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BIOSYNTHESIS OF MAGNESIUM OXIDE NANOPARTICLES VIA *KALANCHOE PINNATA*: NOVEL UTILIZATION AS ANTIBACTERIAL, ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL

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ABSTRACT

This research project delves into the multifaceted properties of magnesium oxide nanoparticles synthesized through a green method using fresh leaves of the *Kalanchoe pinnata* plant. The study meticulously characterizes these nanoparticles employing UV spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). The synthesized nanoparticles exhibit promising antibacterial attributes against both gram-positive and gram-negative bacterial strains. The evaluation of antibacterial activity reveals a noteworthy potential for applications in combating bacterial infections. Additionally, the nanoparticle's antioxidant properties are assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Phosphomolybdenum (PM) assays, showcasing their competence in scavenging free radicals and thereby contributing to potential therapeutic applications. Furthermore, the study explores the anti-inflammatory aspects of the green-synthesized magnesium oxide nanoparticles through the protein denaturation method. This investigation sheds light on the nanoparticles' capability to mitigate inflammation, offering insights into their potential as anti-inflammatory agents. The research amalgamates the realms of nanotechnology, biomedicine, and plant-based synthesis, paving the way for future developments in environmentally friendly nanoparticle synthesis and their diverse applications in medicine and healthcare.

KEYWORDS: Green synthesis, *Kalanchoe pinnata*, MgONPs-KP, Antibacterial, In-Vitro Antioxidant, Anti-inflammatory.

1. INTRODUCTION

The term "nanoparticles" is used to describe a particle with a size in the range of 1nm-1000nm, at least in one of the three possible dimensions. In this size range, the physical, chemical, and biological properties of the nanoparticles change in fundamental ways from the properties of both individual atoms/molecules and the corresponding bulk materials. Metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon, and biomolecules are the most common materials that can be used for developing nanoparticles. Nanoparticles may exist in several kinds of shapes, including spheres, cylinders, platelets, tubes, and more. Generally, the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. The enormous diversity of the nanoparticles arising from their wide chemical nature, shape, and morphologies, the medium in which the particles are present, the state of dispersion of the particles, and most importantly, the numerous possible surface modifications the nanoparticles can be subjected to make this an important active field of science nowadays.[1,2,3,4]

Magnesium oxide nanoparticles, with their diminutive size and distinctive properties, have emerged as a compelling focal point in medical research and applications. These nanoparticles, typically ranging from 1 to 1000 nm, exhibit unique characteristics owing to their nanoscale dimensions. The discovery of magnesium oxide nanoparticles is rooted in the advancements in nanotechnology and materials science. Various synthesis methods, including sol-gel processes and thermal decomposition, enable controlled fabrication, allowing researchers to manipulate the size and properties of these nanoparticles.[5] This deliberate control over synthesis methods opens avenues for tailoring magnesium oxide nanoparticles for specific medical uses. The importance of magnesium oxide nanoparticles in medicine lies in their biocompatibility, low toxicity, and versatile properties. These nanoparticles are being explored for drug delivery systems, diagnostic imaging, and

therapeutic interventions. In drug delivery, their ability to encapsulate and release therapeutic agents precisely offers a potential breakthrough in improving treatment efficacy while minimizing side effects. In diagnostic imaging, their tunable properties make them promising candidates for developing contrast agents, enhancing precision in medical imaging techniques.[6]

1.2 Plant Profile

Kalanchoe is botanically classified with two main Latin names that refer to the same plant: *Bryophyllum pinnatum* and *Kalanchoe pinnatum* (as well as various synonyms). The plant contains alkaloids, phenolics, tannins, macro elements (magnesium, calcium, potassium, phosphorus, sodium), microelements, (iron, zinc), and vitamins (ascorbic acid, riboflavin, thiamine, niacin).[7] "Leaves contain astragalin, rutin, kaempferol, and quercetin. Fresh leaves of the plant contain three new constituents, bryophyllol, bryophollone, and bryophollenone". Three new compounds, bryophytes, and two phenanthrene are also present. The leaf contains amino acids i.e. thiamine, pyridoxine, ascorbic acid, glycine, cysteine, casein, nicotinamide, bufadienolides like bryophyllin A and C. Food contents are also present i.e. carbohydrates, protein, lipids; minerals i.e. sodium, calcium, potassium, phosphorus, magnesium, ferrous, copper, zinc and sugars i.e. raffinose, lactose, sucrose, glucose. Alkaloids, flavonoids, glycosides, steroids, organic acids, and bufadienolides are among the identified active components. Modern pharmacological investigations have generally confirmed the traditional uses of Kalanchoe pinnata and their extracts in severe sicknesses, inflammations, painful ulcers, fungal infections, viral diseases and microbial attacks, impaired immune systems, diabetes mellitus, spasms, and insecticidal properties.[8,9]

Fig 1: Kalanchoe Pinnata.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of *Kalanchoe pinnata* Leaves

The plant *Kalanchoe pinnata* leaves were collected from a medicinal garden, at PPG College of Pharmacy, Coimbatore, (Medicinal Garden accession number PPG 72/2023). It was taxonomically identified and authenticated (BSI/SRC/5/23/2023/-24/Tech-498) by the Botanical Survey of India, TNAU Campus, Coimbatore, Tamil Nadu, India.

2.2 Preparation of Fresh Leaf Juice of *K. Pinnata*

The dust and dirt particles were completely removed from the fresh *Kalanchoe pinnata* leaves by thoroughly washing them using tap water. 50 g of leaves was crushed in a mechanical grinder without the use of any solvent. After squeezing the pulp with a muslin cloth, the filtrate was more than once filtered through Whatman filter paper No. 1.[10,11]

2.3 Green Synthesis of Magnesium Oxide Nanoparticles (MgONPs)

20 ml of plant juice was placed in a 500-ml conical flask and stirred with a magnetic stirrer for the green synthesis of MgO nanoparticles. In the burette, a 0.05 M magnesium nitrate hexahydrate solution was prepared and dropped into the conical flask containing the plant juice. After that, 2 M NaOH was added, and precipitate formation occurred rapidly. The entire process was carried out in 1 hour and 30 minutes at 75°C. Yellow colloidal particles were formed by adding sodium hydroxide. It was filtered and washed with ethanol. The washed precipitates were dried in a hot air oven at 110°C for 30 minutes before being calcined in the Muffle Furnace at 600°C for 3 hours. White precipitates were obtained following calcination.[12]

2.4 Qualitative Characterization Techniques

Synthesized Magnesium Oxide nanoparticles (MgONPs) were characterized by Visual interpretation, UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), and Fourier Transform Infrared (FT-IR).

2.5 Antibacterial Activity

The conventional disc diffusion method was used to evaluate the synthesized Magnesium oxide nanoparticle's antibacterial activity against *Bacillus subtilis, Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae, Escherichia coli* (Gram-negative). Bacteria were cultured on nutrient agar. After pouring the medium into the petri dish, it was allowed to solidify for 30 minutes. the fresh overnight cultures of inoculum of four different cultures were spread onto solidified nutrient agar plates. Sterile paper discs made of Whatman filter paper, 5 mm diameter. The disc dipped in Magnesium Oxide nanoparticles (50µg/ml and 100µg/ml), plant extract, and the standard solution (ciprofloxacin) were placed in each plate. For 24 hours, the cultivated agar plates were incubated at 37°C. Following incubation, the plates were examined to evaluate if an obvious inhibition zone had developed around each well, signifying antibacterial activity. By measuring the widths of the inhibition zones surrounding the well, the zone of inhibition was calculated.[13,14,15,17]

2.6 Antioxidant Activity

2.6.1 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay

Various concentrations of Standard solution (ascorbic acid) and Magnesium Oxide nanoparticles (50, 100, 150, 200, and 250 µg/ml) were prepared and added to 1 ml of methanolic solution of DPPH (0.2 mM) for the DPPH free radical scavenging activity. An equal volume of standard phosphate buffer was added to the reaction mixture without the nanoparticle sample and the Standard solution acted as a control. The mixture was shaken and left to remain at room temperature for 30 minutes. At 517 nm by UV-vis spectrophotometer (SHIMADZU | UV-1780) using triple distilled water as the blank, the absorbance of the reaction mixtures was measured.[12,16,20] The % scavenging activity of various concentrations was calculated, and the following formula gives the % scavenging activity.

DPPH scavenging activity (%) = Absorbance of Control – Absorbance of Sample or Standard / Absorbance of Control × 100,

2.6.2 PM (Phosphomolybdenum) Assay

84 mg of sodium phosphate were dissolved in 25 ml of distilled water to make 28 mm of sodium phosphate, and the preparation of 4 mm of ammonium molybdate involved dissolving 124 mg of ammonium molybdate in 25 ml of distilled water and 0.6 mm of sulphuric acid, which was then mixed to formulate the reagent mixture and various concentrations (50, 100, 150, 200, and 250 µg/ml) of MgONPs and the standard (L-ascorbic acid) were prepared. Using a separate vial for the reagent solution, 2 ml of the reagent solution was combined with the same volume of MgONPs solution and standard. The mixture was kept at 95°C for one hour and thirty minutes in a water bath. After cooling, the absorbance was then measured at 695 nm by UV-vis spectrophotometer (SHIMADZU | UV-1780) using triple distilled water as the blank and compared to a control of the reagent suspension.[18,19,21,22] The percentage of molybdenum inhibition was calculated using the formula.

(%) molybdenum inhibition = Absorbance of Control – Absorbance of Sample or Standard / Absorbance of Control × 100,

2.7 Anti-Inflammatory Activity

2.7.1 Preparation of 1% Egg Albumin Solution

To prepare a 1% Egg albumin solution Fresh hen's eggs or egg albumin powder that is readily accessible in stores can be used. Making egg-albumin solution using a fresh hen's egg properly involves carefully cracking an egg, transferring 1 ml of the translucent portion to 100 ml of distilled water, and stirring thoroughly. When preparing the solution, water has to be cooled. Boiling water will lead it to coagulate.[24]

2.7.2 Protein Denaturation Assay

The anti-inflammatory activity of MgO nanoparticles can be determined in vitro by the denaturation of egg albumin (protein). 0.2 ml of 1% egg albumin solution (from fresh hen's egg), 2 ml of MgO nanoparticles or standard (Diclofenac sodium) at varying concentrations (50, 100, 150, 200, and 250 µg/ml) and 2.8 ml of phosphate-buffered saline (pH 6.4) were mixed to form a reaction mixture of a total volume of 5 ml. A total volume of 5 ml of the control was created by combining 2 ml of triple-distilled water, 0.2 ml of 1% egg albumin solution, and 2.8 ml of phosphate-buffered saline. The reaction mixtures were then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°C for 15 min. After cooling, the absorbance was measured at 660nm by UV-vis spectrophotometer (SHIMADZU | UV-1780) using triple distilled water as the blank.[12,23] The percentage of protein denaturation was calculated by using the following formula.

(%) protein denaturation = Absorbance of Control – Absorbance of Sample or Standard / Absorbance of Control × 100,

3. RESULTS AND DISCUSSION

3.1 Qualitative Characterization Techniques

3.1.1 Visual interpretation

Visual identification of the colour change is used as a preliminary confirmatory test of nanoparticle synthesis. For the green synthesis of MgO nanoparticles, the juice colour turned yellow by adding sodium hydroxide, and yellow colloidal particles were formed. It was filtered and washed with ethanol. The washed precipitates were placed in the oven for drying at 110°C for 30 min, followed by calcination. white precipitates were obtained after calcination. Figure 3 represents the colour change obtained after the calcination of Magnesium Oxide nanoparticles.[12]

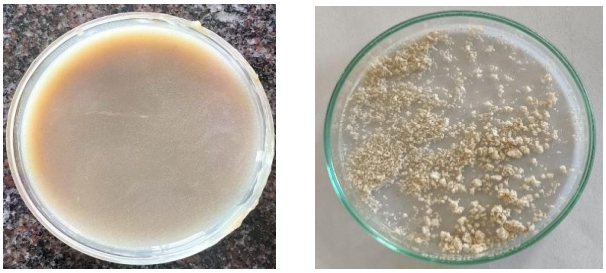


Fig 3: Colour change obtained after the calcination of Magnesium Oxide nanoparticles.

3.1.2 UV-vis spectroscopy

The magnesium oxide nanoparticles mediated by *Kalanchoe pinnata* were characterised using UV-visible spectroscopy (SHIMADZU | UV-1780) to determine the structural characteristics of MgO nanoparticles. Figure 3 illustrates the absorbance spectra of biosynthesized magnesium oxide nanoparticles after 24 hours, which vary from 260 to 330 nm. The strong absorbance in the visible light region is indicated by the absorbance peak of MgONPs-*KP*, which was found at 303 nm. The UV result from previously conducted studies appears to align with this.

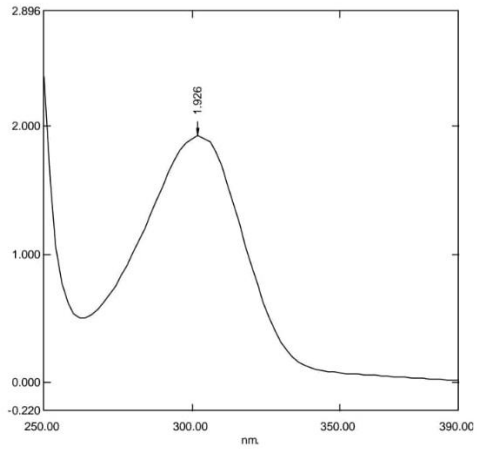


Fig 3: The absorbance spectra of biosynthesized magnesium oxide nanoparticles after 24 hours.

3.1.3 Dynamic Light Scattering (DLS)

Particle Size Distribution

The particle size distribution of biosynthesized MgO nanoparticles was shown based on intensity. Figure 4 shows the average particle size (z-average) of MgO nanoparticles is found to be 738.3 nm. Particle size analysis showed the presence of nanoparticles with a polydispersity index (PDI) value of 0.715 with an intercept of 0.987.

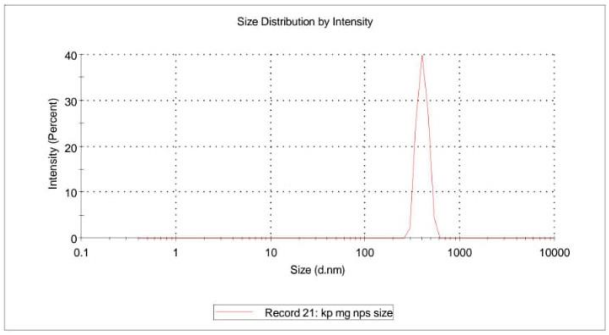


Fig 4: The average particle size (z-average) of MgO nanoparticles.

Zeta Potential

The Zeta potential of biosynthesized MgO nanoparticles was found to be -22.4 mV in Figure 5. A negative value in the zeta potential of nanoparticles might be possible due to the presence of hydroxyl (OH^-) functional groups adsorbed on the surface of nanoparticles during capping which is responsible for increasing stability owing to electrostatic repulsion between each nanoparticle.

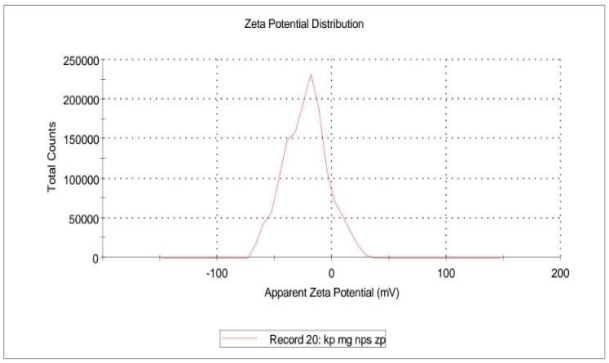


Fig 5: The Zeta potential of biosynthesized MgO nanoparticles.

3.1.4 Scanning Electron Microscopy (SEM)

The nanoparticles were characterized for their structure and morphology by SEM analysis. Figure 6 SEM images of biosynthesized MgO nanoparticles were taken using EDAX-FEI, QUANTA 200. The size of nanoparticles (MgONPs) is less than 100 nm and the shape is spherical.

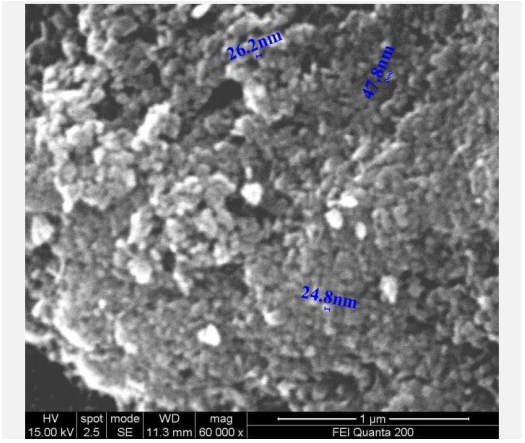


Fig 6: SEM images of biosynthesized MgO nanoparticles.

3.1.5 Fourier Transform Infrared Spectroscopy (FT-IR)

Figure 7 shows the FT-IR of synthesized MgO nanoparticles peaks at 3865.35, 2978.09, 2368.59, 2314.58, 1419.61, and 462.92 cm^{-1}. The interactions between *Kalanchoe pinnata* and the supporting materials were characterized by analysing the samples with FT-IR spectroscopy at room temperature. The FT-IR spectrum for the synthesized Mg $(OH)_2$ and calcined MgO nanoparticles is depicted in Figure 7. In the FT-IR spectrum of MgO nanoparticles the sharp and intense peak at 3865.35cm^{-1} is due to the –OH group in *Kalanchoe pinnata.* It is well known that H_2O and CO_2 molecules are easily chemisorbed onto the MgO surface when exposed to the atmosphere. In the spectrum of the calcined sample, the sharp band at 3865.35 cm^{-1} is associated with the OH stretching vibrations of surface-adsorbed water molecules, while those at 1419.61 cm^{-1} are associated with their bending vibrations. In addition, 2368.59, 2314.58 cm^{-1} is associated with the CH

stretching vibrations of surface-adsorbed carboxylate (O–C=O) molecule. The adsorption carboxylate (O–C=O) is visible around 2368.59, and 2314.58 cm^{-1} and CO_3 is visible at around 1419.61 cm^{-1} The bands that appeared at low frequencies of 864.11 and 648.08 cm^{-1} correspond to stretching vibrations of Mg–O–Mg bonding. Showed the presence of a stretching frequency peak at 462.92 cm^{-1}, confirming the Mg–O bond. The surface analysis revealed the cluster form of the nanoparticles. The carboxylate groups proved the presence of flavanones or terpenoids that are adsorbed on the surface of metal nano-sized particles by interaction through π-electrons in the carbonyl groups in the absence of sufficient concentration of chelating agents. It was also confirmed that the carbonyl group from the protein and amino acid had a stronger ability to bind with metal nanoparticles or act as capping and stabilizing agents The band at 3865.35cm^{-1} in the FTIR spectrum of the dried sample completely disappears after calcination, which is due to the decomposition of Mg $(OH)_2$ to MgO nanoparticles.

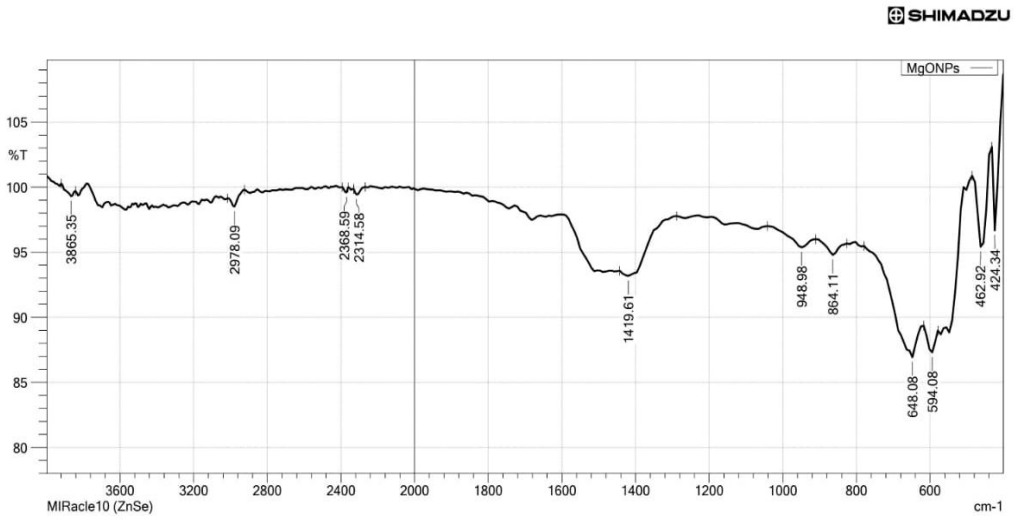


Fig 7: Shows the FT-IR of synthesized MgO nanoparticles peaks.

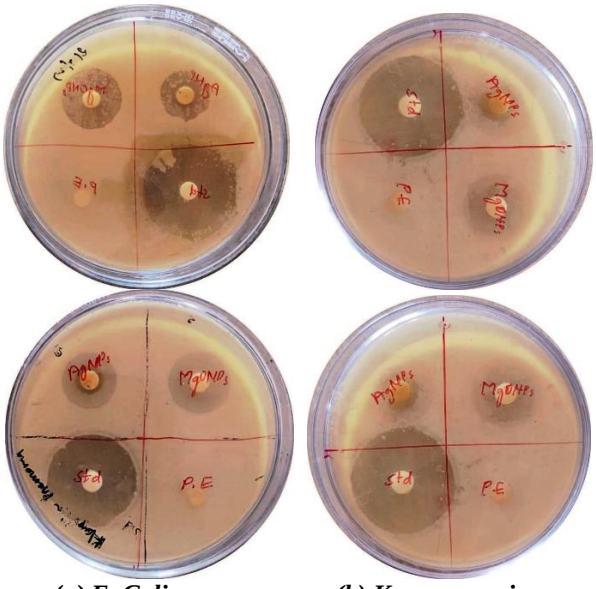
3.2 Antibacterial Activity

The Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and the Gram-negative bacteria *Klebsiella pneumoniae*, *Escherichia coli*, were significantly inhibited by magnesium oxide nanoparticles (50µg/ml and 100µg/ml). The zone of inhibition exhibited by nanoparticles was larger than that of a plant extract. Table 1 and Figure 8 show the results. The disruption of the cell membrane by reactive oxygen species (ROS), binding to the cell membrane, lipid peroxidation, and disruption of the DNA, RNA, and protein synthesis process are a few of the processes linked to the antibacterial action of nanoparticles that have been identified.

Table 1: The zone of inhibition against bacteria species.

Bacteria	Minimum Inhibitory Concentration (MIC) in mm			
	Ciprofloxacin (std)	KP leaf juice	MgONPs-KP (50mcg/ml)	MgONPs-KP (100mcg/ml)
E. Coli	21	4	9	17
Klebsiella pneumoniae	24	5	10	19
Staphylococcus aureus	20	5	9	17
Bacillus subtilis	19	4	8	17

KP- Kalanchoe pinnata, MgONPs-KP - Magnesium Oxide nanoparticles of Kalanchoe pinnata.



(a) ***E. Coli*** ***(b) K. pneumoniae***
(c) S. aureus ***(d) Bacillus subtilis***

Fig 8: (a) (b) (c) (d) The zone of inhibition of both Gram-positive and Gram-negative bacteria.

3.3 Antioxidant Activity

3.3.1 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Assay

The DPPH test is used in this study to evaluate the biologically synthesized MgO nanoparticle's ability to function as an antioxidant. DPPH is often reduced to DPPH-H by the antioxidants in the sample. The level of discolouration was used to measure the antioxidant compound's scavenging capacity. Deep purple in hue, DPPH exhibits high absorption at 517 nm. After reacting with the antioxidant, this colour becomes colourless or pale yellow. The results of the scavenging ability of the standard (ascorbic acid) and Green synthesized magnesium Oxide nanoparticles are listed in Table 2, Magnesium Oxide nanoparticles scavenge 47.58, 55.36, 59.22, 69.71 and 73.02 percent of conventional ascorbic acid at concentrations of 50, 100, 150, 200, and 250 µg/ml, respectively, while standard ascorbic acid scavenges 48.15, 54.55, 61.16, 70.57 and 75.59 percent at comparable concentrations listed in Figure 9.

Table 2: The scavenging ability of the standard and synthesized MgO nanoparticles.

Concentration (mcg/ml)	% Scavenging of free radical	
	Vitamin C (Std)	MgONPs-KP
50	48.15	47.58
100	54.55	55.36
150	61.16	59.22
200	70.57	69.71
250	75.59	73.02

MgONPs-KP - Magnesium Oxide nanoparticles of Kalanchoe pinnata.

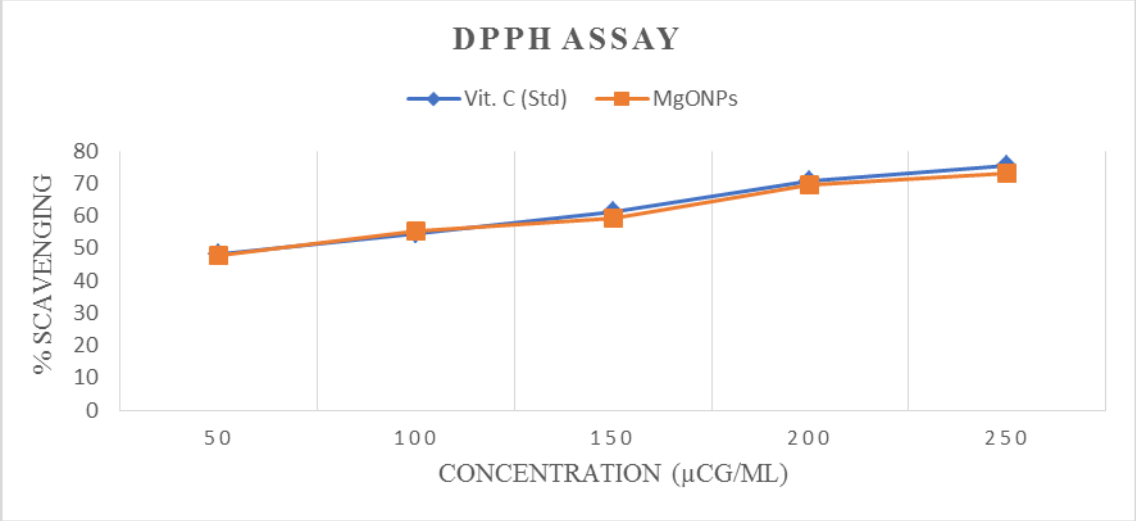


Fig 9: Graphical representation of DPPH free radical scavenging of Std and MgONPs.

3.3.2 PM (Phosphomolybdenum) Assay

The molybdenum inhibition capacity of green synthesized MgO nanoparticles is also assessed using this technique. This procedure is called a Phosphomolybdenum (PM) assay because it uses a phosphomolybdate reagent. The phosphomolybdate reagent changes colour when the plant extract is applied, indicating a decrease in Phosphomolybdenum. Table 3 and Figure 10 show the percentage inhibition for both the standard (ascorbic acid) and sample Magnesium Oxide nanoparticles (MgONPs). Ascorbic acid is scavenged up to 8.69% at a content of 50 mcg/ml and up to 75.36% at a dose of 250 mcg/ml. (figure 11) MgO nanoparticles scavenged up to 4.34% at 50 mcg/ml and 67.14% at 250 mcg/ml.

Table 3: % Molybdenum inhibition of Std and MgO nanoparticles.

Concentration (mcg/ml)	Absorbance of control	Absorbance of standard (Vit. C)	The absorbance of sample (MgONPs)	% Scavenging of standard	% Scavenging of sample
50	0.207	0.189	0.198	8.69	4.34
100	0.207	0.152	0.169	26.57	18.35
150	0.207	0.124	0.134	40.09	35.26
200	0.207	0.086	0.10	58.45	51.69
250	0.207	0.051	0.068	75.36	67.14

MgONPs-KP - Magnesium Oxide nanoparticles of Kalanchoe pinnata

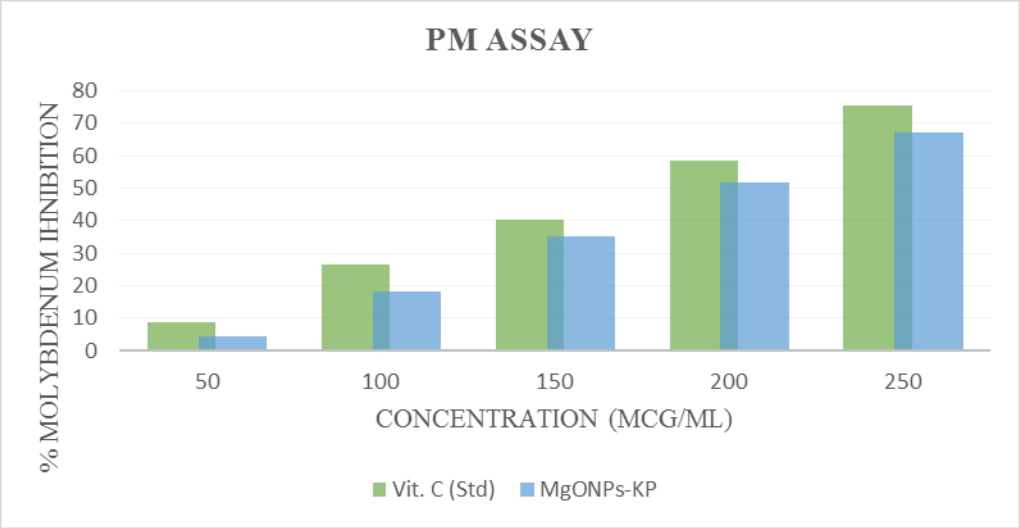


Fig 10: Graphical representation of % molybdenum Inhibition.

3.4 Anti-inflammatory Activity

3.4.1 Protein denaturation Assay

The anti-inflammatory assessment of green synthesized MgO nanoparticles was evaluated to check their ability to reduce inflammation. Table 4 and Figure 11 show the percentage inhibition of both the standard drug (diclofenac sodium) and MgO nanoparticles. Diclofenac sodium reduced protein denaturation by up to 21.87% at 50 µg/ml and up to 88.32% at 250 µg/ml. MgO nanoparticles reduced protein denaturation up to 28.12% at a concentration of 50 µg/ml and up to 91.87% at a concentration of 250 µg/ml.

Table 4: % protein denaturation of Standard (Diclofenac Sodium) and MgONPs-KP.

Concentration (mcg/ml)	Absorbance of control	The absorbance of standard (Diclofenac sodium)	The absorbance of sample (MgONPs)	% Scavenging of standard	% Scavenging of sample
50	0.352	0.275	0.253	21.87	28.12
100	0.352	0.202	0.184	42.61	47.72
150	0.352	0.141	0.132	59.94	62.50
200	0.352	0.0841	0.0703	76.10	80.02
250	0.352	0.0411	0.0286	88.32	91.87

MgONPs-KP - Magnesium Oxide nanoparticles of Kalanchoe pinnata

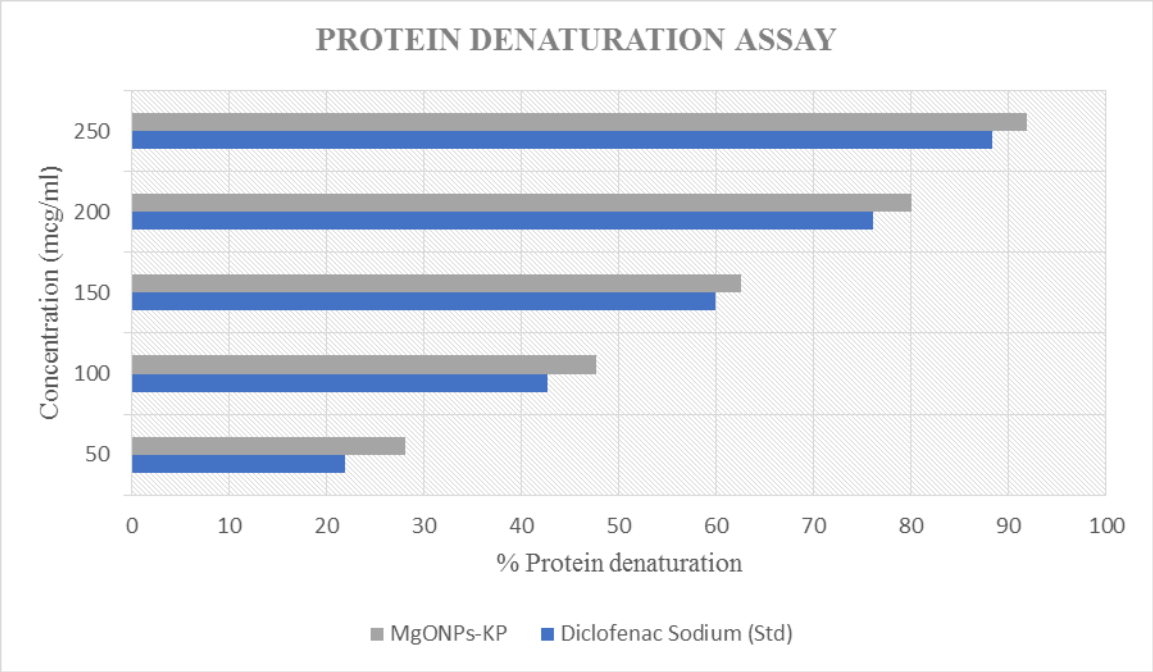


Fig 11: Graphical representation of % Protein denaturation.

4. CONCLUSION

Plant extracts provide a greener, more affordable, and environmentally friendly way to synthesize magnesium oxide nanoparticles than existing approaches. The capacity of *Kalanchoe pinnata* to synthesise MgO nanoparticles at ideal temperature conditions was demonstrated. When nanoparticles are formed, the plant itself serves as a capping and reducing agent. Advanced techniques such as UV spectroscopy for absorption analysis, DLS for particle size determination, SEM for morphological characterization, and FT-IR for revealing molecular interactions were employed in the process.

By using conventional microbiological methods, the antibacterial activity is evaluated against two bacterial strains: one gram-positive and one gram-negative. When applied to selected microorganisms, magnesium oxide nanoparticles exhibit strong antibacterial properties.

The PM assay, which assesses the nanoparticles' ability to lower molybdenum, and the DPPH assay, which measures the nanoparticles' ability to neutralize free radicals, are used to determine their antioxidant capability. As concentration increased, so did the percentage of scavenged standard and magnesium oxide nanoparticles. The biosynthesized MgO nanoparticles' DPPH assay scavenging potential is 73.02%, while a standard concentration at the same concentration exhibits 74.52% scavenging. MgO nanoparticles exhibit 67.14% molybdenum inhibition in the PM assay, while a standard concentration exhibits 75.36% molybdenum inhibition.

The anti-inflammatory investigation demonstrates that MgONPs-KP similar to the reference anti-inflammatory medicine efficiently prevented the denaturation of egg albumin in vitro. Protein denaturation is 91.87% in MgO nanoparticles and 88.32% in standard at a comparable concentration. Consequently, the study concludes that these nanoparticles hold significant promise as multifaceted agents for antibacterial, antioxidant, and anti-inflammatory interventions. This research contributes substantively to the burgeoning field of green nanotechnology and positions plant-mediated MgO nanoparticles as compelling candidates for advanced biomedical applications.

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