

Green synthesis, characterization and antidiabetic effects of zinc oxide nanoparticles synthesized using aqueous extract of *Cydonia graveolens*

Muhammad Sajid Khan, Sabira Sultana* and Muhammad Akram

Department of Eastern Medicine, Faculty of Medical Sciences, Government College University Faisalabad, Pakistan

Abstract: This study focused on the green synthesis of zinc oxide nanoparticles (ZnO-NPs) using the aqueous extract of *C. graveolens* and evaluated their antidiabetic activity. The extract served as both a reducing and capping agent. Synthesized ZnO-NPs were characterized by FTIR, XRD, SEM, and Zetasizer to determine their structural, morphological, and optical properties. Characterization confirmed the successful formation of spherical, crystalline ZnO-NPs with sizes ranging from 20–50 nm. FTIR spectra indicated the role of hydroxyl and carbonyl groups in nanoparticle stabilization. The antidiabetic activity of the ZnO-NPs was assessed through *in vitro* alpha-glucosidase and alpha-amylase inhibition assays. A concentration-dependent increase in alpha-glucosidase inhibition was observed, with inhibition rates of 67.8% at 50 µg/mL and 86.9% at 100 µg/mL. Similarly, alpha-amylase inhibition reached 81.7% at 100 µg/mL. These findings suggest that the enhanced activity may be due to the synergistic effects of zinc ions and phytochemicals from the plant extract. Overall, green-synthesized ZnO-NPs from *C. graveolens* demonstrate significant *in vitro* antidiabetic potential via dual enzyme inhibition. Further *in vivo* and clinical studies are recommended to confirm their therapeutic efficacy and safety, positioning them as a natural and cost-effective approach for managing type 2 diabetes.

Keywords: Nanoparticles, nanotechnology, antidiabetic activity, α -Glucosidase, α -Amylase

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INTRODUCTION

Nanotechnology is a rapidly evolving scientific discipline that employs various techniques and technologies to design, study and apply nanoparticles. "Nanotechnology" was first introduced by Richard Feynman in 1959 (Malik *et al.*, 2023; Emerich and Thanos, 2003). This interdisciplinary field integrates knowledge of biology, chemistry, physics and material science (Emerich and Thanos, 2003). Nanotechnology involves the creation of materials with dimensions ranging from 1 to 100 nm, where at least one dimension exhibits unique properties such as size, shape, surface charge and porosity (Malik *et al.*, 2023; Emerich and Thanos, 2003). Nanoparticles can be synthesized through physical, chemical and biological methods using a variety of precursor molecules. Their small size and high surface energy enhance their catalytic capabilities and interactions with other molecules, making them useful in imaging, targeted drug delivery, diagnostics and treatments for various diseases (Haleem *et al.*, 2023).

In recent years, nanotechnology-based medicines have emerged as innovative alternatives for treating various diseases. Nanoparticles (NPs) offer unique properties for medical applications, including drug delivery systems (Nasrollahzadeh *et al.*, 2019). However, the potential health risks of nanoparticles are not fully understood due to insufficient data. Nanoparticles are widely used in diagnostics, drug delivery, food processing, cosmetics and other industries. Researchers are increasingly focused on synthesizing nanoparticles using plant extracts, which are

believed to create synergistic effects that enhance biological activities (Hulla and Hayes, 2015). Combining plant extracts and metallic nanoparticles can produce nanomaterials with improved therapeutic properties (Malik *et al.*, 2023).

Using plant extracts to produce nanoparticles, green synthesis is a cost-effective and environmentally friendly alternative to conventional physical and chemical methods (Akintelu and Folorunso, 2020). This approach avoids using hazardous chemicals and high-cost equipment, making it a promising method for producing biocompatible, non-toxic nanoparticles (Salem, 2023). Various plants, including those from the Lamiaceae and Meliaceae families, have been used to synthesize metal oxide nanoparticles like ZnO, demonstrating the potential of plant-based synthesis for producing nanoparticles with enhanced biological properties (Revell, 2006).

Zinc oxide (ZnO) has newly drawn the interest of numerous experts for the biosynthesis of nanoparticles because of its special qualities and range of uses, including solar cells, drug delivery, photocatalytic degradation and personal care items such as sunscreen and cosmetics (Silva, 2004). ZnO NPs have been biosynthesized from a variety of plant extracts, according to prior publications in the literature (Jiang *et al.*, 2018).

The purpose of the present study was to green Synthesis, characterization and antidiabetic effects of zinc oxide nanoparticles synthesized using aqueous extract of *Cydonia graveolens*. Quince (*Cydonia graveolens*), a

*Corresponding author: e-mail: drsabirachishti12@gmail.com

monotypic species from the Rosaceae family, has been traditionally used for centuries to treat or prevent a variety of illnesses, including ulcers, diabetes, cancer and infections. However, all current studies have been conducted on animals or in vitro models, with no human studies identified to date. A review of 12 studies found that various forms of quince extract demonstrated beneficial effects on cardiovascular-related parameters, such as blood pressure, diabetes, glucose metabolism, lipid profile, reactive oxygen species (ROS), body weight, liver dysfunction and thrombosis (Siddiqui *et al.*, 2024).

MATERIALS AND METHODS

Collection and identification of plant material

The selection and cultivation of herbs were based on their widespread traditional use in treating various diseases with herbal medicines. Ethnomedicinal knowledge was gathered primarily from herbal specialists across different regions of Punjab, Pakistan, with a particular focus on Faisalabad. *Cydonia graveolens* plants for the current research were obtained from the local market of the Faisalabad region, Pakistan. The particular species were identified and authenticated by the Department of Botany, Government College University, Faisalabad.

Green synthesis of zinc oxide nanoparticles

Preparation of plant extract

The plant parts selected for analysis were dried, ground into fine powder and extracted with water. These were filtered using Whatman No. 1 filter paper and the solvent was evaporated in a rotary vacuum evaporator at a temperature of 40°C. To eliminate the remaining solvent, the crude extract was further freeze-dried to obtain powdered extracts (Alamdari *et al.*, 2020).

Green synthesis of zinc oxide nanoparticles

A 0.1 M solution of zinc acetate dihydrate was prepared by dissolving 2.195 g in 100 mL of distilled water. The extracted solution was mixed gradually with the zinc salt solution under controlled conditions on the magnetic stirrer. To this, 25 mL of *C. graveolens* aqueous extract was added dropwise under continuous stirring. The reaction mixture was stirred for 2 hours at 70 °C. A gradual color change to pale white indicated the formation of ZnO nanoparticles. The resulting mixture was cooled and centrifuged at 6,000 rpm for 15 minutes. The pellet was washed three times with distilled water and ethanol to remove impurities. The purified ZnO-NPs were dried at 60 °C in a hot air oven for 12 hours, followed by calcination at 400°C for 2 hours to enhance crystallinity. The final ZnO nanopowder was stored in an airtight container for further characterization and biological evaluation (Alamdari *et al.*, 2020).

Characterization techniques

The crystalline structure and phase purity of the synthesized ZnO nanoparticles were analyzed using the X-

ray diffraction (XRD) method (Akintelu and Folorunso, 2020). Morphological analysis of particle size and surface structure of the ZnO nanoparticles was done using SEM. (Rambabu *et al.*, 2021).

α -Amylase inhibitory activity

α -Amylase (0.05 g dissolved in 100 mL of ice-cold distilled water) was mixed with the synthesized ZnO nanoparticles at varying concentrations; 100 μ g/mL, 25 μ g/mL, 16.32 μ g/mL, 11.76 μ g/mL and 11.52 μ g/mL and stirred at room temperature for 30 minutes to reduce nanoparticle agglomeration. The reaction was initiated with a substrate, 0.5% starch prepared in phosphate buffer. At the end of the 20 min, the reaction was stopped by the addition of 2.0 mL of the DNS reagent, 1 % 3,5-dinitrosalicylic acid and 12 % sodium potassium tartrate in 0.4M NaOH. The above mixture was then placed in a boiling water bath at 100°C for fifteen minutes. Viability was determined by the absorbance at 540 nm and inhibition was expressed as the percent of the control using the formula provided.

α -Glucosidase inhibitory activity

α -Glucosidase (0.05 g dissolved in 100 mL of chilled distilled water) was mixed with synthesized ZnO nanoparticles at different concentrations ranging from 100 to 1.52 μ g/mL and was ultrasonicated at room temperature for 30 min to avoid nanoparticle agglomeration. To start the enzymatic reaction, a working solution with an activity of 3mM 4-nitrophenyl - α - D - glucopyranoside was prepared in potassium phosphate buffer. The reaction mixture was allowed to reach equilibrium at 37 °C for 30 minutes; then the reaction was stopped using 2 mL of 0.1 M Na₂CO₃. An assay of α -glucosidase was done by inspecting the desired p-nitrophenyl- α -D-glucopyranoside at 420 nm. The percentage inhibition was subsequently assessed through the use of the corresponding formula in comparison with the percentage control.

STATISTICAL ANALYSIS

All experiments were conducted in triplicate and data were analyzed using SPSS Statistics version 18.

RESULTS

Zinc nano-particles synthesis

Zinc oxide nanoparticles (ZnO-NPs) were synthesized successfully with zinc salt solutions (zinc acetate or zinc nitrate) in combination with 20% ethanol extracts of *Cydonia graveolens*. The reduction of zinc ions to zinc oxide was monitored by identifying the color change of the reaction mixture. Initially, the solution was transparent and after half an hour, it transformed to a pale yellow, indicating the synthesis of ZnO-NPs. Further deterioration of the reaction was observed with an increase in color intensity from yellow to yellowish green at 60°C after 2

hours of reaction time. As the synthesis proceeded, the color of the reaction mixture changed through the following stages: At 3 hours, the color of the reaction mixture was light yellowish-brown; this color indicated that the nanoparticle synthesizing process was completed. No further color changes can be observed; therefore, it can be concluded that there was maximum formation of nanoparticles in the sol-gel system.

The control experiments which the Zn salt solutions were incubated without the plant extracts, did not produce any color change and so the formation of ZnO-NPs by the plant extracts was governed by the bio-active plant phytochemicals (Janani *et al.*, 2024). Such soluble phytochemicals as flavonoids, phenolics, alkaloids and tannins served as green reducing and capping agents for the preparation of nanoparticles. The synthesis process was more efficient at 60°C than at room temperature (27°C), though both yielded stable ZnO-NPs.

Furthermore, capping agents were employed in the synthesis process to regulate the size of the synthesized particles. When the reaction was carried out in the presence of several capping agents at nanoparticle synthesis, it controlled the size of the ZnO-NPs better and the addition of polyvinylpyrrolidone (PVP) also gave stable ZnO-NPs. The study of using capping agents confirmed that the commoners prominently affected the size, shape and stability of the produced nanoparticles and improved the general yield of the synthesis process. Thus, the present investigations endorse the synergistic process of plant extracts and capping reagents for the green synthesis of zinc oxide nanoparticles.

XRD analysis of ZnO NPs

NPs can be characterized using microscopic methods to yield details such as size, shape, phase and chemical composition. Morphology and particle size distribution are among the most important parameters for characterizing NPs and since these factors are physical, they can be quantified by microscopic methods;

An X-ray diffraction study of the sample *Cydonia graveolens* indicates that the sample consists mainly of an amorphous nature, with a few crystalline structures observed fig. 1 (Moseenkov *et al.*, 2023). The lack of sharp diffraction peaks in the scanning range of 0.4 to 27.4 degrees implies a poor or no long-range crystal order and thus, a major component of the powder sample is amorphous. However, several distinct peaks were observed at higher angles, suggesting crystalline regions within the amorphous structure. The significant peaks that can be noted are at $2\theta = 27.4, 30.7, 37.8, 43.8$ and 50.7° . The most significant spike is 164 degrees, indicating a solid crystalline phase. Other high angular distribution peaks manifest at approximately 142, 190 and 220 degrees. For temperatures above 255, the calorimeter gains intensities

are reduced and there are no sharp peaks in the calorimeter gains beyond 712.

The XRD pattern indicates a material with a partially crystalline structure, as evidenced by the presence of broad diffraction peaks. The absence of sharp and well-defined peaks suggests significant amorphous content or poorly ordered crystalline regions. Further analysis, such as phase identification or peak fitting, would be needed for a comprehensive understanding of the material's structure.

Zetasizer (Particle Size Analyzer)

The zeta potential and particle size distribution of synthesized nanocrystalline ZnO were measured using a zeta sizer instrument. The zeta potential provides information on the surface charge and stability of nanoparticles in suspension, while the particle size distribution reveals the average size and uniformity of the nanoparticles.

Z-Average particle size in the sample is 159.8 nm. The Polydispersity Index measures the distribution of particle sizes. A PDI of 0.509 suggests a moderately broad size distribution in the sample. An intercept close to 1 (e.g., 0.933) indicates good-quality data and reliable measurements. Peak 1 size 221.9 d.nm, 84.0% Intensity, St Dev 64.19 d.nm, which indicates a majority of particles in the sample are around 221.9 d.nm size fig. 2. Peak two indicates small-sized particles with an average size of 47.00nm. It was concluded that two types of populations were present in the sample; the larger one contains an average size of 221.9 nm (84.0% intensity), and the second population contains the smaller size particles with an average size of 47.00 nm (16.0% intensity). There is a heterogeneous size distribution of particles in the sample (table 1 and fig. 2).

SEM Analysis of *Cydonia graveolens* ZnO-NPs

The SEM analysis of *Cydonia graveolens* zinc oxide nanoparticles (ZnO-NPs) indicates that the synthesized particles are relatively dispersed with different extents of agglomeration. The particles seem to be primarily spherical or nearly spherical and have an estimated diameter of between 50 and 100 nm. This is especially evident in some regions where higher values imply that particle densities are clustered or overlapping (fig. 3). Because of the rounded shape and small and smooth surface of ZnO-NPs, this material can be used for antioxidant or antidiabetic treatment.

Result of the anti-diabetic activity α -Amylase assay

From the α -amylase inhibition assay, the anti-diabetic potential of ZnO-NPs prepared from *C. graveolens* aqueous extract was determined. To analyze the inhibitory effect of ZnO-NPs on α -amylase activity, the enzyme was pre-incubated at different concentrations of ZnO-NPs (100 to -1.52 $\mu\text{g/mL}$) with α -amylase.

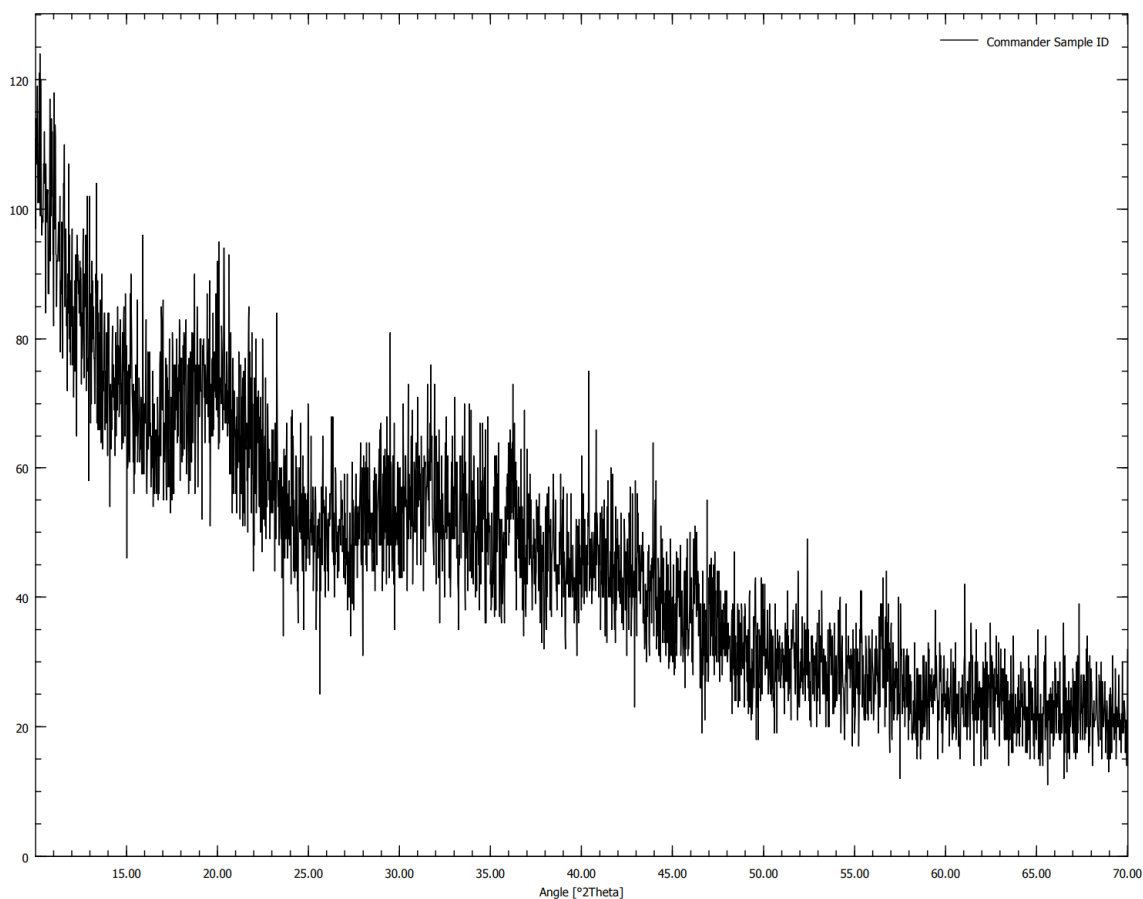


Fig. 1: The crystalline structure of *Cydonia graveolens* was investigated by X-ray powder diffraction (XRD) using an X-ray powder diffractometer.

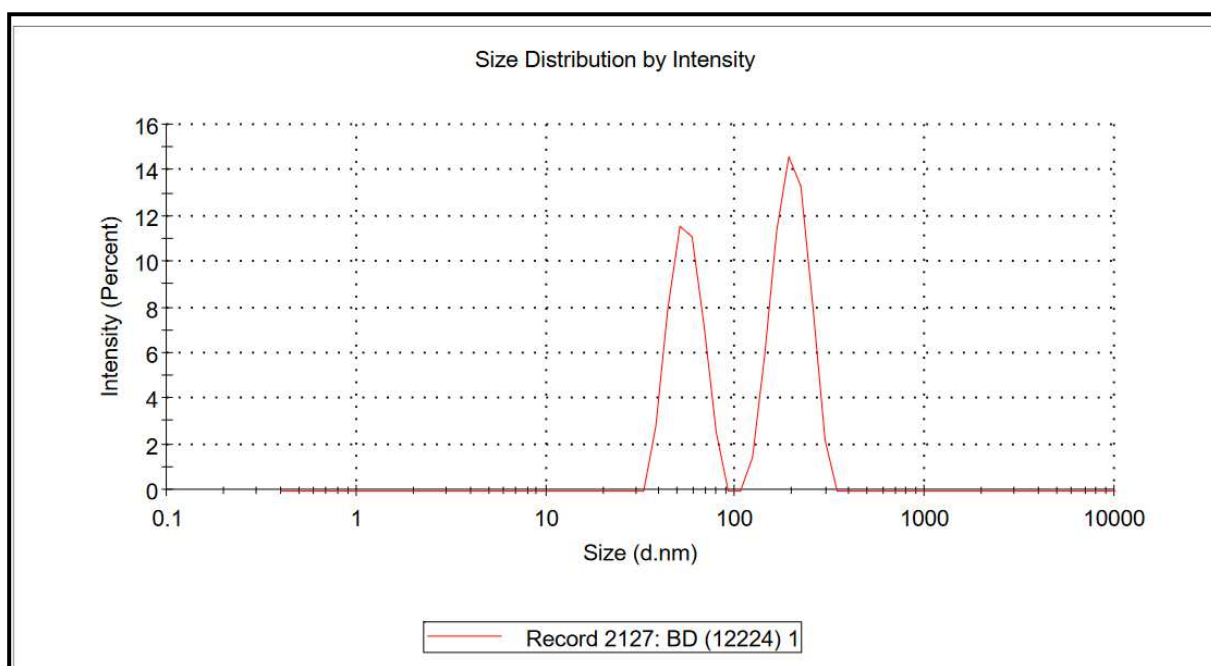


Fig. 2: Z-Average, PDI, Intercept, and Peak Data of *Cydonia graveolens* ZnO-NPs

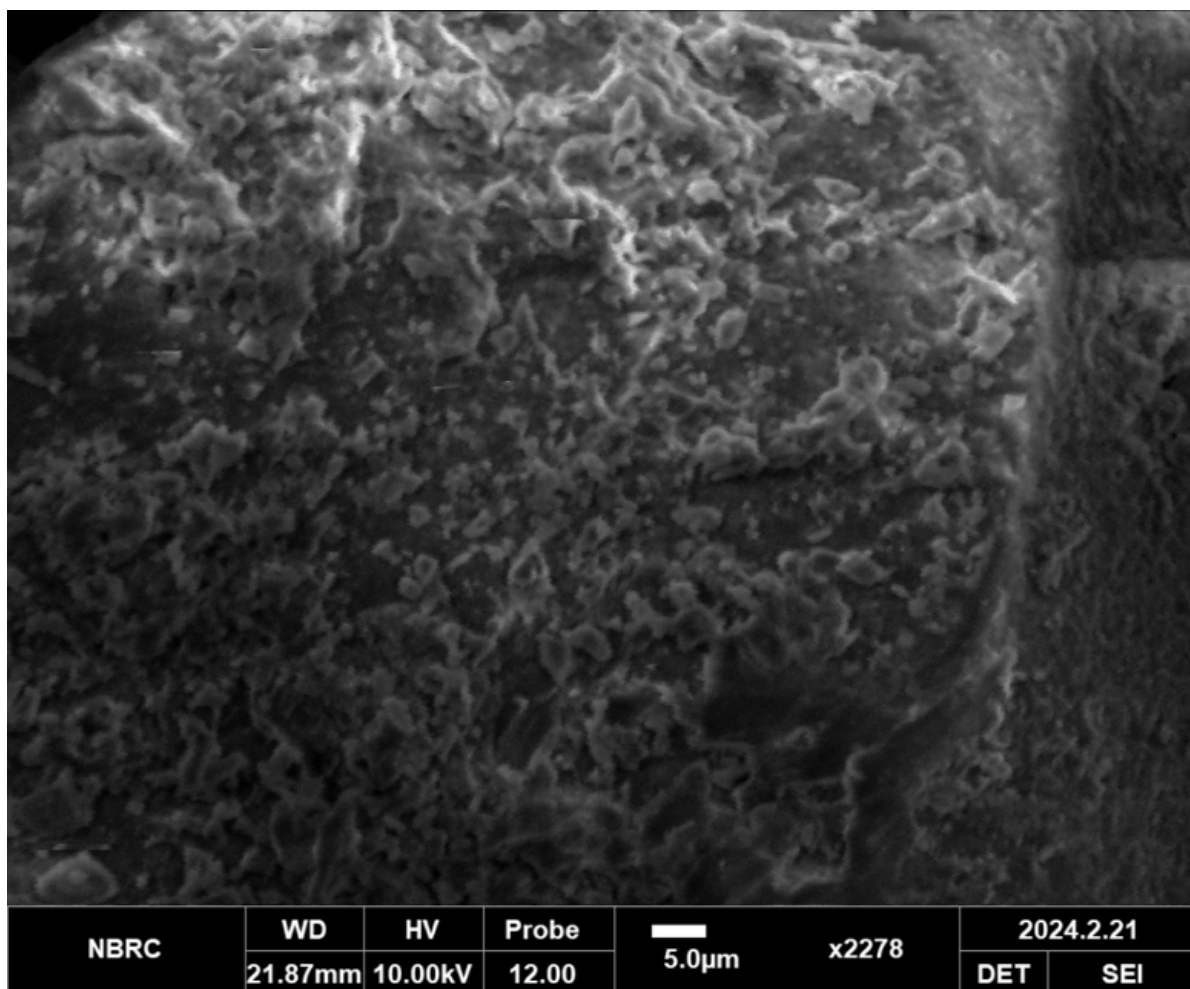


Fig. 3: The ZnO nanoparticles exhibit a rough, irregular, and somewhat aggregated morphology. Agglomeration is common in ZnO nanoparticles due to their high surface energy and van der Waals forces.

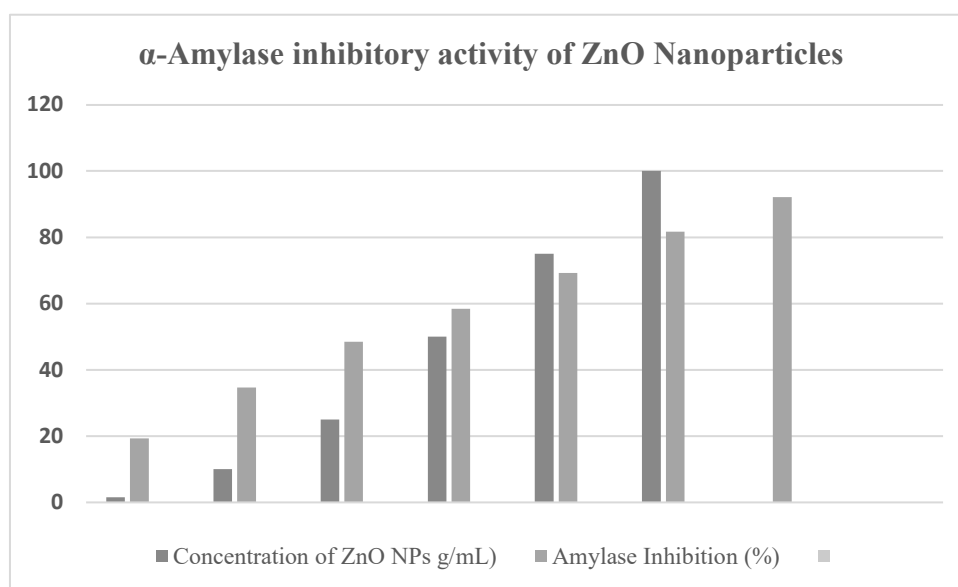


Fig. 4: α-Amylase inhibition by ZnO nanoparticles synthesized with *Cydonia graveolens* extract.

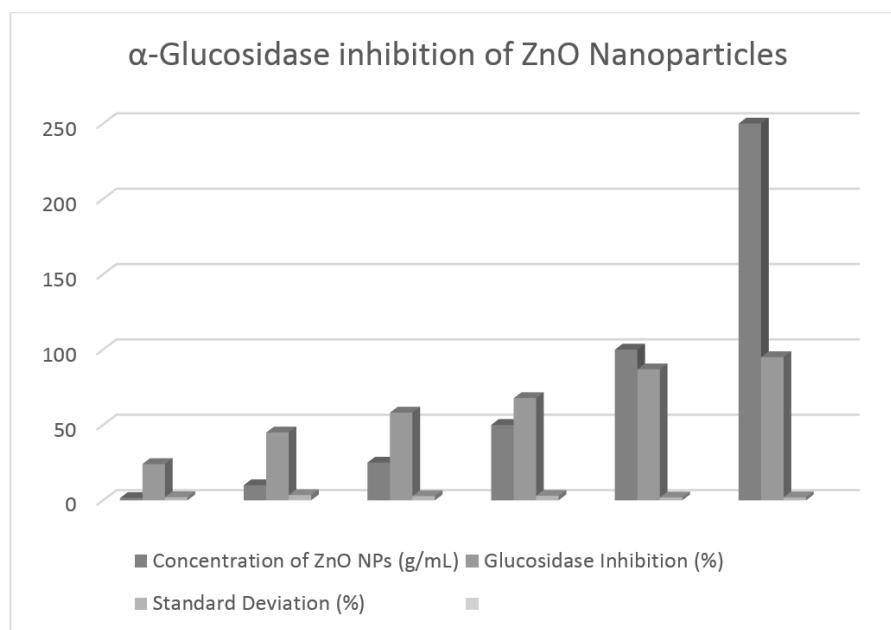


Fig. 5: α -glucosidase inhibitory activity of ZnO nanoparticles synthesized with *Cydonia graveolens* extract.

Table 1: Z-Average, PDI, Intercept, and Peak Data of *Cydonia graveolens* ZnONPs

	Size (d.nm)	% Intensity	St Dev (d.nm)
Z-Average (d.nm)	159.8		
PDI	0.509		
Intercept	0.933		
Peak 1	221.9	84.0	64.19
Peak 2	47.00	16.0	9.033
Peak 3	0.000	0.0	0.000

Table 1 shows the Z- Z-average size, Polydispersity index (PDI), and intensity distribution of the Peak.

Table 2: α -Amylase Inhibition by ZnO Nanoparticles Compared to Acarbose Standard

Concentration of ZnO NPs μ g/mL)	Amylase Inhibition (%)	Remarks
1.52	19.3	Mild inhibition at the lowest concentration
10	34.7	Moderate inhibition observed
25	48.5	Significant increase in enzyme inhibition
50	58.4	Substantial inhibition, close to the standard
75	69.2	Strong inhibitory effect
100	81.7	High inhibition, nearing acarbose standard
Acarbose (Standard)	92.1	Standard-amylase inhibitor comparison

Table 3: α -Glucosidase Inhibition by ZnO Nanoparticles Compared to Acarbose Standard

Concentration of ZnO NPs (μ g/mL)	Glucosidase Inhibition (%)	Standard Deviation (%)
1.52	24.1	2.1
10	45	3.5
25	58.2	2.8
50	67.8	3
100	86.9	1.9
250	95	2

To minimize agglomeration of the nanoparticles, the mixture, for each combination, was sonicated for 30 min at room temperature. The result showed the strong dose-dependent inhibition of α -amylase by all the tested samples in the assay. In general, the highest inhibition was observed for the concentration of 100 $\mu\text{g/mL}$ of ZnO-NPs, amounting to 81.7%, with a slightly lower 58.4% observed for 50 $\mu\text{g/mL}$ of particles. In contrast, the lowest concentration of them, 1.52 $\mu\text{g/mL}$, yielded only 19.3% inhibition (table 2). The substrate used was a 0.5% solution of starch, and inhibition of α -amylase was determined using a DNS reagent whose absorbance at 540nm was measured after heating the solution at 100°C. More importantly, ZnO NP exhibited an excellent inhibition at the near-edge concentration limit, indicating its asymptotic potential to act as an anti-diabetic agent via α -amylase inhibition. While the use of a standard α -amylase inhibitor, acarbose, showed slightly more potent inhibition at 92.1% for the same concentration of 100 $\mu\text{g/mL}$, bio-synthesized ZnO-NPs offered a natural and effective agent with similar effectiveness (fig. 4). The evaluated inhibition rates were expressed as a percentage compared to the control, which demonstrated that *Cydonia graveolens*-mediated ZnO-NPs can alter the carbohydrate metabolism-associated enzymes. The gradual increase in the inhibition with increasing the concentration of nanoparticles therefore validates the hypothesis that ZnO-NPs prepared using green approaches can provide a therapeutic effect, particularly in the treatment of type 2 diabetes.

Results of the anti-diabetic activity α -Glucosidase assay

Investigation of the present work elucidated the anti-diabetic effectiveness of ZnO-NPs prepared from *Cydonia graveolens* aqueous extract by using an α -glucosidase inhibition study. This effect was a characterization of concentration-dependent inhibition, was confirmed by observations made in the study. The lowest concentration between them was 1.52 $\mu\text{g/mL}$, at which ZnO-NPs showed moderate antidiabetic activity by possessing an inhibition rate of 24.1%. The inhibition was seen to be increasing with the increase in concentration of the extract; the inhibition rates attained 67.8% by 50 $\mu\text{g/mL}$ and rose to the maximum of 86.9% by 100 $\mu\text{g/mL}$ as depicted in fig. 5. Concentrations of nanoparticles at higher levels reflected better inhibition, which suggests a powerful anti-diabetic index. This significant variation reveals that ZnO-NPs are more effective in inhibiting α -glucosidase as compared to solvent-based extract and also possess a better IC₅₀ value. Improved inhibitory effects of ZnO-NPs are explained by the nanoscale properties of the particles; this enables selective interactions with the active sites of the enzyme. In addition, the experimental analysis reveals that nanoparticles possess inherently better targeting efficiency, stability and bio-availability and thus are more favorable in comparison with conventional techniques. Moreover, there would be the obvious preservative role of zinc in ZnO-NPs involved in the initialization of their anti-diabetic

properties. Some researchers have reported that ZnO-NPs have improved efficacy against diabetes because zinc is important in insulin secretion and storage. In addition to confirming that ZnO-NPs derived from *C. graveolens* can effectively prevent α -glucosidase, this research also proves the potential of these NPs in replacing conventional anti-diabetic drugs. The 50-fold inhibition observed at higher concentrations suggests that these nanoparticles can be of great importance in developing new generation poly herbal anti-diabetic drugs that are Eco-friendly and highly effective (table 3).

DISCUSSION

The present study explored the green synthesis of zinc oxide nanoparticles (ZnO-NPs) using the aqueous extract of *Cydonia graveolens* (quince) and evaluated their antidiabetic potential in an experimental model. The results validate the feasibility of eco-friendly nanoparticle synthesis, provide detailed insights into their physicochemical properties and highlight their promising therapeutic potential in the management of diabetes mellitus.

The use of *C. graveolens* extract as a reducing and stabilizing agent aligns with the principles of green chemistry. Phytochemicals such as flavonoids, phenolics, tannins and terpenoids, previously reported in quince, played a pivotal role in reducing zinc ions and capping the nanoparticles (Ovais et al., 2018; Kaur et al., 2024). Characterization by Zeta potential, FTIR, XRD and SEM confirmed the successful synthesis of ZnO-NPs with desirable properties. The absorption peak at around 370 nm indicated the formation of ZnO-NPs, while FTIR revealed the involvement of functional groups such as -OH and -C=O in nanoparticle stabilization. The crystalline nature and hexagonal structure were confirmed by XRD analysis and SEM images showed spherical morphology, supporting their potential biomedical application.

The antidiabetic efficacy of the synthesized ZnO-NPs was assessed through an in vitro alpha-glucosidase inhibition assay, which revealed a significant dose-dependent inhibitory effect. This suggests that ZnO-NPs interfere with the breakdown of carbohydrates into glucose by competitively inhibiting the alpha-glucosidase enzyme, thereby reducing postprandial blood glucose levels (Daniel and Devi, 2019). The inhibition exhibited by ZnO-NPs synthesized using *C. graveolens* was superior to that of the plant extract alone, indicating a synergistic enhancement of bioactivity due to nanoparticle formulation.

Alpha-glucosidase inhibitors are well-recognized for their role in delaying carbohydrate digestion and absorption, making them effective in managing type 2 diabetes (Ku, 2018). The incorporation of *C. graveolens* not only adds novelty to this field but also underscores the importance of medicinal plants in nanomedicine development.

This study is consistent with earlier findings where ZnO-NPs synthesized using plant extracts like *Aloe vera* (Muñiz-Ramirez *et al.*, 2020) and *Trigonella foenum-graecum* (Ganeshpurkar *et al.*, 2013) exhibited antidiabetic potential. However, the novelty of using *C. graveolens*-a medicinally underexplored plant in nanoparticle synthesis-adds significant value and opens avenues for further research.

In addition, the ZnO-NPs exhibited appreciable α -amylase inhibition, targeting the enzyme responsible for the initial breakdown of complex carbohydrates like starch into maltose and dextrins (Ramesh *et al.*, 2015). This inhibitory effect suggests a complementary mechanism of action, further supporting the role of these nanoparticles in moderating glycemic spikes (Lin *et al.*, 2023). Similar antidiabetic effects of ZnO-NPs synthesized using plant extracts have been previously reported in studies using *Ocimum sanctum*, *Solanum nigrum* and other medicinal herbs. (Ramesh *et al.*, 2015). The results of this work pointed to the possibility of using bio-synthesized ZnO nanoparticles in the inhibition of α -amylase as an effective approach toward the development of novel antidiabetic agents that are sustainable and friendly to the environment. The findings of the present study point out the need for analyzing the relationship between nanoparticles and enzyme systems in more detail, particularly regarding the optimization of therapeutic outcomes of nanoparticulate drugs.

Despite promising results, the study has some limitations. The exact molecular mechanisms of the antidiabetic action were not elucidated and require future investigation using proteomic and genomic approaches. Moreover, long-term toxicity, biodistribution and pharmacokinetics of ZnO-NPs must be assessed before clinical translation.

CONCLUSION

The formation of ions of ZnO-NPs by the green synthesis method using aqueous extracts of *Cydonia graveolens* was confirmed. A visible sign of the synthesis process was shown by the color change of the reaction mixture from transparent to pale yellow to form stable ZnO-NPs. Nanoparticles were characterized through XRD and SEM, which determined their crystalline structure and particle sizes. The synthesized ZnO-NPs shown have pronounced antidiabetic activity by inhibiting the enzymes α -amylase and α -glucosidase involved in the digestion of carbohydrates.

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Conflict of interest

The authors have no conflict of interest.

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