Green Synthesis and Comparative Evaluation of Calcium Oxide, Potassium Oxide, and Magnesium Oxide Nanoparticles using Annona squamosa Seed Extract

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Abstract

With the increasing need for sustainability in nanomaterial synthesis, researchers have been developing new approaches for environment friendly synthesis and without the use of toxic substances, yet still maintain the properties of metal oxides nanoparticles. In this research work, the green synthesis and comparative evaluation of calcium oxide (CaO), potassium-based, and magnesium oxide (MgO) nanoparticles are done via the use of Annona squamosa seed extract as a natural reducing and stabilizing agent. The synthesized nanoparticles have been analyzed using UV-Vis spectroscopy, FTIR spectroscopy, XRD analysis, SEM analysis, and DLS to assess their optical, structural, morphological and colloidal properties. The biological activities of the synthesized nanoparticles have been comparatively evaluated using antibacterial, antioxidant, anti-ulcer, and antifungal activity tests. The results from characterization indicate successful synthesis of stable and crystallized nanoparticles with different physicochemical properties, while the biological activity assessments showed good bioactivity for all the synthesized nanomaterials.

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1. Introduction

Increased interest in the sustainable nature of nanotechnology has promoted green techniques that are more effective in synthesizing nanoparticles of metal oxides with improved functionality. Green synthesis is one of the widely used, cost-efficient, non toxic, and non-hazardous techniques for the preparation of simple metal oxides [1]. The green technique stands out as one of the most interesting alternatives to the traditional techniques of synthesis since it employs natural phytochemicals as reducing and stabilizing agents, using fewer toxins, energy, and harmful byproducts in the process [2]. CaO, potassium and MgO nanoparticles have been of great interest due to their antimicrobial, antioxidant, catalytic and biomedical applications. Traditional techniques of synthesis are associated with high costs of equipment and reagents as well as unfavorable reaction conditions, hence making such processes unsustainable.

Plant-based synthesis methods for metal oxide nanoparticles using various medicinal plants and agricultural waste have been extensively studied. However, the benefits of these methods include increased biocompatibility, decreased adverse effects on the environment, and increased biological performance of the produced nanoparticles. Despite the numerous researches carried out in the field, most research works have considered only one kind of nanoparticle prepared from various plant extracts. Comparative research on the effects of different metal precursors on the characteristics of the nanoparticle is not yet available.

To overcome this limitation, the present study proposes a green synthesis approach utilizing the extract of Annona squamosa seeds for the synthesis of CaO, potassium-based, and MgO nanoparticles. Annona squamosa L. (Annonaceae), commonly known as sitaphal, is a small evergreen tree distributed throughout tropical and subtropical regions and is widely recognized in traditional medicine [3]. Phytochemical investigations have revealed that the plant contains a rich array of bioactive compounds, including alkaloids, flavonoids, coumarins, tannins, cardiac glycosides, saponins, terpenoids, phenolics, and carbohydrates, which are responsible for its documented antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, hepatoprotective, neuroprotective, and antifungal properties [4]. These phytochemicals also serve as natural reducing and stabilizing agents during nanoparticle synthesis, making A. squamosa an excellent candidate for green synthesis. The synthesized CaO, potassium-based, and MgO nanoparticles will be characterized through physicochemical analyses and evaluated for their antimicrobial, antioxidant, ulcerogenic, and antifungal activities. This approach provides a systematic basis for comparing the physicochemical and biological properties of different metal oxide nanoparticles synthesized using the same biomaterial under similar green synthesis conditions.

2. Related Works

The green synthesis is now a highly efficient and eco-friendly process to synthesize nanoparticles of metal oxides using various natural phytochemicals from plants as reducing and stabilizing agents. Green synthesis reduces hazardous chemicals and increases the efficiency of nanoparticles in their functionality and biocompatibility [5], [6].

The Annona squamosa has a rich amount of biologically active substances, which help in the formation and stabilization of nanoparticles. The CaO nanoparticles formed through Annona squamosa seed extract have been found to be having highly effective antibacterial, antioxidant, and anti-ulcer effects. Likewise, the use of Annona species in the formation of silver nanoparticles is also well-documented [7], [8].

Plant extracts have been used in the preparation of nano-MgO, nano-ZnO, and nano-CuO with superior antimicrobial and antioxidant properties. Some recent reports indicated that green-synthesized MgO nanoparticles showed enhanced antibacterial, antifungal, photocatalytic, and biomedical activities, whereas CuO and ZnO nanoparticles exhibited excellent biological activities due to their phytochemical-based synthesis [9], [10].

Metal oxide nanoparticles generated by green techniques have found numerous applications in antimicrobial treatments, antioxidants, tissue engineering, environmental treatment, and sustainable agriculture [11], [12]. The high surface area, chemical stability, and biocompatibility of these nanoparticles make them suitable for biomedical and environmental applications [13]. In addition, plant-derived nanobactericides have proven useful in microbial infection control with reduced environmental impact compared to traditional antimicrobial drugs [14], [15].

Despite several studies carried out by researchers to examine the synthesis of metal oxide nanoparticles such as CaO, MgO and other metal oxides, comparative studies between the CaO, potassium and MgO nanoparticles using Annona squamosa seed extract under identical conditions are still in the nascent stage. This is why the current study is carried out to investigate these particles.

3. Materials and Methods

3.1 Materials

Reduction and stabilization of metal ions through phytochemicals represent the basic principle of nanoparticle preparation using plants, where biomolecules play an important role in controlling nucleation, crystal formation, and surface passivation. In case of A. squamosa, it is rich in phenols, flavonoids, tannins, alkaloids, and other secondary metabolites which act as reducing and capping agents, whereas pure metal salts are used to control the amount of precursor used. List of chemicals, plants, and their functions used in experiment is given in Table 1.

Table 1. Materials and reagents used for green synthesis of metal oxide nanoparticles.

Material/Reagent	Chemical Formula	Purity/Grade	Function
Annona squamosa seeds	–	Fresh, mature seeds	Natural reducing and stabilizing agent
Calcium chloride	$CaCl_2$	Analytical grade	Calcium precursor for CaO nanoparticles
Potassium chloride	KCl	Analytical grade	Potassium precursor for potassium-based nanoparticles
Magnesium chloride	$MgCl_2$	Analytical grade	Magnesium precursor for MgO nanoparticles
Sodium hydroxide	NaOH	Analytical grade	pH adjustment and hydroxide formation
Distilled/Deionized water	H_2O	Laboratory grade	Solvent and washing medium
Ethanol	C_2H_5OH	Analytical grade	Washing and purification of nanoparticles

3.2 Green Synthesis of Metal Oxide Nanoparticles

Nanoparticle synthesis via plant-mediated green technology provides an environmentally friendly approach to synthesize metal oxide nanoparticles by using phytochemicals available in nature that serve as reducing, complexing, and stabilizing agents. Phytochemicals existing in Annona squamosa seed extract, such as phenols, flavonoids, tannins, alkaloids, and other oxygenated metabolites, aid in the formation of metal oxide nanoparticles from metal ions through electron transfer and nucleation process. At the same time, phytochemicals get adsorbed onto the surface of the nanoparticles, thus controlling the crystal growth and preventing aggregation. Following this approach, Annona squamosa seed extract was used for the synthesis of CaO, potassium-based, and MgO nanoparticles.

Figure 1. Annona squamosa seed extract preparation

The prepared seed extract used throughout the synthesis process is presented in Figure 1. Fresh Annona squamosa seeds were obtained and washed with distilled water to clean off any adhered impurities and dried under natural conditions to dry out any moisture present. The dried seeds were crushed into fine powder form by using a laboratory grinder. About 10 grams of the crushed material was added to 100 mL of distilled water and heated between 60 to 70 ºC for half an hour continuously stirred to get the active phytochemicals extracted. It was then cooled to room temperature and filtered through Whatman No. 1 filter paper to separate any insoluble particles from the solution. The obtained aqueous solution, as shown in Figure 1, was preserved at 4ºC for further applications as the biogenic reductant and stabilizer for nanoparticle formation.

3.3 Experimental Workflow

A systematic experiment procedure was devised to ensure consistent, repeatable, and unbiased analysis through the entire course of nanoparticle synthesis and characterization. This includes the preparation of plant extracts, synthesis of nanoparticles using precursors, physicochemical analysis, and biological evaluation. The integration of all these procedures into one scheme helps in reducing variability in the experimental setup for all three nanoparticles. Under similar synthesis and analysis conditions, it is now possible to compare the characteristics of CaO, potassium, and MgO nanoparticles. The complete experimental sequence adopted in this study is illustrated in Figure 2.

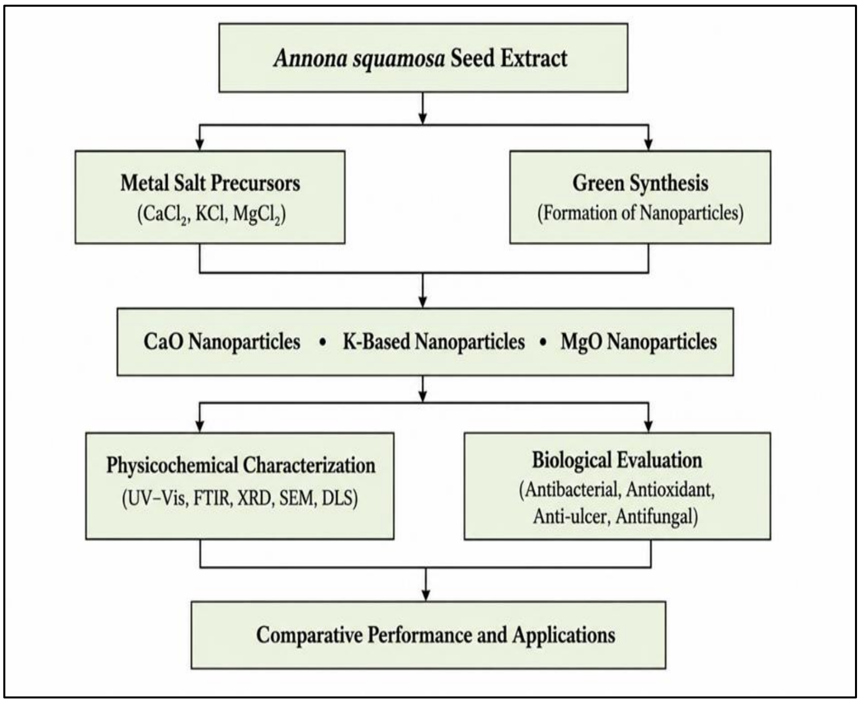

Figure 2. Experimental workflow of green nanoparticle synthesis

3.4 Characterization and Biological Evaluation

A complete physicochemical investigation was carried out to ensure the successful synthesis of CaO, potassium-containing, and MgO nanoparticles and to assess the parameters responsible for their biological activity. The presence of nanoparticles was confirmed by UV–Vis spectroscopy on the basis of optical absorbance features, whereas FTIR spectroscopy was carried out to establish the nature of functional groups participating in phytochemical-assisted reduction and stabilization. The XRD technique was used to identify the crystalline phase as well as the crystallite size, while SEM and DLS were carried out to examine their morphology and particle size distribution.

The biological applications of the prepared nanoparticles were analyzed comparatively by performing antimicrobial, antioxidant, antiulcer, and antifungal experiments to measure their biological efficiency. The antimicrobial and antifungal tests were carried out to measure the inhibitory nature of the nanoparticles for selected microbes, whereas the antioxidant test was performed with the help of free radical scavenging experiment. Antiulcer study was carried out to assess the therapeutic applicability of the prepared nanoparticles. Through these methods, the systematic comparative analysis of the structural characteristics and biological efficiency of the prepared nanoparticles was performed.

4. Results and Discussions

4.1 UV–Visible Spectral Analysis

The UV-Visible absorption spectrum of the extract from Annona squamosa seeds and the CaO, potassium, and MgO nanoparticles synthesized are depicted in Figure 3. The extract had characteristic absorption peaks indicating the presence of phytochemical components like phenols and flavonoids that function as reducing and stabilizing agents in the synthesis of nanoparticles. Distinct absorption peaks were seen for all synthesized nanoparticles after the reaction with their metal precursors.

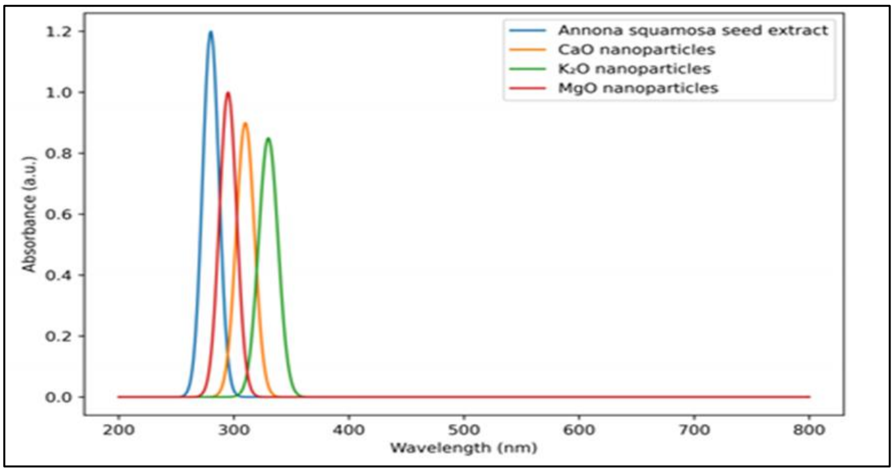

Figure 3. UV–Visible spectra of green-synthesized metal oxide nanoparticles

The absorption spectrum of the prepared nanoparticles was influenced by the metal precursor, and hence there were changes in the optical and electronic properties of the nanoparticles. Differences in the shapes of peaks and absorption edges indicate differences in the crystalline nature, size distribution of particles, and surface properties due to the effect of plant phytochemicals in nucleating and growing the nanoparticles. Out of the prepared nanoparticles, MgO has a relatively sharp absorption spectrum, while CaO and potassium-containing nanoparticles have relatively broad absorption spectra.

4.2 FTIR Analysis

The FTIR spectra confirmed the presence of hydroxyl, carbonyl, and other phytochemical functional groups that played an important role in the reduction and stabilization of green-synthesized MgO nanoparticles, which is consistent with the observations reported previously [17]. Figure 4 shows differences in peak position and intensity between the three nanoparticles, indicating differences in the interaction of the phytochemical components with the metallic ions during the synthesis process.

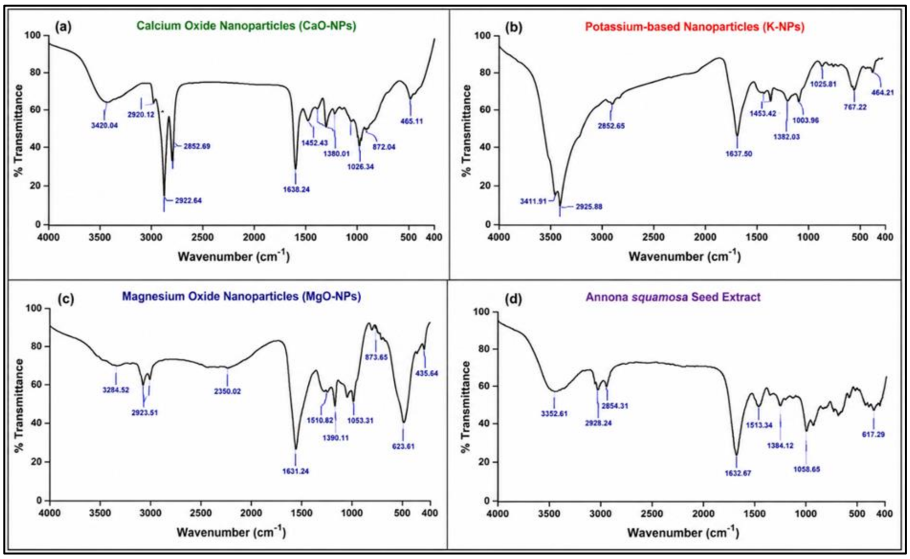

Figure 4. Comparative FTIR analysis of green-synthesized metal oxide nanoparticles

The fact implies that the type of metal ion used has an impact on the surface properties of the nanoparticles due to the presence of different surface functionalization properties. This is also evident from the list of functional groups and their vibrational assignment shown in Table 2, showing the development of biogenic nanoparticles using the components of Annona squamosa.

Table 2. Functional group assignments from FTIR analysis

Wave number (cm-1)	Functional group	Vibrational mode	Interpretation
3200–3400	O–H	Stretching	Presence of phenols and alcohols in seed extract; indicates phytochemical capping
~2920	C–H	Stretching	Aliphatic hydrocarbons from biomolecules 1630–1650
1630–1650	C=O / C=C	Stretching	Flavonoids, aromatic compounds,

			proteins
1380–1450	C–N /C–H	Bending	Amine groups and biomolecular residues
1000–1100	C–O	Stretching	Alcohols, ethers, phenols
<700	Mg–O / Ca–O / K–O	Stretching	Confirms formation of MgO, Ca_2O and K_2O nanoparticles

4.3 X-ray Diffraction (XRD) Analysis

The crystalline nature of the synthesized MgO nanoparticles is consistent with previous reports, where green-synthesized MgO nanoparticles exhibited a polycrystalline cubic structure confirmed by XRD analysis [16]. The X-ray diffraction (XRD) profiles of the green-synthesized CaO, potassium-based, and MgO nanoparticles have been given in Figure 5 whereas the crystallographic data is provided in Table 3 below. It can be seen from the diffraction peaks that the formation of crystalline nanoparticles of the metal oxides has been successfully achieved. The lack of any significant impurity peaks confirms the efficiency of the synthesis procedure in achieving the formation of the required crystalline phases.

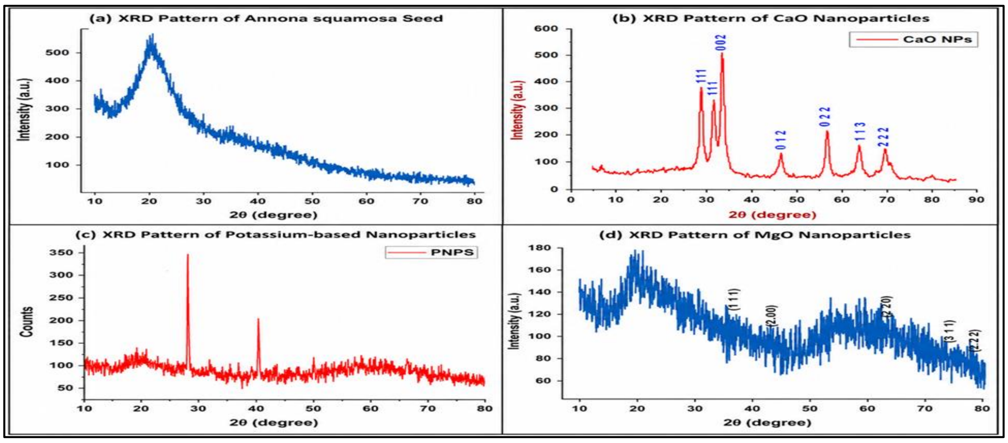

Figure 5. XRD patterns of green-synthesized metal oxide nanoparticles

From the comparative study, it was observed that there were disparities in terms of the intensity and sharpness in the peaks of the synthesized nanoparticles, signifying differences in their degree of crystallization and crystal growth. This is due to the chemical interaction between the metal precursors and the bioactive components of the Annona squamosa seed extract. Moreover, the values of the crystallite size and other structural parameters, as shown in Table 3, prove the formation of stable nanocrystals.

Table 3. Crystallographic parameters of green-synthesized metal oxide nanoparticles.

Sample	2θ (major peaks)	Crystal structure	Crystallite size (nm)
Annona squamosa seed extract	Broad hump	Amorphous	–
CaO nanoparticles	32.2°, 37.4°, 53.9°, 64.2°	Cubic	28
K_2O nanoparticles	29.6°, 36.1°, 42.8°, 61.4°	Cubic	34
MgO nanoparticles	36.9°, 42.9°, 62.3°, 74.7°	Cubic	21

4.4 Scanning Electron Microscopy (SEM) Analysis

The surface morphology of the biologically synthesized CaO, potassium based, and MgO nanoparticles were analyzed using the scanning electron microscopy, and the micrographs obtained for these nanoparticles are shown in Figure 6. These observations about the surface morphology show that nanoscale particles were successfully produced with unique surface structures. Differences in the surface morphology such as the surface structure, particle shapes, and agglomeration were found between the produced nanoparticles owing to the effect of metal precursors and the biological synthesis technique.

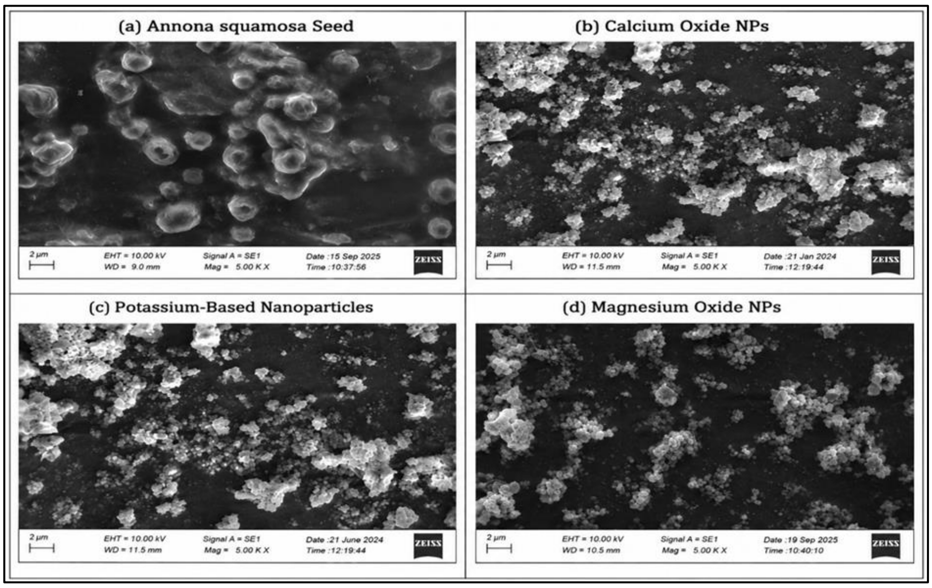

Figure 6. SEM micrographs of green-synthesized metal oxide nanoparticles.

Morphological differences and particle size measurements are presented in Table 4. Particle shape differences are associated with differences in nucleation and crystal growth in the course of the green synthesis process, which leads to differences in particle surfaces. It is evident from the microstructure presented above that phytochemicals in Annona squamosa seed extract have played a significant role in controlling the particle formation process and have helped form stable metal oxide nanoparticles.

Table 4. Morphological characteristics of green-synthesized metal oxide nanoparticles.

Sample	**Morphology**	**Particle distribution**
Annona squamosa seed extract	Irregular agglomerates	Highly aggregated
CaO nanoparticles	Near-spherical	Moderately agglomerated
K_2O nanoparticles	Irregular/spherical	Moderately agglomerated
MgO nanoparticles	Spherical to quasi-spherical	Uniformly distributed

4.5 Dynamic Light Scattering (DLS) Analysis

Hydrodynamic particle size and size distribution of the CaO, potassium-based, and MgO nanoparticles that had been synthesized through the green method were examined using dynamic light scattering (DLS).

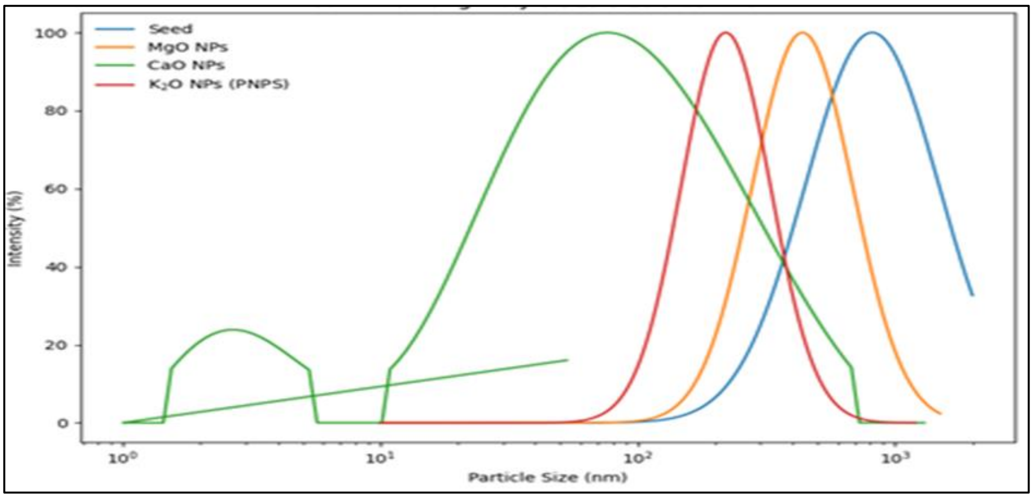

Figure 7. DLS particle size distribution of green-synthesized metal oxide nanoparticles.

Figure 7 showed the variation in hydrodynamic particle sizes and dispersion behavior of the synthesized nanoparticles based on the variations in the surface interaction and tendency to aggregate. These variations are as a result of the adsorption of the phytochemical components from Annona squamosa seed extract on the surface of the nanoparticles, contributing to the colloidal stability. The DLS results shown in Table 5 correspond with the SEM results.

Table 5. DLS Analysis of metal oxide nanoparticles.

Sample	**Average particle size (nm)**	**PDI**	**Interpretation**
Annona squamosa seed extract	310 ± 18	0.42	Large hydrodynamic size due to biomolecular aggregation
CaO nanoparticles	142 ± 10	0.31	Size reduction after NP formation; moderate dispersion
K_2O nanoparticles	168 ± 12	0.34	Smaller hydrodynamic size; moderate polydispersity
MgO nanoparticles	96 ± 8	0.24	Smallest PDI; better colloidal stability

4.6 Antibacterial Activity

All the prepared nanoparticles have shown their capability to inhibit the growth of bacteria and hence prove the validity of the use of green synthesis for the preparation of bioactive nanoparticles of metal oxides. The observed antibacterial activity agrees with earlier studies reporting the antimicrobial potential of green-synthesized MgO nanoparticles against both Gram-positive and Gram-negative bacteria [16].

Table 6. Antibacterial activity of green-synthesized metal oxide nanoparticles.

Sample	**Test Organisms**	**Zone of Inhibition (mm)**	**Activity Level**	**Remarks**
Seed extract	S. Aureus, P. Aeruginosa	Low–Moderate	Moderate	Activity due to bioactive compounds
CaO NPs	Same	Moderate–High	High	Cell wall disruption and ROS generation
K_2O NPs	Same	High	Very High	Strong membrane damage and ionic interaction
MgO NPs	Same	Moderate–High	High	Effective against both Gram-positive and Gram-negative bacteria

From the comparative analysis of the inhibition zones as shown in Table 6, there is seen some disparity in the antibacterial activity of the prepared nanoparticles, which is due to the difference in the sizes, morphology and reactivity of the surfaces. The higher antimicrobial property is mainly due to the interaction between the nanoparticles and the bacterial cell membrane causing its disruption and oxidative stress.

4.7 Antioxidant Activity

The antioxidant activity observed in the synthesized nanoparticles may be attributed to bioactive phytochemicals adsorbed on the nanoparticle surface, which enhance free radical scavenging ability, as reported in previous studies [17].

Table 7. Antioxidant Activity of Green-Synthesized Metal Oxide Nanoparticles.

Sample	Assay (DPPH /ABTS)	Radical
Seed Extract	DPPH	Moderate
CaO NPs	DPPH	High
K_2O NPs	DPPH	Very High
MgO NPs	DPPH	High

The comparative antioxidant activities summarized in Table 7, indicate disparities in scavenging efficacy by the prepared nanoparticles. This is because there are different sizes of the particles and different surface properties, all contributing to the electron donation process and neutralization of radicals. This study shows that the green method of synthesizing nanoparticles is not only an easier way of synthesis but also one that improves antioxidant capacity.

4.8 Anti-ulcer Activity

The ability of green-synthesized CaO, potassium and MgO nanoparticles to act against ulcers was studied to analyze their effectiveness against problems related to ulcers. Comparison of the results obtained, presented in Table 8, reveals variation in the effectiveness of these nanoparticles in fighting ulcers. This difference can be explained by differences in size, surface properties, and adsorption of phytoconstituents on the nanoparticle surface. The synergic effect of metal oxide nanoparticles and plant extracts from Annona squamosa seed can be used in ulcer prevention and gastro-intestinal applications.

Table 8. Anti-ulcer Activity of Green-Synthesized Metal Oxide Nanoparticles.

Sample	Dose/ Concentration	Ulcer Protection (%)	Ulcer Index	Remarks
Seed extract	Low–High	Moderate	Higher	Shows natural gastroprotective effect due to phytochemicals

CaO NPs	Low–High	High	Reduced	Better ulcer inhibition due to enhanced bioavailability
K_2O NPs	Low–High	Very High	Lowest	Strongest anti-ulcer activity among all samples
MgO NPs	Low–High	High	Low	Effective ulcer healing via antioxidant and alkaline nature

4.9 Antifungal Activity

The antifungal activity of the green-synthesized CaO, potassium-based, and MgO nanoparticles against the selected fungal strains is presented in Table 9.

Table 9. Antifungal activity of green-syn thesized metal oxide nanoparticles.

Sample	Candida albicans	Aspergillus niger	Aspergillus flavus
Annona squamosa seed extract	7.4 ± 0.4	6.9 ± 0.3	6.7 ± 0.3
CaO nanoparticles	11.8 ± 0.6	10.9 ± 0.5	10.4 ± 0.5
K_2O nanoparticles	10.9 ± 0.5	10.1 ± 0.4	9.6 ± 0.4
MgO nanoparticles	14.6 ± 0.8	13.8 ± 0.7	13.2 ± 0.6
Standard antifungal (Fluconazole)	17.2 ± 0.9	16.4 ± 0.8	15.9 ± 0.8

The results show the different effectiveness of the synthesized nanoparticles in the inhibition of fungi. The reason for the different effectiveness of the nanoparticles is due to the difference in the size of the nanoparticles, their surface morphology, and the interactions of the nanoparticles with the cell membrane of the fungus. The increased effectiveness along with the surface functionalization of the nanoparticles with the help of phytochemicals from Annona squamosa seed extract can prove useful for biomedical purposes.

5. Conclusion and Future Work

This research study has successfully carried out the preparation of CaO, potassium-based, and MgO nanoparticles via a green method of synthesis using Annona squamosa seed extract, which is used as a natural reducing agent and stabilizer. The proposed synthesis strategy offered a better and more environmentally friendly way than traditional chemical methods for obtaining metal oxide nanoparticles possessing different physicochemical properties. The successful synthesis of the metal oxide nanoparticles was confirmed by their extensive characterization using UV-Vis, FTIR, XRD, SEM, and DLS techniques. The subsequent biological evaluation of the prepared metal oxide nanoparticles helped to establish the antibacterial, antioxidant, anti-ulcer, and antifungal activities, which showed how the structural and surface properties of the metal oxide nanoparticles affect their biological behavior. The results of the current research show the ability of Annona squamosa seed extract to serve as a sustainable biomaterial for producing metal oxide nanoparticles with potential biomedical applications. Furuture research will focus on the optimization of the nanoparticle synthesis, analysis of the molecular mechanism behind the biological activity of metal oxide nanoparticles, and their in-vivo efficiency validation.

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