INHIBITORY EFFECT OF WATER FRACTION OF THE LEAVES OF *Lonchocarpus cyanescens* ON ALPHA AMYLASE AND ALPHA GLUCOSIDASE

BY

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CERTIFICATION

We, the undersigned, hereby certify that **OSARIEMEN EMMANUEL ELOGHOSA (BMS2101449)** carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City, and we approve the same as adequate in scope and quality for the reward of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

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DEDICATION

This seminar is dedicated to God Almighty and my parents, Mr and Mrs Osariemen**,** whose steadfast support has been a source of strength and inspiration. To my father, whose physical absence has never diminished the impact of his presence in my life, your presence has been deeply felt in every step I take through your words, prayers, and most importantly, your actions, they continue to direct my path and shape me into the man I am becoming. To my mother, whose meek spirit, unwavering prayers, quiet resilience, and steadfast hope for my success have been both my anchor and a constant source of encouragement. Thank you both for providing the basis upon which I stand. This milestone is as much yours as it is mine.

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia due to impaired insulin secretion or action. Inhibiting carbohydrate-digesting enzymes such as α-amylase and α-glucosidase is a recognized strategy to reduce

postprandial glucose spikes. Lonchocarpus cyanescens is a medicinal plant widely used in West African ethnotherapy with reported antidiabetic activity. This study aimed to evaluate the inhibitory effects of the water fraction of L. cyanescens on α-amylase and α-glucosidase and compare its efficacy with acarbose. The water fraction of L. cyanescens leaves was prepared and tested at concentrations of 0.1–0.5 mg/mL using standard in vitro enzyme inhibition assays, and the results were statistically analysed using one-way ANOVA followed by LSD post hoc tests to compare treatment groups and assess significance relative to acarbose. The water fraction demonstrated concentration-dependent inhibition of both enzymes, with α-amylase inhibition ranging from 76.79% to 89.26% and α-glucosidase inhibition from 36.60% to 61.22% ($p < 0.01$). Although acarbose produced consistently higher inhibition values (>90%), statistical comparison using an independent samples t-test (equal variance assumed) confirmed that its inhibitory activity was significantly greater than that of the water fraction ($p < 0.001$). Nonetheless, the water fraction exhibited substantial inhibition of both enzymes, and overall, the findings indicate that it possesses significant α-amylase and α-glucosidase inhibitory activity, supporting its traditional use and potential as a natural complementary therapy for diabetes management.

CHAPTER 1

1.0 INTRODUCTION

Diabetes mellitus is a multifaceted metabolic disorder arising from pancreatic dysfunction that disrupts the normal processing of carbohydrates, proteins, and fats due to impaired insulin secretion, impaired insulin action, or a combination of both (Eluehike and Onoagbe, 2018). These disturbances lead to persistent elevations in blood glucose, predisposing individuals to systemic complications such as dyslipidaemia, hypertension, atherosclerosis, retinopathy, neuropathy, and nephropathy (Eluehike and Onoagbe, 2018). Despite extensive global education and prevention campaigns, the prevalence of diabetes continues to rise. According to the IDF Diabetes Atlas, an estimated 589 million adults were living with diabetes in 2024, with projections reaching 853 million by 2050. Diabetes accounted for approximately 3.4 million deaths in 2024, and over 40% of affected adults (about 252 million people) remain undiagnosed (IDF, 2025). Type 2 diabetes (T2DM) constitutes the majority of cases, affecting roughly 530 million adults. Gestational diabetes mellitus (GDM) also contributes significantly to the global disease burden, with an estimated 18.9 million live births affected in 2024. Additionally, the prevalence of secondary diabetes is rising, contributing to the recognition of Type 5 diabetes (T5DM), which affects 20–25 million individuals and is associated with early-life malnutrition (IDF, 2025).

Carbohydrate-digesting enzymes, particularly α-amylase and α-glucosidase, represent key therapeutic targets in diabetes management. α-Amylase catalyses the breakdown of complex starches into oligosaccharides, while α-glucosidase, located on the small intestinal

brush border, hydrolyses these intermediates into absorbable glucose. Inhibition of these enzymes slows carbohydrate digestion and reduces postprandial hyperglycaemia, a critical strategy in diabetes control (Jaber, 2023; Olayinka *et al*., 2016).

Acarbose, a pseudo-oligosaccharide and established α-glucosidase inhibitor, is commonly used to manage postprandial glucose spikes by competitively inhibiting intestinal α-glucosidases and partially inhibiting pancreatic α-amylase, thereby delaying carbohydrate digestion and absorption (Yousefi *et al.*, 2023). Its clinical use, however, is often limited by gastrointestinal side effects, such as flatulence, bloating, and diarrhoea, resulting from the fermentation of undigested carbohydrates (Zhu *et al*., 2020). Newer agents, including semaglutide and tirzepatide, offer improved glycaemic control through incretin-based mechanisms, but they also cause nausea, vomiting, gastrointestinal discomfort, and occasional gallbladder complications, and may not be tolerated by all patients (Wilding *et al*., 2021; Del Prato *et al*., 2022). These limitations, combined with high costs, highlight the need for safer, affordable, and accessible plant-derived enzyme inhibitors as alternative or complementary therapies.

L*onchocarpus cyanescens,* a climbing shrub native to West Africa, has long been used in ethnomedicine for the management of diabetes. Experimental studies support its traditional uses: leaf extracts exhibit potent inhibition of α-amylase and α-glucosidase, with IC_{50} values of 3.69 mg/mL and 0.13 mg/mL, respectively, and significantly reduce postprandial glucose levels in starch-loaded rats (Kazeem and Davies, 2015). Its phytochemical composition, including flavonoids, chalcones, rotenoids, pterocarpans, and triterpenoids, likely contributes to this bioactivity. Toxicological evaluations have also

reported minimal hepatotoxicity across a wide dose range of aqueous leaf extracts (Onyeije *et al*., 2024), supporting its safety for therapeutic use. Given its enzyme-inhibitory potential, ethnomedicinal relevance, and favourable safety profile, *L. cyanescens* represents a promising candidate for further investigation, particularly its water fraction for managing T2DM and potentially GDM.

1.1 Aim of Study

This study aims to investigate the inhibitory effects of the water fraction of *Lonchocarpus cyanescens* leaves on α-amylase and α-glucosidase by evaluating its concentration-dependent activity and comparing its inhibitory potency with acarbose at corresponding concentrations

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus represents a multifaceted group of metabolic disorders characterized by persistently elevated blood glucose levels resulting from disturbances in insulin production, insulin action, or both. It has a significant impact on the endocrine system, particularly the pancreas, which produces enzymes involved in carbohydrate metabolism (amylase), protein metabolism (trypsin, chymotrypsin, carboxypeptidase), and fat metabolism (lipase, phospholipase) (Eluehike and Onoagbe, 2018; Zaworski *et al*., 2025). The disease is marked by an increase in both fasting and postprandial glucose levels (Anu *et al*., 2020). The pancreas plays a central role in glucose homeostasis, as beta cells in the islets of Langerhans are responsible for the production and release of insulin (Morales-Brown, 2025). According to Zheng, Ley, and Hu (2018), diabetes develops when the intricate balance between insulin secretion and peripheral insulin sensitivity is disrupted, resulting in metabolic abnormalities that progressively affect vascular, renal, neural, and ocular systems. Globally, diabetes is a major cause of morbidity and mortality.

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes worldwide and is primarily characterized by insulin resistance and an inadequate compensatory insulin response. Goyal, Singhal, and Jialal (2023) describe T2DM as a condition in which peripheral tissues, such as muscle, liver, and adipose tissue, become less responsive to insulin, compelling beta cells to increase insulin output, a response that is eventually unsustainable. The condition may result from a combination of genetic and lifestyle factors,

including obesity, poor dietary choices, physical inactivity, and environmental exposures (Zheng, Ley, and Hu, 2018). T2DM typically develops gradually, and many individuals remain undiagnosed until complications arise, underscoring the importance of early detection and prevention. Treatment often involves lifestyle modifications, pharmacotherapy, and regular physical activity. However, some antidiabetic drugs are associated with toxicity and adverse effects, prompting research into plant pharmacology and phytochemistry as alternative or complementary therapeutic approaches.

The global burden of diabetes is substantial and rising. The International Diabetes Federation (IDF) Diabetes Atlas (2025) estimated that approximately 589 million adults aged 20 to 79 years were living with diabetes in 2024, with projections reaching 853 million by 2050. Data from 138 countries indicate that the global prevalence of diabetes in high, middle, and low-income nations was 10.4%, 9.5%, and 4.4%, respectively (IDF, 2024; Olamoyegun *et al*., 2024). In Nigeria, the prevalence of T2DM is rapidly increasing. A systematic review and meta-analysis by Olamoyegun *et al.* (2024), which analysed 60 studies spanning the period from 1989 to 2024, reported a pooled prevalence of 7.0% (95% CI: 6.4–9.6%), with a mean participant age of 48.55 ± 11.21 years. This corresponds to approximately 8.02 million Nigerian adults living with T2DM, almost double the previous modelled IDF estimate of 3.6% in 2021. Regionally, the South-South region of Nigeria exhibited the highest pooled prevalence. Further research in Nigeria identified major risk factors for T2DM, including family history, socioeconomic status, physical inactivity, urban residence, obesity (particularly BMI > 25), and low vegetable consumption (Olamoyegun *et al*., 2024).

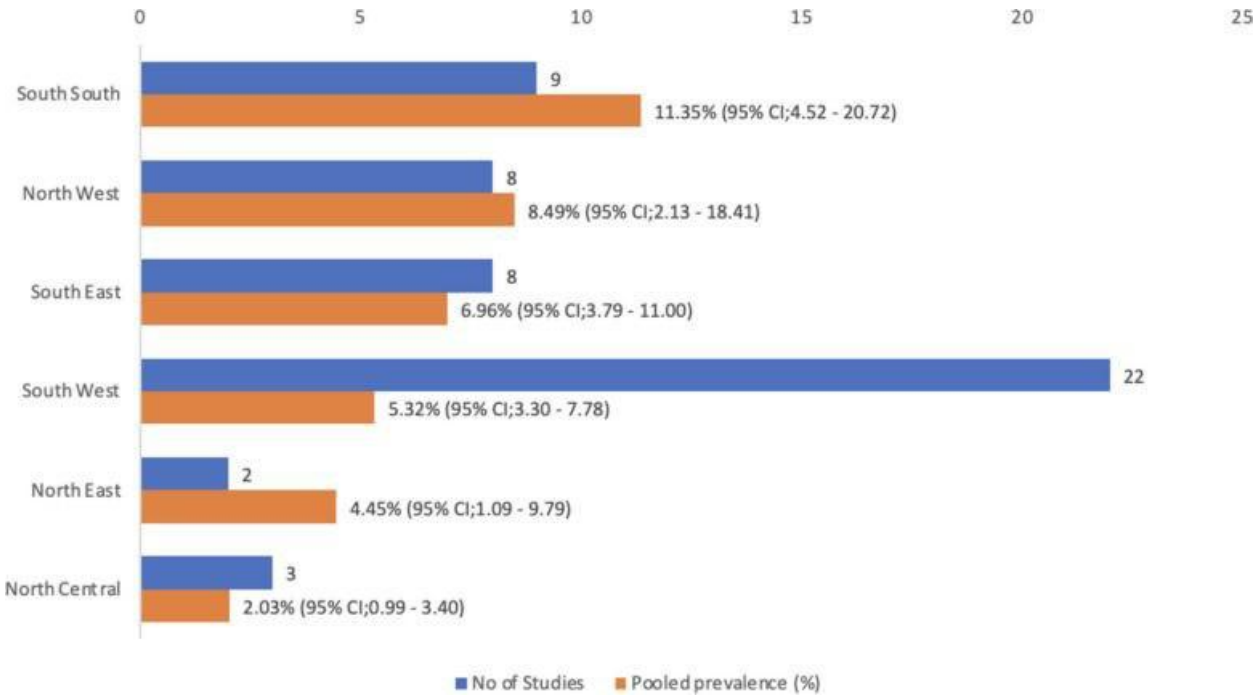

Figure 2.1: Pooled Prevalence of Type 2 Diabetes Mellitus and Study Counts by Geopolitical Zone in Nigeria (Olamoyegun *et al.,* 2024)

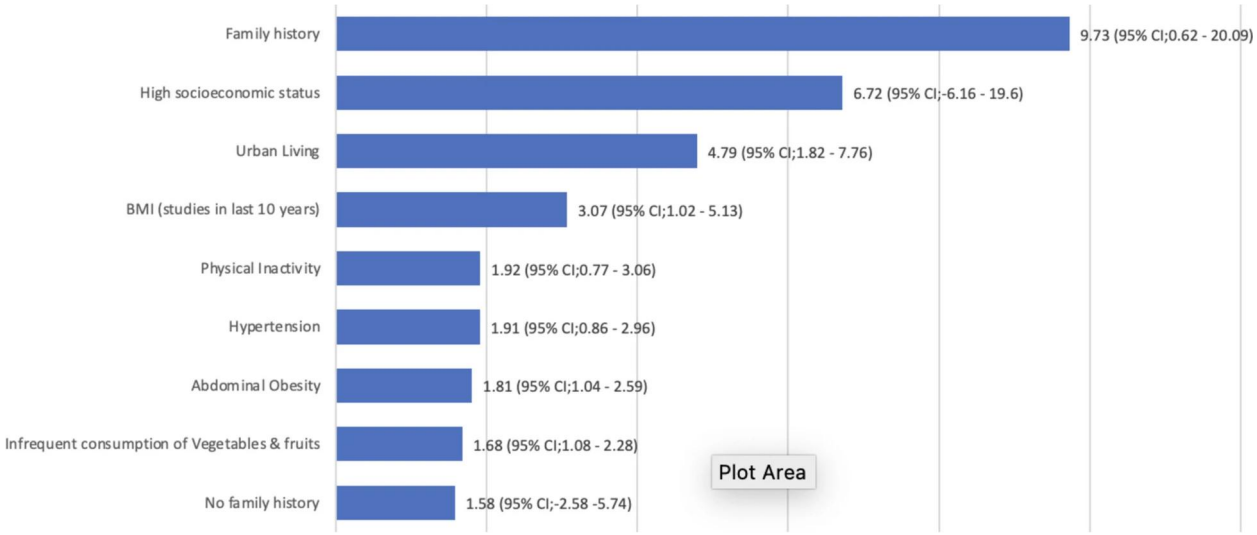

Figure 2.2: Forest Plot of Pooled Odd Ratios for the Association Between Risk Factors and Type 2 Diabetes Mellitus in Nigeria (Olamoyegun *et al.,* 2024)

Gestational diabetes mellitus (GDM) refers to glucose intolerance first identified during pregnancy. Although temporary in many cases, it reflects underlying metabolic vulnerability and is linked to insulin resistance aggravated by pregnancy-associated hormonal changes. According to Goyal, Singhal and Jialal (2023), women with GDM have an increased likelihood of developing type 2 diabetes later in life, while the condition also poses risks to maternal and fetal health. Olayeye *et al* (2024). Risk factors such as obesity, advanced maternal age, and family history of diabetes are particularly prominent among Nigerian women. Because pregnancy unmasks latent defects in insulin sensitivity, gestational diabetes serves as an important clinical indicator of future metabolic disease. It highlights the need for targeted interventions during and after pregnancy.

2.2 Enzymes and standard reference compounds in Antidiabetic screening.

2.2.1 α-Amylase (EC 3.2.1.1)

α-Amylase is a key endo-hydrolase involved in the initial stages of dietary carbohydrate breakdown. It catalyses the cleavage of internal α-1,4-glycosidic bonds in starch and glycogen to release maltose, maltotriose, and limit dextrins. In humans, two major isoforms occur: salivary α-amylase (AMY1), which initiates starch digestion in the oral cavity, and pancreatic α-amylase (AMY2), which continues hydrolysis in the small intestine. Both isoforms share high structural similarity but differ in their site of secretion and relative activity under physiological conditions (Swe *et al*., 2019). Their complementary actions ensure efficient conversion of complex carbohydrates into oligosaccharides, providing substrates for subsequent α-glucosidase-mediated terminal digestion (Zheng, Ley and Hu, 2018).

Structure and Catalytic Mechanism

Human α-amylase belongs to the glycoside hydrolase family GH13, characterised by a conserved $(\beta/\alpha)_8$ TIM barrel that houses the catalytic residues. Its three-domain architecture comprises:

1. Domain A – a central TIM barrel containing the catalytic triad responsible for glycosidic bond cleavage;

2. Domain B – a calcium-binding loop that provides thermostability and structural rigidity;

3. Domain C – a β-sheet-rich C-terminal domain implicated in substrate binding and product release.

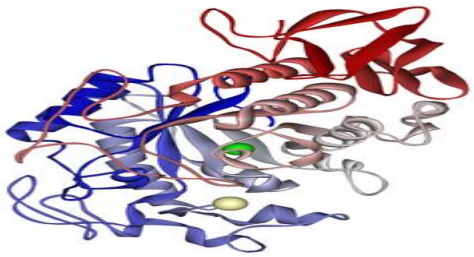

Figure 2.3: Ribbon diagram of human salivary alpha-amylase (PDB 1SMD) (Fvasconcellos, 2007)..

Catalysis proceeds via a double-displacement (retaining) mechanism, involving formation of a covalent glycosyl enzyme intermediate and an oxocarbenium ion-like transition state (Brayer *et al.*, 2020). Chloride ion binding modulates the active-site geometry, enhancing catalytic turnover and affecting inhibitor interactions.

Biochemical Properties and Physiological Role

α-Amylase exhibits optimal activity at near-neutral pH (6.5–7.0 in saliva and ~7.2 in the duodenum). The enzyme requires calcium ions for structural stability and chloride ions for catalytic activation. These cofactors significantly influence the performance of in vitro assays, particularly in studies evaluating inhibitor potency (Hedrington and Davis, 2019). Physiologically, pancreatic α-amylase plays a central role in postprandial glucose homeostasis by regulating the rate at which complex carbohydrates are converted into absorbable monosaccharides (Moelands *et al*., 2018).

Amylase activity is also clinically relevant. Elevated serum α-amylase serves as a biomarker for acute pancreatitis, while reduced activity may accompany advanced diabetic states due to exocrine pancreatic dysfunction. Experimental studies, including those evaluating antidiabetic tannins from Spondias mombin, have reported altered serum amylase activity in diabetic models, supporting a link between pancreatic enzyme dysregulation and hyperglycaemia-related metabolic disturbances.

Assay Considerations

Multiple analytical methods are used for assessing α-amylase activity and inhibition, including:

● DNSA assay, which quantifies reducing sugars released during starch hydrolysis;

● Chromogenic substrates such as p-nitrophenyl-maltoside, which yield coloured products measurable spectrophotometrically;

● Dyed-starch assays, which provide high sensitivity for screening natural-product inhibitors.

Modern kinetic analyses increasingly employ non-linear regression models to derive accurate Km, Vmax, and Ki values and to classify inhibitors as competitive, non-competitive, or mixed-type (Zhen *et al.*, 2017).

Implications for Inhibitor Screening and Therapeutic Development

α-Amylase inhibition is a validated strategy for attenuating postprandial hyperglycaemia. However, complete inhibition is associated with gastrointestinal discomfort due to undigested starch fermentation. Therefore, moderate inhibition is often preferred in antidiabetic drug discovery (Hayward *et al.*, 2019). Natural products, particularly polyphenols and tannin-rich plant extracts, frequently exhibit such balanced inhibitory profiles.

2.2.2 α-Glucosidase (EC 3.2.1.20)

α-Glucosidases are exo-acting glycoside hydrolases localised on the brush border membrane of small intestinal enterocytes, where they catalyse the terminal steps of carbohydrate digestion. These enzymes hydrolyse α-1,4 glycosidic linkages at the non-reducing ends of oligosaccharides such as maltose, maltotriose, and limit dextrins, releasing free glucose, the only monosaccharide directly absorbable in significant

quantities across the intestinal epithelium (Hedrington and Davis, 2019). In humans, two major complexes constitute brush-border α-glucosidase activity:

1. Maltase-Glucoamylase (MGAM): optimised for long linear substrates;

2. Sucrase-Isomaltase (SI): responsible for sucrose breakdown and hydrolysis of α-1,6 branch points. Together, these complexes ensure efficient post-amylase digestion and strongly influence postprandial glycaemic excursions, making them a central therapeutic target in Type 2 diabetes mellitus (Zheng, Ley and Hu, 2018).

Structural Features and Catalytic Mechanism

Human intestinal α-glucosidases belong mainly to GH31, although catalytic principles overlap with GH13 enzymes. Crystallographic and structural analyses reveal a conserved catalytic motif comprising acidic residues that function as proton donors and nucleophiles. Depending on the specific isoform, catalysis may proceed via:

- a retaining mechanism, which forms and resolves a covalent glycosyl–enzyme intermediate, or
- an inverting mechanism, which directly inverts the anomeric configuration during hydrolysis (Brayer *et al.*, 2020).

Despite overarching similarities, subtle variations in the geometry, electrostatics, and topology of active site pockets account for differences in substrate specificity and underpin the selective binding behaviour seen with various inhibitor classes.

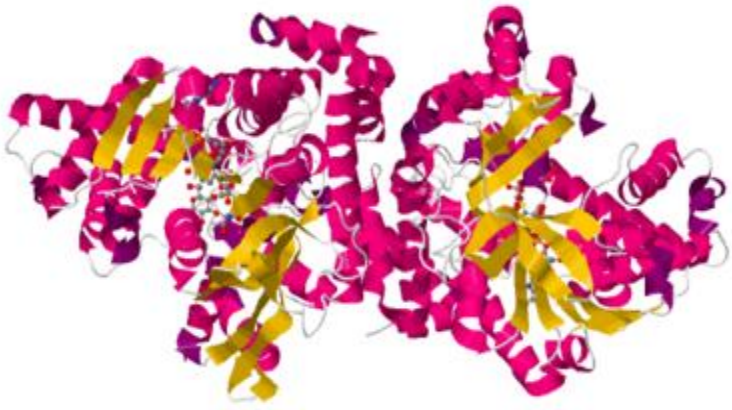

Figure 2.4: Alpha-glucosidase in complex with maltose and NAD+ (Ccfreeny, 2012)..

Assay Approaches for Activity and Inhibition Studies

- Functional characterisation of α-glucosidase frequently employs chromogenic model substrates, most commonly p-nitrophenyl-α-D-glucopyranoside (pNPG), which produces a quantifiable yellow product upon cleavage. Although convenient and highly sensitive, assay outcomes vary considerably depending on enzyme source:
- Yeast α-glucosidase is widely used due to its affordability and availability. However, its structural divergence from human enzymes limits physiological relevance and can overestimate inhibitor potency.
- Recombinant human MGAM or SI, though more expensive, provide superior translational accuracy in screening antidiabetic compounds (Zhen *et al.,* 2017).

Buffer composition, pH, ionic strength, and presence of cofactors must be standardised across assays, particularly when comparing inhibitory extracts, polyphenols, or synthetic molecules.

Inhibitor Classes, Mechanisms, and Structure Activity Relationships

α-Glucosidase is a major pharmacological target in diabetes management, and several structurally distinct inhibitor groups have been identified:

- Pseudo-oligosaccharides, such as acarbose, which mimic natural substrates and competitively occupy the active site;
- Iminosugars (e.g., 1-deoxynojirimycin), characterised by nitrogen substitution that stabilises transition-state-like conformations;
- Polyphenols and flavonoids, which frequently demonstrate mixed or noncompetitive inhibition, interacting at allosteric or peripheral binding regions (Hayward *et al.*, 2019);
- Protein-based inhibitors derived from plant sources, including legume-associated proteins with emerging relevance in metabolic regulation (Moreira *et al.*, 2024).

Structure-activity analyses consistently show that hydroxylation, glycosylation, prenylation, and ring conjugation significantly influence inhibitory strength and selectivity. Many natural inhibitors also exhibit multi-target effects, combining α-glucosidase inhibition with antioxidant, anti-inflammatory, or α-amylase-modulating activities. However, despite strong in vitro potency, several phytochemicals have limited in vivo effectiveness due to poor bioavailability, rapid phase-II metabolism, and gastrointestinal degradation. Recent advances in formulation science such as nanoencapsulation, phytosome complexes, and

gut-microbiota-stabilising delivery systems are increasingly shaping the evaluation of α-glucosidase inhibitors for therapeutic application (Moreira *et al*., 2024).

2.2.3 Acarbose as a Reference Standard (Positive Control)

Acarbose is a pseudo-oligosaccharide produced by Actinoplanes species and is widely used as a prototypical α-glucosidase inhibitor. It competitively inhibits intestinal brush-border α-glucosidases, and to a lesser extent pancreatic α-amylase, thereby attenuating postprandial glucose excursions. Clinical use of acarbose has shown modest reductions in glycosylated haemoglobin (HbA1c) and improvements in post-meal glycaemia. However, its gastrointestinal tolerability remains a significant limitation. The most commonly reported adverse effects include flatulence, abdominal discomfort, and diarrhoea, which are caused by bacterial fermentation of undigested carbohydrates in the colon.

Because of this, when evaluating new α-glucosidase or amylase inhibitors, it is essential to compare them directly to acarbose in identical assay conditions, so that their efficacy can be meaningfully benchmarked against this clinical standard

2.2.4 Dimethyl Sulfoxide (DMSO) (Negative Control)

Dimethyl sulfoxide (DMSO) is a polar aprotic solvent commonly used in enzyme assays to dissolve hydrophobic test compounds, including crude plant extracts, flavonoids, and synthetic inhibitors with limited aqueous solubility. Its high dielectric constant, strong hydrogen-bond-accepting

capacity, and miscibility with water allow stable solubilisation of bioactive constituents without precipitation in assay buffers (Hedrington and Davis, 2019). Although indispensable in α-amylase and α-glucosidase inhibition studies, DMSO must be used at controlled concentrations because excessive levels can disrupt enzyme structure, catalytic behaviour, or substrate interactions, potentially altering apparent inhibitor potency. To avoid such artefacts, most assays restrict DMSO to ≤1% v/v, ensuring that solvent effects do not independently influence enzyme activity (Zhen *et al.*, 2017), especially when comparing novel inhibitors to standards like acarbose (Moreira *et al.*, 2024). In phytochemical assays, DMSO supports the preparation of concentrated stock solutions, prevents aggregation in tannin- or flavonoid-rich fractions, and maintains compatibility with chromogenic substrates such as pNPG, thereby enhancing accuracy and reproducibility in antidiabetic screening protocols.

2.3 *Lonchocarpus cyanescens*

Lonchocarpus cyanescens Benth. (Fabaceae), commonly known as Yoruba indigo or "elu" among the Yoruba people of southwestern Nigeria, is a perennial climbing shrub distributed across West and Central Africa. It is best recognised as a source of indigo dye but has also been widely incorporated into traditional medicine (Burkill, 1995; Momoh *et al.,* 2014).

Figure 2.5:*Lonchocarpus cyanescens* (NMPPDB, 2025)

2.3.1 Taxonomy.

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Lonchocarpus

Species: *L. cyanescens* Benth.

2.3.2 Botanical description.

The plant is a vigorous twining shrub, often extending several metres in length. Leaves are pinnate with 5 –7 ovate to elliptic leaflets, flowers are violet to purple and borne in racemes, and fruits are flattened pods containing several seeds (Hutchinson and Dalziel, 1958).

2.3.3 Ethnopharmacological and Phytochemical Relevance

L*onchocarpus cyanescens* has been used traditionally for the management of various ailments. Ethnomedical applications include treatment of gastrointestinal disorders such as diarrhoea and dysentery, fever, malaria-like symptoms, skin infections, and postpartum cleansing (Isyaka *et al*., 2025). Ethnobotanical surveys indicate that decoctions of its leaves are sometimes administered for blood sugar regulation and as mild laxatives. A common decoction prepared from the leaves and roots is traditionally given to women during or after childbirth to cleanse and strengthen the uterus (Isyaka *et al*., 2025; Moronkola *et al*., 2013).

Phytochemical studies reveal that L. *cyanescens* is rich in bioactive secondary metabolites with potential therapeutic properties:

- Flavonoids: Known for their antioxidative, superoxide scavenging, aldose reductase inhibitory, and antidiabetic activities (Ajani *et al*., 2017; Muthu *et al*., 2006).
- Tannins and Phenolic Compounds: These compounds may inhibit α-glucosidase and α-amylase, reduce oxidative stress, and lower blood glucose levels (Ajani *et al*., 2017).

- Alkaloids: Alkaloids can influence insulin secretion and inhibit carbohydrate-digesting enzymes, contributing to antidiabetic effects (Sonibare *et al*., 2012).
- Triterpenoids: Triterpenoids may have insulin-sensitizing effects, influence glucose metabolism, and exhibit purgative and uterotonic properties (Moronkola *et al*., 2013; Ajani *et al*., 2017).
- Saponins and Glycosides: Saponins, a polar class abundant in legumes, can reduce intestinal glucose absorption and modify transporter expression. Their amphipathic nature allows partial retention in aqueous extracts, supporting their activity in traditional decoctions (Liu *et al*., 2013).
- Steroids: Plant steroids have been reported to modulate carbohydrate metabolism, improve insulin sensitivity, and exert anti-inflammatory effects, which may complement the antidiabetic potential of L. *cyanescens* (Sonibare *et al*., 2012).

2.4 Review of Previous Studies on Enzyme-Inhibition by Medicinal Plants

2.4.1 Moringa *oleifera*

Leaves of Moringa *oleifera* are among the most studied in antidiabetic research. In vitro assays have demonstrated that various fractions of the leaf extract strongly inhibit α-glucosidase, with some fractions achieving IC_{50} values comparable to or even lower than acarbose. This potent inhibition has been attributed to the presence of flavonoids, particularly quercetin and kaempferol glycosides, which not only inhibit enzyme activity but also provide antioxidant protection (Olayinka *et al*., 2016; Patintingan *et al*., 2023).

2.4.2 Camellia *sinensis* (Green Tea)

Polyphenol-rich extracts from Camellia *sinensis* (green tea) are well-known to inhibit both α-amylase and α-glucosidase. Among the active compounds, galloylated catechins, especially epigallocatechin gallate (EGCG), show particularly strong inhibitory activity. Computational (in silico) docking studies corroborate these findings, demonstrating that these catechins bind with high affinity to the active site of α-glucosidase (Zhu *et al*., 2020).

2.4.3 Phaseolus *vulgaris* (Common Bean)

Phaseolus *vulgaris* produces a proteinaceous α-amylase inhibitor (α-AI1) that has been shown to significantly reduce starch digestion in vitro and in vivo. Clinical trials indicate that this inhibitor may help with glycaemic control and weight management, although the efficacy varies depending on bean variety and how the beans are processed (Barrett and Udani, 2011; López-García *et al*., 2019).

2.4.4 Quercus *coccifera*

Jaber (2023) conducted both in vitro and in vivo studies on Quercus *coccifera* leaves to assess their potential to inhibit α-amylase and α-glucosidase. Multiple extraction methods were used, including methanol, boiled water, and microwave water. The methanolic extract was found to be the most active, with IC_{50} values of 0.17 mg/mL for α-amylase and 0.38 mg/mL for α-glucosidase, surpassing the activity of acarbose. In animal models (diabetic mice), this extract reduced blood glucose levels over 28 days, while also maintaining healthy liver markers (ASAT, ALAT) and renal markers (urea, creatinine). The study highlights how the extraction method and solvent polarity strongly influence the

yield of bioactive compounds, and demonstrates that even water-based extracts can significantly modulate carbohydrate-digesting enzymes.

2.4.5 Lonchocarpus *cyanescens*

Research directly investigating the antidiabetic enzyme-inhibitory properties of *Lonchocarpus cyanescens* provides strong support for its traditional use. Kazeem and Davies (2015) demonstrated that leaf extracts, particularly the ethanol extract, significantly inhibited both α-amylase and α-glucosidase, with reported IC_{50} values of 3.69 mg/mL and 0.13 mg/mL, respectively. Notably, the α-glucosidase inhibitory potency of the ethanol extract (IC_{50} = 0.13 mg/mL) was stronger than that of the aqueous extract (IC_{50} = 0.21 mg/mL) and exceeded the activity of the standard acarbose (IC_{50} = 0.63 mg/mL), indicating a remarkable capacity to suppress intestinal carbohydrate digestion. Enzyme-kinetic analyses revealed competitive inhibition of α-amylase and non-competitive inhibition of α-glucosidase, while in vivo experiments showed that treatment of starch-loaded Wistar rats significantly reduced postprandial blood glucose, confirming physiological relevance.

Beyond carbohydrate-hydrolysing enzymes, *L. cyanescens* has also demonstrated inhibitory activity against aldose reductase (AR), a key enzyme associated with diabetic complications such as neuropathy and cataract formation. Ajani *et al*. (2017) reported significant AR inhibition across multiple fractions, with the aqueous and chloroform fractions showing the strongest effects (IC_{50} = 0.06 ± 0.02 mM^{-1} and 0.09 ± 0.01 mM^{-1}, respectively). Phytochemical analyses identified phenols, flavonoids, tannins, and phlobatannins as major

constituents, which are widely recognised for their antioxidant and enzyme-modulating properties. Variation in inhibition modes, competitive in ethyl acetate, hexane, and methanol fractions, and mixed in aqueous and chloroform fractions, suggests diverse active phenolic compounds.

Safety evaluations further support the pharmacological potential of the plant. Onyeije *et al.* (2024) reported that sub-chronic administration of aqueous leaf extract (200–5000 mg/kg) for 42 days in Wistar rats produced only mild vascular dilatation and Kupffer cell activation, with no significant hepatic damage. These findings indicate low toxicity and justify further biochemical and therapeutic investigations, particularly in the context of enzyme-inhibition–based antidiabetic strategies.

2.4.6 Annona *muricata* (Soursop)

Agu, Eluehike and colleagues (2019) conducted a detailed investigation into the inhibitory effects of different solvent extracts, methanol, ethyl acetate, and dichloromethane obtained from the leaf, fruit pulp, stem bark, and root bark of *Annona muricata* on key carbohydrate-hydrolyzing enzymes. Their in vitro findings revealed that several extracts displayed markedly stronger inhibition of both α-amylase and α-glucosidase than the standard drug, acarbose. Notably, the stem-bark methanol extract and the fruit-pulp ethyl acetate extract showed pronounced α-amylase inhibitory activity, while the leaf methanol and fruit-pulp ethyl acetate extracts demonstrated significant α-glucosidase inhibition, in many cases surpassing acarbose. All extracts exhibited an uncompetitive mode of inhibition, indicating that they bind preferentially to the enzyme–substrate complex rather than the free enzyme. Molecular docking and compound isolation further identified 15-acetyl-guanacone

acetogenin as a prominent bioactive constituent, displaying strong affinity for the active sites of the target enzymes.

CHAPTER 3

3.0 MATERIAL AND METHODOLOGY

3.1 Chemicals and Reagents

Ethanol, distilled water, soluble starch, 3,5-dinitrosalicylic acid (DNSA), sodium phosphate buffer, sodium carbonate, p-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, and dimethyl sulfoxide (DMSO) were obtained from standard chemical suppliers. The enzymes α-amylase (porcine pancreas) and α-glucosidase (yeast) were also procured commercially.

3.2 Plant Collection, Identification, and Preparation of Extracts

Fresh leaves of *Lonchocarpus cyanescens* were collected from a local herb dealer in Lagos State, Nigeria. The plant was authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria, with voucher number UBH-F029, by Prof. H.A. Akinnibosun. The leaves were thoroughly washed, air-dried under shade at room temperature (~25°C) for three weeks, and then pulverized into a fine powder using a clean mechanical grinder. The powdered plant material (2,500 g) was extracted by maceration in 10 L of ethanol for 72 hours. The mixture was filtered through muslin cloth, and the filtrate was concentrated using a rotary evaporator and subsequently freeze-dried to obtain the crude ethanolic extract (CEE). The extract was stored in an airtight container at 4°C until use.

3.3 Fractionation of Crude Extract

Fractionation of the CEE was carried out using solvent-solvent partitioning as described by Jamil *et al*. (2012). Briefly, 50g of CEE was dissolved in 250 mL of ethanol (w/v) and transferred into a fractionating column, followed by fractionation with distilled water. The process was repeated 6 times. All fractions were freeze-dried and stored at 4°C for further experiments.

Figure 3.1: Solvent partitioning of L. *cyanescens* with Distilled water (left), Acetone and Hexane

3.4 Enzyme Inhibition Assays

3.4.1 α-Amylase Inhibitory Assay

The α-amylase inhibitory activity of the crude extract of *L. cyanescens* was determined according to Kolawole *et al*. (2020). Briefly, 500 µL of the extract (0.1-0.5 mg/mL) was

mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9, containing 0.006 M NaCl) containing 0.5 mg/mL α-amylase, and incubated at room temperature for 10 minutes. Subsequently, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) was added, and the mixture was incubated at 25°C for 10 minutes. The reaction was stopped by adding 1.0 mL of 96 mM DNSA reagent. Test tubes were heated in a boiling water bath for 5 minutes and then cooled. Absorbance was measured at 540 nm. The control was prepared by replacing the extract with DMSO, while acarbose was used as the standard. Percentage inhibition was calculated using the formula: %Inhibition = $\frac{Acontrol - Aextract}{A\ control} * 100$

3.4.2 α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity of each fraction was measured using the method of Apostolidis *et al*. (2007). The substrate solution, p-nitrophenyl-α-D-glucopyranoside (pNPG), was prepared in 0.2 M phosphate buffer (pH 6.9). 1ml of α-glucosidase was incubated with 500 µL of the extract at different concentrations for 10 minutes at 25°C. Then, 500 µL of freshly prepared 5 mM pNPG solution was added. The reaction mixture was incubated for 5 minutes at 25°C and stopped by adding 2 mL of 30.1 mM Na_2CO_3. Absorbance was measured at 405 nm using a spectrophotometer. The control was prepared by replacing the extract with DMSO, while acarbose was used as the standard. Percentage inhibition was calculated using the formula %Inhibition = $\frac{Acontrol - Aextract}{A\ control} * 100$

3.5 Statistical Analysis

Data were analyzed using GraphPad Prism v10. Results were expressed as mean ± SEM of three replicates. Statistical significance was evaluated by one-way analysis of variance (ANOVA), followed by a two-sample t-test assuming equal variances and LSD post hoc test. Differences were considered significant at $p < 0.05$.

CHAPTER 4

4.0 RESULTS

4.1 α-Amylase

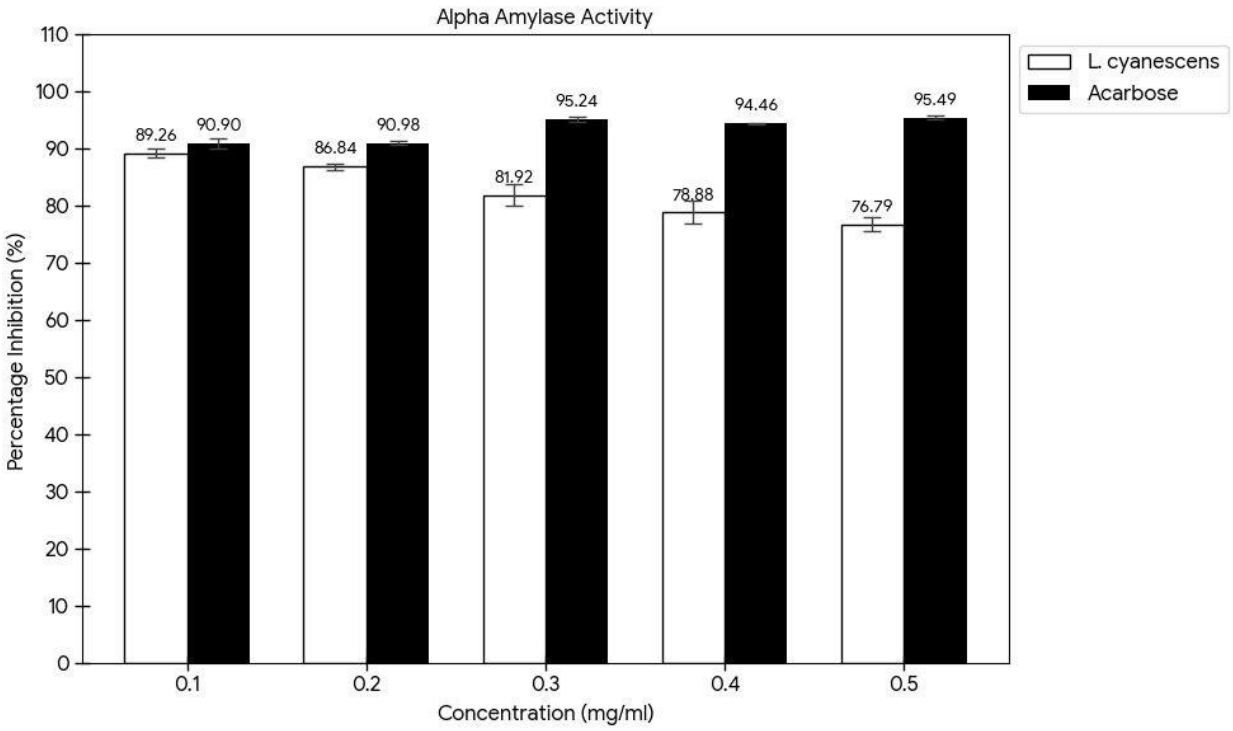

Figure 4.1 Inhibitory effect of L. *cyanescens* and Acarbose on alpha-amylase activity.

α-Amylase inhibitory activity of L. *cyanescens* water fraction compared with Acarbose within the concentration range of 0.1-0.5 mg/mL. Data are expressed as Mean ± SEM. The

assay was conducted against a control absorbance (A_0) of 0.813. One-way ANOVA revealed significant differences among all sample groups ($F(9,20) = 39.80$, $p < 0.001$). An independent samples t-test further confirmed a significant difference between the inhibitory activity of the L. *cyanescens* water fraction and Acarbose ($t(8) = 4.17$, $p = 0.003$).

4.2 α-Glucosidase

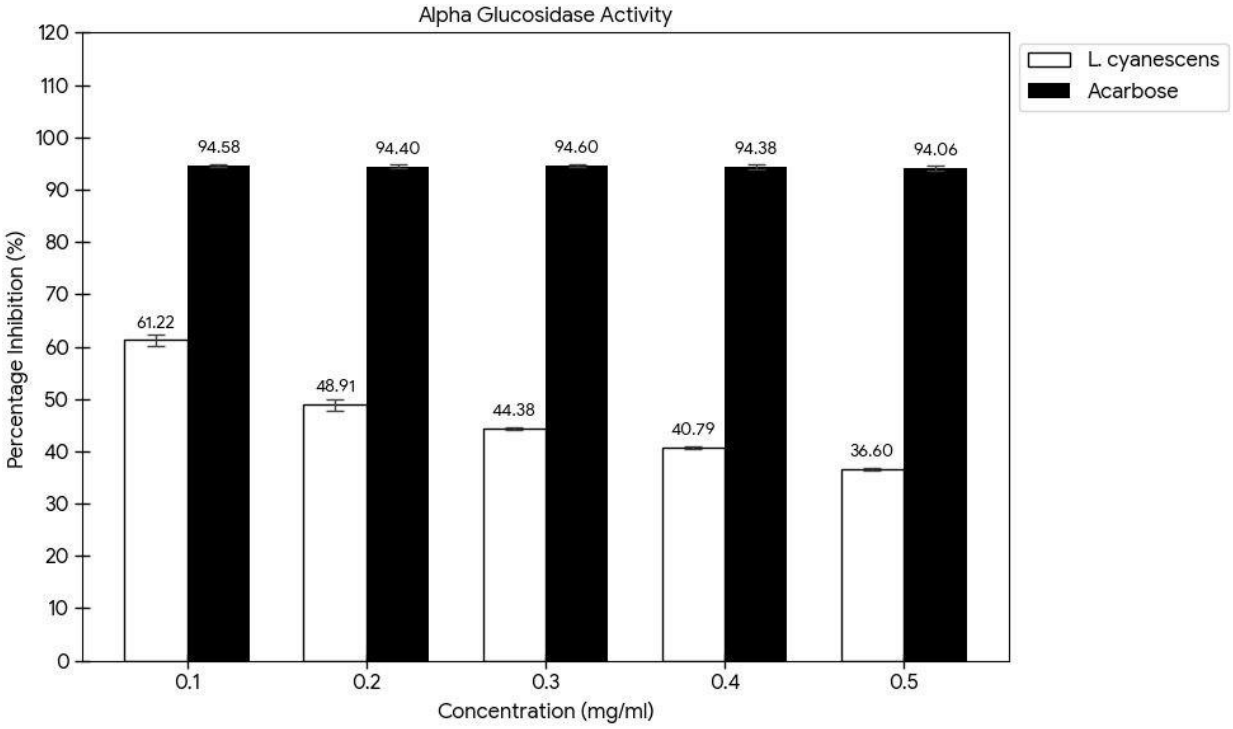

Figure 4.2: Inhibitory effect of L. *cyanescens* and Acarbose on alpha-glucosidase activity.

α-Glucosidase inhibitory activity of L. *cyanescens* water fraction compared with Acarbose across concentrations ranging from 0.1-0.5 mg/mL. Data are presented as Mean ± SEM. The assay was performed against a control absorbance (A_0) of 1.346. One-way ANOVA indicated significant differences among the sample groups ($F(9,20) = 2119.16$, $p < 0.001$).

An independent samples t-test showed a significant difference between the overall inhibitory effect of the L. *cyanescens* water fraction and Acarbose ($t(8) = 11.36$, $p < 0.001$), with Acarbose exhibiting significantly higher α-glucosidase inhibition.

CHAPTER 5

5.1 Discussion

α-Amylase and α-glucosidase are key enzymes involved in carbohydrate digestion, catalyzing the breakdown of starches into absorbable glucose. As such, they represent important therapeutic targets for managing postprandial hyperglycemia (Jaber, 2023; Olayinka *et al*., 2016). Inhibition of these enzymes slows glucose absorption, contributing to improved glycemic control in diabetes mellitus. L*onchocarpus cyanescens* has a history of traditional use for diabetes management, and previous studies have demonstrated that its leaf extracts inhibit both α-amylase and α-glucosidase. This activity is likely mediated by bioactive constituents such as flavonoids, chalcones, rotenoids, and triterpenoids, which also confer antioxidant protection (Kazeem and Davies, 2015; Onyeije *et al*., 2024). These findings support the potential of L. *cyanescens* as a culturally accepted, natural antidiabetic agent.

The present study evaluated the inhibitory potential of the water fraction of L. *cyanescens* against α-amylase and α-glucosidase, with comparisons to acarbose. The water fraction exhibited significant, concentration-dependent inhibition for both enzymes. Mean α-amylase inhibition ranged from 76.79 ± 1.24% to 89.26 ± 0.77%, while α-glucosidase inhibition ranged from 36.60 ± 0.24% to 61.22 ± 1.01%. Although acarbose demonstrated

consistently higher inhibition (α-amylase: 90.90–95.49%; α-glucosidase: 94.06–94.60%), the water fraction's activity was statistically significant ($p < 0.01$) and aligns with the study's aim of identifying potential natural inhibitors of carbohydrate-digesting enzymes.

The water fraction of *Lonchocarpus cyanescens* exhibited dose-dependent inhibition of α-amylase, albeit with an inverse concentration-response trend. Maximal inhibition occurred at the lowest concentration (0.1 mg/mL), decreasing progressively with higher concentrations up to 0.5 mg/mL. This non-linear pattern may indicate a critical threshold for optimal enzyme-inhibitor complex formation, where higher concentrations could lead to partial precipitation of active constituents or reduced availability of free inhibitory compounds in the reaction mixture. This contrasts with the potent ethanol extract of *L. cyanescens*, which displayed conventional competitive α-amylase inhibition (IC_{50} = 3.69 mg/mL) (Kazeem and Davies, 2015), highlighting the influence of solvent polarity on inhibitory phytochemical profiles. Similar inverse dose-dependent effects have been observed in plant-derived inhibitors from *Moringa oleifera* and *Phaseolus vulgaris* (Olayinka *et al*., 2016; Barrett and Udani, 2011). LSD post hoc analysis confirmed statistically significant differences among water fraction concentrations (e.g., A1 vs. A5; A2 vs. A4; A2 vs. A5, $p < 0.05$), indicating meaningful variation in inhibitory strength. Comparisons with acarbose revealed that the water fraction consistently exhibited lower potency across all concentrations, affirming the superior inhibitory efficiency of the pharmaceutical standard.

The water fraction demonstrated moderate α-glucosidase inhibition, following a similar inverse concentration-dependent trend as observed for α-amylase. Inhibition decreased from 61.22 ± 1.01% at 0.1 mg/mL to 36.60 ± 0.24% at 0.5 mg/mL. This activity is consistent with previous reports on *L. cyanescens* aqueous extracts (IC_{50} = 0.21 mg/mL) (Kazeem and Davies, 2015) and other polyphenol-rich plant extracts such as *Camellia sinensis* and *Annona muricata* (Zhu *et al.*, 2020; Eluehike, 2018), where flavonoids and tannins mediate α-glucosidase suppression. LSD post hoc analysis confirmed a statistically significant decline in inhibition with increasing concentration (e.g., A1 vs. A5; A1 vs. A4; A2 vs. A5, $p < 0.05$). Across all concentrations, the water fraction was significantly less potent than acarbose (e.g., A1 vs. AA1, $p < 0.05$), whereas acarbose maintained consistent and robust inhibition (>94%) with non-significant variation between concentrations, validating its role as a stable reference α-glucosidase inhibitor.

Overall, the aqueous fraction of L. *cyanescens* demonstrates promising inhibitory activity, supporting its traditional use in managing T2DM and potentially gestational diabetes mellitus (GDM). Its moderate but effective inhibition suggests that incorporation into dietary interventions such as decoctions, teas, or functional food preparations could confer glycemic benefits with a lower risk of gastrointestinal side effects compared to pharmacological agents like acarbose or incretin-based therapies (e.g., semaglutide, tirzepatide) (Yousefi *et al*., 2023; Wilding *et al*., 2021; Del Prato *et al.*, 2022). Its natural origin, safety profile, and ethnomedicinal acceptance make it a culturally and economically viable option, particularly in regions with limited access to conventional drugs.

A key limitation of this study is the lack of in vivo evaluation. While in vitro enzyme inhibition provides mechanistic insight, the pharmacokinetics, bioavailability, and systemic effects in animal or human models remain untested. Therefore, conclusions regarding clinical efficacy, therapeutic dosing, and potential side effects are preliminary. Future studies should investigate the antidiabetic potential of the water fraction in animal models of T2DM and GDM, including dose-response analysis, long-term safety, and incorporation into food-based formulations. Combinatorial studies with other bioactive plant extracts or standard antidiabetic agents may also enhance efficacy while minimizing adverse effects, providing a foundation for integrative diabetes management strategies.

5.2 Conclusion

The water fraction of *Lonchocarpus cyanescens* exhibits significant, concentration-dependent inhibition of both α-amylase and α-glucosidase in vitro. This activity supports its traditional use as a natural antidiabetic agent and suggests potential application in managing T2DM, GDM, and possibly prediabetic states. Although less potent than acarbose, its moderate enzyme inhibition, natural origin, and ethnomedicinal acceptance highlight its potential as a complementary or dietary-based therapy. Further in vivo studies are required to confirm efficacy, determine optimal dosing, and assess safety, which could ultimately support the development of culturally appropriate, plant-based interventions for glycemic control.

REFERENCES

Agu, K.C., Eluehike, N., Ofeimun, R.O. and others. (2019). Possible anti-diabetic potentials of *Annona muricata* (soursop): Inhibition on alpha-amylase and alpha-glucosidase activities. *Clinical Phytoscience* 5:21.

Ajani, E.O., Sabiu, S., Odufuwa, K.T., Ibrahim, T.B. and Salau, B.A. (2017). Evaluation of lens aldose reductase inhibitory and free radical scavenging potential of fractions of *Lonchocarpus cyanescens*: Potential for cataract mediation. *Pharmacognosy Journal* 9(1):62–69.

Apostolidis, E., Kwon, Y.I. and Shett, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science and Emerging Technologies* 8:46–54.

Barrett, M.L. and Udani, J.K. (2011). A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): A review of clinical studies on weight loss and glycemic control. *Nutrition Journal* 10:24.

Brayer, G.D., Luo, Y. and Withers, S.G. (1995). The structure of human pancreatic alpha-amylase at 1.8 Å resolution and comparisons with related enzymes. *Protein Science* 4(9):1730–1742.

Burkill, H.M. (1995). *The Useful Plants of West Tropical African*. Vol. 3, Families J-L. Royal Botanic Gardens, Kew.

Ccfreeny. (2012). Alpha-glucosidase in complex with maltose and NAD$^+$ [Image]. Wikimedia Commons.

Del Prato, S., Kahn, S.E., Purnell, J.Q., Hu, G., Trischitta, V., Weyer, C. and SURPASS-4 Investigators. (2021). Tirzepatide versus insulin glargine in type 2 diabetes and increased cardiovascular risk (SURPASS-4): A randomised, open-label, parallel-group, multicentre, phase 3 trial. *The Lancet* 398(10313):1811–1824.

Eluehike, N. and Onoagbe, I. (2018). Changes in organ and body weight, serum amylase and antidiabetic effects of tannins from *Spondias mombin* on streptozotocin-induced diabetic rats. *Journal of Metabolic Health* 3(1):a40.

Fvasconcellos. (2007). Ribbon diagram of human salivary alpha-amylase (PDB 1SMD) [Image]. Wikimedia Commons.

Goyal, R., Singhal, M. and Jialal, I. (2025). Type 2 diabetes. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing.

Hayward, N.J., McDougall, G.J., Fyffe, S., Farag, S. and Stewart, D. (2019). Bioactive polyphenols from berries modulate carbohydrate-digesting enzymes: A systematic review. *Critical Reviews in Food Science and Nutrition* 59(14):2262–2275.

Hedrington, M.S. and Davis, S.N. (2019). Considerations when using $alpha$-glucosidase inhibitors in the treatment of type 2 diabetes. *Expert Opinion on Pharmacotherapy* 20(18):2229–2235.

Hutchinson, J. and Dalziel, J.M. (1958). *Flora of West Tropical Africa*. Vol. 1, 2nd edn. London: Crown Agents for Overseas Governments: 632

International Diabetes Federation. (2024). *IDF Diabetes Atlas*, 11th edn. Brussels: International Diabetes Federation.

Isyaka, S.M., Olayemi, O.A. and Abdullahi, M.A. (2025). Phytochemistry and medicinal uses of *Lonchocarpus* species: Systematic review of a promising medicinal plant. *South Asian Research Journal of Natural Products* 8(1):32–44.

Jaber, M.A., Al-Otaibi, M.F. and Al-Ajmi, A.H. (2023). Analysis of the management and therapeutic performance of diabetes mellitus employing special target receptors. *International Journal of Environmental Research and Public Health* 20(2):1421.

Jamil, M., Mirza, B., Yasmeen, A. and Khan, M.A. (2012). Pharmacological activities of selected plant species and their phytochemical analysis. *Journal of Medicinal Plants Research* 6(37):5013–5022.

Kazeem, M.I. and Davies, A.I. (2015). In vitro antioxidant and antidiabetic potential of methanolic leaf extract of *Lonchocarpus cyanescens* (Schumach. and Thonn.) Benth. *African Journal of Traditional, Complementary and Alternative Medicines* 12(3):40–47.

Kolawole, O.T. and Akiibinu, M.O. (2013). Inhibition of baker's yeast $alpha$-glucosidase and rat pancreatic alpha-amylase by the methanolic extract of *Newbouldia laevis* leaves. *African Journal of Biochemistry and Molecular Biology Research* 1(1):18–24.

Liu, Y., Sun, C., Li, J., Xu, G. and Meng, Y. (2013). Saponins as potential antidiabetic and anti-obesity agents: A review of in vitro and in vivo studies. *Journal of Functional Foods* 5(4):1698–1709.

López-García, E., Blázquez, I., Busto, V., Culebras, J.M. and Villar, J.M. (2019). Potential application of non-flavonoid phenolics in diabetes: Anti-inflammatory effects. *Nutrients* 11(10):2322.

Moelands, S.V.L., Lucassen, P.L.B.J., Akkermans, R.P., De Grauw, W.J.C. and Van de Laar, F.A. (2018). Alpha-glucosidase inhibitors for prevention or delay of type 2 diabetes mellitus and its associated complications in people at increased risk of developing type 2 diabetes mellitus. *Cochrane Database of Systematic Reviews* Issue 12:CD005061.

Momoh, M.A., Adedokun, M.O., Mora, A.T. and Agboke, A.A. (2014). Antidiabetic activity and acute toxicity evaluation of aqueous leaf extract of *Vernonia amygdalina*. *African Journal of Biotechnology* 13(50):4586–4593.

Morales-Brown, P. (2025). How is the pancreas involved in diabetes? *Medical News Today*.

Moreira, T.F.M., da Silva, L.R.S., de Oliveira, M.A.L., Assis, D.M., Costa, C.S., Vieira, L.G.E. and da Silva, F.A. (2024). Legume-derived peptides and proteins with antidiabetic properties: Mechanisms and prospects. *Trends in Food Science and Technology* 139:385–400.

Moronkola, D.O. and Oladosu, I.A. (2013). Chemical compositions of *Lonchocarpus cyanescens* Benth. (Fabaceae) case study of its volatile oils and two triterpenoids. *American Journal of Plant Sciences* 4:1653–1659.

Muthu, C., Ayyanar, M., Raja, N. and Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in the Kancheepuram District of Tamil Nadu, India. *Journal of Ethnobiology and Ethnomedicine* 2:43.

NMPPDB. (n.d.). *Lonchocarpus cyanescens* [Image]. Nigerian Medicinal Plants Project Database.

Olamoyegun, M.A., Alare, K., Afolabi, S.A., Adewole, A.D., Awolola, O.O., Osayemi, A.O., Osanyin, V. and Osakwe, C.O. (2024). A systematic review and meta-analysis of the prevalence and risk factors of type 2 diabetes mellitus in Nigeria. *Clinical Diabetes and Endocrinology* 10(43):1–14.

Olayeye, T.O., Bodunwa, O.K. and Adewole, A.I. (2024). Prevalence and risk factors of diabetes mellitus among women using the multinomial logistic regression model. *FUDMA Journal of Sciences* 8(1):195–200.

Olayinka, A.A. and Anthony, I.O. (2016). Evaluation of anti-diabetic potential of aqueous extract of *Moringa oleifera* leaf in alloxan-induced diabetic rats. *African Scientist* 17(1):1–8.

Onyeije, B.P., Iyoha, E. and Augustine, O.O. (2024). The effect of aqueous extract of *Lonchocarpus cyanescens* leaf on the histology of the liver of Wistar rats. *Sokoto Journal of Medical Laboratory Science* 9(3):168–172.

Patintingan, C.G., Louisa, M., Juniantito, V., Arozal, W., Hanifah, S., Wanandi, S.I. and Thandavarayan, R. (2023). *Moringa oleifera* leaves extract ameliorates doxorubicin-induced cardiotoxicity via its mitochondrial biogenesis modulator activity in rats. *Journal of Experimental Pharmacology* 15:307–319.

Sonibare, M.A., Umukoro, S. and Shonibare, E.T. (2012). Antipsychotic property of aqueous and ethanolic extracts of *Lonchocarpus cyanescens* (Schumach and Thonn.) Benth. (Fabaceae) in rodents. *Journal of Natural Medicines* 66(1):127–132.

Swe, M.T., Thant, A.A., Park, E.Y. and Lee, S. (2019). Functional insights into human alpha-amylase and inhibitors. *Applied Biological Chemistry* 62:65.

Wilding, J.P.H., Batterham, R.L., Calanna, S., Davies, M., Van Gaal, L.F., Lingvay, I., McGowan, B.M., Rosenstock, J., Tran, M.T.D., Wadden, T.A., Wharton, S., Yokote, K., Zeuthen, N. and Kushner, R.F. (2021). Once-weekly semaglutide in adults with overweight or obesity. *New England Journal of Medicine* 384(11):989–1002.

Yousefi, N., Jafari, M. and Khoshdel, S. (2023). Medicinal plants and phytochemicals targeting type 2 diabetes: A comprehensive review of recent findings and future perspectives. *Current Diabetes Reviews* 19(5):e210822207902.

Zaworski, K., Wychowański, P., Szkopek, D., Woliński, J., Donaldson, J., Pierzynowski, S. and Pierzynowska, K. (2025). The regulatory role of pancreatic enzymes in the maintenance of small intestinal structure and enterocyte turnover with special reference to alpha amylase. *International Journal of Molecular Sciences* 26(1):249.

Zhen, J., Villani, T.S. and Zempleni, J. (2017). Alpha-glucosidase inhibition by plant extracts: A review of current evidence. *Critical Reviews in Food Science and Nutrition* 57(14):3020–3031.

Zheng, Y., Ley, S.H. and Hu, F.B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology* 14:88–98.

Zhu, H., Wang, J. and Li, Q. (2020). Cyanide content and detoxication in the leaves of *Lonchocarpus cyanescens* (Schumach. and Thonn.) Benth. *African Journal of Biotechnology* 19(11):779–786.