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IN VITRO ANTI-DIABETIC, ANTI-OBESITY, AND ANTIOXIDANT-RELATED ENZYME INHIBITORY POTENTIALS OF GREEN-SYNTHESISED SILVER NANOPARTICLES OF *Zingiber officinale* RHIZOME ALKALOIDS

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ABSTRACT

Globally, diabetes and its complications are becoming a major health burden. However, treatment of these conditions has become unrealistic, expensive and in some cases inaccessible with its attendant side effects. Natural products with phytochemicals like alkaloids, flavonoids, quercetin, among others, have shown some therapeutic effects, however, the nanoparticles of alkaloids from ginger are yet to be investigated. Hence, this study investigated the potential therapeutic ability of silver nanoparticles of ginger rhizome alkaloids (SNPGR) to inhibit the major enzymes implicated in diabetes and obesity. Alkaloids were extracted from the ginger rhizomes, subsequently followed by qualitative tests to confirm the presence of alkaloids. The SNPGR was then synthesized, and the inhibitory potentials of the synthesized nanoparticles were tested against α-amylase, α-glucosidase, lipase and xanthine oxidase. Alkaloids presence was confirmed qualitatively and spectrophotometric wavelength scan revealed the synthesis of the nanoparticle at 294 nm. The SNPGR at various concentrations (10 to 100 µg/mL) significantly inhibited the activities of α-amylase (10 to 75%), α-glucosidase (40 to 70%), xanthine oxidase (60 to 80%) and lipase (40 to 75%). These results revealed the therapeutic potentials of SNPGR as a likely solution to diabetes and its complication, and therefore, call for further studies.

1. INTRODUCTION

Diabetes mellitus is a chronic and multifaceted metabolic disorder; they are associated with persistent hyperglycemia. Factors that influence this condition are either impaired insulin secretion or insulin action, or both. This can arise from carbohydrate, protein, and lipid metabolism and disturbance. In most cases, it leads to some long-term complications such as retinopathy, cardiovascular disease, nephropathy, and neuropathy (Ajayi et al., 2016; American Diabetes Association, 2024). In the recent years, global prevalence of diabetes has increased greatly due to dietary pattern, aging population, sedimentary life style, lack of physical exercise and urbanization. In 2025, the International Diabetes Federation (IDF), reported that approximately 589 million adults were living with diabetes, with a projection of about 853 million by 2050. This health concern is rising sharply in the developing countries, especially Kenya, South Africa and Nigeria Sub-Saharan Africa. The healthcare system in these countries is often inadequate with either obsolete or no equipment to manage the metabolic diseases (Atun et al., 2017; Bruce et al., 2025).

The general ways of diabetes management had been reported to include modification of life style as well as therapeutic regimen, such as oral hypogenic and insulin agents like α-glucosidase inhibitors, Metformin® (a biguanide), and sulfonylureas (ADA, 2024). In developed countries like America, Finland and Germany, many facilities like integrated care systems, more equipment and latest therapeutic discoveries have been used to reduce complication rate, and to achieve glycemic control. The contrast is the case in developing countries, especially in Africa, where many countries do not have enough medical personnel or low expertise among medical personnels, limited health facilities, drugs, outdated diagnostic equipment, as well as high cost of treatment, which ultimately leads to delayed or low-quality treatment (Eseadi et al., 2023: Viswanathan et al., 2020: Hall et al., 2011). Furthermore, all of these factors in developing countries contribute to high rates of diabetes complications, including renal impairment and diabetic foot ulcers (Zulfiqarali et al., 2025; Mekonnen et al., 2024; WHO, 2024; Ogbera and Ekpebegh, 2014). Nigeria has one the largest diabetes prevalence in Africa, with over 3.6 million people living with diabetes in 2025 (IDF, 2025; Uloko et al., 2018). Hence, the need for complementary and alternative therapeutic solutions that are affordable, accessible, and culturally acceptable.

Key metabolic enzymes like lipase, α-glucosidase, α-amylase, and xanthine oxidase contributes to diabetes mellitus. Carbohydrate metabolic digestive enzymes, α-amylase and α-glucosidase, hydrolyse dietary polysaccharides into absorbable simple sugars thereby contributing directly to postprandial hyperglycemia (Mitra et al., 2026; Zeng et al., 2025; Hasaninezhad et al., 2020; Krentz and Bailey, 2005). As such, inhibition of these enzymes could serve as a major reason for discovery or development of therapeutic drugs. Also, pancreatic lipase, which is responsible for lipid digestion, affects insulin secretion by modulating the circulating free fatty acids (Subramaniyan et al., 2025: Shetty et al., 2021: Birari and Bhutani, 2007); whereas xanthine oxidase (XO) is a key enzyme of purine metabolism, which catalyzes the conversion of hypoxanthine to uric acid with reactive oxygen species as the end product. Invariably resulting into insulin resistance, oxidative stress and endothelial dysfunction. These are major contributors to diabetes and its complications (Hasan et al., 2022; Hernandez-Hernandez et al., 2022; Battelli et al., 2016; Desco et al., 2002). Therefore, inhibition of XO represents a promising molecular target for reducing oxidative damage and metabolic dysregulation associated with diabetes.

Structurally diverse bioactive compounds have been found in natural products, presenting with great beneficial effects in the treatment of metabolic disorders (Latif and Nawaz, 2025; Noce et al., 2021). Most phytochemicals like alkaloids, phenolic, and flavonoids compounds have shown antidiabetic effects (Muema et al., 2023; Ardalani et al., 2021). The mechanisms of action for these promising compounds includes inhibition of oxidative stress-causing enzymes, and carbohydrate-digesting enzymes, improvement of uptake of peripheral

glucose and reduction of inflammation (Modak et al., 2007; Tiwari and Rao, 2002). Advances in nanotechnology have enabled the development of metal nanoparticles as novel therapeutic systems, with silver nanoparticles (AgNPs) receiving particular attention due to their high surface area, physicochemical stability, and bioactivity (Iravani et al., 2014). Biogenic synthesis of AgNPs using plant-derived alkaloids offers dual functionality, as these compounds act as both reducing and capping agents while imparting pharmacological properties such as antioxidant and enzyme inhibitory activities (Ahmed et al., 2016). These features suggest that alkaloid-mediated AgNPs may enhance the therapeutic efficacy of natural products as an alternative way of managing diabetes and its complications.

The present rate of increase in the prevalence of diabetes, and the different limitations associated with the current treatment pharmacotherapy, there is an urgent need to develop cost-effective antidiabetic agents and multifunctional antidiabetic agents derived from natural sources. Ginger (*Zingiber officinale*) rhizome contains bioactive constituents with established antioxidant, anti-inflammatory, and metabolic regulatory properties (Ali et al., 2008). This study, therefore, investigates the therapeutic potential of silver nanoparticles synthesized from Ginger rhizome alkaloids by evaluating their inhibitory potentials on enzymes involved in diabetes pathophysiology (α-amylase, α-glucosidase, lipase, and xanthine oxidase) and their possible applications as novel nanotherapeutic agents.

2.0 **MATERIALS AND METHODOLOGY**

2.1. Ginger (*Zingiber officinale*)

Ginger rhizomes were collected from Oja-Oba market, Osogbo, Osun State, Nigeria. The authentication was carried out at the Department of Botany, Obafemi Awolowo University, Nigeria with voucher number IFE/18417.

2.2. Chemicals

All the chemicals or reagents used in this study were of analytical grade. Silver nitrate ($AgNO_3$), Chloroform, Ethanol, Sulphuric acid (H_2SO_4), Conc. ammonium hydroxide (NH_4OH), Methanol, Xanthine, Allopurinol, Xanthine oxidase, Ginger, Phosphate buffer, P-nitrophenol, Metformin®, α-amylase, α-glucosidase, and lipase.

2.3. Extraction of alkaloid from ginger rhizome

Alkaloids were extracted from air-dried ginger rhizome using method of Abdelouaheb et al. (2006) with little modifications. 10 g of ginger powder was weighed and soaked with 40 mL of ethanol and distilled water. The mixture was then stirred with the aid of magnetic stirrer, poured into a jar and left for 2 hours. A clean sieve was then used to filter the mixture into a clean Jar while the shaft was thrown away. The pH of the solution was then adjusted to between pH 1 and 2 with the aid of H_2SO_4. The solution was then separated using a separating funnel with the addition of eluting solvent chloroform two times. From the two distinct layers formed, the lower layer was decanted and the upper layer discarded. The pH of the resulting solution was then adjusted to

between pH 9 and 10 using ammonium hydroxide solution. Mixture of chloroform and methanol in ratio 70:30 and then followed by chloroform alone. The lower part was then collected, dried and kept for further studies.

2.4 Test for Alkaloids

A small portion (0.1 g) of tannic acid was added to 9mL of distilled water in a volumetric flask and then shaken thoroughly to dissolve. 5 mL of alkaloid of ginger rhizome extract solution was added to a test tube and the tannic acid solution was then poured into the test tube, and the mixture was shaken well. Immediately, a pale-yellow colour change was observed and white turbidity formation, which indicates presence of an alkaloid (Al-Naqqash et al., 2014).

2.5 Synthesis of AgNPs of Ginger Rhizome alkaloids

AgNPs of ginger rhizome alkaloids were synthesized by mixing 1mL of the ginger rhizome alkaloids with 4mL of 0.0017M of silver nitrate ($AgNO_3$) solution. The mixture in a beaker was kept in a water bath for 10 min at 60 ºC. Thereafter, stored in a dark environment for more than 24 hours.

2.6 UV- Spectrophotometric characterization of Silver Nanoparticles of Ginger Rhizome Alkaloids

The synthesized silver nanoparticle of ginger rhizome alkaloids was analysed using UV-Visible spectroscopy, distilled water was used as the blank for calibration. The absorbance peak (290- 350 nm range) confirmed the formation of the nanoparticle.

2.7 Antidiabetic *In vitro* Assays

2.7.1 *In vitro* α-amylase inhibitory assay

Starch azure (2 mg) was dissolved in 0.2 mL of 0.5 M Tris-NaOH buffer (pH 9.5) containing 0.01 M $CaCl_2$ to prepare the substrate solution. The mixture was boiled for 5 minutes and pre-incubated at 37°C for 5 minutes. Silver nanoparticles synthesized from ginger rhizome alkaloids were dissolved in DMSO to obtain concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. Thereafter, 0.2 mL of each sample concentration was added to the substrate solution, followed by 0.1 mL of porcine pancreatic α-amylase (2 U/mL) prepared in Tris-NaOH buffer.

The reaction mixture was incubated at 37°C for 10 minutes and terminated by adding 0.5 mL of 50% acetic acid. The mixture was centrifuged at 3000 rpm for 5 minutes at 4°C, and the absorbance of the supernatant was measured at 595 nm using a spectrophotometer. Acarbose was used as the standard α-amylase inhibitor according to the method of Sani and Nair (2017). Percentage inhibition was calculated using the formula below:

α-amylase inhibitory activity=Abs of Test/Abs of Control×100

2.7.2 *In vitro* α-glucosidase inhibitory assay

The capacity of silver nanoparticle alkaloid of ginger rhizome extracts to inhibit α-glucosidase was measured in a 96-well microplate reader at 405 nm (Kazeem et al., 2013). Each well contained a 50 µL sample and 100 µL enzyme (1 U/mL) solved in buffer (12.5 mM Na_2HPO_4, 3.3 mM NaH_2PO_4; pH = 6.9). After 10 minutes of incubation at room

temperature, 50 µL pNPG (3 mM) was added and incubated at 37 °C for 15 minutes (absorbance readings took place every 5 min since the addition of substrate). Control wells contained 50 µL of solvent. The inhibition was calculated using the following formula:

$$\textbf{Inhibition (\%)} = \left\{ \frac{\textbf{(Abs control − Abs sample)}}{\textbf{Abs control}} \right\} \textbf{x100} \quad \textbf{……equation (1)}$$

2.7.3 *In vitro* lipase inhibitory assay

Type II lipase from pancreases (Sigma Co., St. Louis, USA) was prepared in a solution of 10 mM and 1 mM EDTA (pH 6.8) was incubated with the silver nanoparticles of ginger rhizome alkaloids (25,50 and 100µg/ml) in a Tris (hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH 7.0) containing 100 mM Tris-HCl and 5mM $CaCl_2$ for 15 minutes. A substrate solution (10 mM p-nitrophenyl butyrate) in acetonitrile was added to the mixture. After 30 minutes of incubation at 37ºC, the absorbance of the formed p-nitrophenol was read at 405 nm. Orlistat® (F. Hoffmann-La Roche AG, Basel, Switzerland) was used as the reference compound (Kim et al., 2010). The percentage of lipase inhibition was calculated using equation (1)

2.7.4 *In vitro* xanthine oxidase inhibitory assay

Xanthine oxidase inhibitory activity was determined using the spectrophotometric method of Noro et al. (1983). Silver nanoparticles of *Zingiber officinale* rhizome alkaloid were prepared at concentrations of 10, 20, 40, 60, 80, and 100 µg/mL, while 100 µg/mL Allopurinol in methanol served as the standard. Each sample was mixed with 1.3 mL of 0.05 M phosphate buffer (pH 7.5) and 0.2 mL xanthine oxidase solution (0.2 U/mL), followed by incubation at room temperature (25°C) for 10 minutes. Thereafter, 1.5 mL of 0.15 M xanthine substrate solution was added, and the reaction mixture was further incubated at 25°C for 30 minutes. Absorbance was measured at 293 nm against a blank containing 0.5 mL methanol, 1.3 mL phosphate buffer, and 0.2 mL xanthine oxidase solution using a spectrophotometer. Percentage inhibition of xanthine oxidase activity was calculated using the formula below:

Percentage inhibition (%) of xanthine oxidase= {(A-B) – (C-D)}/(A-B)} × 100

Where:

A is the activity of the enzyme without the compound,

B is the control of A without the compound and enzyme,

C and D are the activities of the compound with or without XO, respectively.

3.0 RESULTS AND DISCUSSION

3.1 Results

The SNPGR was initially monitored *via* visual inspection. Upon the addition of the aqueous silver nitrate solution to the isolated alkaloid fraction, a distinct and rapid color transition from a light yellow to a deep, dark brown was observed. This macroscopic change serves as a primary, qualitative indicator of Ag^{+} reduction to metallic silver (Ag^{0}) and the subsequent excitation of surface plasmon resonance (SPR) as shown in Figures 1.

Figure 1: (a) $AgNO_3$ with ginger rhizome alkaloid-rich extract (b) Colourchange that indicated nanoparticles synthesis

These nanostructures were confirmed by UV-Visible spectrophotometric analysis. The electronic absorption spectrum of the reaction mixture exhibited a prominent, well-defined SPR absorption maximum at λ = 294 nm. This sharp spectral peak aligns with the characteristic optical signatures of localized surface plasmon resonance for nanoscale silver, verifying the successful reduction, stabilization, and capping of the nanoparticles by the ginger rhizome alkaloidal constituents (Figures 2).

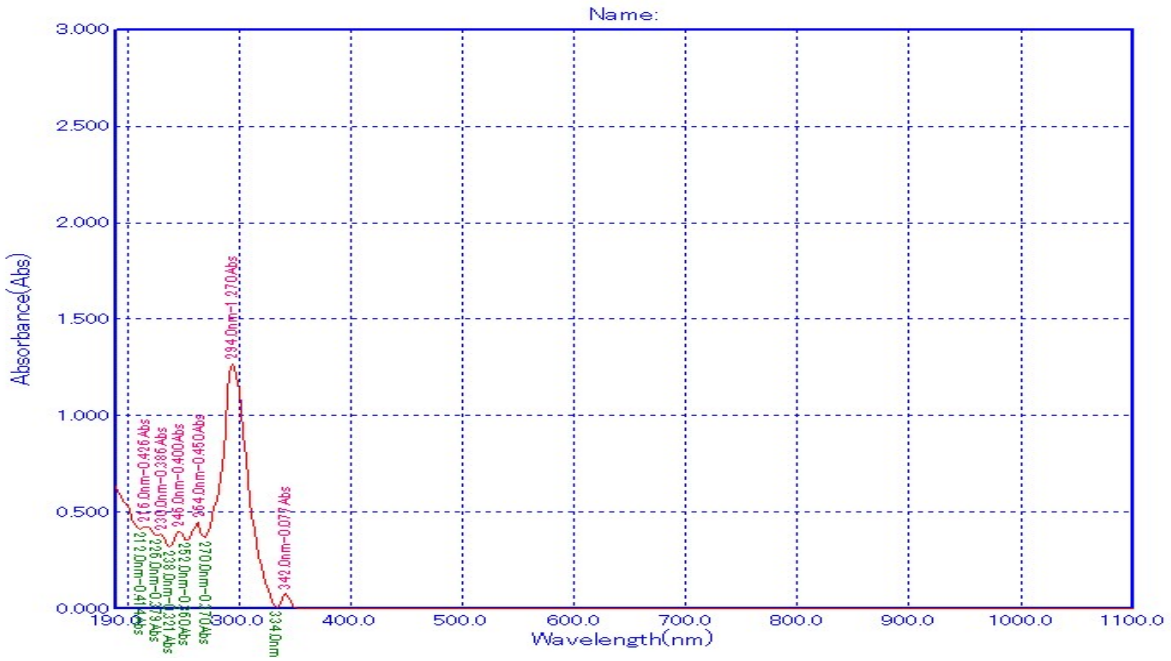

Figure. 2: Wave length scan detection of silver nanoparticles of ginger rhizome alkaloids at 294.0 nm (Abs 1.270)

The synthesized SNPGR demonstrated potent, concentration-dependent inhibitory profiles against key metabolic enzymes implicated in diabetes and obesity, namely α-amylase, α-glucosidase, pancreatic lipase, and xanthine oxidase. The nanoparticles exhibited substantial inhibitory activity against α-amylase across the tested concentration range of 10 to 100 µg/mL with inhibition percentages escalating from 10% at the lowest concentration to 75% at the peak concentration (Figure 3).

Similarly, robust biocatalytic suppression was observed for both α-glucosidase and pancreatic lipase within the 10 to 60 µg/mL range. Specifically, SNPGR mediated a significant, dose-dependent reduction in α-glucosidase activity, achieving an inhibitory range of 40% to 70%, while concurrently suppressing lipase activity by 40% to 75% (Figure 4-6).

In contrast, the inhibitory dynamics against xanthine oxidase revealed a distinct kinetic profile; the most pronounced and statistically significant suppression of the enzyme occurred at the minimum concentration of 10 µg/mL, contributing to an overall inhibition band of 60% to 80% across the treatment spectrum (Figure 7).

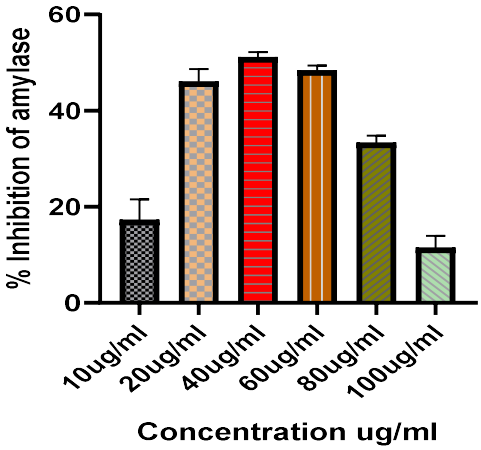

Figure 3: Silver nanoparticles of ginger rhizome alkaloids inhibitory activities on α-amylase

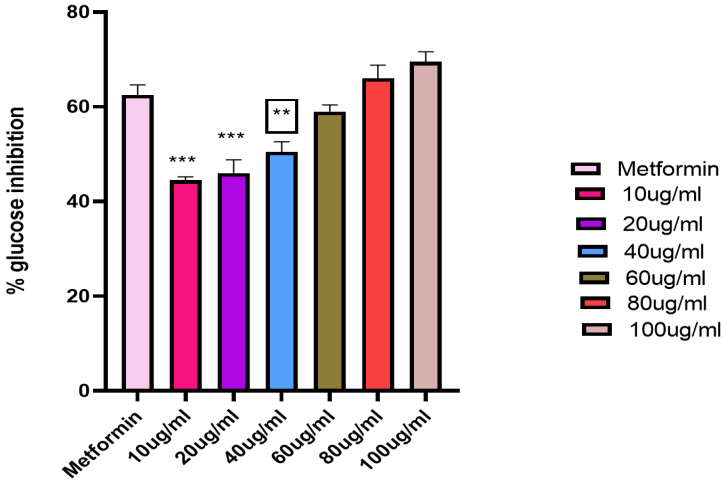

Figure 4: Silver nanoparticles of ginger rhizome alkaloids inhibitory activities on α-glucosidase

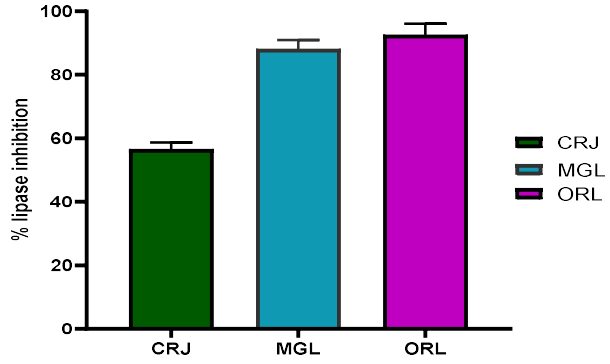

Figure 5: Comparison of lipase inhibitory abilities of popular herbal supplements against the standard drug, Orlistat®

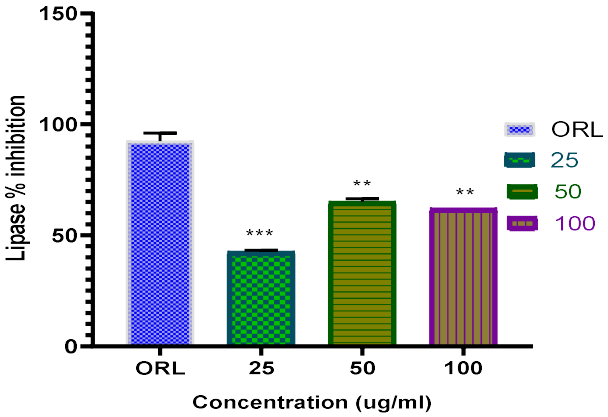

Figure 6: Silver nanoparticles of ginger rhizome alkaloids inhibitory activities against lipase

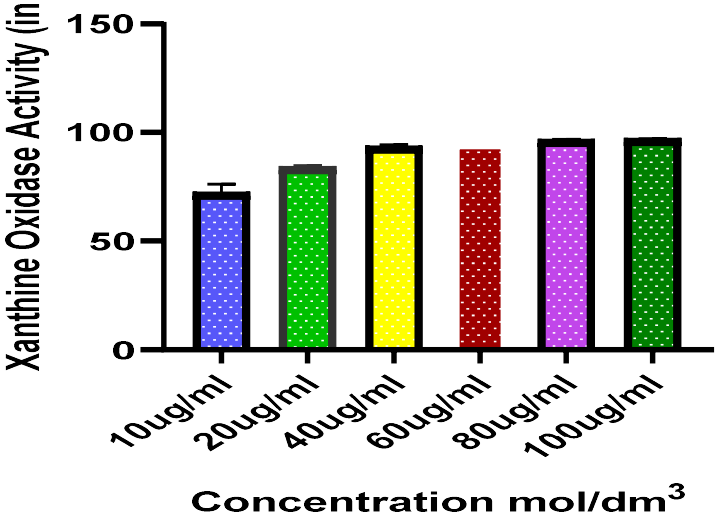

Figure 7: Silver nanoparticles of ginger rhizome alkaloids inhibitory activities against xanthine oxidase

3.2 Discussion

Diabetes is a multi-dimensional, -factorial and -systemic metabolic complication. Worldwide, the attendant public health effects, financial burden, and number of growing reported cases is of great concern (Chhajed and Arora, 2022; IDF, 2025). Recent reports suggest that the rate of increase of diabetes and its complications in Africa requires urgent attention (Davies et al., 2022; Motala et al., 2022; Godman et al., 2020). In West Africa, Nigeria is reported to have the highest number of cases of diabetes with an exponential rate of complication of DFU (Ugwu et al., 2019). However, it is logical to hypothesize that if the incidence of diabetes is biochemically reduced, it would improve world economy and make life bearable.

The confirmation of the presence of alkaloids extracted from ginger rhizomes suggests that this vegetal part can be useful for its anti-inflammatory, anti-diabetes and anti-bacterial purposes. In the report of Alqahtani et al. (2020), zinc nanoparticles of ginger peels showed ~~a~~ great antibacterial and antifungi activities. Also, Wan et al. (2019) established the inhibitory effect of the alkaloid extract of *Corydalis yanhusuo* (Corydalis) on vascular endothelial growth factor (VEGF)-induced angiogenesis in both *in-vitro* and animal models. As shown in Figure 1 and 2 above, the formation of the silver nanoparticles of ginger rhizome alkaloids was confirmed at 294 nm by the UV- Vis spectroscopy. Although, the formation of silver nanoparticles is usually detected between 400 and 550 nm, in this case, the presence of alkaloids as a reducing agent may have shifted the wavelength at which the reduction was achieved (Ahmed et al., 2016). This signals the possibility of an enhanced therapeutic use of the AgNPs possessing miniature size advantage achieved when the positive charge of the Ag^{+} ion became reduced to Ag^{0}. Therefore, improving both the access and absorption of the synthesized nanoparticles at the target site to elicit a positive therapeutic effect. In 2025, Chang et al. reported a similar trend of results.

Furthermore, α-amylase (in the mouth) and α-glucosidase (in the stomach) have been reported to have a key role in diabetes management. If these enzyme activities are controlled, the over-production or high concentration of the glucose within the systemic circulation would be controlled or managed for optimum glycemic control (Fariha et al., 2023; Nariman et al., 2023; Kim et al., 2022). As shown in Figure 3 above, the AgNPs showed a significant inhibition potential on α-amylase, which is a major enzyme for starchy food digestion that affects hyperglycemia from which insulin resistance and diabetes may arise. This suggest that the hydrolyzation of starch into glucose will be inhibited, thus curtailing the glycemic index of a carbohydrate meal. This ultimately results in the reduction of glucose availability or its non-availability as the case may be. As shown in figure 4 above, the silver nanoparticles of ginger rhizome alkaloids equally inhibited (59 to 69.5 %) the activities of α-glucosidase with no significant difference between Metformin®, the standard drug and the AgNPs at

various concentrations (40-100 µg/mL). This may lead to the inhibition of the breakdown of complex carbohydrates into glucose in the small intestine which ultimately reduces glucose availability to systemic circulation, thereby reducing post-prandial blood glucose level. These results support the claims of Ahmed et al. (2016), Hasya et al. (2022) and Uddin et al. (2022). If the AgNPs can normalize systemic glucose levels, the incidences of diabetes can be reduce as also postulated by the findings of Sonia (2021), Hasya et al. (2022), Fariha et al. (2023), and Uddin et al. (2022).

Additionally, in order to determine the most suitable standard drug that can effectively control obesity, and underlying metabolic condition of diabetes, Figure 5 showed a comparative *in vitro* inhibitory activity assay result of already established supplements conducted against porcine lipase. The supplements, Magilim® (MGL) and Cordy Royal Jelly® (CRJ) were compared to Orlistat® (ORL). The ORL gave the best inhibitory activity (92.5%) followed by MGL (88%) and then CRJ (56.5%). The comparable inhibitory activities observed for these nutraceuticals may be due to the different nutritional composition, *viz*: 250 mg of CRJ comprises of *Cordyceps*militaris (100 mg), Royal Jelly freeze-dried powder (40 mg), oligomeric proanthocyanidin (30 mg), *Foliumginkgo* (Gingko leaf extracts, 30 mg), and red rice substance (50 mg); while 300 mg capsule of MGL comprises of akonjac glucomannan (204.2 mg), chistosan (83 mg),Vitamin C (10 mg), and chromium-accumulated yeast (2.8 mg); whereas ORL is a synthetic hydrogenated form of lipstatin, which acts by binding intestinal lipases. In the small intestine and stomach, ORL forms a covalent bond with the active serine site of lipases. With reference to Figure 6, AgNPs inhibited lipase at various concentrations tested from 25 to 100 µg/mL thus showing highest inhibitory potential, especially at 100 µg/mL being comparable to the activity of the standard drug. This suggests that AgNPs have anti-obesity property in agreement with the findings of Haguet et al. (2023), Uddin et al. (2022), Ajayi et al. (2016), and Kim et al. (2010) on the therapeutic inhibition of porcine pancreatic lipase *in vitro*. Also, the inhibitory activities of the AgNPs were investigated against xanthine oxidase, an enzyme that when inhibited leads to the reduction of the production of oxidative stress, which may slow down the progression of diabetes complications. As reported in Figure 7, the silver nanoparticles of ginger rhizome alkaloids showed a promising inhibitory ability against xanthine oxidase at various concentrations ranging from 10 to 100 µg/mL, with most potent activity at 80 µg/mL. This aligns with the work of Zang et al. (2024), Floris et al. (2021), and Ajayi et al. (2016) on the therapeutic inhibition of the enzyme *in vitro*.

4.0 **CONCLUSION**

Collectively, these findings of this study underscore the multi-target therapeutic efficacy of the alkaloid-capped nanostructures in modulating carbohydrate, lipid, and purine metabolic pathways. It can be concluded that the formulated AgNPs of *zingiber officinale* rhizome alkaloid exhibited antidiabetic, anti-obesity and reduction of reactive oxygen species.

This implies that, it has therapeutic potential in the treatment of diabetes, diabetes complications and other related disease conditions. However, further in vivo and topical application of the nanoparticles is highly recommended.

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