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Antidiabetic Activity of Green-Synthesized Silver Nanoparticles from Ziziphus mucronata Leaves Extract

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Abstract:

Green-synthesized silver nanoparticles (AgNPs) offer sustainable, cost-effective therapeutic potential in nanomedicine due to their unique physicochemical properties. This study explores Ziziphus mucronata (Z. mucronata) leaf extract-synthesized AgNPs (ZM-AgNPs) for in vitro antidiabetic activity via in vitro assay. Phytochemical composition was analyzed using qualitative method and GC-MS, ZM-AgNPs were synthesized using methanol extract and confirmed via UV-vis spectroscopy and characterized using SEM, FT-IR, and XRD. Antidiabetic activity was assessed through α-glucosidase and α-amylase inhibition assays at varying concentrations (20–100 µg/mL). The result of phytochemical analysis reveals the presence of alkaloid, flavonoid, saponin, anthraquinone, phenols, tannins, steroid, glycoside and phlobatannins. UV-vis spectra showed highest surface plasmon resonance peak at 412 nm, confirming AgNPs formation. FT-IR identified bioactive compounds (phenolics, and flavonoids) involved in reduction and stabilization. XRD and SEM revealed crystalline, spherical nanoparticles (20–50 nm). ZM-AgNPs exhibited dose-dependent inhibition, with maximal α-glucosidase (88.05%, IC_{50}: 1.56 mg/mL) and α-amylase (68.09 %, IC_{50}: 61.31mg/mL) activity, surpassing both crude extract (80.59 %, 44.34 %) and Acarbose (83.32 %, 52.24 %) respectively. ZM-AgNPs demonstrate superior antidiabetic potential attributed to enhanced bioavailability and synergistic phytochemical interactions. This underscores their promise as a natural alternative for diabetes management.

Keywords: AgNPs, α-amylase, α-glucosidase, Ziziphus mucronata, green-synthesis, diabetes mellitus.

Introduction

Nanotechnology is a rapidly advancing field within modern science. It involves the study and application of nanomaterials, particularly nanoparticles, which are relevant across diverse disciplines (Mohammed et al., 2024). Engineering materials at the nanoscale enhances their functional properties, including electronic, catalytic, magnetic, and optoelectronic behaviours. The unique attributes of nanoparticles are largely determined by factors such as shape, size, and surface morphology (Joudeh & Linke, 2022). Manipulating atoms and molecules at sub-microscopic dimensions allows researchers to control and enhance their physicochemical performance (Chaturvedi et al., 2024). In recent years,

nanoscience and nanotechnology have demonstrated remarkable progress, particularly in the diagnosis, prevention and treatment of various diseases worldwide (Haleem et al., 2023; Malik et al., 2023). The use of silver for wound healing and infection control dates back to ancient civilizations; however, the emergence of silver nanoparticles AgNPs has renewed scientific interest due to their exceptional physicochemical properties. These distinctive features have positioned AgNPs as one of the most promising metallic nanoparticles in biomedical applications (Nqakala et al., 2021). The synthesized AgNPs exhibit outstanding features such as excellent chemical stability, strong catalytic activity, and high electrical conductivity. In addition, they demonstrate significant biological activities, including antimicrobial, antidiabetic, and anti-inflammatory effects (Habeeb Rahuman et al., 2022). Their broad therapeutic potential has been well documented, as AgNPs possess multiple mechanisms of antidiabetic action, show effectiveness against drug-resistant microorganisms, and present relatively low systemic toxicity, making them a promising option for biomedical applications (Habeeb Rahuman et al., 2022).

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by persistently high blood glucose levels resulting from defective insulin secretion or action. It represents a major public health issue globally and is related with severe complications such as cardiovascular diseases, kidney failure, limb amputation, stroke, and blindness (Galicia-Garcia et al., 2020). In many parts of the world, most especially in developing regions, medicinal plants are widely used in the management of diabetes due to their accessibility, affordability, and efficacy (Payab et al., 2020).

Ziziphus mucronata subsp. mucronata, commonly called the buffalo thorn, is a medium-sized deciduous tree widely recognized for its distinctive berry-like fruits, which ripen between March and September. During its flowering season, the plant attracts nectar-feeding organisms, contributing to its ecological significance. Traditionally, various parts of Z. mucronata, particularly the stem bark and roots, have been utilized in African ethnomedicine for managing ailments such as rheumatism, syphilis, gonorrhea, gastrointestinal infections (including dysentery and diarrhea), and snakebites (Mongalo et al., 2020; Ngaradoum, 2018). Pharmacologically, species of the Ziziphus genus have showed a wide spectrum of biological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antimalarial, and anthelmintic effects, as well as anticancer, antiulcer, analgesic, sedative, and antipyretic properties (Sakna et al., 2023). The used of nanotechnology with plant-derived compounds has therefore opened new frontiers in diabetes research. The biosynthesis of AgNPs using medicinal plant extracts offers a sustainable and biocompatible approach in developing potent antidiabetic agents. This green nanotechnology approach enhances the stability and bioactivity of nanoparticles and also provides a cost-effective method suitable for developing countries, including Nigeria, where diabetes prevalence and the cost of synthetic drugs pose significant challenges.

Materials and Methods

Drugs, Chemicals, and Reagents

Acarbose was obtained from a local pharmaceutical outlet. Analytical-grade methanol (CH_3OH) used for plant extraction was supplied by Sigma-Aldrich (Germany). The α-amylase and α-glucosidase, as well as their respective substrates potato starch and p-nitrophenyl α-D-glucopyranoside (pNPG) were bought from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical purity.

Plant Material

Fresh leaves of Ziziphus mucronata (locally known as Kurna) were collected from farmland located in the Bado area of Sokoto State in Nigeria. The species was authenticated at the Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto by a taxonomist. A voucher specimen (No. UDUS/ANS/0832) was given and deposited at the herbarium for future reference.

Preparation of Ziziphus mucronata Leaf Extract.

Freshly collected leaves (20 g) were washed thoroughly with distilled water and air-dried. The dried samples were ground into fine powder using a laboratory blender. The powdered leaves were extracted with 100 mL of methanol it was kept overnight at room temperature. The extract was filtered and concentrated by evaporation at 60°C using a water bath. The resulting methanolic extract was stored in airtight containers and used for nanoparticle synthesis.

Phytochemical Screening of Z. mucronata Leaf Extract

The methanolic extract of Z. mucronata leaves was qualitatively screened for the presence of major phytochemical groups including flavonoids, alkaloids, saponins, anthraquinones, phenols, steroids, phlobatannins, tannins and glycosides using standard methods described by (Nicholas et al., 2016)

Synthesis of Z. Mucronata base Silver Nanoparticles (ZM - AgNPs)

A 1 mM silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.085 g of $AgNO_3$ in 100 mL of distilled water. An extract of Z. mucronata was then added dropwise to this solution with continuous stirring at room temperature. The reaction mixture was incubated on a rotary shaker for 4 hours. A visible colour change from colourless to yellowish-brown confirmed the formation of silver nanoparticles (ZM-AgNPs). The synthesised nanoparticles were characterised using UV–Visible spectroscopy, XRD (X-ray Diffraction), FTIR (Fourier Transform Infrared spectroscopy), and EDAX (Energy Dispersive X-ray Analysis) according to the procedure of (Prashar, 2023).

In vitro antidiabetic activities

α-Amylase Inhibition Assay.

The α-amylase inhibitory activity of the synthesized ZM-AgNPs was determined following the method of Egbuna et al., (2021) with few modifications. Different volumes of ZM-AgNPs (20–100 µL) were pre-incubated with 100 µL of α-amylase solution (0.1 mg/mL) at room temperature for 30 minutes. Subsequently, addition of 100 µL of 1% (w/v) starch solution and incubated for 10 minutes. The reaction was stopped by adding 100 µL of 96 mM **3,5-dinitrosalicylic acid (DNSA)** reagent, followed by heating in a water bath for 5 minutes. The control mixture has the same reagents with the enzyme replacement by sodium phosphate buffer (pH 6.8). Absorbance was measured **at 540 nm** using a UV–Visible spectrophotometer. Acarbose (25 mg dissolved in 0.5 mL distilled water) served as the positive control. Each assay was conducted in triplicate, and the percentage inhibition was calculated using the following formula: % inhibition = Abs Control – Abs Sample/ Abs Control × 100

α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory potential of the synthesized ZM-AgNPs was evaluated according to the method of Attia et al. (2021). Various concentrations of the nanoparticles (20–100 µg/mL) were prepared, and 0.2 mL of each sample was mixed with 1.0 mL of 0.1 M potassium phosphate buffer (pH 6.8) and 0.2 mL of α-glucosidase enzyme solution. The reaction mixture was pre-incubated for 5 minutes at temperature of 37°C, followed by the addition of substrate 0.3 mL of 10 mM p-nitrophenyl α-D-glucopyranoside (pNPG). After incubation for 30 minutes, the reaction was stopped by adding 2.0 mL of 100 mM sodium carbonate (Na_2CO_3). The absorbance of the released p-nitrophenol was measured at 400 nm using a spectrophotometer. the experiment was carried out in triplicate, and the inhibitory activity was calculated as: % inhibition = Abs Control – Abs Sample/ Abs Control × 100

Result and Discussion

Phytochemical composition of ziziphus mucronata leaves extract

The methanolic leaf extract of Ziziphus mucronata contained abundant alkaloids, saponins, phenols and tannins, with flavonoids present in moderate amounts, and steroids, glycosides, anthraquinones and phlobatannins in trace amounts (Table 1). This phytochemical profile provides a plausible biochemical basis for both the green synthesis and the expected bioactivity of the silver nanoparticles (AgNPs) produced from the extract (Olajuyigbe & Afolayan, 2018)

Table 1: Phytochemical constituents of methanol leave extract of Ziziphus mucronata.

s/n	Test	Result
1.	Flavonoids	+
2.	Saponin	++
3.	Anthraquinone	+
4.	Phenols	++
5.	Alkaloids	++
6.	Tannins	++
7.	Steroids	+
8.	Glycoside	+
9.	Phlobatannins	+

Keys: + = Present in trace, ++ = Present in abundance ND = Not detected

Characterization of Silver Nanoparticles

Various approaches are employed in the characterization of AgNPs like biological and chemical methods (Garg et al., 2020). UV as shown in figure 1a with the highest peak at 412nm. Figure 1b shows Fourier transform infrared spectroscopy of AgNps. To determine the purity and nature of the silver nanoparticles, an infrared study was conducted. (Tamilselvan et al., 2022). The peak was observed at 3250.2 cm^{-1} represents O-H group due to the stretching and deformation, respectively assign to phenolic functional groups (Azeez & Shenbagaraman, 2025), and similarly the peaks occur at (2922.2 cm^{-1} representing C–H stretching for alkanes functional group), (1707.1 cm^{-1} interpreted as C=O stretching for carbonyl functional group), (1617.7 cm^{-1} representing C=C stretching possibly aromatic compounds) (Abiola et al., 2019), and (1297.1 cm^{-1}, 1036.2 cm^{-1} and 797.7 cm^{-1} representing C–O stretching for esters or phenols, C–O–C stretching for ethers or secondary alcohols, Aromatic C–H bending for aromatic compounds such as benzene) respectively. These functional groups that are present in the synthesized particle indicate that it can be used for various application purposes. This is in line with the findings of (Azeez & Shenbagaraman, 2025). The elemental signals by EDAX showed the presence of silver in higher concentration and oxides in the synthesized AgNPs with other trace element which might be contaminant (Amare et al., 2020)

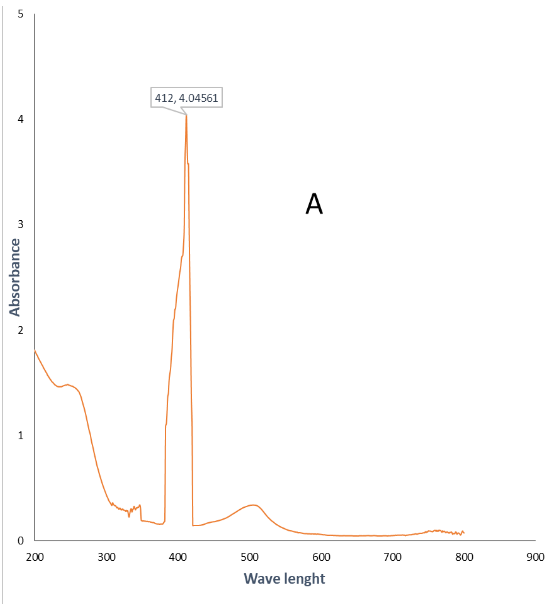

Figure 1a: Uv-vis of ZM-AgNPs

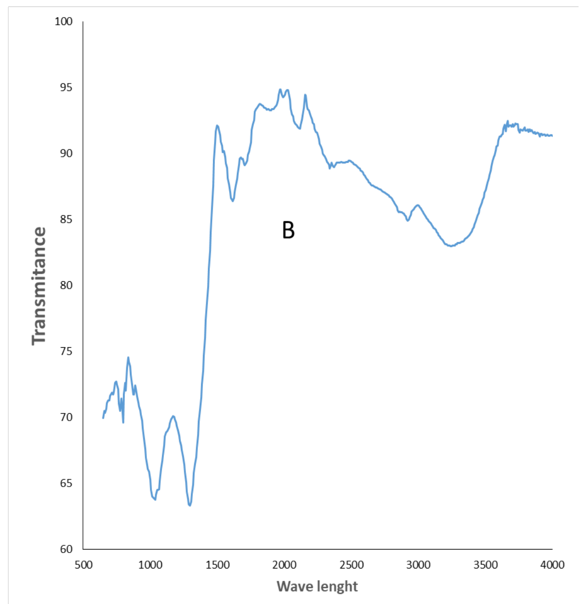

Figure 1b: FTIR of ZM-AgNPs

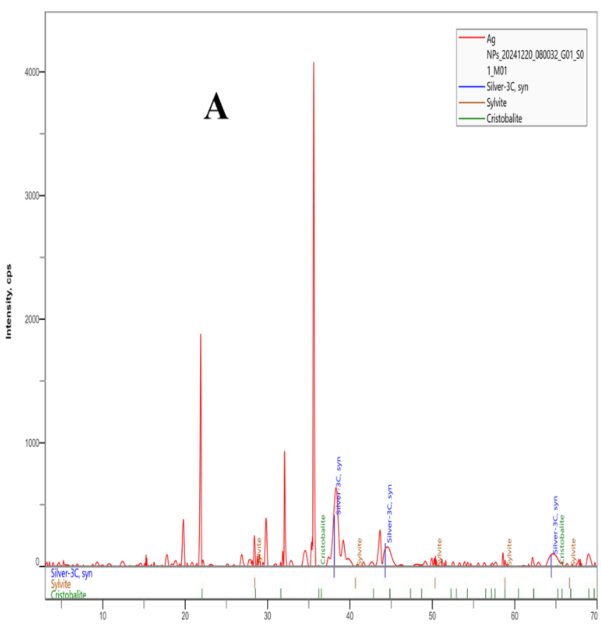

Figure 2a: XRD of ZM-AgNPs

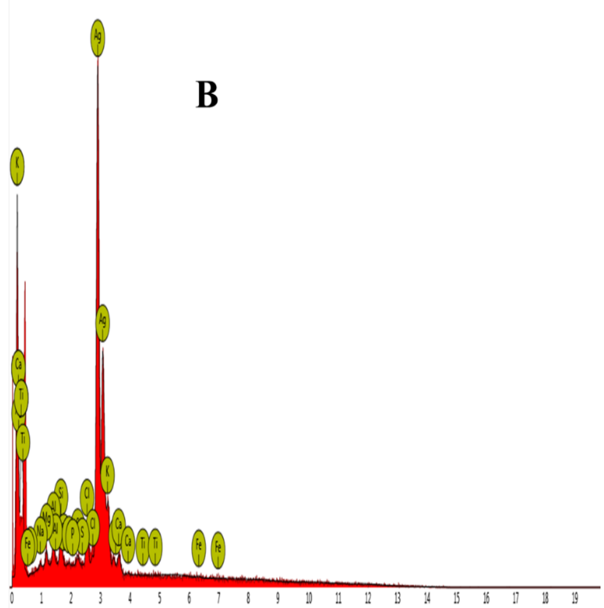

Figure 2b: EDAX of ZM-AgNPs

Invitro anti-diabetic effect

Alpha-amylase and Alpha-glucosidase inhibitory effect of Zn-AgNPs

For α-amylase inhibition (Figure 3A), ZM-AgNPs exhibited the highest percentage inhibition across all concentrations when compared with ME-ZM and acarbose. The IC_{50} value of ZM-AgNPs (61.31 µg/mL) indicates a potent inhibitory effect, which is lower than that of ME-ZM, suggesting that nanoparticle synthesis enhanced the bioactivity of the plant extract. Similarly, in the α-glucosidase inhibition assay (Figure 4A), ZM-AgNPs again demonstrated stronger inhibition than ME-ZM and acarbose, with an IC_{50} value of 1.56 µg/mL, reflecting a higher potency and potential for glucose-lowering action.

These results imply that biosynthesized ZM-AgNPs significantly enhanced the inhibitory potential of Z. mucronata on carbohydrate-hydrolyzing enzymes compared to the crude extract, likely due to nanoparticle-mediated improvement in bioavailability, stability, and surface reactivity. Providing an insight for the recent use of nanoparticle into the drug delivery systems to improve the bio-availability of anti-diabetic drugs (Chauhan et al., 2024). This can also be attributed to antioxidant properties of silver nanoparticles by reducing oxidative stress markers in diabetic patients, highlighting their potential in mitigating the oxidative damage associated with diabetes mellitus (Alkhalaf et al., 2020)

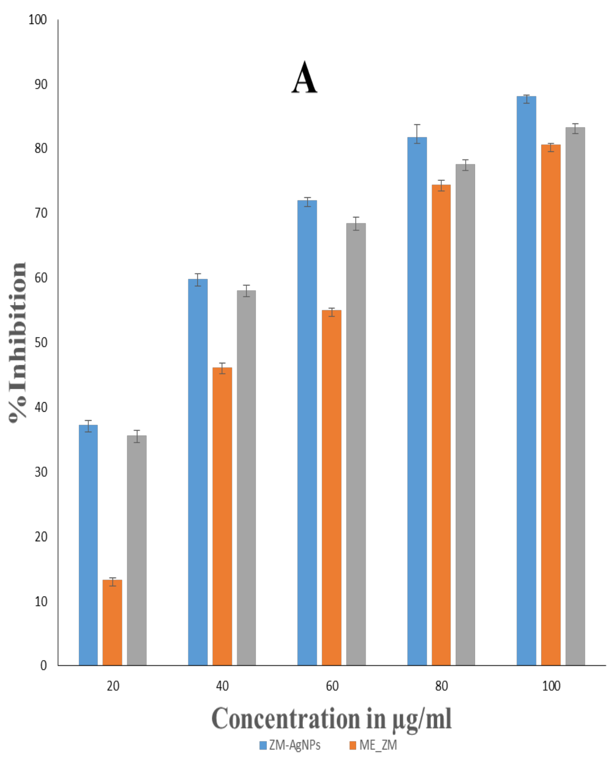

Figure 3a: Effect on alpha-glucosidase inhibition

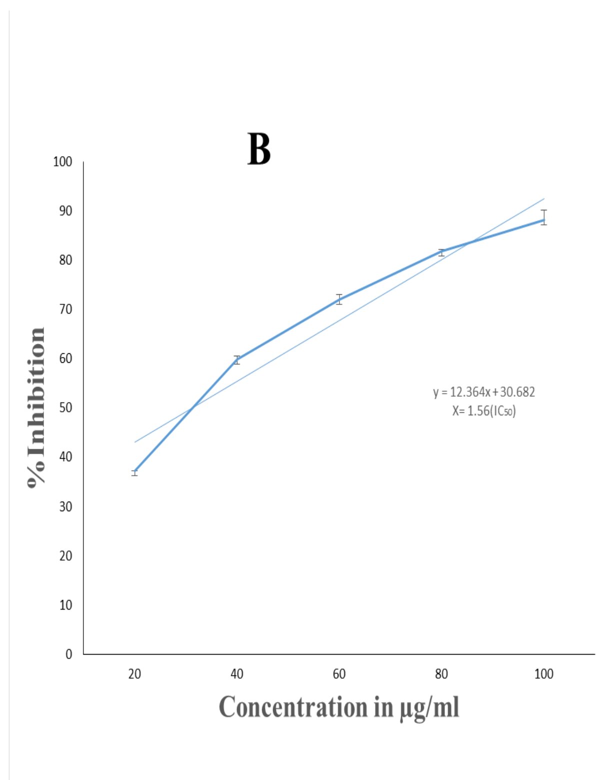

Figure 3b: IC_{50} of Zm-AgNPs on Alpha-glucosidase

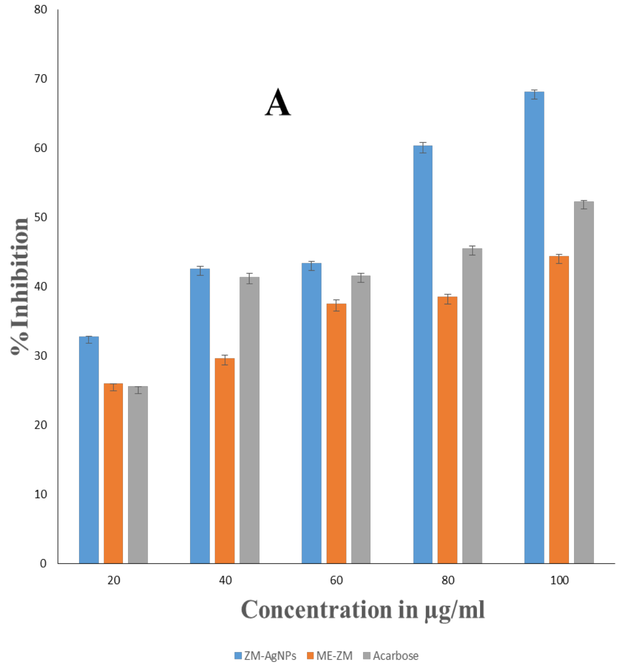

Figure 4a: Effect on alpha-amylase inhibition

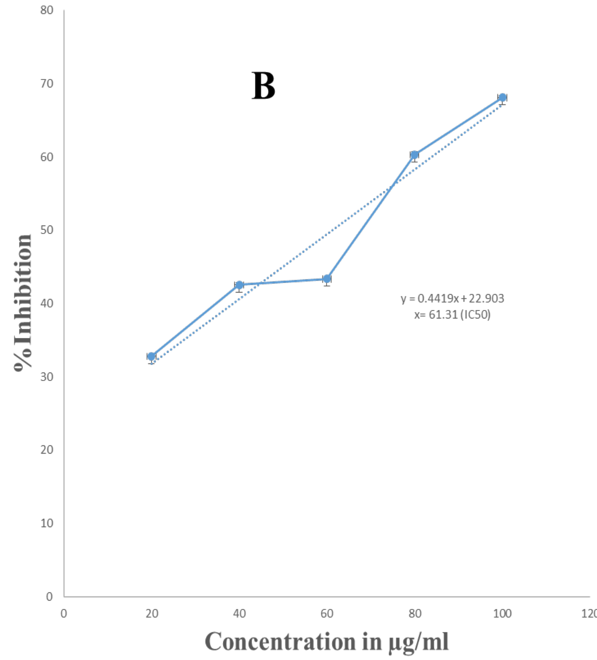

Figure 4b: IC_{50} of Zm-AgNPs on Alpha-amylase

Discussion

The abundant of phytocompounds present in the methanolic leaf extract provide plausible reducing and capping functions during AgNP synthesis. Phenolic and flavonoid hydroxyl groups can donate electrons to reduce Ag^+ to Ag^0, while polar groups (OH, C=O, amine functionalities) bind to the nascent nanoparticle surface to limit growth and aggregation. This mechanism is consistent with established plant-mediated (green) synthesis pathways (Kinayyigit, 2021).

The superior inhibitory activity of ZM-AgNPs compared to crude ME-ZM suggests that nanoparticle synthesis enhances the bioactivity of phytochemicals in Z. mucronata. This improvement is likely due to increased surface area, stability, and more effective enzyme–ligand interactions facilitated by silver nanoparticle conjugation, as reported in previous studies on plant-mediated nanoparticles

The strong α-amylase and α-glucosidase inhibitory activities observed for ZM-AgNPs are consistent with previous reports indicating that metallic nanoparticles synthesised using medicinal plant extracts can enhance enzyme inhibition and antidiabetic efficacy (Khanal, 2023). The improved activity of ZM-AgNPs compared to the methanolic extract suggests that phytochemicals from Z. mucronata including flavonoids, phenolics, and saponins not only served as reducing and capping agents during nanoparticle synthesis but also enhanced the enzyme binding affinity of the resulting nanocomposite.(Malik et al., 2025)

The α-amylase and α-glucosidase inhibitory actions are key mechanisms in managing postprandial hyperglycaemia, as these enzymes are responsible for carbohydrate digestion and glucose release.(Ćorković et al., 2022) The lower IC_{50} values of ZM-AgNPs in both assays therefore indicate their higher efficiency in delaying carbohydrate hydrolysis and absorption, mimicking the pharmacological effect of acarbose but with potentially fewer side effects, as noted in other green-synthesized AgNP studies (Parthasarathy, 2024).

Comparable findings were reported by (Dhanalekshmi et al., 2022), in which silver nanoparticles synthesised from Ziziphus spina-christi extract showed significant inhibitory effects on α-glucosidase and α-amylase. Similarly, (Nate, 2018) demonstrated that plant-mediated AgNPs derived from Azadirachta indica and Moringa oleifera significantly outperformed their crude extracts in antidiabetic assays, confirming that nanoparticle synthesis enhances bioefficacy through nanoscale interaction and increased enzyme affinity.

Conclusion

In conclusion, the higher inhibitory potency of ZM-AgNPs suggests a synergistic effect between silver ions and bioactive metabolites, which may act by competitive binding to the enzyme's active site or altering enzyme conformation. Such enhanced performance reinforces the therapeutic potential of nanobiotechnology in developing cost-effective and biocompatible antidiabetic agents, especially from indigenous medicinal plants like Z. mucronata, which is traditionally used in African ethnomedicine for managing diabetes and related metabolic disorders.

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