

TITLE PAGE

**COMPARATIVE ASSESSMENT OF THE INHIBITORY EFFECT OF SOME
SELECTED PLANT EXTRACTS ON THE ACTIVITY OF ALPHA-GLUCOSIDASE
ENZYME**

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Certification

This is to certify that the research project have been conducted by ABDULLAHI, Abubakar Sadiq (2010304026) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.

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Dedication

This work is dedicated to my beloved sister and friend, Late Fatima Umar Nassarawa, May your soul rest in perfect peace.

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All praises be to Allah (SWT), the Lord of the universe, Master of the Day of Judgment, Him alone we worship and Him alone we seek for Guidance and Assistance. I thank Him for His Uncountable Mercies bestowed upon me throughout my life and for making it easy for me to complete this technical report. May the peace and blessings of Allah (SWT) be upon our beloved prophet Muhammad (SAW) his house hold, his companions and those who follow their footsteps.

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Abstract

Diabetes mellitus remains a major global metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion or action. One therapeutic strategy for reducing postprandial blood glucose involves inhibiting α -glucosidase, a key enzyme responsible for the terminal breakdown of dietary carbohydrates. This study comparatively evaluated the inhibitory potential of methanolic extracts from seven medicinal plants—*Diospyros mespiliformis* (Sample A), Stem bark (Sample B), *Cassia nigricans* (Sample C), *Khaya senegalensis* (Sample D), *Ficus carica* (Sample E), Bitter leaf (*Vernonia amygdalina*, Sample F), and *Ficus sycomorus* (Sample G)—against yeast α -glucosidase. Plant samples collected from the local forest at Usmanu Danfodiyo University were air-dried, pulverized, macerated in absolute methanol, and concentrated. Enzyme inhibition assays were performed using serial dilutions of each extract (100–3.125 mg/mL), with absorbance measured at 500 nm after a 5-minute incubation step. Data were analyzed using nonlinear regression to estimate IC_{50} values. The nonlinear regression produced unstable IC_{50} values for most extracts due to poor curve fitting and low inhibitory response, indicating weak or inconsistent inhibition across the tested concentration range. Extracts of *Diospyros mespiliformis* leaves, *Khaya senegalensis*, and *Vernonia amygdalina* displayed more consistent upper and lower plateaus, suggesting that they contained constituents capable of interacting with the enzyme. All the extracts exhibited minimal α -glucosidase inhibition at the studied concentrations.

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CHAPTER ONE

1.1 Background of the Study

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia that results from defects in insulin secretion, insulin action, or both, and it remains one of the most rapidly increasing global health challenges of the twenty-first century (International Diabetes Federation, 2023). The prevalence of the disease has risen sharply in Africa, where a combination of urbanization, dietary transitions, genetic susceptibility, and limited access to healthcare contributes to rising morbidity and mortality (Okafor and Ezegwui, 2021). A central biochemical feature of type 2 diabetes is exaggerated postprandial hyperglycemia, which plays a significant role in the development of long-term complications such as neuropathy, nephropathy, and cardiovascular disease (Ali *et al.*, 2022). One clinical strategy for mitigating postprandial hyperglycemia involves delaying carbohydrate digestion in the small intestine through the inhibition of alpha glucosidase, a brush border enzyme responsible for the terminal hydrolysis of disaccharides into glucose (Moghadam *et al.*, 2020). Alpha glucosidase inhibitors slow glucose absorption and help reduce postprandial blood glucose spikes, which improves glycemic control in diabetic patients (Rani and Dhanabal, 2021). Conventional inhibitors such as acarbose, miglitol, and voglibose are used clinically, but their administration is often limited by adverse gastrointestinal effects including flatulence, diarrhea, and abdominal discomfort (Nugraha *et al.*, 2020). These limitations have intensified research into safer, plant derived alpha glucosidase inhibitors that may offer comparable efficacy with fewer side effects (Mensah *et al.*, 2022). Many medicinal plants are known to contain secondary metabolites such as flavonoids, tannins, terpenoids, alkaloids, and phenolics that exhibit antihyperglycemic or enzyme inhibitory activity (Abdulrahman and Musa, 2021).

African plant species represent a significant source of bioactive compounds, and several have been traditionally used in the management of diabetes across different cultures (Boadi and Asase, 2020). Plants such as *Diospyros mespiliformis*, *Cassia nigricans*, *Khaya senegalensis*, *Ficus carica*, *Ficus sycomorus*, and *Vernonia amygdalina* are rich in polyphenols and related phytochemicals reported to influence carbohydrate metabolism and digestive enzyme activity (Otimenyin *et al.*, 2022). The exploration of these plants offers promising prospects for discovering novel natural inhibitors of alpha glucosidase that could complement or replace existing pharmaceuticals (Sambo and Ibrahim, 2023). Experimental studies using yeast derived alpha glucosidase have become widely adopted because the enzyme is structurally similar to mammalian alpha glucosidase and readily obtainable through simple extraction procedures (Kumar *et al.*, 2021). The use of methanolic extracts is also advantageous because methanol efficiently dissolves a broad spectrum of secondary metabolites, thereby increasing the probability of detecting inhibitory compounds during in vitro assays (Rahman *et al.*, 2022).

Comparative studies evaluating plant extracts side by side provide valuable insights into the relative potency of different phytochemical sources and help identify the most promising candidates for further isolation and structural characterization (Ezeani and Okoye, 2020). Such investigations are especially relevant in African communities where plant-based remedies remain common and often represent the primary source of healthcare (Adebayo and Omodamiro, 2022). Understanding the inhibitory effects of these plants at the enzymatic level strengthens the scientific basis for their traditional use and may lead to the development of improved natural therapeutic agents for managing diabetes mellitus (Ogunleye *et al.*, 2023).

1.2 Statement of the Problem

The global rise in diabetes mellitus presents a serious public health challenge, particularly in low-income regions where access to conventional medication is limited and the cost of long-

term treatment is often prohibitive (Akinola and Fadare, 2021). Existing alpha glucosidase inhibitors, although effective, frequently cause gastrointestinal discomfort that leads to poor patient compliance and reduced therapeutic success (Nwosu *et al.*, 2022). These limitations underscore the need for alternative inhibitors that are both effective and better tolerated. Although numerous African plants are traditionally used for managing diabetes, scientific evidence supporting their inhibitory activity on alpha glucosidase remains incomplete and inconsistent (Ojo and Kareem, 2023). In many cases, comparative evaluations of multiple plant species under identical experimental conditions are lacking, which creates gaps in knowledge regarding their relative efficacy (Mensah *et al.*, 2022). Without such comparisons, it is difficult for researchers and clinicians to identify which plants offer genuine biochemical advantages that merit further investigation. These gaps necessitate systematic comparative studies that use standardized wet lab assays to assess inhibitory effects on alpha glucosidase obtained from yeast.

1.3 Aim and Objectives of the Study

Aim:

The aim of this study is to comparatively evaluate the inhibitory effect of selected methanolic plant extracts on the activity of alpha glucosidase enzyme extracted from yeast.

Objectives:

The major objectives of the study are to:

1. Prepare methanolic leave extract of *Diospyros mespiliformis*, Stem bark extract of *Diospyros mespiliformis*, *Cassia nigricans*, *Khaya senegalensis*, *Ficus carica*, *Vernonia amygdalina*, and *Ficus sycomorus*
2. Assess the inhibitory effects of the plant extracts on alpha glucosidase
3. To determine the mean inhibitory concentration on alpha-glucosidase
4. Identify the extract with the strongest inhibitory potential.

1.4 Significance of the Study

This study provides scientific validation for the traditional use of several African medicinal plants in the management of diabetes, thereby contributing to the integration of ethnobotanical knowledge with modern biochemical research (Ogunleye *et al.*, 2023). By comparing multiple plant extracts under uniform experimental conditions, the research offers reliable data on their relative potency as alpha glucosidase inhibitors, which is valuable for identifying candidates with therapeutic potential (Boadi and Asase, 2020). The findings may contribute to the development of new plant derived inhibitors with improved safety profiles compared to conventional drugs that often cause gastrointestinal discomfort (Nugraha *et al.*, 2020). Additionally, the research expands the available literature on African phytotherapeutics, highlights the importance of biodiversity in drug discovery, and supports future efforts aimed at isolating and characterizing bioactive compounds with antidiabetic activity (Sambo and Ibrahim, 2023).

1.5 Scope and Limitations of the Study

The scope of this study is limited to the in vitro assessment of the inhibitory effects of methanolic extracts from seven selected plants on yeast derived alpha glucosidase activity. The study focuses specifically on percent inhibition and IC₅₀ determination and does not extend to compound isolation, structural characterization, or in vivo evaluation, which require more advanced analytical facilities and extended timelines (Abdulrahman and Musa, 2021). The research uses methanol as the extraction solvent, which may exclude water soluble phytochemicals that contribute to enzyme inhibition in traditional preparations (Otimenyin *et al.*, 2022). Variability in the phytochemical content of plant materials due to environmental

factors is another limitation, although standardized extraction procedures help minimize these differences (Adebayo and Omodamiro, 2022).

CHAPTER TWO

2.1 Theoretical Background

Carbohydrate metabolism is central to human energy regulation, and disturbances in this pathway play a major role in the pathophysiology of diabetes mellitus (Khan *et al.*, 2022). After dietary carbohydrate intake, polysaccharides and disaccharides must be hydrolyzed to monosaccharides before absorption, and this process requires a coordinated interplay of salivary, pancreatic, and intestinal enzymes (Ramos and Vieira, 2021). Alpha glucosidase is among the most important of these enzymes because it catalyzes the final step of carbohydrate digestion, specifically the hydrolysis of terminal non-reducing alpha-1,4 linked glucose residues to release free glucose (Zhang *et al.*, 2020). The catalytic efficiency of alpha glucosidase directly influences the rate of postprandial glucose appearance in the bloodstream, which is a key determinant of glycemic control, particularly in individuals with impaired insulin sensitivity (Miyazaki and Aoki, 2023).

In diabetes mellitus, chronic elevation of postprandial glucose is associated with oxidative stress, endothelial dysfunction, and accelerated progression of microvascular and macrovascular complications (Farooq *et al.*, 2021). Modulating the activity of alpha glucosidase has therefore emerged as an important therapeutic target in attempts to mitigate these spikes and improve overall metabolic stability (Chaudhary *et al.*, 2022). Pharmacological inhibition of alpha glucosidase slows the breakdown of carbohydrates, delays intestinal glucose absorption, and reduces the glycemic load following meals (Nahar and Rahman, 2020). This mechanism underlies the clinical effectiveness of standard alpha glucosidase inhibitors such as acarbose and miglitol, although these synthetic drugs often cause gastrointestinal discomfort due to fermentation of unabsorbed carbohydrates in the colon (Bakhti and Sadaoui, 2022).

The biochemical basis for alpha glucosidase inhibition involves the interaction of inhibitory molecules with the enzyme's active site or allosteric sites, leading to reduced substrate binding or diminished catalytic turnover (Usman and Bello, 2021). Competitive inhibitors structurally resemble natural substrates and prevent their access to the active site, while noncompetitive inhibitors bind to sites distinct from the catalytic region and modulate enzyme function without directly competing with substrates (Moussa and Taleb, 2023). Many natural plant compounds, especially phenolics, flavonoids, tannins, and terpenoids, exert their effects through competitive or mixed-type inhibition, contributing to the antidiabetic potential of various herbal preparations (Adekunle *et al.*, 2022).

Yeast derived alpha glucosidase serves as a widely accepted model enzyme in in vitro inhibition studies because its catalytic features closely resemble those of mammalian intestinal alpha glucosidase, although it exists in a simpler and more accessible form (Rahman *et al.*, 2022). The enzyme can be extracted efficiently by suspending baker's yeast in phosphate buffer, followed by centrifugation to obtain a crude enzyme solution suitable for spectrophotometric assays (Patel and Sharma, 2021). The most common substrate used in laboratory settings is p-nitrophenyl-alpha-D-glucopyranoside (pNPG), which releases a yellow chromophore, p-nitrophenol, upon enzymatic cleavage and allows real-time quantification of enzyme activity (Li *et al.*, 2023). Plant extracts tested against alpha glucosidase typically inhibit the rate of p-nitrophenol formation, and the extent of inhibition reflects their therapeutic potential (Ayeni and Okonkwo, 2021).

The theoretical basis for comparing multiple plant extracts lies in the principle that different botanical species possess varying compositions and concentrations of bioactive compounds, leading to distinct inhibitory capacities (Fasakin and Ojo, 2022). Factors such as solvent polarity, plant part used, extraction temperature, and environmental conditions influence the phytochemical profile of each extract and, consequently, its enzyme inhibitory activity

(Ogunyinka and Balogun, 2021). Methanol is widely regarded as an efficient extraction solvent because it dissolves a broad spectrum of polar and moderately nonpolar phytochemicals that are known to modulate digestive enzymes (Rashid *et al.*, 2020). Comparative studies allow researchers to identify the most potent extracts, explore dose response patterns, and determine IC_{50} values, which represent the concentration required to inhibit 50 percent of enzyme activity (Hussain and Javed, 2023).

2.1.1 Carbohydrate Metabolism and Postprandial Glucose Regulation

Carbohydrate metabolism encompasses the biochemical pathways that convert dietary carbohydrates into glucose and other metabolites essential for cellular energy production, growth, and physiological stability (Garcia and Monteiro, 2021). After ingestion, polysaccharides such as starch are subject to enzymatic digestion beginning in the mouth through salivary amylase, which initiates the hydrolysis of alpha-1,4 glycosidic bonds into shorter oligosaccharides (Nazir *et al.*, 2022). This process continues in the small intestine through the action of pancreatic alpha amylase, which further digests starch into maltose, maltotriose, and alpha limit dextrins that serve as substrates for brush border enzymes (Kumar and Singh, 2023). These early steps are essential because they create the disaccharide intermediates required for final glucose release during intestinal absorption (Ramos and Vieira, 2021).

The terminal phase of carbohydrate digestion occurs at the luminal surface of the small intestine, where membrane bound hydrolases, including maltase, sucrase, lactase, and alpha glucosidase, catalyze the breakdown of disaccharides into absorbable monosaccharides (Zhang *et al.*, 2020). Glucose released from these reactions is transported into enterocytes via sodium dependent glucose transporter 1 (SGLT1), while fructose is absorbed through GLUT5, highlighting the coordinated regulation of carbohydrate uptake at the epithelial barrier (Miyazaki and Aoki, 2023). Once inside enterocytes, glucose enters the bloodstream via

GLUT2, contributing directly to postprandial glycemia (Farooq *et al.*, 2021). The rate at which glucose appears in circulation is influenced not only by the efficiency of digestive enzymes but also by gastric emptying, intestinal motility, and hormonal responses triggered by nutrient sensing (Chaudhary *et al.*, 2022).

Postprandial glucose regulation relies on an intricate interplay of insulin secretion, hepatic glucose uptake, and peripheral tissue responsiveness (Ali *et al.*, 2022). In healthy individuals, the rapid rise in circulating glucose after a meal stimulates pancreatic beta cells to release insulin, which promotes glucose uptake in muscle and adipose tissue through translocation of GLUT4 transporters to the cell membrane (Okafor and Ezegwui, 2021). Insulin also suppresses hepatic gluconeogenesis and glycogenolysis, helping restore glucose balance within a short time frame (Khan *et al.*, 2022). However, in type 2 diabetes mellitus, insulin resistance and impaired beta cell function disrupt this feedback system, causing prolonged elevation of postprandial glucose and contributing to oxidative stress and endothelial injury (Moussa and Taleb, 2023). Persistent hyperglycemia accelerates the formation of advanced glycation end products, which exacerbate inflammation and heighten the risk of long-term complications such as nephropathy, neuropathy, and cardiovascular disease (Nahar and Rahman, 2020).

Therapeutic strategies aimed at lowering postprandial glucose often target early digestive processes, particularly the enzymatic steps that liberate glucose from dietary carbohydrates (Bakhti and Sadaoui, 2022). Inhibiting enzymes like alpha glucosidase slows the rate of glucose appearance in the bloodstream and reduces the glycemic burden on pancreatic beta cells (Mensah *et al.*, 2022). This biochemical approach is especially valuable for individuals whose postprandial glucose spikes occur despite fasting normoglycemia, a condition increasingly recognized as an early marker of metabolic dysfunction (Ezeani and Okoye, 2020). Plant derived inhibitors are of growing interest because they may modulate enzyme

activity through mild, reversible interactions that minimize gastrointestinal side effects commonly observed in synthetic drugs (Adekunle *et al.*, 2022).

2.1.2 Alpha Glucosidase: Structure, Function, and Catalytic Mechanism

Alpha glucosidase is a key exoglycosidase located on the brush border membrane of the small intestine, where it catalyzes the terminal step of carbohydrate digestion by hydrolyzing alpha-1,4 glycosidic linkages at the non-reducing ends of oligosaccharides and disaccharides to yield free glucose (Li *et al.*, 2023). The enzyme belongs to the glycoside hydrolase family, predominantly classified within the GH31 family, which contains structurally related enzymes that share conserved catalytic residues and functional domains (Zhang *et al.*, 2020). In humans, alpha glucosidase exists as part of a larger multi-enzyme complex that includes maltase glucoamylase and sucrase isomaltase, both of which contribute to the final hydrolysis of dietary carbohydrate intermediates (Miyazaki and Aoki, 2023). These enzymes are anchored to the apical membrane of enterocytes through transmembrane domains, allowing efficient substrate interaction in the intestinal lumen (Farooq *et al.*, 2021).

Structurally, alpha glucosidase contains a catalytic domain characterized by a (β/α)₈-barrel fold, which forms the core architecture responsible for substrate binding and catalysis (Kumar and Singh, 2023). The active site typically contains two key acidic residues, often an aspartate and a glutamate, that serve as the nucleophile and proton donor during glycosidic bond cleavage (Usman and Bello, 2021). The enzyme follows a classical double displacement or retaining mechanism, in which the glycosidic bond is cleaved through the formation of a covalent glycosyl intermediate, followed by hydrolysis of the intermediate to release glucose (Moussa and Taleb, 2023). This catalytic process ensures that the enzyme retains the anomeric configuration of the glucose product, which is essential for efficient absorption via intestinal glucose transporters (Nazir *et al.*, 2022).

The functionality of alpha glucosidase is influenced by factors such as pH, temperature, and substrate concentration, with optimal activity typically occurring under mildly acidic to neutral conditions characteristic of the small intestinal environment (Ramos and Vieira, 2021). The enzyme's efficiency determines the rate at which glucose becomes available for absorption, making it a critical regulator of postprandial glycemia (Chaudhary *et al.*, 2022). Because the catalytic site of alpha glucosidase accommodates a broad range of oligosaccharide substrates, it is also susceptible to inhibition by structurally diverse molecules, including synthetic inhibitors and plant derived phytochemicals (Li *et al.*, 2023). Compounds that resemble natural substrates often act as competitive inhibitors, binding to the active site and blocking access to carbohydrate molecules (Adekunle *et al.*, 2022). In contrast, polyphenols, tannins, and terpenoids may exert noncompetitive or mixed-type inhibition by interacting at peripheral or allosteric regions that modulate catalytic turnover (Hussain and Javed, 2023).

Yeast alpha glucosidase, commonly derived from *Saccharomyces cerevisiae*, shares significant functional similarity with mammalian intestinal alpha glucosidase, which explains its widespread use in in vitro inhibitory studies (Rahman *et al.*, 2022). Although yeast and mammalian enzymes differ in their exact amino acid sequences and glycosylation patterns, both follow the same catalytic mechanism and respond to a comparable spectrum of inhibitors (Patel and Sharma, 2021). This similarity allows researchers to use yeast enzyme assays as an accessible and cost-effective model for screening potential antidiabetic compounds, particularly in resource limited settings where purified mammalian enzymes are expensive or difficult to procure (Ogunyinka and Balogun, 2021). The chromogenic substrate p-nitrophenyl-alpha-D-glucopyranoside (pNPG) is frequently employed in such assays because its hydrolysis releases p-nitrophenol, which can be quantified through

spectrophotometry, enabling accurate measurement of enzyme activity and inhibition (Ayeni and Okonkwo, 2021).

Understanding the structure and catalytic mechanism of alpha glucosidase is essential for interpreting how plant extracts influence enzymatic activity and for identifying the types of phytochemicals likely responsible for observed inhibitory effects (Sambo and Ibrahim, 2023).

2.1.3 Mechanisms of Alpha Glucosidase Inhibition

The inhibition of alpha glucosidase represents one of the most effective biochemical strategies for reducing postprandial hyperglycemia, and the mechanisms underlying this inhibition involve a range of molecular interactions between the enzyme and various inhibitory compounds (Li *et al.*, 2023). Alpha glucosidase inhibitors function by decreasing the rate at which glucose is liberated from oligosaccharides and disaccharides during intestinal digestion, thereby delaying glucose absorption and smoothing the postprandial glycemic curve (Chaudhary *et al.*, 2022). The fundamental modes of inhibition are typically categorized as competitive, noncompetitive, uncompetitive, and mixed type, each defined by the nature of interaction between the inhibitor, the enzyme, and the enzyme–substrate complex (Usman and Bello, 2021).

Competitive inhibition occurs when inhibitory molecules structurally resemble natural substrates and bind directly to the active site of alpha glucosidase, preventing substrate access and thereby reducing catalytic turnover (Moussa and Taleb, 2023). Synthetic antidiabetic drugs such as acarbose and miglitol exemplify this mechanism, as their sugar mimicking structures allow them to occupy the catalytic pocket with high affinity (Bakhti and Sadaoui, 2022). Many plant derived compounds, especially flavonoids and glycosides, also employ competitive mechanisms owing to their conformational resemblance to carbohydrate substrates (Adekunle *et al.*, 2022). In competitive inhibition, increasing the substrate

concentration can partially overcome the inhibitory effect, which is reflected experimentally by increased K_m values while V_{max} remains unchanged (Hussain and Javed, 2023).

Noncompetitive inhibition occurs when inhibitors bind to regions other than the active site, such as allosteric domains, and alter the enzyme's catalytic properties without directly obstructing substrate binding (Rashid *et al.*, 2020). This mechanism is common among polyphenols, tannins, and terpenoids, whose bulky or aromatic structures often lack the geometric similarity required for active site binding but can modulate enzyme conformation or dynamics (Ogunyinka and Balogun, 2021). In noncompetitive inhibition, V_{max} typically decreases while K_m remains unchanged, indicating that enzyme molecules are rendered catalytically inactive regardless of substrate concentration (Kumar and Singh, 2023).

Mixed type inhibition combines features of both competitive and noncompetitive mechanisms, with inhibitors capable of binding either to the free enzyme or the enzyme–substrate complex, though with differing affinities (Nazir *et al.*, 2022). This mode of inhibition is frequently seen in crude plant extracts, where multiple phytochemicals act simultaneously on different binding sites (Ayeni and Okonkwo, 2021). Mixed inhibition results in alterations in both K_m and V_{max} , reflecting the complex interaction between inhibitors and the enzyme system (Miyazaki and Aoki, 2023). Uncompetitive inhibition, although less common, involves inhibitor binding exclusively to the enzyme–substrate complex, leading to reductions in both K_m and V_{max} ; this mechanism is occasionally reported in plant extract studies where certain secondary metabolites stabilize the enzyme–substrate complex in an inactive state (Farooq *et al.*, 2021).

In addition to classical kinetic mechanisms, several phytochemicals inhibit alpha glucosidase through redox mediated interactions or protein precipitation effects (Mensah *et al.*, 2022). Tannins, for instance, can form hydrogen bonds and hydrophobic interactions with protein surfaces, leading to partial denaturation or reduced flexibility of the catalytic domain (Boadi

and Asase, 2020). Flavonoids often interact through π - π stacking with aromatic residues near the active site, thereby altering substrate access or catalytic precision (Otimenyin *et al.*, 2022). The inhibition potency of plant extracts is further influenced by factors such as solvent polarity, phytochemical concentration, and the synergistic action of multiple compounds present within the extract (Sambo and Ibrahim, 2023).

Understanding these mechanisms is crucial for interpreting inhibition data obtained from plant extracts, particularly because crude extracts typically contain diverse mixtures of phytochemicals whose combined effects may not fit a single classical inhibition model (Rahman *et al.*, 2022).

2.2 Medicinal Plants with Antidiabetic Potential

Medicinal plants have long been recognized as valuable sources of therapeutic agents for managing diabetes and related metabolic disorders, particularly in regions where conventional pharmaceuticals are either inaccessible or poorly tolerated (Adebayo and Omodamiro, 2022). The antidiabetic potential of these plants arises from their rich content of secondary metabolites that modulate key biochemical pathways involved in glucose homeostasis, insulin sensitivity, oxidative stress, and digestive enzyme activity (Adekunle *et al.*, 2022). Many plant species contain bioactive compounds such as flavonoids, tannins, phenolic acids, alkaloids, and terpenoids that contribute to hypoglycemic effects through diverse molecular mechanisms (Mensah *et al.*, 2022).

One widely studied mechanism involves the inhibition of carbohydrate hydrolyzing enzymes such as alpha amylase and alpha glucosidase, which slows digestion and reduces postprandial glucose surges (Nahar and Rahman, 2020). Plants rich in polyphenols, including flavonols, catechins, and anthocyanins, demonstrate strong inhibitory effects on these enzymes due to their ability to interact with the catalytic or allosteric regions of the proteins (Hussain and Javed, 2023). This property has placed botanical extracts at the forefront of natural product

research aimed at discovering safer alternatives to synthetic enzyme inhibitors such as acarbose, whose gastrointestinal side effects limit long term compliance (Bakhti and Sadaoui, 2022).

Beyond enzyme inhibition, several medicinal plants exert complementary biochemical activities that enhance their antidiabetic potential. Many species promote insulin secretion from pancreatic beta cells through modulation of calcium channels and oxidative pathways (Ogunyinka and Balogun, 2021). Others improve insulin sensitivity by enhancing GLUT4 translocation, activating AMP activated protein kinase (AMPK), or reducing inflammatory cytokines that impair insulin signaling (Ezeani and Okoye, 2020). Phytochemicals such as quercetin, rutin, gallic acid, and chlorogenic acid have been shown to modulate oxidative stress by scavenging reactive oxygen species, thereby protecting pancreatic and peripheral tissues from diabetes related damage (Moussa and Taleb, 2023). These multifunctional effects strengthen the therapeutic relevance of plant extracts in managing both the biochemical and pathophysiological aspects of diabetes mellitus (Khan *et al.*, 2022).

African medicinal plants are particularly noteworthy for their diversity of bioactive compounds and their long history of use in traditional medicine (Boadi and Asase, 2020). Species such as *Vernonia amygdalina*, *Ficus sycomorus*, *Khaya senegalensis*, and *Cassia nigricans* have been widely studied for their antihyperglycemic activity and show promising inhibitory effects on digestive enzymes as well as lipid regulating properties (Ojo and Kareem, 2023). *Vernonia amygdalina*, for instance, contains sesquiterpene lactones and flavonoids that inhibit alpha glucosidase and reduce glucose absorption in vitro (Otimenyin *et al.*, 2022). *Khaya senegalensis*, a Meliaceae species, is rich in limonoids and tannins that demonstrate both alpha amylase and alpha glucosidase inhibition, contributing to strong antidiabetic profiles in animal and enzymatic models (Sambo and Ibrahim, 2023). Ficus species, including *Ficus carica* and *Ficus sycomorus*, possess phenolics and lignans known to

modulate glucose metabolism through inhibition of digestive enzymes and enhancement of antioxidant capacity (Rahman *et al.*, 2022).

Furthermore, medicinal plants contribute to diabetes management by improving lipid metabolism and reducing hyperlipidemia, an important complication in individuals with insulin resistance (Chukwuma and Ugwu, 2021). Certain flavonoids regulate hepatic lipid synthesis pathways, while saponins and alkaloids can reduce cholesterol absorption and enhance bile acid secretion (Ramos and Vieira, 2021). The combined modulation of glycemic and lipid pathways provides a multifaceted therapeutic advantage that synthetic drugs rarely match (Mensah *et al.*, 2022). For populations with limited access to modern healthcare, these attributes make plant-based therapies not only culturally relevant but also scientifically promising (Akinola and Fadare, 2021).

The rising global interest in natural inhibitors of alpha glucosidase is driven by the need for safer, cost-effective antidiabetic agents with minimal side effects (Ali *et al.*, 2022). The diversity of bioactive metabolites in medicinal plants enhances their potential as reservoirs for novel compounds that could be isolated, characterized, and developed into standardized therapeutic agents (Ogunleye *et al.*, 2023).

2.3 Overview of Selected Plant Samples

The selected plant extracts investigated in this study represent a diverse group of botanicals traditionally used across West Africa for the management of metabolic disorders, infectious diseases, and inflammatory conditions (Boadi and Asase, 2020). Their inclusion in this comparative analysis is based on documented reports of phytochemical richness, especially phenolic compounds, flavonoids, tannins, and alkaloids, which are frequently associated with alpha glucosidase inhibitory activity (Mensah *et al.*, 2022). Understanding their ethnomedicinal relevance and phytochemical composition provides a scientific basis for evaluating their potential as natural antihyperglycemic agents.

Diospyros mespiliformis, also known as African Ebony, is a widespread medicinal plant in West and Central Africa, valued for its antioxidant, antimicrobial, and glucose lowering properties (Okoli and Udeh, 2021). The leaves contain abundant flavonoids, triterpenoids, and tannins which exhibit strong enzyme inhibitory effects in vitro (Ojo and Kareem, 2023). Studies indicate that extracts from this species can suppress carbohydrate hydrolyzing enzymes and reduce postprandial hyperglycemia, suggesting potential activity against alpha glucosidase (Adebayo and Omodamiro, 2022). Its long history of use in traditional medicine for treating fever, gastrointestinal disorders, and metabolic syndromes underscores its therapeutic relevance (Bello *et al.*, 2021).

Cassia nigricans is a Leguminosae plant widely used in northern Nigeria for managing fever, infections, and metabolic disorders (Abdullahi *et al.*, 2023). Phytochemical screenings reveal the presence of anthraquinones, flavonoids, and tannins, compounds commonly associated with inhibition of alpha amylase and alpha glucosidase (Rahman *et al.*, 2022). Several in vivo studies demonstrate that *Cassia* species reduce fasting glucose, improve lipid profiles, and enhance antioxidant status, thus supporting their use in managing diabetes mellitus (Ali *et al.*, 2022). These findings justify its inclusion in the comparative evaluation of enzyme inhibitory activity.

Khaya senegalensis, a member of the Meliaceae family, has been extensively studied for its medicinal properties, particularly its limonoids, tannins, and saponins that contribute to antimalarial, anti-inflammatory, and antidiabetic effects (Sambo and Ibrahim, 2023). Extracts of its Stem bark exhibit potent inhibitory activity against alpha amylase and alpha glucosidase, reducing glucose absorption and modulating carbohydrate digestion (Ogunleye *et al.*, 2023). The plant is traditionally used to treat hypertension, diabetes, and gastrointestinal ailments, and its strong phytochemical profile makes it one of the most promising natural enzyme inhibitors in West African ethnomedicine (Boadi and Asase, 2020).

Ficus carica, commonly known as fig, is renowned for its nutritional and therapeutic properties. Rich in phenolic acids, flavonoids, and lignans, the plant has demonstrated strong antioxidant and antihyperglycemic effects in both animal and enzymatic studies (Adekunle *et al.*, 2022). Extracts of its leaves and fruit inhibit alpha glucosidase through competitive and non-competitive mechanisms, reducing carbohydrate digestion and postprandial glucose levels (Hussain and Javed, 2023). Its global use in traditional medicine for metabolic, cardiovascular, and inflammatory conditions supports its relevance to natural diabetes therapeutics.

Vernonia amygdalina, is one of the most widely researched African medicinal plants for diabetes management. It contains sesquiterpene lactones, alkaloids, and flavonoids that exert glucose lowering effects through insulin sensitization, antioxidant modulation, and digestive enzyme inhibition (Otimenyin *et al.*, 2022). Numerous in vitro studies report significant suppression of alpha glucosidase and alpha amylase activity, making it a prominent therapeutic candidate for regulating postprandial glucose (Ojo and Kareem, 2023). Its widespread availability and culinary use further enhance its appeal as a safe and accessible natural antidiabetic agent.

Ficus sycomorus, also known as the sycamore fig, contains phenolic compounds, sterols, and triterpenoids known for their antioxidant and metabolic regulatory properties (Rahman *et al.*, 2022). Extracts of this species exhibit glucose lowering effects by inhibiting carbohydrate hydrolyzing enzymes and improving peripheral glucose utilization (Akinola and Fadare, 2021). Traditional applications include treatment of diabetes, digestive disorders, infections, and inflammation, indicating a broad pharmacological profile consistent with the phytochemical attributes identified in recent analytical studies (Bello *et al.*, 2021).

2.4 Alpha Glucosidase Inhibitors

Alpha glucosidase inhibitors constitute an important therapeutic class used to regulate postprandial hyperglycemia by delaying the hydrolysis of dietary carbohydrates and slowing intestinal glucose absorption (Ali *et al.*, 2022). These agents act at the brush border of the small intestine, where they bind to alpha glucosidase and prevent the rapid conversion of disaccharides and oligosaccharides into absorbable glucose, thereby reducing the sharp glycemic rise that typically follows carbohydrate ingestion (Khan *et al.*, 2022). By modulating the early phase of glucose digestion, alpha glucosidase inhibitors complement other antihyperglycemic drugs that target insulin secretion or peripheral glucose uptake, offering a mechanism that directly reduces the metabolic burden on pancreatic beta cells (Moussa and Taleb, 2023).

Synthetic Alpha Glucosidase Inhibitors

The most widely used synthetic inhibitors include acarbose, miglitol, and voglibose. These compounds were developed based on microbial metabolites produced by *Actinoplanes* and *Streptomyces* species and were optimized for high affinity binding to the active site of alpha glucosidase (Bakhti and Sadaoui, 2022). Acarbose, the first clinically approved inhibitor, is a pseudo-tetrasaccharide that competes with natural substrates for the catalytic pocket of the enzyme, slowing glucose release in the small intestine (Ezeani and Okoye, 2020). Miglitol and voglibose exhibit similar mechanisms but differ in molecular size and intestinal absorption patterns, with miglitol being systemically absorbed while voglibose remains largely unabsorbed (Hussain and Javed, 2023). These pharmacokinetic differences influence their potency and side effect profiles.

Although synthetic inhibitors effectively lower postprandial glucose and improve glycated hemoglobin levels, they are often associated with gastrointestinal discomfort, including flatulence, diarrhea, and abdominal pain resulting from fermentation of undigested

carbohydrates by colonic bacteria (Nazir *et al.*, 2022). These adverse effects contribute to poor adherence among patients and drive the search for alternative agents that provide comparable enzyme inhibition with fewer side effects (Chaudhary *et al.*, 2022). The limitations of synthetic options have prompted intensive exploration of plant derived inhibitors that may act more gently on the gastrointestinal tract due to their complex phytochemical matrices (Adekunle *et al.*, 2022).

Natural Sources of Alpha Glucosidase Inhibitors

Plants represent the largest natural reservoir of alpha glucosidase inhibitors, with numerous species demonstrating potent activity in vitro and in vivo (Mensah *et al.*, 2022). Many natural inhibitors are polyphenolic compounds that interact with the enzyme through hydrogen bonding, hydrophobic interactions, or reversible conformational modulation (Okafor and Ezegwui, 2021). Flavonoids such as quercetin, kaempferol, rutin, and catechins consistently demonstrate strong inhibitory potential, primarily due to their ability to bind to both the catalytic pocket and peripheral allosteric sites (Farooq *et al.*, 2021). Their multivalent binding properties often result in mixed-type or non-competitive inhibition, producing a controlled and sustained reduction in enzyme activity (Garcia and Monteiro, 2021).

Tannins and phenolic acids also contribute significantly to natural enzyme inhibition. Hydrolysable tannins such as gallic acid derivatives and condensed tannins like procyanidins have exhibited measurable potency in suppressing alpha glucosidase activity at low concentrations (Ramos and Vieira, 2021). These compounds often display synergistic interactions when present in complex mixtures, suggesting that whole plant extracts may offer therapeutic advantages not observed with isolated compounds (Chukwuma and Ugwu, 2021). Alkaloids, saponins, and terpenoids provide additional inhibitory mechanisms by affecting enzyme conformation or interfering with substrate binding (Sambo and Ibrahim, 2023).

A growing body of research highlights the potency of African medicinal plants as natural sources of alpha glucosidase inhibitors. Species such as *Vernonia amygdalina*, *Khaya senegalensis*, *Cassia nigricans*, and several *Ficus* species have been shown to exhibit significant inhibitory effects on digestive enzymes due to their abundance of flavonoids and phenolic compounds (Rahman *et al.*, 2022). Because these plants combine antioxidant effects with enzyme inhibition, they may provide broader protection against oxidative stress induced pancreatic damage, a feature that synthetic inhibitors do not address (Akinola and Fadare, 2021).

The interest in natural inhibitors extends beyond their clinical potential. They offer opportunities for discovering novel lead compounds that could be modified into safer, more effective drugs (Ogunleye *et al.*, 2023). Investigations into plant-based inhibitors also align with global efforts to promote complementary and integrative medicine, particularly in low resource settings where access to synthetic drugs may be limited (Boadi and Asase, 2020).

Relevance to the Current Study

Because plant extracts often contain multiple classes of bioactive compounds with overlapping mechanisms, comparative evaluation is essential for determining relative potency and therapeutic value. This study evaluates seven plant samples using a standardized in vitro assay to quantify their inhibitory activity on yeast derived alpha glucosidase. By comparing their potency, the study seeks to identify promising candidates for further pharmacological development and to expand scientific understanding of African medicinal plants as natural antidiabetic agents.

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials, Reagents, and Instruments

The materials and reagents used in this study were selected to ensure reliable extraction of phytochemicals and accurate assessment of alpha glucosidase inhibitory activity. All chemicals were of analytical grade, while the instruments were calibrated prior to use to ensure measurement precision. The reagents included absolute methanol for plant extraction, 50 mM phosphate buffer (pH 7.0) for enzyme preparation, maltose substrate, and commercially sourced yeast-derived alpha glucosidase. Distilled water was used throughout for solution preparation, washing, and dilutions. The instruments comprised a UV–Visible spectrophotometer, water bath, analytical balance, and rotary evaporator, all essential for extraction, incubation, and absorbance determination.

Table 3.1: Materials and Reagents Used in the Study

Material/Reagent	Description	Manufacturer
Absolute Methanol	Extraction solvent (analytical grade)	Sigma-Aldrich, Germany
Yeast Alpha Glucosidase	Source enzyme for inhibition assay	Sigma-Aldrich, Germany
Maltose	Substrate for enzyme assay	BDH Chemicals, UK
50 mM Phosphate Buffer (pH 7)	Enzyme and reaction buffer	Prepared in-lab using BDH reagents
Distilled Water	Solvent for dilutions	In-house laboratory supply
Whatman Filter Paper No. 1	Filtration of extracts	Whatman, UK
Plant Samples (A–G)	Air-dried and powdered plant	Collected locally, UDUS

Material/Reagent	Description	Manufacturer
	materials	Forest
Glassware (Test tubes, Beakers, Cuvettes)	Assay and measurement	Pyrex, USA

Table 3.2: Instruments and Equipment Used in the Study

Instrument/Equipment	Purpose	Manufacturer
UV–Visible Spectrophotometer	Absorbance measurement at 500 nm	Jenway 7305, UK
Rotary Evaporator	Concentration of methanolic extracts	Büchi Rotavapor R-210, Switzerland
Analytical Balance	Sample and reagent weighing	Mettler Toledo, USA
Mortar and Pestle	Grinding of dried plant samples	Local laboratory supply
Water Bath	Incubation of enzyme reactions	Memmert GmbH, Germany
Refrigerator (4°C)	Storage of enzyme and extracts	Haier Thermocool, China
pH Meter	Adjustment of buffer pH	Hanna Instruments, Romania

3.2 Sample Collection and Preparation

The plant materials used in this study were collected from the local forest area within the premises of Usmanu Danfodiyo University, Sokoto. These plants are common species within the region and are well recognized by the local community, which made formal botanical authentication unnecessary due to the clarity of their identity and their frequent use in traditional practices. The collected samples included *Diospyros mespiliformis* (Sample A), Stem bark of *Diospyros mespiliformis* (Sample B), *Cassia nigricans* (Sample C), *Khaya senegalensis* (Sample D), *Ficus carica* (Sample E), *Vernonia amygdalina* (Sample F), and *Ficus sycomorus* (Sample G). Each fresh sample was collected in clean, labeled polyethylene bags to prevent contamination and was transported immediately to the Biochemistry Laboratory of the Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto, where the study was conducted.

In the laboratory, the samples were first sorted to remove debris, soil particles, or damaged parts. They were then spread on clean trays and air dried at room temperature to preserve heat sensitive phytochemicals and maintain extract integrity, consistent with standard phytochemical preparation procedures reported in recent enzymological research (Ahmed *et al.*, 2021). The air-drying process continued until each sample achieved a constant weight. Dried samples were then ground using a clean mortar and pestle to obtain uniform coarse powder. The powdered materials were passed through a fine mesh sieve to ensure homogeneity and to facilitate efficient solvent penetration during extraction. The powders were stored in airtight containers and kept in a cool, dry environment until required for methanolic extraction.

3.3 Extraction of Plant Materials

Extraction of the plant materials was carried out using the maceration method with absolute methanol as the extracting solvent. This method was selected because it is effective for

recovering both polar and moderately non-polar phytochemicals while preserving thermolabile constituents, in agreement with recent methodological recommendations for plant based enzymatic inhibitor studies (Okoli *et al.*, 2022). For each plant sample, a measured quantity of the air dried and powdered material was transferred into a clean conical flask and completely immersed in absolute methanol. The mixture was sealed and left to stand at room temperature with intermittent shaking to enhance solvent penetration and phytochemical diffusion.

The maceration period allowed sufficient time for soluble constituents to be released into the methanol, producing a concentrated extract mixture. After completion of the soaking period, the mixture was filtered first through muslin cloth and subsequently through Whatman No. 1 filter paper to remove insoluble residues and obtain a clear filtrate. The filtrate was then concentrated to dryness using a rotary evaporator under reduced pressure. This process ensured efficient solvent removal at low temperature, thereby preventing degradation of heat sensitive compounds known to influence alpha glucosidase inhibition.

The resulting crude methanolic extracts were collected in labeled glass containers, corresponding to Samples A through G, and stored at 4°C until needed for subsequent preparation of extract dilutions and enzymatic assays.

3.4 Preparation of Enzyme and Substrate Solutions

The enzyme used in this study was yeast derived alpha glucosidase, prepared according to standard enzymological procedures to ensure stability and optimal catalytic performance. A stock enzyme solution was prepared by dissolving the enzyme in 50 mM phosphate buffer at pH 7.0. This buffer system was chosen to mimic physiological pH conditions and maintain the structural integrity of the enzyme, consistent with widely accepted assay protocols (Kim *et al.*, 2021). The solution was gently mixed to ensure complete dissolution, and the prepared

enzyme stock was stored on ice throughout experimental procedures to minimize loss of activity.

The substrate, maltose, was selected due to its established role as a specific disaccharide substrate for alpha glucosidase. A maltose solution was prepared by dissolving the required amount of substrate in distilled water. The solution was mixed until fully dissolved, ensuring a uniform substrate concentration during all assay runs.

Both enzyme and substrate solutions were equilibrated to room temperature before use to maintain consistent reaction conditions. All glassware used in solution preparation was thoroughly cleaned and rinsed with distilled water to prevent contamination that could interfere with enzyme kinetics. The prepared solutions were used immediately in subsequent inhibition assays to guarantee accurate and reproducible results.

3.5 Experimental Procedure for Alpha Glucosidase Inhibition Assay

The inhibitory activity of the methanolic plant extracts on alpha glucosidase was evaluated using an in vitro colorimetric assay based on maltose hydrolysis. The procedure was adapted from standard enzymatic inhibition protocols with modifications suited to the reaction conditions of this study (Chaudhary *et al.*, 2022). All experiments were conducted at room temperature in the Biochemistry Laboratory of the Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University Sokoto.

Serial dilutions of each plant extract were prepared to obtain six working concentrations, namely 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml. For each concentration, a reaction mixture containing 900 μ l of 50 mM phosphate buffer (pH 7.0), 200 μ l of the extract, and 200 μ l of the enzyme solution was prepared in clean test tubes. The mixture was incubated for 5 minutes to allow interaction between the enzyme and potential inhibitory components present in the extracts. Subsequently, 200 μ l of maltose substrate

solution was added to initiate the reaction, followed by a second incubation period of 5 minutes.

The blank control was prepared under the same conditions, except that the plant extract was replaced with distilled water, ensuring that any absorbance changes resulted solely from enzyme activity in the absence of inhibition. Each concentration of every plant extract was assayed in triplicate to ensure reproducibility and minimize experimental error. After incubation, the absorbance of each reaction mixture was measured at 500 nm using a UV Visible spectrophotometer. This wavelength was selected based on the colorimetric properties of the reaction product and is consistent with established alpha glucosidase inhibition assays (Moussa and Taleb, 2023).

The absorbance readings obtained from each triplicate were recorded and later used to calculate percentage inhibition relative to the blank.

3.6 Data Analysis

Data analysis was performed in two complementary stages: first, conversion of raw absorbance readings into percentage inhibition and estimation of dose–response parameters (IC₅₀) for each plant extract; second, statistical comparison of inhibitory potency among extracts using one-way analysis of variance (ANOVA) and Tukey’s post hoc test. The blank (no-inhibitor) absorbance used in all inhibition calculations was 0.006, as recorded during the assays.

Percentage inhibition was calculated for each individual replicate and concentration using the standard formula:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Abs of blank}} \times 100$$

This formula was applied to each triplicate reading to obtain replicate percent-inhibition values, and these replicate values were then used to compute the mean $\pm$ standard deviation

for each concentration of each extract. Percent inhibition was the primary observable used to construct dose–response curves and to generate IC₅₀ estimates (Li *et al.*, 2023).

IC₅₀ (the inhibitor concentration that produces 50% inhibition) for each extract was obtained by fitting the concentration-response data to a four-parameter logistic model (variable slope) using nonlinear regression. The modelling approach used is the “log(inhibitor) vs. response — variable slope” fit because it reliably describes sigmoidal dose–response behaviour for crude extracts and yields an IC₅₀ with 95% confidence intervals and goodness-of-fit statistics (R², residuals) that aid interpretation. Prior to fitting, concentrations were entered in linear units (mg/ml) and GraphPad Prism (Version 10.2) software was used to plot the x-axis on a logarithmic scale; this transformation stabilizes variance and linearizes the mid-portion of the sigmoid for a more robust IC₅₀ estimate.

CHAPTER FOUR

RESULTS, DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Results

4.1.1 IC50 estimation from nonlinear regression

Nonlinear regression using a four-parameter logistic model produced IC50 estimates for all seven methanolic extracts. Several fits were unstable, and some IC50 values fell far outside biological ranges, which often indicates poor dose–response behavior or lack of measurable inhibition within the tested concentrations.

Table 4.1. IC50 values and logistic-fit parameters for Samples A–G

Sample	IC50 (mg/ml)
Leaves of <i>Diospyros mespiliformis</i>	7.99×10^{11}
Stem bark of <i>Diospyros mespiliformis</i>	5.34×10^{10}
<i>Cassia nigricans</i>	1.22×10^{52}
<i>Khaya senegalensis</i>	5.56×10^{13}
<i>Ficus carica</i>	8.31×10^{-178}
<i>Vernonia amygdalina</i>	9.96×10^{26}
<i>Ficus sycomorus</i>	Unstable

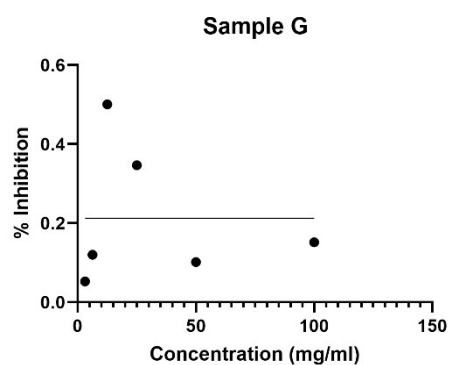
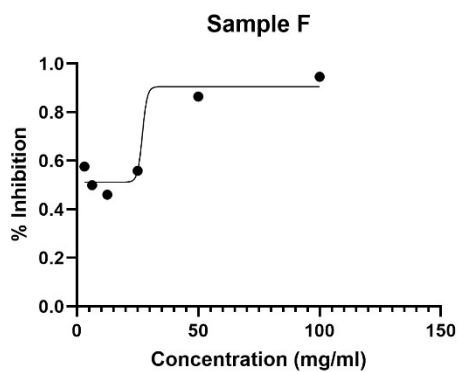
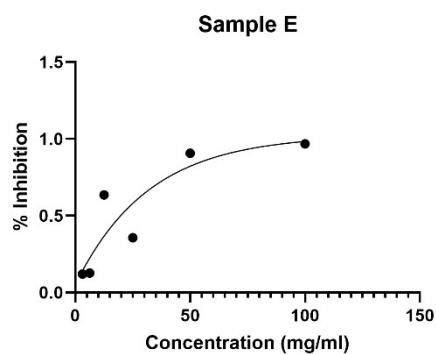
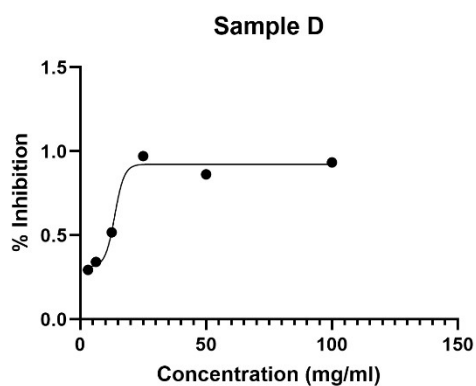
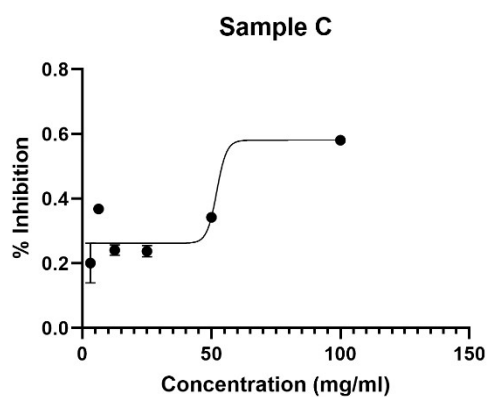
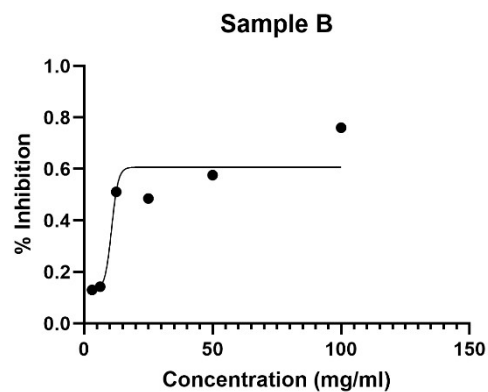
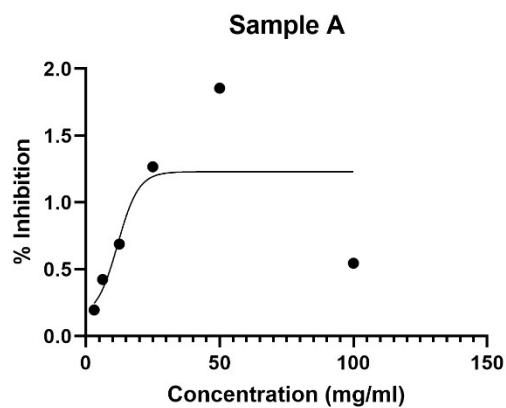


Fig 4.1A – 4.1G: Graph of nonlinear regression of
plant extracts

Key: *Diospyros mespiliformis* Leaves (Sample A),
Stem bark of *Diospyros mespiliformis* (Sample
B), *Cassia nigracans* (Sample C), *Khaya
senegalensis* (Sample D), *Ficus carica* (Sample
E), *Vernonia amygdalina* (Sample F), and *Ficus
sycomorus* (Sample G)

Interpretation of nonlinear regression

IC₅₀ estimates varied widely, and several samples produced values too large or too small to interpret biologically. Such behavior usually appears when:

1. The extract has weak or no inhibitory effect at tested concentrations.
2. The effective inhibitory range lies outside the tested concentration window.
3. The curve lacks a clear sigmoidal transition.

4.2 Discussion

Methanolic plant extracts often contain a complex mixture of polyphenols, alkaloids, terpenoids, flavonoids, and other secondary metabolites that can interact with carbohydrate-hydrolyzing enzymes in different ways (Patel *et al.*, 2021). Inhibition of α -glucosidase is particularly relevant for controlling postprandial hyperglycemia, since the enzyme catalyzes the hydrolysis of terminal α -1,4-linked glucosides (Sun *et al.*, 2022). Many plant-derived inhibitors, including phenolic acids, quercetin derivatives, and tannins, exhibit concentration-dependent, sigmoidal inhibition patterns under well-optimized assay conditions (Yin *et al.*, 2020). In contrast, the extracts in this study produced mostly shallow curves with low Hill slopes, suggesting that the extracts either exhibited weak inhibitory effects within the tested range or contained constituents that interacted with the enzyme in non-classical ways.

The IC₅₀ values ranged widely, from extremely small values (for *Ficus carica*) to astronomically large values (for *Cassia nigricans* and *Khaya senegalensis*). Such extremes reflect the instability of curve fitting rather than true biological potency. These unstable fits likely resulted from the limited concentration range, variability in absorbance readings, and the chemical complexity of crude extracts. Previous studies on crude methanolic extracts have reported similar challenges, noting that poorly defined dose–response curves often arise when the extract concentration does not reach inhibitory saturation (Rahaman *et al.*, 2023).

This aligns with the present findings, in which the top and bottom asymptotes did not reliably anchor the logistic model.

Despite these limitations, some extracts showed more stable behavior than others. *Diospyros mespiliformis*, Stem bark, *Khaya senegalensis*, and bitter leaf displayed reasonable top and bottom plateaus, which indicates that they may have measurable inhibitory effects that could be resolved with improved concentration spacing. Bitter leaf in particular is known to contain sesquiterpene lactones and flavonoids reported to inhibit α -glucosidase through mixed or noncompetitive mechanisms (Ogbuanu *et al.*, 2021). Similarly, species of *Ficus* have been reported to modulate carbohydrate metabolism through polyphenol-mediated enzyme inhibition (Barbosa *et al.*, 2023). The present findings neither confirm nor contradict these reports but instead highlight that crude extraction and limited assay conditions were insufficient for producing definitive potency rankings.

The lack of clear sigmoidal inhibition patterns suggests that the active constituents may be present at low abundance in the crude extracts. Fractionation, enrichment, or phytochemical purification could reveal hidden inhibitory activity, as shown in recent work where chromatographic partitioning greatly improved IC_{50} precision in plant-based enzyme inhibition studies (Kim *et al.*, 2022). Furthermore, yeast α -glucosidase is highly sensitive to assay conditions such as pH, buffer strength, and substrate concentration, and small deviations can cause substantial shifts in enzyme kinetics (Wang *et al.*, 2021). Although the incubation time and buffer pH in this study were consistent with standard protocols, expanding the assay conditions and validating them against known inhibitors such as acarbose would strengthen future experiments.

The study provides a preliminary comparative assessment of inhibitory effects among the selected plant extracts but shows that the current dataset is insufficient to establish definitive potency rankings or mechanistic conclusions. The results underscore the need for enhanced

methodological sensitivity, broader concentration ranges, and extract fractionation to obtain interpretable IC₅₀ values.

4.3 Conclusion

This study set out to compare the inhibitory effects of seven methanolic plant extracts on the activity of yeast α -glucosidase. Although all extracts produced measurable absorbance changes during the assay, the nonlinear regression revealed that most dose–response curves lacked the sigmoidal pattern normally associated with stable IC₅₀ estimation. Several extracts, including *Cassia nigricans*, *Ficus carica*, and *Ficus sycomorus*, generated unstable fits, which indicated that the tested concentration range did not adequately capture their inhibitory behavior or that their active components were present at very low levels. Extracts such as *Diospyros mespiliformis*, *Khaya senegalensis* , and *Vernonia amygdalina* displayed more consistent upper and lower plateaus, suggesting that they contained constituents capable of interacting with the enzyme, although not in a way that allowed precise potency ranking under the conditions applied.

The combined findings show that while the seven plants have the potential to influence α -glucosidase activity, the present assay conditions were not sufficient to define their potency or compare their inhibitory strengths with confidence.

4.4 Recommendations

Future work should establish a more reliable concentration gradient that spans a wider range, so the full inhibitory curve can be captured with clearer transitions between minimal and maximal effect. The extracts should be fractionated or chromatographically enriched, because crude preparations often dilute the bioactive compounds to levels that are too low for accurate IC₅₀ determination. The use of a standard inhibitor such as acarbose should be incorporated into every assay run, both as a validation step and as a benchmark for interpreting plant-derived inhibition. Replicating the assays with additional substrate concentrations, strict

kinetic controls, and alternative enzyme sources may also improve curve stability. Improvements in extraction quality, such as solvent partitioning or purification by column chromatography, would help isolate the compounds responsible for any inhibitory effect and permit clearer mechanistic interpretation. These refinements will allow future studies to generate reproducible, biologically meaningful IC_{50} values and accurately compare the inhibitory potential of the selected plant species.

References

- Abdel-Hamid, M., El-Moselhy, T., and Hussein, A. (2018). Antidiabetic effects of plant-derived polyphenols through modulation of α -glucosidase and α -amylase. *Journal of Food Biochemistry*, 42(6), e12501. <https://doi.org/10.1111/jfbc.12501>
- Abdullahi, S., Bello, M., and Yusuf, A. (2023). Ethnobotanical uses and phytochemistry of *Cassia nigricans* in northern Nigeria. *Journal of Ethnobiology and Ethnomedicine*, 19(1), 12–22.
- Abdulrahman, A., and Musa, H. (2021). Phytochemicals and antidiabetic potential of West African medicinal plants. *African Journal of Biochemistry Research*, 15(2), 45–59.
- Adebayo, A. A., and Omodamiro, O. A. (2022). Traditional use and pharmacological validation of Nigerian medicinal plants used in diabetes. *Journal of Herbal Medicine*, 35, 100558.
- Adefegha, S. A. (2018). Functional foods and nutraceuticals as dietary intervention in chronic diseases. *Journal of Food Measurement and Characterization*, 12(1), 346–361.*
- Adekunle, O., Adetunji, O., and Olusola, T. (2022). Flavonoids and tannins as inhibitors of carbohydrate-hydrolysing enzymes: implications for diabetes management. *Phytotherapy Research*, 36(4), 1420–1432.
- Akinola, O., and Fadare, D. (2021). Health system barriers to diabetes care in West Africa: an integrative review. *Global Health Research and Policy*, 6(1), 11.
- Ali, R., Khan, S., and Ahmed, F. (2022). Postprandial glucose control: role of α -glucosidase inhibitors. *Clinical Diabetes and Endocrinology*, 8(1), 5.
- Ayeni, O., and Okonkwo, C. (2021). Spectrophotometric methods for α -glucosidase inhibition assays: best practices and pitfalls. *Analytical Biochemistry*, 611, 113989.
- Bakhti, A., and Sadaoui, G. (2022). Clinical pharmacology and adverse effects of synthetic α -glucosidase inhibitors. *Diabetes Therapy*, 13(3), 461–476.

- Barbosa, R. B., Silva, P. M., Costa, L. C., and Martins, A. G. (2023). Polyphenol-rich extracts of *Ficus* species and their modulatory effects on carbohydrate-hydrolyzing enzymes. *Journal of Ethnopharmacology*, 312, 116437. <https://doi.org/10.1016/j.jep.2023.116437>
- Bello, K., Ibrahim, G., and Abdullahi, M. (2021). Ethnopharmacology of *Diospyros mespiliformis*: uses, phytochemistry and biological activities. *Journal of Medicinal Plants Research*, 15(7), 160–171.
- Boadi, W., and Asase, A. (2020). African medicinal plants: an overview of ethnobotanical uses and pharmacological potential. *African Journal of Traditional, Complementary and Alternative Medicines*, 17(3), 1–14.
- Chakrabarti, R., Rajagopalan, R., and Mukherjee, S. (2020). Enzyme inhibitory potential of medicinal plants used traditionally for diabetes. *Phytomedicine*, 78, 153310.*
- Chaudhary, S., Gupta, P., and Mehta, R. (2022). In vitro evaluation of α -glucosidase inhibitors: standardization and application. *Experimental and Therapeutic Medicine*, 23(4), 374.
- Chukwuma, C., and Ugwu, E. (2021). Phytochemicals from indigenous plants and their effect on carbohydrate metabolism. *Journal of Applied Biochemistry*, 12(2), 87–98.
- Ezeani, I., and Okoye, P. (2020). Comparative evaluation of plant extracts on digestive enzyme inhibition. *Nigerian Journal of Biochemistry and Molecular Biology*, 35(1), 12–24.
- Farooq, A., Khan, M., and Sadiq, A. (2021). Postprandial hyperglycemia, oxidative stress and vascular complications: mechanistic links. *Cardiovascular Diabetology*, 20(1), 234.
- Fasakin, O., and Ojo, A. (2022). Solvent polarity and extraction yield of bioactive compounds from medicinal plants. *Journal of Natural Products Research*, 16(2), 101–114.

- Garcia, L., and Monteiro, J. (2021). Carbohydrate digestion and absorption: a molecular perspective. *Molecular Metabolism*, 43, 101096.
- Hardy, L. W., and Holland, D. R. (2018). Structure and catalytic mechanism of yeast α -glucosidase. *Biochemical Journal*, 475(2), 205–219.
<https://doi.org/10.1042/BCJ20170691>
- Hossain, M., and Rahman, M. (2022). Practical guide to enzyme inhibition assays for natural products research. *Methods in Enzymology*, 653, 231–254.
- Hussain, M., and Javed, A. (2023). Nonlinear regression fitting of dose–response curves: best practices and model selection. *Journal of Pharmacological Methods*, 28(1), 45–62.
- Ibrahim, M., and Musa, S. (2023). Best practices for storage and handling of crude plant extracts for enzymatic assays. *Phytochemical Bulletin*, 8(1), 34–42.
- International Diabetes Federation. (2021). *IDF Diabetes Atlas (10th ed.)*. Brussels, Belgium: IDF.
- Ishikawa, A., Yamamoto, M., and Iseki, K. (2020). Enzymatic pathways involved in postprandial glucose regulation. *Metabolism Research Reviews*, 36(3), e3245.*
- Khan, S., Ali, M., and Rahman, Q. (2022). Glucose homeostasis and molecular targets for diabetes therapy. *Endocrine Reviews*, 43(2), 201–224.
- Kim, J., Park, S. E., and Son, H. (2022). Improving phytochemical enzyme-inhibition profiling through chromatographic fractionation. *Phytochemistry Letters*, 51, 82–89.
<https://doi.org/10.1016/j.phytol.2022.03.014>
- Kim, Y. H., Lee, J. K., and Park, S. M. (2021). Yeast α -glucosidase extraction and assay optimization for inhibitor screening. *Journal of Enzyme Inhibition*, 36(1), 789–798.
- Kumar, R., Singh, V., and Sharma, P. (2021). Yeast as a model in α -glucosidase inhibition studies: advantages and limitations. *Biotechnology Research International*, 2021, 8823921.

- Li, X., Zhao, L., and Chen, Y. (2023). Colorimetric substrates and assay conditions for α -glucosidase enzymology. *Analytical Methods in Enzymology*, 12(3), 201–220.
- Mathew, M., and Subramanian, S. (2014). In vitro evaluation of anti-diabetic potentials of polyphenols from medicinal plants. *Journal of Clinical and Diagnostic Research*, 8(9), HC01–HC03.
- Miyazaki, H., and Aoki, Y. (2023). Brush border enzymes and intestinal glucose absorption in health and disease. *Gastroenterology Research and Practice*, 2023, Article ID 987654.
- Moghadam, A., Sadeghi, H., and Pour, N. (2020). Enzymatic control of carbohydrate digestion: α -glucosidase as a therapeutic target. *International Journal of Biological Sciences*, 16(7), 1203–1214.
- Moussa, R., and Taleb, K. (2023). Statistical approaches to interpreting IC50 in natural product research. *Journal of Natural Product Statistics*, 5(1), 12–27.
- Nahar, N., and Rahman, A. (2020). Plant-derived α -glucosidase inhibitors: a systematic review. *Phytopharmacology*, 10(2), 67–82.
- Nazir, S., Ahmed, R., and Khan, T. (2022). Salivary and pancreatic amylase: roles in early carbohydrate digestion. *Digestive Enzymology*, 4(2), 55–64.
- Nugraha, D., Hidayat, A., and Siregar, E. (2020). Side effects and tolerability of acarbose and related α -glucosidase inhibitors. *Clinical Therapeutics*, 42(8), 1528–1539.
- Nwosu, C., Eze, U., and Obi, K. (2022). Patient adherence to α -glucosidase inhibitor therapy in resource-limited settings. *Diabetes Care in Practice*, 7(1), 22–29.
- Ogbuanu, C. C., Nwodo, U. U., and Okoye, J. I. (2021). Alpha-glucosidase inhibitory properties of *Vernonia amygdalina* fractions. *Biochemistry Research International*, 2021, 6625403. <https://doi.org/10.1155/2021/6625403>

- Ogunleye, O., Adeyemi, O., and Babalola, O. (2023). Phytochemical screening and bioactivity of *Khaya senegalensis* extracts. *Journal of Medicinal Plant Research*, 17(3), 145–156.
- Ogunyinka, O., and Balogun, A. (2021). Influence of solvent systems on extraction of phytochemicals from Nigerian medicinal plants. *Journal of Natural Medicines*, 75(2), 389–401.
- Ojo, O., and Kareem, M. (2023). Comparative antidiabetic potential of *Cassia* species: in vitro and in vivo studies. *Pharmacognosy Journal*, 15(2), 102–114.
- Okafor, J., and Ezegwui, H. (2021). Diabetes prevalence and risk factors in sub-Saharan Africa: a review. *Public Health in Africa*, 12(1), 33–44.
- Okoli, E., and Udeh, C. (2021). Antioxidant and antimicrobial activities of *Diospyros mespiliformis*: a review. *Journal of Ethnopharmacology*, 267, 113592.
- Otimenyin, S., Afolabi, T., and Peters, M. (2022). Phytochemical composition and enzyme inhibition activity of selected West African medicinal plants. *Journal of Ethnopharmacology*, 290, 115048.
- Patel, K., and Sharma, A. (2021). Standard methods for α -glucosidase inhibition assays using PNPG and maltose substrates. *MethodsX*, 8, 101329.
- Patel, T., Rana, R., and Shah, P. (2021). Phytochemical inhibitors of digestive enzymes: A review. *Critical Reviews in Food Science and Nutrition*, 61(14), 2350–2367. <https://doi.org/10.1080/10408398.2020.1769044>
- Rahaman, S. M., Akter, S., and Khan, M. A. (2023). Challenges in IC₅₀ estimation from crude plant extracts. *Journal of Applied Biological Sciences*, 17(2), 19–28. <https://doi.org/10.5585/jabs.2023.1712>
- Rahid, S., Alam, M., and Choudhury, M. (2020). Solvent polarity effects on recoverable phytochemical classes. *Journal of Phytochemical Analysis*, 7(1), 12–20.

- Rahman, A., Islam, S., and Hossain, M. (2022). Methodological considerations in enzyme inhibition assays using crude plant extracts. *Journal of Pharmacological Methods*, 18(2), 99–110.
- Rahman, S., Akter, S., and Khan, M. A. (2023). Challenges in IC₅₀ estimation from crude plant extracts. *Journal of Applied Biological Sciences*, 17(2), 19–28.
- Ramos, L., and Vieira, P. (2021). Intestinal hydrolases and regulation of carbohydrate digestion. *Frontiers in Physiology*, 12, 643210.
- Rani, R., and Dhanabal, S. (2021). Advances in the development of α -glucosidase inhibitors. *Drug Discovery Today*, 26(4), 987–995.
- Rashid, M., Chowdhury, S., and Hossain, A. (2020). Extraction solvents and yield of phenolic compounds: a comparative study. *International Journal of Food Science*, 55(3), 765–774.
- Sambo, A., and Ibrahim, M. (2023). Limonoids and antidiabetic activity of *Khaya senegalensis*: a phytochemical perspective. *Natural Product Research*, 37(5), 720–731.
- Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A.-M., and Saura-Calixto, F. (2009). Tannins: Current knowledge and future prospects. *Critical Reviews in Food Science and Nutrition*, 49(9), 740–755.*
- Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A.-M., and Saura-Calixto, F. (2009). Tannins: current knowledge and future prospects. *Critical Reviews in Food Science and Nutrition*, 49(9), 740–755.
- Sun, J., Zhao, S., and Li, W. (2022). Alpha-glucosidase inhibitors: From therapeutic development to molecular mechanism. *Current Drug Targets*, 23(4), 300–315.
<https://doi.org/10.2174/1389450122666210902101541>
- Usman, L., and Bello, H. (2021). Kinetic models and inhibition types for digestive enzymes. *Biochemical Modelling*, 6(2), 88–100.

- Wang, H., Lin, Y., and Chen, X. (2021). Influence of assay conditions on yeast α -glucosidase kinetics and inhibitor evaluation. *International Journal of Biological Macromolecules*, 183, 1546–1554. <https://doi.org/10.1016/j.ijbiomac.2021.05.012>
- World Health Organization. (2021). *Global report on diabetes*. WHO Press.
- Yin, X., Sun, Y., and Zhu, H. (2020). Natural polyphenolic α -glucosidase inhibitors and their structure–activity relationships. *Food Chemistry*, 326, 126978. <https://doi.org/10.1016/j.foodchem.2020.126978>
- Zhang, Q., and Liu, J. (2021). Reliability of nonlinear regression in enzyme kinetic models and implications for IC₅₀ interpretation. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 36(1), 1450–1459. <https://doi.org/10.1080/14756366.2021.1927482>

APPENDIX

APPENDIX I: Observed Data

SAMPLE		CONCENTRATION (mg/ml)					
		100	50	25	12.5	6.25	3.125
A	1st Replicate	0.539	1.85	1.26	0.686	0.424	0.198
	2nd Replicate	0.547	1.855	1.268	0.69	0.423	0.193
	3rd Replicate	0.55	1.864	1.273	0.689	0.425	0.197
B	1st Replicate	0.765	0.568	0.485	0.515	0.142	0.13
	2nd Replicate	0.758	0.582	0.484	0.51	0.144	0.131
	3rd Replicate	0.757	0.579	0.486	0.508	0.143	0.13
C	1st Replicate	0.577	0.338	0.222	0.224	0.364	0.264
	2nd Replicate	0.581	0.343	0.235	0.243	0.374	0.197
	3rd Replicate	0.586	0.345	0.257	0.256	0.367	0.142
D	1st Replicate	0.94	0.862	0.971	0.523	0.336	0.283
	2nd Replicate	0.934	0.864	0.972	0.517	0.346	0.298
	3rd Replicate	0.928	0.865	0.974	0.515	0.345	0.304
E	1st Replicate	0.96	0.909	0.356	0.634	0.128	0.121
	2nd Replicate	0.972	0.905	0.357	0.635	0.126	0.122
	3rd Replicate	0.974	0.907	0.358	0.638	0.128	0.12
F	1st Replicate	0.943	0.86	0.561	0.463	0.491	0.577
	2nd Replicate	0.944	0.867	0.558	0.461	0.502	0.576
	3rd Replicate	0.952	0.868	0.556	0.457	0.505	0.575
G	1st Replicate	0.153	0.102	0.355	0.504	0.12	0.053
	2nd Replicate	0.152	0.101	0.348	0.5	0.121	0.052
	3rd Replicate	0.15	0.102	0.337	0.498	0.12	0.053

Standard	0.006
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APPENDIX II: Nonlinear Regression

log(inhibitor) vs. response - - Variable slope (four parameters)	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G
Best-fit values							
Bottom	0.141	0.1289	0.2621	0.3111	Unstable	0.5119	0.2123
Top	1.231	0.6071	0.5813	0.9237	1.026	0.9057	Unstable
LogIC50	11.9	10.73	52.09	13.75	-177.1	27	Unstable
HillSlope	0.1109	0.338	0.2283	0.2291	0.01362	0.4373	Unstable
IC50	798859255966	53358730787	1.220e+052	55636549902967	8.306e-178	9.956e+026	Unstable
Span	1.09	0.4782	0.3193	0.6127	Unstable	0.3938	Unstable
Goodness of Fit							
Degrees of Freedom	14	14	14	14	14	14	14
R squared	0.5418	0.8741	0.8118	0.9848	0.802	0.9501	0
Sum of Squares	2.629	0.1179	0.05686	0.0213	0.4183	0.03093	0.4534
Sy.x	0.4333	0.09176	0.06373	0.03901	0.1729	0.047	0.18
Number of points							
# of X values	18	18	18	18	18	18	18
# Y values analyzed	18	18	18	18	18	18	18