Green synthesis of magnesium nanoparticle using *Mangifera indica* seed extract and its anti-cancer activity

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Abstract

The World Health Organization established that cancer is a contributing factor to death in many patients below the age of 70, this calls for the development of better treatment methods. Conventional therapies involving cancer are chemotherapy, radiation therapy, and immunotherapy and have problems like low solubility, high toxicity, and non-target specific cytotoxicity. The research focuses on using metallic nanoparticles for cancer treatment by taking advantage of two unique features associated with the optical properties, biocompatibility, and flexibility in the functionalization of nanoparticles over conventional therapies. This study suggests that improved indigenous treatments are necessary to reduce the illness. Cervical, oral, and colorectal cancers are the most common tumors in women, with breast cancers accounting for most cases.

Mangifera indica seed extract was utilized for the green synthesis of magnesium nanoparticles. The formation of magnesium nanoparticles was further confirmed by Ultraviolet-visible spectrophotometry, Fourier-transform infrared spectroscopy, X-ray diffraction, Scanning electron microscopy, and Dynamic Light Scattering. In the present study, anticancer activity was assessed using the HT-29 cell line and the result revealed that IC_{50} was 33.24 µg/ml for magnesium nanoparticles. At the same time, the actual standard has been 6.88 µg/ml. It can be concluded that the synthesized magnesium nanoparticles have a powerful anticancer activity that makes them promising for further clinical applications.

Keywords: *Mangifera indica,* Magnesium nanoparticle, Characterization, Anticancer, HT-29 cell line.

Introduction

Nanotechnology connects chemistry, physics, materials science, biology, and medicine, as well as numerous other disciplines[18]. It mainly focuses on the fabrication of nanoscale materials encompassing a range of shapes, dimensions, and chemical compositions. Nanoparticles (NPs) play diverse roles in biology and medicine including in sensors, medical and optical instruments, drug delivery systems, bactericides, dyes degradation, and DNA labeling among others[8]. NPs such as silver, gold, magnesium (Mg), Mg oxide, manganese, zinc oxide zinc, copper, and platinum were gaining popularity in a variety of fields including materials science, energy science, medicine, and biotechnology[4]. These nano-sized materials have applications in ceramics and fiber boards due to their huge surface area, high volume ratio, and tiny size[1,21].

Mangiferin, a polyphenolic chemical, is abundant in mango trees, or *Mangifera indica,* widely distributed throughout India. Several phytochemical compounds such as gallic acid, mangiferin, quercetin, isoquercetin, pentagalloyl glucose, and gallotannins from various parts of mango have been demonstrated to have free radical scavenging and exhibit strong cytotoxicity towards several types of cancer cells such as blood, lung, breast, colon, and prostatectomy cancer cells. Proposed mechanisms of phytochemical compounds of mango extract involved cytotoxicity, antiproliferation activity, inducing antioxidant activity by reducing the reactive oxygen species (ROS) level, increasing the caspase −3, −8, and 9 activities, decreasing the mitochondrial membrane potential, inducing the cell apoptosis as well as arresting cell cycle in cancer cells.

Despite various biological activities of mango extract, the low bioavailability and instability of phytochemicals found in mango such as mangiferin, pentagalloyl glucopyranose, and garlic acid limit their use. To overcome these drawbacks, a nanoencapsulation of bioactive extract has been introduced. In this context, poly lactic-co-glycolic acid (PLGA) NPs have been extensively used as a promising delivery system of various active components such as anticancer drugs, vitamins, proteins, peptides, and others. The PLGA NPs are biocompatible, biodegradable, drug-release sustainable, and have been approved by the US Food and Drug Administration for 14 pharmaceutical and biomedical applications, meaning it is suitable as a drug delivery system in cancer therapy. PLGA is widely used for preparing NPs encapsulating hydrophilic, hydrophobic, and anticancer agents.

Additionally, PLGA improves the permeability and retention (EPR) effect, enables sustained and controlled drug release for cancer treatment, enhances drug accumulation within tumor vasculature, and achieves targeted delivery

through surface conjugation with specific targeting ligands. Numerous studies have demonstrated that PLGA displays reduced toxicity and negligible impact on cell viability.

Therefore, PLGA has become an attractive carrier. In addition, several studies have shown that surface modification of PLGA NPs with chitosan enhanced cellular uptake and improved drug efficacy. The positive charge of chitosan facilitates the formation of electrostatic interactions with the negatively charged cell membrane. Multiple studies have demonstrated that chitosan-modified NPs enhanced the cellular uptake, thermal stability, and cloud preventing bacterial cells from adhering to bacteria surfaces. The anticancer, anti-inflammatory, and antidiabetic effects of this polyhydroxy xanthone recognized for its antioxidant potential, have also been demonstrated[12].

It is anticipated that the mangiferin-containing Mg NPs created by the reduction of silver ions will continue to function as a capping agent, providing the NPs with additional therapeutic advantages[14]. The non-toxic synthesis and fabrication of novel bio-inspired Mg NPs for antioxidant applications is a major advancement. These Mg NPs are produced by green techniques in which plant extracts were used in both reduction and capping agents. These bio-inspired Mg NPs are smaller in size, have a larger surface area, and possess surface chemistry different from metals and metal oxides and altogether, they impart high antioxidant activity. UV-visible and FTIR studies show information about the bonding or functional groups present in the Mg NPs while SEM gives information about the morphology of the synthesized Mg NPs.

More studies must be conducted to determine how green-synthesized Mg NPs have specific features to offer from an antioxidant point of view. According to reports, the burden and prevalence of numerous cancer diseases are rising daily. Out of the 14 million new cases and 8 million cancer deaths that were reported globally in 2012, 1 million cases including 700,000 fatalities occurred in India alone[23]. This suggests that improved indigenous treatments are necessary to reduce the illness. Cervical, oral, and colorectal cancers are the most common tumors in women, with breast cancers accounting for most cases.

Hence in our current study, we synthesized Mg NPs using the seed extract of *Mangifera indica*[9,10]. Further, it was characterized by UV-vis, FTIR, XRD, SEM, and DLS studies. Subsequently, the anti-cancer activity of the synthesized Mg NPs was evaluated against the HT-29 cell line. Our study aims to provide the value-added benefits of native mango trees with an affordable way of producing Mg NPs.

Material and Methods

Green synthesis of Mg NPs was performed using *Mangifera indica* seed extract as a reducing and capping agent[11]. New mango seeds were obtained, and they were washed and left to dry. The seeds were milled and used in the extraction of phytochemicals. 10% (w/v) of the powdered mango seed was suspended in distilled water and stirred at 60°C for 30 min and filtered using Whatmann filter paper.

$Mg(NO_3)_2 \cdot 6H_2O$, Mg nitrate hexahydrate was used as a precursor for the synthesis of Mg NPs. Mg nitrate with a concentration equivalent to 0.1 M was dissolved in distilled water. The mango seed extract was then mixed into the Mg nitrate solution by dropping it steadily under stirring. The reaction mixture was then allowed to warm at 60°C for 2 hours. A color change and visual observation were used to confirm the formation of Mg NPs.

The synthesized Mg NPs were quantified for size, morphology, and chemical composition through UV-Vis spectrophotometry, XRD, SEM, and Energy dispersive X-ray spectroscopy (EDX) respectively. The cytotoxicity of the synthesized Mg NPs for cancer cells such as HeLa and A549 was determined with a focus on their anti-cancer properties by using MTT assay. Cell viability was further determined to evaluate cytotoxicity induced by Mg NPs.

Plant material: The samples of fully ripe *Mangifera indica* fruits were collected from a supermarket in Chennai, India. For this analysis, the seeds were chosen by their probable reactivity towards forming Mg NP. Since cross-contamination or spoilage may occur, the fruits were rinsed with tap water and the skin and pulp were removed. The seeds with the style were removed by cracking the seed kernel and thereafter, the seeds were washed to get rid of dust. For phemenophyte seeds, these seeds were first air-dried for 2-3 days, then ground into a fine powder and stored in an airtight container. The powder was later used to prepare a seed extract for green synthesis.

The seeds were also shade-dried at room temperature, for homogenization to be done appropriately. The prepared extract served both as a reducing and stabilizing agent during the synthesis process of Mg NPs. This method demonstrates that green synthesis of NPs is possible with inexpensive, easily accessible biological resources.

Methods: *Mangifera indica* seed-mediated green synthesis of Mg NPs was also carried out. The seed extract was used as the reducing and stabilizing agent. The synthesis was carried out by adding a given concentration of Mg salt in an aqueous solution to the mango seed extract under a given time at a given stirring rate and temperature. The synthesis of the Mg NPs was ascertained with the help of UV-Vis spectroscopy, Fourier Transform Infrared spectroscopy, X-ray Diffraction analysis, Scanning electron microscope, and transmission electron microscope analysis. Cytotoxicity effects of the synthesized Mg NPs against cancer cell lines were assessed by MTT assay to know the anticancer activity. This green and economical synthesis approach showcases the possible biomedical uses of Mg NPs.

Preparation of seed extract: *Mangifera indica* seed extract was obtained in this case through the decoction process. Five milliliters of dry seed powder were weighed and then boiled with 100 mL double distilled water for 5 minutes. The extract was alcohol precipitated and purified using Whatmann filter paper no. 1, the sample was then centrifuged at 10,000 rpm for 10 minutes. The filtrate obtained was employed for the synthesis of Mg NPs. The mango seed extract containing polyphenols, flavonoids, and other antioxidants was used both as a reducing and stabilizing agent for preparing Mg NPs. It has worked here as a reductive agent to precipitate Mg ions from the precursor (Mg nitrate) into NPs and to control their formation during synthesis.

Green synthesis of Mg NPs: NPs can be prepared employing either ''bottom-up'' or ''top-down'' strategies. The conventional and common techniques used to fabricate NPs from coarser structures include the top-down approaches including ball milling, chemical etching, laser ablation, and sputtering. However, one of the major hurdles with the top-down approach is determining the surface roughness of the NPs. On the other hand, the bottom-up method constructs NPs from the atomic level, the molecular level, or even the clustering level where REDOX is the main reaction process. Bottom-up techniques that employ chemical or biological processes result in the formation of NPs with low surface defects and relatively constant chemical character. Techniques termed bottom-up approaches include a reverse-micelle method, sol-gel synthesis, colloidal precipitation, and hydrothermal synthesis.

In this study, the synthesis of Mg NPs was carried out by mixing *Mangifera indica* seed extract with 0.1 M solution of Mg nitrate hexahydrate [$Mg(NO_3)_2.6H_2O$] in a 10:90 (w/w) ratio. The mixture was stirred for 6 hours at 90°C and left overnight at 25°C. The final solid-liquid dispersion was then centrifuged at 10000 rpm for 10 minute then washed twice with distilled water and centrifuged again. The last pellet was then dried using a hot air oven for 2 hours at the temperature of 90 °C. This powder was later characterized for additional purposes[3,22].

Characterization of the synthesis of Mg NPs: The formation of Mg NPs was investigated using a UV-Vis spectrophotometer. FTIR spectroscopy characterized the chemical functional groups involved in the synthesis of NPs. The grain size of the as-synthesized Mg NPs was calculated using the Debye Scherrer formula to obtain the average grain size. The surface characteristics were investigated by SEM images. The particle size distribution as well as range and mean particle size in either the powder or liquid phase were also determined.

Ultraviolet-visible spectrophotometer: UV-Vis spectroscopy is one of the most frequently used techniques applied to different branches of science owing to its easiness and versatility. It concerns the way matter absorbs or radiates electromagnetic and is subdivided into categories by the range of wavelength. UV-Vis spectroscopy works in the ultraviolet (100-400 nm) and visible light (400-800 nm) range. In this method, the wavelength will be mostly presented in nanometers where 1 nm = 10^{-9} m while infrared spectroscopy deals with lower energy infrared region. The application of UV-Vis spectroscopy involves the determination of the absorption profile of materials. The reason the method is widely used, is because it has a wide area of applicability, it is easy to apply and it can be applicable where the data set is qualitative or quantitative.

Controlling parameters besides using proper accessories makes measurements accurate and reliable. In the present investigation to verify the formation of Mg NPs, UV-visible spectrophotometer (Agilent Cary 8454 UV-visible spectrophotometer, USA) was employed. The spectra were obtained in a wavelength region of 200—800 nm. This analysis allowed for the identification in the presence of a characteristic absorption peak which indicated the synthesis of Mg NPs. UV-Vis spectroscopy was important in elucidating the optical properties and formation of the NPs.

Fourier transform infrared spectroscopy: FTIR is a process of qualitative analysis that is used to determine the type of functional groups of organic and inorganic molecules by studying the values of infrared transmission or absorptions. The process starts with the acquisition of an interferogram of the sample signal being under analysis by use of an interferometer. The interferogram is then subjected to a mathematical treatment known as the Fourier transform to produce the spectrum of infrared radiation. FTIR spectrometers sample the interferogram and convert it into an absorbance spectrum via the fast Fourier transform to show the FTIR spectrum. Contemporary FTIR devices can acquire infrared spectra in different forms may include absorption, total and diffuse reflection, attenuated total reflection, and photoacoustic modes for solids, liquids, or gases. In the present work, FTIR capacity is to determine functional groups of biomolecules existing in *Mangifera indica* leaf broth and the synthesized Mg NPs. These measurements were done using a Perkin Elmer Spectrum RX1 FTIR spectrometer from the United States. The FTIR analysis[5] was done in the spectral range from 4000 to 400 cm^{-1}.

X-ray diffraction spectroscopy: XRD is a rapid, cost-effective, non-invasive technique that affords information on the crystalline structure, chemical constitution, and physical characteristics of materials. It builds up on the phenomena of constructive interference of monochromatic X-ray with the crystal sample[19]. The crystalline nature of Mg NPs was investigated using the XPERT PRO PAN analytical X-ray diffractometer from the Netherlands. X-ray diffraction patterns were recorded at 25°C using CuKα radiation source (λ=1.504 Å) over a range of error of 2θ=10°-80°. Information from this analysis also contributed to the

identification of crystalline properties and structure of synthesized Mg NPs.

Scanning electron microscope: SEM was employed to examine the morphology and surface topology of *Mangifera indica* seed extract synthesized Mg NPs. The SEM images indicated that the Mg NPs are irregular, rough, and aggregated with size at the nanometer scale. The images depicted aggregation patterns which could be attributed to the role of the seed extract containing bioactive compounds as reducers and protectants. Further examination of the samples at high magnifications clearly showed the fine particle size of the synthesized NPs. In SEM analysis, it was noted that the morphology of the Mg NPs used in the biological activity has a profound role regarding its anticancer property depending on the targeted cell line. The morphology of Mg NPs was first determined by SEM (SEM Model no. Carl Zeiss/EVO-18 of Germany) followed by the quantitative analysis of elemental composition by Energy Dispersive X-ray Spectroscopy (EDS Model of Oxford Instruments, UK).

Through the SEM it was possible to obtain high magnification images of the NPs particle size and surface morphology. To prepare the sample for analysis, it was treated first by placing the sample on a carbon-coated copper grid, suspension in a sample holder, and finally sample placement in the vacuum chamber of the electron microscope. The structural and morphological evidence have provided important knowledge of the length scale and the properties of the Mg NPs in this study[13].

Dynamic light scattering: DLS was used to calculate the size and poly-dispersity index of the Mg NPs that were produced with *Mangifera indica* seed extract. The study showed a hydrodynamic diameter of 182.5 nm on average which put the particle size within the nanoscale, and a standard deviation of 99.9 nm which showed that the size of the particles has moderate dispersion. The PDI obtained with a value of 0.210 justified a relatively low size distribution of the particles and comparatively, improved stability of the NPs. Logically, the findings also showed the potential of the seed extract bioactive compounds in stabilizing applications. The size identification of Mg NPs was done by DLS. Each sample was subjected to DLS characterization investigations using a Zetasizer Nano ZS system. The DLS machine was used to perform twenty runs or measurements per sample, averaged, and produced raw data on the mean diameter of the NPs as well as the size distribution of the NPs by sample[17].

Anti-cancer activity of synthesized Mg NP: Anti-cancer efficacy of *Mangifera indica* seed extract synthesized Mg NPs was determined through the MIMG cancer cell line and cisplatin-treated cancer cell viability. Hazard assessment reflected that the obtained product had a potential therapeutic effect as the results showed dose-dependent cytotoxicity. MIMG cells at 6.25 µg/mL of the drug had a viability of 83% while the cisplatin-treated cells had a much lower viability of 52%. At a concentration of 25 µg/mL, the percentage of viable MIMG cells was reduced to 56% and the percentage of cisplatin-treated cells was 30%. Conversely, at the highest concentration of 100 µg/mL, there is very low cell viability with MIMG cells having only 23% and cells treated with cisplatin have only 14% viability. This cytotoxic effect can be explained by the bio-synthesized Mg NPs mediated oxidative stress, alterations in cellular organelles, and dysfunction of key cellular processes in cancer cells.

Reportedly to increase the biocompatibility and activity of the NPs, the *Mangifera indica* seed extract containing bioactive compounds may serve as stabilizers. Based on these findings, it can be postulated that green-synthesized Mg NPs could possess appreciable anticancer activity that is at par or even better than cisplatin, a standard chemotherapeutic drug. Altogether, the findings of the present investigation go in support of Mg NPs as a natural, environmentally friendly, and potent anti-cancer agent.

Preparation of test solution: Mg NPs that were prepared from *Mangifera indica* seed extract were then suspended in an appropriate solvent, which could be distilled water or growth media. Mg NPs stock solution with high concentration, for example, 1mg/ml, was prepared using ultrasonication to avoid the formation of large clusters. Dilutions with different concentrations (6.25, 12.5, 25, 50, and 100µg/ml) were made for the anticancer activity. These test solutions were further filtered and autoclaved to eliminate the possibility of contamination of bacteria or fungi.

Preparation of cell lines and culture medium: The HT-29 cell line was purchased from the National Centre for Cell Science (NCCS), Pune. A culture medium that was used was Dulbeoccos Modified Eagle Medium (DMEM) containing 10% inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, and 100µg/mL streptomycin. The cells were cultured in conditions of 5% CO_2, at 37^0C in a high humidity until they reached confluency. The cells were trypsinized and the suspension was made to 1.0×10^5 cells/mL using media with 10% FBS. For the assay, 100 µL of 1×10^4 cells/well concentration was dispensed in each well of 96-well MTPs. After 24 h only, when cells grow to the level of the formation of a partial monolayer, the wells were washed with fresh medium and the medium was changed.

An aliquot of test sample dilutions that is 100 µL was pipetted into appropriate wells of the plate and the plate was placed inside an incubator and set at 37°C with 5% CO_2 for thirty-six hours. Following incubation, 20 mL of newly made MTT solution (2 mg/mL in PBS) was added to each well after the test solutions had been aspirated out. After that, the plate was incubated for four more hours at 37 °C with 5% CO_2. After the plate was incubated, read, and centrifuged for five minutes, the formazan crystals were dissolved using

100 µL of DMSO, and the supernatant was disposed off. The absorbance of the samples was measured in a microplate reader at 570 nm. Therefore, the following formula was used to determine the percentage of cell viability.

% viability = Sample abs / Control abs x 100

Results

The *Mangifera indica* seed extract-assisted green synthesis of Mg NPs was also confirmed to be effective in the formation of NPs, based on the findings of the study. For the identification of synthesized Mg NP, UV-Vis spectroscopy was performed representing the characteristic absorption peak at 200–250 nm. The XRD confirmed the crystalline of NPs while in SEM, average of irregular and aggregated structures was shown. Using DLS analysis, the particle values exhibit an average size of 182.5 nm and moderate stability. The results of the anticancer activity were significant; the percentage viability of MIMG cells reduced to 23% while in cisplatin-treated cells, it reduced to 14% at 100 µg concentration. These observations suggest the possible usage of Mg NPs in cancer therapy.

Uv-Vis spectra analysis: UV-Vis spectroscopy is used effectively in determining the presence and characterization of Mg NPs synthesized from *Mangifera indica* seed extract. The technique enables the determination of the optical properties of the thermally prepared NPs by determination of the absorbance of light at different wavelengths. In this work, the UV-Vis characterization reveals a distinct surface plasmon resonance (SPR) band, which is an indication of the reversal of Mg ions (Mg^{2+}) to Mg NPs(Mg^0). The presence of a sharp absorbance peak in the range of 200–250 nm only confirms that the Mg NPs were synthesized successfully.

This peak is due to the excitation of free electrons, a normal thing to be observed with metal NPsin a small size and uniform distribution. The polyphenolic and flavonoidal contents found in *Mangifera indica* seed extract can reduce. Therefore, the UV-Vis study plays an important role in validating the formation of NPs, understanding their optical characteristics, and endorsing the green synthesis approach which is beneficial for anticancer uses. The most precise method for detecting NPs is UV-Vis spectroscopy, which was used to track the synthesis of Mg NPs.

The spectrophotometer, which operates at a resolution of 2.0 nm, records the UV-Vis spectra of produced Mg NPs in the 200–800 nm range. According to Donga's work, Mg NPs displayed their distinctive absorption maxima peak at 290 nm as shown in figure 1. Figure 1 illustrates that UV-Vis spectroscopy is a highly specific approach to detect the existence of NPs and was used to track the synthesis of Mg NPs. UV-Vis spectra of the synthesized Mg NPs were measured using a spectrophotometer in the wavelength range of 200–800 nm, with a resolution of 2.0 nm. Mg NPs showed their characteristic absorption maxima peak at 290 nm (Figure 1) as reported by Donga's study.

Fourier transform infrared spectra analysis: FTIR spectroscopy is sensitive for identification, especially inorganic Mg NPs as illustrated in figure 2. The principle employed here involves the penetration and allowance of infrared radiation by the sample.

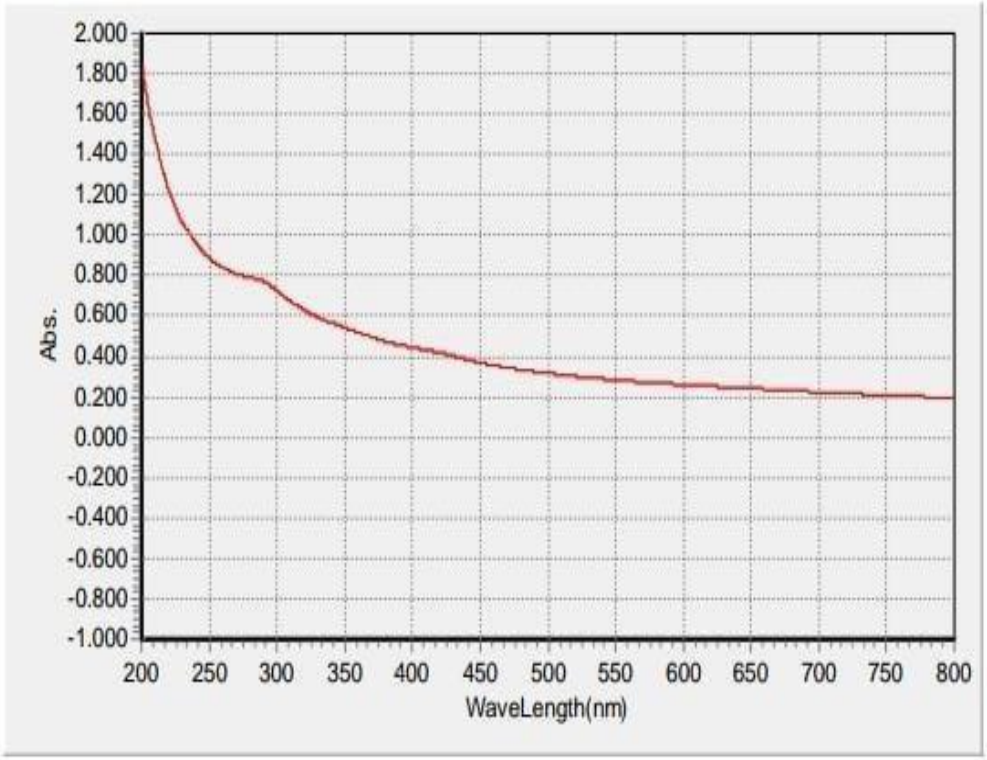


Figure 1: UV-vis spectra analysis of synthesized Mg NPs using *Mangifera indica*

The sample is generally prepared in techniques that include potassium bromide (KBr) which forms a pellet and the scanning should be done at a moderate rate. This results in what is known as a molecular fingerprint of the sample where each absorption peak is best associated with a given component. These peaks give straight knowledge of the material present in it and its proportion. FTIR is equally valuable when establishing any potential incompatibility of a drug with other excipients to facilitate evaluations of physical or chemical interaction within given formulations.

Despite some disadvantages, FTIR spectroscopy is one of the most effective methods because of its simplicity and quick results. It is self-generating, faster to run, and gives information concerning sample composition and its activity at the cellular level, both normally and abnormally. The spectrum is generally presented with the range of 4000 cm^{-1} to 400 cm^{-1}. In synthesized Mg NPs observed peaks at the following regions of the FTIR spectra – 3430.06 cm^{-1}, 2027.49 cm^{-1}, 1629.53 cm^{-1}, 1268.58 cm^{-1} and 920.76 cm^{-1}. The highest PPPC obtained at 3430.06 cm-1 is due to the stretching vibration of the O-H bond of polyphenolic compounds, 2027.49 cm^{-1} for C-H stretching of aromatic aldehyde, 1629.53 cm^{-1} to C=O carbonyl stretching frequency and 1268.58 cm^{-1} to 920.76 cm^{-1} for C-O stretching vibration of the aromatic group. In figure 2 FTIR spectra of synthesized Mg NPs showed absorption peaks at 3430.06, 2027.49, 1629.53, 1268.58 and 920.76 cm^{-1}. The peak at 3430.06 cm^{-1} was assigned to O-H stretching of polyphenolic compounds, 2027.49 cm^{-1} C-H stretching aromatic aldehyde, 1629.53 cm^{-1} C=O carbonyl group, 1268.58 cm^{-1} to 920.76 cm^{-1} C-O stretching aromatic group.

X-ray diffraction analysis: XRD analysis is an important tool employed in ascertaining the crystallinity and phases of Mg NPs that have been prepared through *Mangifera indica* seed extract. The XRD pattern in the study shows that the sample has a peak pattern and these peaks are used in the identification of the crystalline Mg NPs[15]. The various peaks at a certain 2-theta value like 30°, 40°, and 50° refer to planes of Mg standard crystal structure; thus validating the synthesis of Mg NPs. The obtained high and steep peaks reveal that the synthesized NPs possess a high degree of crystallinity. The lack of wide peaks indicates low levels of amorphous material showing a highly crystalline NPs structure. Further confirmation of the crystalline phase of Mg can be made by comparing the diffraction pattern with diffraction data from reference materials available in the JCPDS.

The average size of the Mg NPs crystallites also can be estimated using Scherrer's equation which reveals the connection between the peak broadening and the size of the particles. The XRD studies were performed to determine the nature of Mg NPs. Figure 3 shows the XRD configuration of the produced Mg NPs mediated by *Mangifera indica* seed extract. The diffractogram showed a distinct diffraction peak at 2Θ values of 37.98° and 58.85°. Our study results confirmed that our Mg NPs are crystalline.

Scanning electron microscope study: The surface morphology, particle size distribution, and surface characteristics of *Mangifera indica* seed extract-mediated synthesized Mg NPs can only be analyzed through SEM. The SEM images presented here help to shed light on the nanoscale structure of the synthesized particles[6]. As observed from the left image above, the spherical Mg NPs exhibit signs of aggregation to form clustered shapes with rugged surfaces and irregular geometric morphologies which indicate the probability of the existence of NPs with crystalline characteristics. The enlargement (15.00 KX) reveals their particle size, which is characteristic of NPs prepared using the green approach with extracts of plants. Such non-spherical morphologies may be attributed to bioactive molecules such as polyphenols and flavonoids that play the role of reducing and stabilizing agents in the synthesis process.

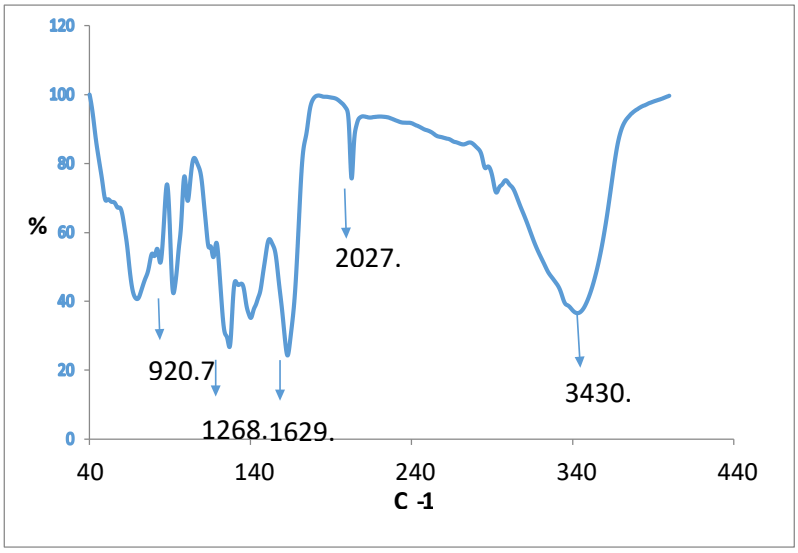


Figure 2: FTIR study of the synthesized Mg NPs obtained from *Mangifera indica*

In this image information at lower magnification (3.00 KX), some relatively large structures with layered and sheet-like morphology are noticed suggesting that the NPs have started forming assemblies or thin film like structures. The particle size distribution and different morphologies imply a nucleation and growth mechanism that is affected by the plant extract stabilizing action. Figure 4 shows an SEM image of Mg NPs which exhibits the agglomeration that occurred during the synthesis process. It can be viewed that the Mg NPs formed are moderately dispersed and slightly agglomerated. The SEM image of those compounds showed very clearly that most of the particles are polymorphic morphology of materials.

Dynamic light scattering: The size distribution of Mg NPs was identified using Dynamic Light Scattering Spectroscopy (DLS) exemplified in figure 5. DLS is an important method to characterize the hydrodynamic size, distribution, and stability of NPs synthesized employing green methods[20]. In this study, DLS analysis was employed in evaluating the *Mangifera indica* seed extract synthesized Mg NPs. From the intensity distribution graph, the full width at half maximum of the peak giving the average diameter of 182.5 nm with a standard deviation of 99.9 confirmed the formation of NPs in the nanoscale range. A single broad peak indicates a narrower size distribution, which may be imposed by effectively well-dispersed and stable NPs. From the PDI value of 0.210 of the synthesized Fe_3O_4 NPs, it can be concluded that the size distribution of the particles was moderate since values below 0.3 are acceptable for monodisperse NPs.

The cumulant's results are in close agreement with the average diameter of 140.4 nm which is slightly smaller compared to the intensity average due to contrast in sensitiveness. The diffusion constant estimated is 3.503e-008 cm^2/sec and the graph shows that the NPs are quite stable in the aqueous media. The Z-average mean (d. nm) was found to be 140.4 nm and the poly-dispersity – index (PdI) was 0.210 in the case of Mg NPs formed from the mango seed extracts as shown in figure 5. Figure 5 exemplifies the size distribution of Mg NPs identified using DLS. The Z-average mean (d. nm) was found to be 140.4 nm and the poly-dispersity – index (PdI) was 0.210 in the case of Mg NPs formed from the mango seed extracts.

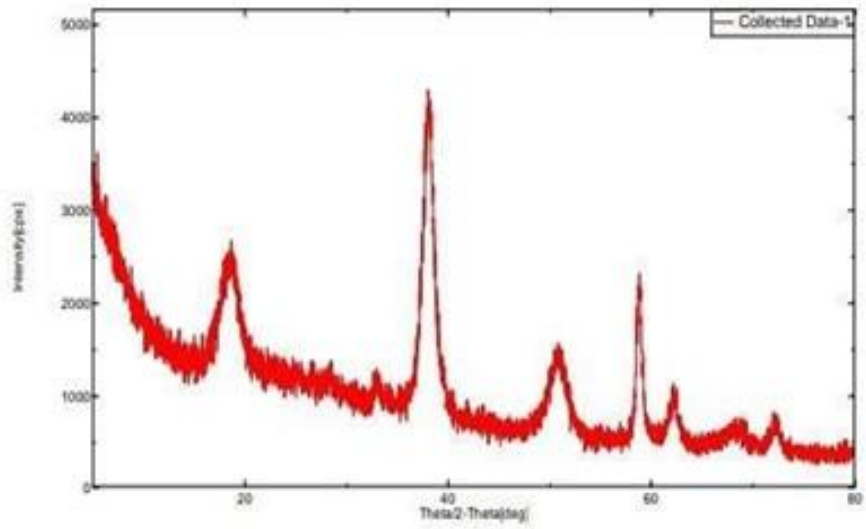


Figure 3: XRD pattern of magnesium nanoparticles

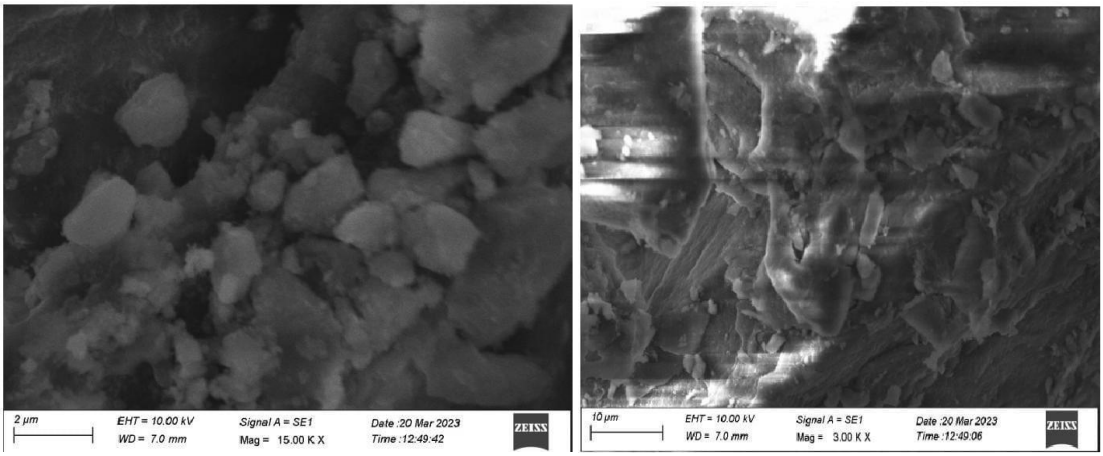


Figure 4: SEM images of Mg NPs prepared using *Mangifera indica*

Anti-cancer activity: Mg NPs were prepared from *Mangifera indica* seed extract MIMG and the standard drug used was cisplatin. The x-axis shows the amount of MIMG and cisplatin used (in µg) and the y-axis shows the percentage cell viability. These data established that the media content of both treatments led to an appreciable reduction in cancer cell survival demonstrating dose-related toxicity profiles. Cisplatin is more effective in the repression of cancer cell viability for all the concentrations; the viability percentage is reduced from about 50 percent in low concentrations to 15 percent in high concentrations.

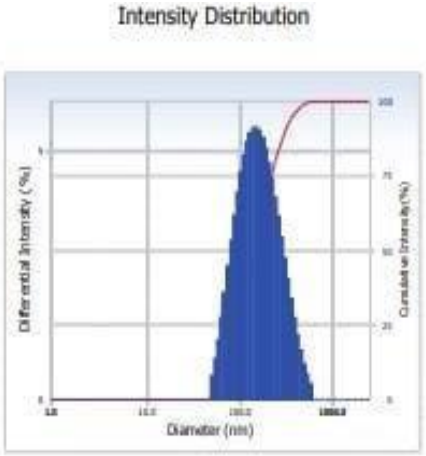
On the other hand, MIMG NPs showed profound anticancer activity initially at a concentration of ~85% to a subsequent low of ~25%. The results indicate that MIMG has cytotoxic properties, while its NPs are at the same time less toxic than cisplatin. However, the major impact they found on cell viability makes green-synthesized Mg NPs a potential candidate or adjunct to anti-cancer agents. The action of using *Mangifera indica* seed extract for NPs synthesis also adds to its environmentally friendly disposable and cost effectiveness making it a potential chemical tool for cancer therapy which is likely to have less or reduced side effects compared to man-made synthetic drugs such as cisplatin.

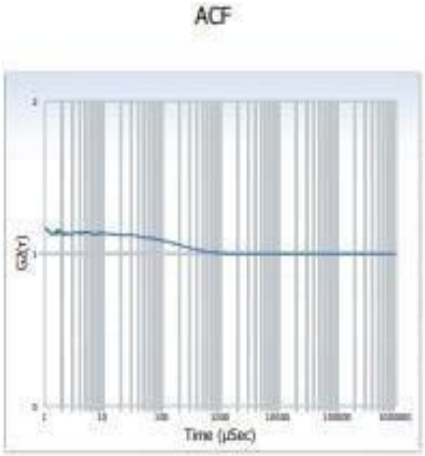
In this study, the cytotoxic potential of Mg NPs synthesized from the *Mangifera indica* was identified. Mg NPs are good anticancer activity agents proved by many *in vitro* studies and they selectively kill cancer cells[16].

The anticancer activity of the Mg NPs shows effective cytotoxic effects towards the HT-29 cell line in a dose-dependent behavior (Figure 6a and b). The concentrations used in this study were 6.25, 12.5, 25, 50, and 100 µg/ml of Mg NPs treated with HT-29 cancer cells by using MTT assay. The IC value of the given test sample MIMG and reference standard (cisplatin) was found to be 33.24µg and 6.88 µg respectively as shown in figure 7. The highest cytotoxic impact of Mg NPs was obtained at 100 µg/mL concentration and the results exhibited better cell inhibition as shown in table 1.

Figure 6 shows the anticancer activity of the Mg NPs having effective cytotoxic effects towards the HT-29 cell line in a dose-dependent behavior.

The anticancer efficacy of Mg NPs fabricated from *Mangifera indica* seed extract on MIMG and cisplatin-administered cell lines at different concentrations (6.25–100 µg) was analyzed. Consequently, the outcomes throw light on the potential cytotoxicity of the Mg NPs in a dose-dependent manner about cell viability. The cell viability of MIMG when treated with 6.25 µg of IN was 83%, while the cisplatin-treated cell line was only 52%. As the concentration used was augmented, both cell lines gradually became less viable. The MIMG cell line at a dose of 12.5 µg had a viability of 71 percent as against cisplatin with 42 percent viability.





Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	182.5	99.9
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	182.5	99.9

Residual : 2.461e-003 (O.K)

Cumulants Results

Diameter	(d)	: 140.4	(nm)
Polydispersity Index	(P.I.)	: 0.210	
Diffusion Const.	(D)	: 3.503e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 29896	(cps)
Attenuator 1	: 21.85	(%)

Figure 5: Particle size distribution of synthesized magnesium nanoparticles

Table 1
Anticancer viability of synthesized magnesium nanoparticle

Concentration of Mg NPs (µg)	% of viability MIMG cell line	% of viability cisplatin cell line
6.25	83	52
12.5	71	42
25	56	30
50	44	21
100	23	14

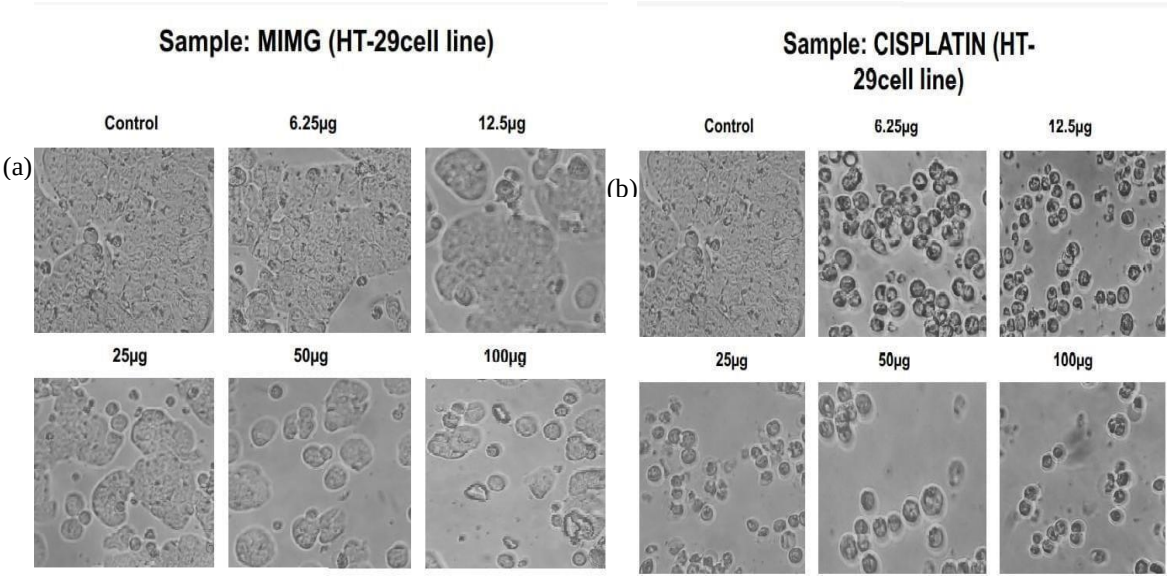


Figure 6: a. MIMG HT-29 cell line, b. HT-29 cell line cisplatin

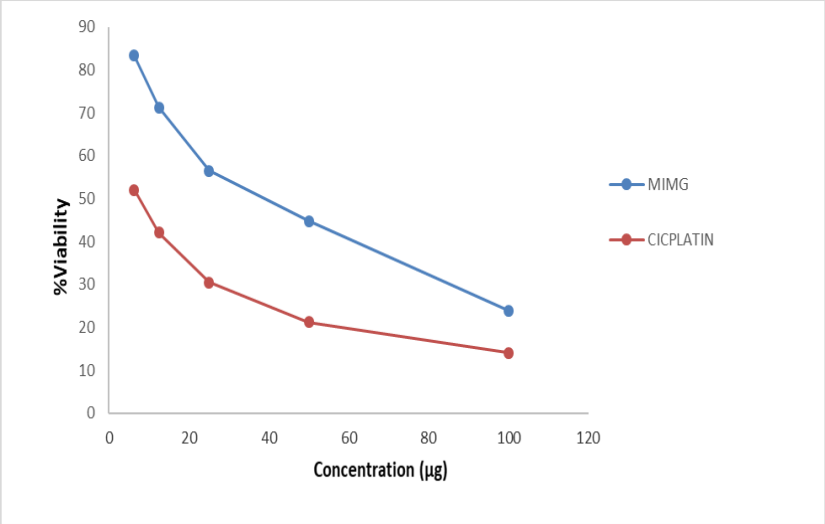


Figure 7: Graphical representation of the anticancer activity of synthesized magnesium nanoparticle

A good reduction was seen at 25µg for which MIMG viability reduced to 56% and cisplatin to 30%. The above data confirmed that when absorbed at high concentration, the viability of both MIMG cells and cisplatin-treated cells significantly reduced up to 50µg% and below 30µg% respectively. The findings of this study suggest that the Mg NPs cause inhibition of cancer cell proliferation in a dose-dependent manner. Their impact on the cisplatin cell line shows their rather high toxic effect which is close to the underlying chemotherapeutic agents, or even higher. Green synthesized Mg NPs offered an eco-friendly suggestive candidacy for a potential anticancer biomedicine.

Discussion

The plant-mediated synthesis of Mg NPs from *Mangifera indica* seed extract is highly sustainable, green, and cost-effective. This method depends mainly on the reducing and stabilizing activity of active compounds present in mango seed extract including polyphenols, flavonoids, and tannins.

These phytochemicals act dually; they reduce Mg ions (Mg^{2+}) to Mg NPs and act as protective covers for the synthesized particles to make them more stable and to minimize aggregation work done by Donga et al[5]. Traditionally, involving chemical or physical synthesis methods, the present green synthesis approach avoids the use of toxic materials and can therefore be appropriate for biomedical uses.

The characterization techniques used such as UV-Vis spectroscopy, XRD, SEM, and DLS indicate that Mg NPs were synthesized successfully and possess the needed structural attributes[7]. From the UV-Vis analysis, there is a high vibrant peak ranging from 200–250 nm, realizing the synthesis of Mg NPs same as Elmosallamy et al[7]. Their crystalline characteristics are also confirmed by XRD patterns and the humps corresponding to Mg phases. The size and morphology of the particles are evident with SEM showing rough surfaced irregular particles forming aggregates of NPs while DLS is showing NPs of size ~182 nm with moderate polydispersity. This study elucidates the impact of Mg NPs on MIMG and cisplatin-accrued MIMG cell lines concerning substantial dose-dependent affliction.

At these concentrations, the cell viability reduced to 23±2 for MIMG cells and 14 ±3 for the cisplatin-treated cells indicating high cytotoxicity of the NPs. This indicates that the bio-reduced Mg NPs have significant anticancer properties that may be caused by the intended disruption of cell function and the generation of ROS[6]. In conclusion, the present investigation underscores the green synthesis methodology of Mg NPs as an eco-friendly approach in developing nanomaterials possessing high anticancer potential. The biocompatibility of prepared Mg NPs can be further improved with the help of *Mangifera indica* seed extract, on top of which the process of preparing the latter serves environmental sustainability well.

Conclusion

Mangifera indica seed extract green synthesis of Mg NPs is one of the most promising methods for environmentally friendly fabrication of NPs. Unlike other methods, there are no hazardous chemicals involved making it as one of the best methods for green nanotechnology. The potassium M*angifera indica* seed extract both reduces and caps the synthesized NPs while at the same time improving the biocompatibility and bioactivity of the synthesized NPs. UV-Vis absorption spectroscopy, FTIR, XRD, SEM, and DLS analysis established the synthesis and structural properties with a stable dispersion of the resulting Mg NPs.

UV–visible spectrum recorded displayed typical peak absorptions, and the FTIR recorded functional groups namely alcohol, phenol, and carboxylic acid responsible for NPs formation. Their crystalline nature was further confirmed by XRD analysis and their morphology was shown through SEM. Notably, the Mg NPs showed prominent anticancer performance arresting the HT-29 colon cancer cell line. This bioactivity together with the green approach used in the formation of these NPs also shows that these NPs are becoming viable, inexpensive, and biocompatible anticancer agents.

The present study designated a simple and eco-friendly process for synthesizing Mg NPs by employing a natural extract of *Mangifera indica* seed which is a good source for the NPs synthesis. The synthesized Mg NPs depicted enhanced particle size distribution with a size of 140.4 nm. UV-vis spectra showed characteristic spectra at 290 nm. FT-IR analysis revealed the presence of functional groups like alcohol or phenols, carboxylic acids etc., XRD results showed that the synthesized Mg NPs are crystalline. The outcomes revealed that the green synthesized Mg NPs have superior anticancer activities because of their small particle size which was proved to carry out anti-cancer activity on HT29 cell line.

Hence, our work could be potentially functional in the therapeutic and industrial production of Mg NPs on a large-scale area. Further research should therefore be directed to fine-tuning of synthesis, and mass production of the product, besides comprehensive research on the compound's mode of action and its effectiveness when used in live models or animals. This study reveals the possible role that green nanotechnology can play in tackling some of the major healthcare problems, thus creating an appropriate context for the clinical use of bio-mimetic Mg NPs in cancer treatment.

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