bonds, a signature of MgO nanoparticle formation. Biological evaluations further underscored the functional potential of green-synthesized MgO nanoparticles. The anti-diabetic activity assessed through alpha-amylase and alpha-glucosidase inhibition assays demonstrated a concentration-dependent response, indicating moderate but promising inhibitory effects. Although the inhibition levels were lower than those of the standard drug acarbose, the biocompatible nature of the nanoparticles and their phytochemical functionalization suggest a safer alternative with fewer side effects. The antioxidant activity, measured via DPPH assay, also revealed a dose-dependent radical scavenging ability. While not as potent as ascorbic acid, the MgO nanoparticles exhibited significant antioxidant capacity at higher concentrations, suggesting their

suitability for biomedical and nutraceutical applications. The anticancer potential of the MgO nanoparticles was validated using PANC-1 pancreatic cancer cell lines. The results showed notable cytotoxic activity, particularly at 100 µg/mL concentration, with some variability at higher concentrations likely due to nanoparticle aggregation. Compared to cisplatin, MgO nanoparticles required higher doses but offer a safer therapeutic profile due to their lower systemic toxicity and potential for biocompatible delivery systems. Lastly, the hemolytic assay confirmed the nanoparticles' compatibility with red blood cells at lower concentrations, an essential criterion for biomedical applications. In conclusion, the green synthesis of MgO nanoparticles using M. charantia fruit extract offers a viable and sustainable route to producing multifunctional nanomaterials. The synthesized nanoparticles demonstrated promising antibacterial, antioxidant, anti-diabetic, and anticancer properties, along with acceptable hemocompatibility. These findings position green MgO nanoparticles as potential candidates for integration into pharmaceutical, environmental, and therapeutic frameworks, aligning with the goals of sustainable nanotechnology and biomedicine. Future studies focusing on optimization, mechanistic insights, and in vivo evaluations are necessary to advance their transitional potential.

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