INVESTIGATING THE EFFECTS OF AQUEOUS EXTRACT OF PICRALIMA NITIDA FRUIT ON ALPHA GLUCOSE ENZYME IN STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR RATS

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BENIN CITY

NOVEMBER, 2025.

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A PROJECT SUBMITTED TOBTHE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE B. Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by DIBIA FERDINAND with matriculation number BMS2101386, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City. The project was conducted in partial fulfillment of the requirements for the award of the Bachelor of Science (B.Sc.) Degree in Medical Biochemistry.

Signed:

..... ……

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External Examiner Date

DEDICATION

This project work is dedicated to God Almighty for His grace and strength all through the process. I am immensely grateful Lord.

ACKNOWLEDGMENT

I give my deepest gratitude to God Almighty for His strength, guidance, and immeasurable mercy throughout the course of this work.

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ABSTRACT

Persistent hyperglycemia is a hallmark of diabetes mellitus, a chronic metabolic condition that increases the risk of major vascular problems such neuropathy, nephropathy, and cardiovascular disease. Inhibition of carbohydrate-digesting enzymes, especially α-glucosidase, has become an effective therapeutic method for controlling postprandial blood glucose. Despite the availability of synthetic α-glucosidase inhibitors like acarbose, their usage is frequently restricted due to gastrointestinal side effects, which has sparked interest in safer, plant-based substitutes. Although the leaves and seeds of the traditional West African medicinal plant Picralima nitida have been shown to have antidiabetic qualities, nothing is known about how its fruit extract affects α-glucosidase.

The purpose of this study was to examine the impact of aqueous Picralima nitida fruit extract on α-glucosidase activity in male Wistar rats with diabetes induced by streptozotocin. Rats were given graded doses of the fruit extract after being acclimated to controlled laboratory conditions and grouped based on body weight. A colorimetric assay based on the hydrolysis of p-nitrophenyl-α-D-glucopyranoside was used to test serum α-glucosidase activity. The enzyme activity was determined spectrophotometrically at 405 nm. Tukey's post hoc test and one-way ANOVA were used to examine the data, which were presented as mean ± SEM. The study's findings are still awaiting. It is anticipated that the study would shed light on whether Picralima nitida fruit extract inhibits α-glucosidase to produce antihyperglycemic effects. It may also help develop safer plant-based medicines for the treatment of diabetes.

CHAPTER ONE

1.1 Background of the Study

Persistent hyperglycemia brought on by decreased insulin secretion or activity is a hallmark of diabetes mellitus, a chronic metabolic disease. Vascular problems like retinopathy, neuropathy, nephropathy, and cardiovascular disease are all exacerbated by persistently high blood sugar. Controlling postprandial hyperglycemia is crucial for managing diabetes because it contributes significantly to the total glycaemic load (Han et al., 2025).

Inhibiting carbohydrate-digesting enzymes, such as α-glucosidase, is one therapeutic strategy that modifies postprandial glucose rise by delaying the breakdown of complex sugars into absorbable monosaccharides (Han et al., 2025; Bhatia et al., 2019). Despite the effectiveness of synthetic α-glucosidase inhibitors like miglitol and acarbose, their usage is restricted due to gastrointestinal adverse effects (Bhatia et al., 2019). More than 290 natural compounds have recently been discovered, demonstrating the increased interest in plant-derived inhibitors with better safety profiles (Dirir et al., 2021).

Picralima nitida, sometimes referred to as “akuamma” or “Osun,” is a traditional West African medicinal plant used to treat gastrointestinal issues, fever, and pain (TheFerns Tropical Plant Database, 2025). Its leaf and seed extracts have been proven to have antidiabetic properties (Ezea et al., 2002; Antioxidant and Antidiabetic Profiles, 2013). Nevertheless, little research has been done on the fruit extract’s direct impact on α-glucosidase.

This study uses streptozotocin-induced diabetic male Wistar rats to examine if Picralima nitida aqueous fruit extract lowers blood glucose through α-glucosidase inhibition. Along with in vitro

enzyme tests on serum or intestinal homogenates, fasting and postprandial glucose will be tracked.

1.2 Aim of the Study

To determine the effects of aqueous Picralima nitida fruit extract on α-glucosidase activity in streptozotocin-induced diabetic male Wistar rats and establish whether its antihyperglycaemic action is linked to enzyme inhibition.

1.3 Scope of the Study

Male Wistar rats with STZ-induced diabetes are used in the study, and only Picralima nitida aqueous fruit extract is assessed. Fasting and postprandial glucose assessments as well as in vitro α-glucosidase assays with serum or intestinal homogenates are among the investigations. During a brief experimental time, extract doses will be examined across graded levels. The study does not cover long-term impacts, additional enzymes, or sophisticated pharmacokinetic or microbial analysis.

1.4 Objectives of the Study

1. To induce diabetes in male Wistar rats with streptozotocin and confirm elevated fasting blood glucose.
2. To prepare and administer graded doses of aqueous Picralima nitida fruit extract.
3. To evaluate α-glucosidase activity in serum
4. To compare enzyme activity and percentage inhibition between groups.
5. To evaluate the relationship between glucose-lowering effects and α-glucosidase inhibition.

1.5 Significance of the Research

Picralima nitida seed and leaf extracts have demonstrated antidiabetic efficacy, however it is yet unknown how the fruit extract affects α-glucosidase. Whether the fruit extract inhibits this important digestion enzyme is directly demonstrated by this investigation. The results may shed light on whether the extract's antihyperglycemic actions are enzyme-mediated by connecting enzyme activity with glycaemic alterations. If successful, Picralima nitida fruit may provide a safer plant-based α-glucosidase inhibitor than synthetic medications. By assisting in the future isolation of active chemicals and advancing scientific knowledge of a plant commonly used in African traditional medicine, the study also advances pharmacognosy.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Diabetes Mellitus

Introduction

The term "diabetes mellitus" describes a collection of metabolic diseases marked by chronic hyperglycemia brought on by decreased insulin secretion, action, or both. The most prevalent varieties of diabetes are Type 1 and Type 2, although gestational and secondary variants also pose a serious threat to public health. Comprehending these categories facilitates the creation of focused therapeutic and preventive approaches.

2.1.1 Definition and Classification of Diabetes Mellitus

Elevated blood glucose caused by deficiencies in insulin production or action characterizes diabetes mellitus, a chronic metabolic disease (American Diabetes Association, 2022). Pancreatic β-cells produce insulin, which controls the intake of glucose. When this insulin fails, it can cause hyperglycemia and long-term problems such neuropathy, nephropathy, and cardiovascular disease (World Health Organization, 2023).

Type 1 diabetes mellitus, Type 2 diabetes mellitus, gestational diabetes mellitus, and secondary or other particular variants are the four primary categories into which the World Health Organization (2023) divides diabetes.

Type 1 Diabetes Mellitus

An autoimmune condition known as type 1 diabetes mellitus (T1DM) causes the death of pancreatic β-cells and a complete lack of insulin (Pociot and Lernmark, 2016). It usually appears

in childhood or adolescence and necessitates insulin medication for the rest of one's life. Its development is influenced by viral infections, environmental factors, and genetic predisposition (Atkinson, Eisenbarth, and Michels, 2014). People who don't have insulin suffer from serious metabolic problems such hyperglycemia and ketoacidosis (American Diabetes Association, 2022).

Type 2 Diabetes Mellitus

Approximately 90% of cases of diabetes worldwide are type 2 diabetic mellitus (T2DM) (International Diabetes Federation, 2023). It is characterized by both a relative insulin deficit and peripheral tissue insulin resistance (DeFronzo et al., 2015). Obesity, inactivity, age, and genetic predisposition are risk factors. B-cell failure and persistent hyperglycemia are ultimately caused by persistent insulin resistance (American Diabetes Association, 2022).

Lifestyle changes, oral antidiabetic drugs, and occasionally insulin are all part of management. Chronic hyperglycemia raises the risk of renal impairment, neuropathy, and cardiovascular disease (DeFronzo et al., 2015).

Gestational Diabetes

Pregnancy-induced insulin resistance is the main cause of gestational diabetes mellitus (GDM), a glucose intolerance identified during pregnancy (Kampmann et al., 2015). More glucose can normally reach the fetus due to decreased maternal insulin sensitivity, but insufficient pancreatic compensation leads to hyperglycemia. GDM typically goes away after giving delivery, although it raises the risk of macrosomia, preeclampsia, and Type 2 diabetes in the future (World Health Organization, 2023).

Obesity, a family history of diabetes, and advanced maternal age are risk factors. Pregnancy outcomes are improved by early screening and nutritional or insulin therapy (Kampmann et al., 2015).

Secondary or Other Specific Types of Diabetes

Conditions include pancreatitis, cystic fibrosis, endocrine illnesses like Cushing's syndrome, hereditary abnormalities, and long-term glucocorticoid medication that affect insulin action or pancreatic function can cause secondary diabetes (American Diabetes Association, 2022).

Additionally, viral infections that harm β-cells or pancreatic surgery may cause it (Alberti and Zimmet, 2014). In addition to glycaemic control, management focuses on treating the underlying cause.

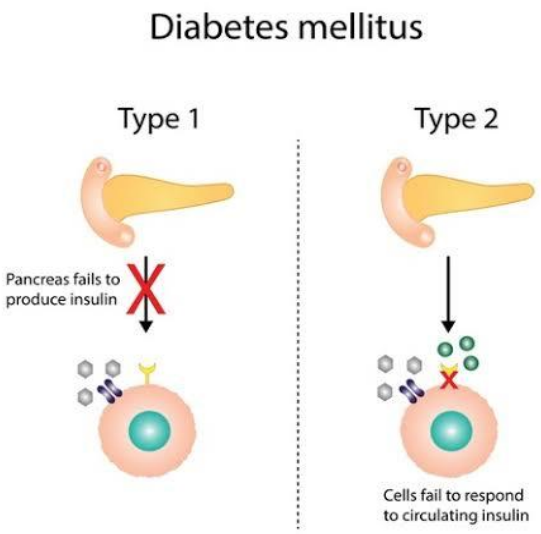

Fig. 1.1 showing Type 1 diabetes

Source: Shutterstock

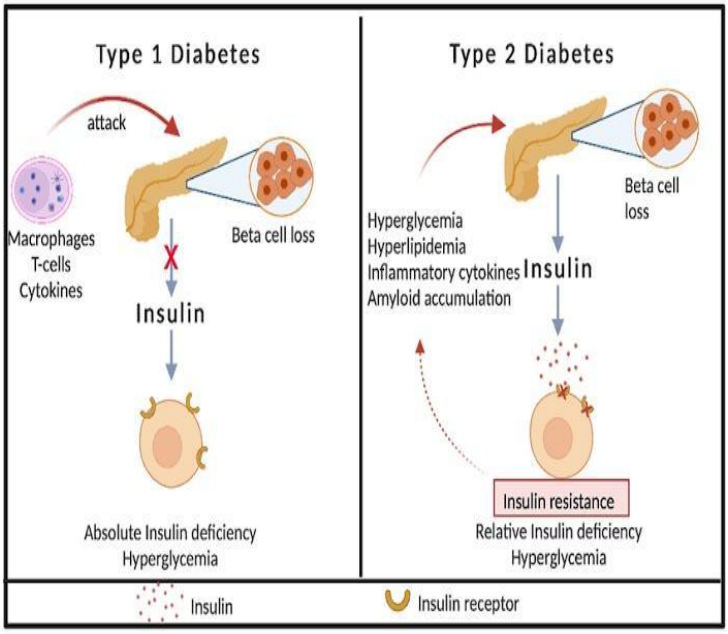

Fig. 1.2 showing pathogenesis of Type 1 and Type 2 diabetes

Source: Researchgate

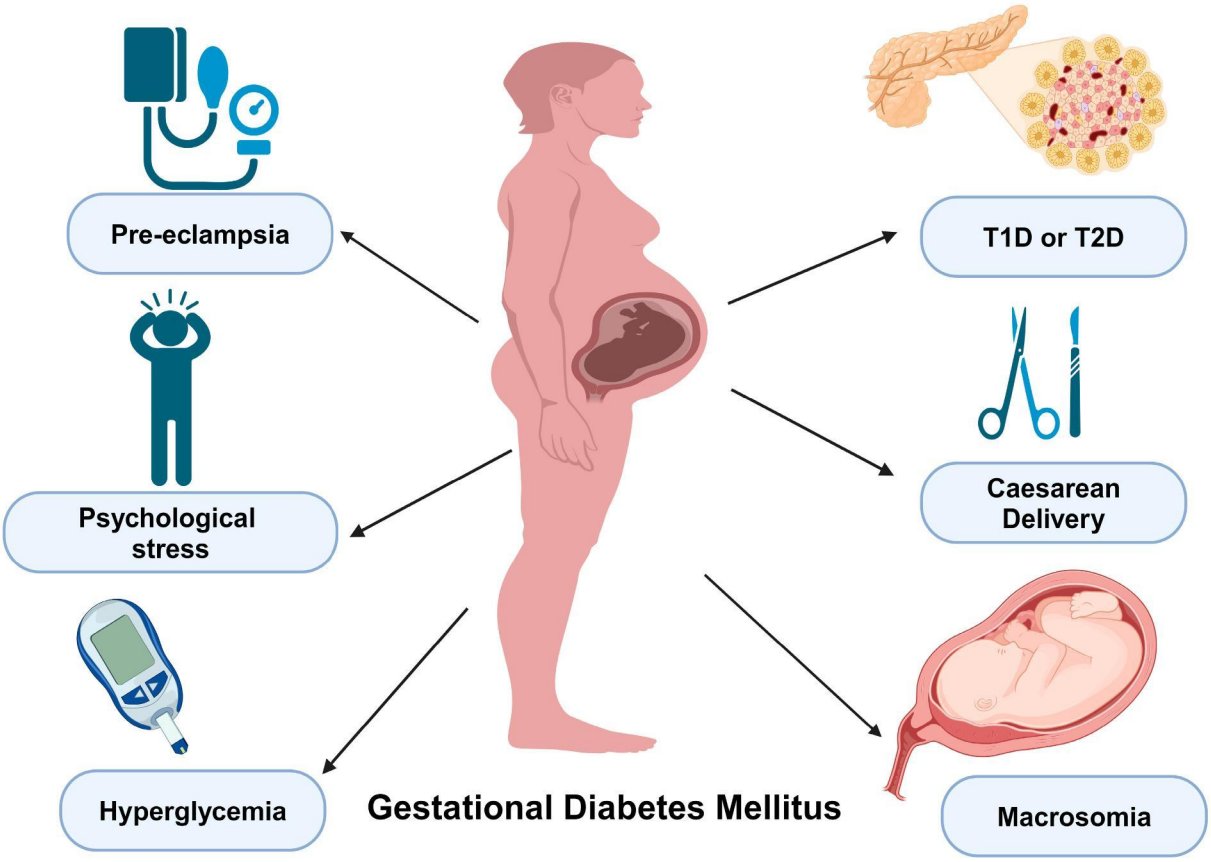

Fig. 1.3 showing pathophysiology of gestational diabetes

Source: MDPI

2.1.2 Pathophysiology of Diabetes Mellitus

Chronic hyperglycemia is caused by genetic, metabolic, and environmental variables in the pathophysiology of diabetes. It is primarily caused by insulin resistance, insufficiency, or both, which impairs the metabolism of proteins, fats, and carbohydrates (DeFronzo et al., 2015).

By encouraging peripheral organs to absorb glucose and reducing hepatic glucose synthesis, insulin secreted by pancreatic β-cells preserves glucose homeostasis (Roden and Shulman, 2019). Blood glucose levels rise steadily when these processes are disrupted.

Insulin Deficiency

Cytotoxic T-cells and autoantibodies like GAD65 and IA-2 cause the autoimmune death of pancreatic β-cells, which leads to insulin insufficiency, a feature of Type 1 diabetes (Atkinson, Eisenbarth, and Michels, 2014). Reduced insulin increases the breakdown of fat and protein and increases the risk of ketoacidosis by preventing cellular glucose uptake (Cersosimo et al., 2018).

Insulin Resistance

A key component of Type 2 diabetes is insulin resistance, which happens when tissues don't react well to insulin. Inflammatory cytokines such TNF-α and IL-6, decreased GLUT4 expression, and compromised insulin receptor signaling are all contributing factors (Samuel and Shulman, 2016). Fasting and postprandial hyperglycemia result from the liver producing more glucose and the muscles absorbing less of it. B-cell fatigue finally results from chronic resistance (Roden and Shulman, 2019).

Impaired Glucose Metabolism

Impaired insulin action in diabetes promotes lipolysis, decreases glycogen storage, and increases hepatic glucose production (Cersosimo et al., 2018). Excess fatty acids and glucose cause oxidative stress and reactive oxygen species, which increase metabolic dysfunction and harm β-cells (Maritim, Sanders, and Watkins, 2003). Complications include neuropathy, nephropathy, and retinopathy are caused by persistent abnormalities (Roden and Shulman, 2019).

2.1.3 Global and Regional Prevalence and Impact of Diabetes

Diabetes is a serious worldwide health issue. According to the International Diabetes Federation (2019), 463 million adults have diabetes, and by 2045, that number is expected to increase to 700 million. According to more recent projections, there are currently 589 million persons with diabetes, of whom 252 million are undiagnosed (International Diabetes Federation, 2024). Due

to urbanization, changing lifestyles, and restricted access to healthcare, the burden is rising most quickly in low- and middle-income nations (World Health Organization, 2023).

The incidence is still increasing in sub-Saharan Africa. With a countrywide prevalence of 7.0% (Uwa et al., 2024) and a pooled rate of 5.77% across regions (Olamoyegun et al., 2024), Type 2 diabetes cases are on the rise in Nigeria. The prevalence of gestational diabetes is likewise high, at 11.0% (Systematic Review, 2021).

Diabetes has serious repercussions, including retinopathy, renal disease, neuropathy, and cardiovascular problems (World Health Organization, 2023). Depending on the population under study, diabetic kidney disease rates in Nigeria vary greatly (Diabetic Kidney Disease Burden Nigeria, 2021).

High treatment expenses, decreased productivity, and stress on health systems are among the economic effects, underscoring the importance of early detection and prevention (International Diabetes Federation, 2024).

2.1.4 Complications Associated with Uncontrolled Diabetes

Acute and long-term problems associated with oxidative stress, endothelial dysfunction, and the production of advanced glycation end products are caused by uncontrolled diabetes (Oputa et al., 2023). Hyperosmolar hyperglycemia and diabetic ketoacidosis are examples of acute consequences (Protein Data Bank-101, 2023).

Microvascular and macrovascular problems are chronic. Retinopathy, nephropathy, and neuropathy are examples of microvascular problems. Up to 70% of people with long-term diabetes have neuropathy, and retinopathy is a major cause of adult blindness (Medical Laboratory Observer Online, 2025). Renal failure may develop from nephropathy.

Cardiovascular disease, stroke, and peripheral artery disease are examples of macrovascular consequences; diabetics are more likely to experience coronary events and amputations (MLO Online, 2025).

Additionally, uncontrolled diabetes raises the risk of several oral health conditions, infections, and poor wound healing (EMedicalHealth, 2023). Reduced quality of life, stress, and higher medical expenses are examples of psychosocial effects (Chuchat et al., 2023).

2.2 Overview of α-Glucosidase Enzyme

2.2.1 α-Glucosidase Enzyme and its Role in Glucose Metabolism

One important intestinal enzyme found in the small intestine's brush border is α-glucosidase. By cleaving α-1,4 glycosidic linkages, it hydrolyzes dietary carbohydrates into absorbable glucose (Bischoff, 1994). Because quick carbohydrate breakdown increases insulin release and glucose absorption, it has a significant impact on postprandial blood glucose levels (Lebovitz, 2001).

Despite normal enzyme activity, poor insulin action in diabetes leads to higher postprandial glucose (van de Laar, 2008).

One well-established method for managing postprandial hyperglycemia is α-glucosidase inhibition. Although they inhibit the digestion of carbohydrates, medications like miglitol and acarbose can have adverse effects on the gastrointestinal tract (Chiasson et al., 2002; Kim et al., 2005).

Plant-derived inhibitors have become more popular as a result. Flavonoids, alkaloids, terpenoids, and phenolics are examples of phytochemicals that exhibit α-glucosidase inhibitory and antioxidant activity, potentially providing safer treatments (Tadera et al., 2006; Liu et al., 2018).

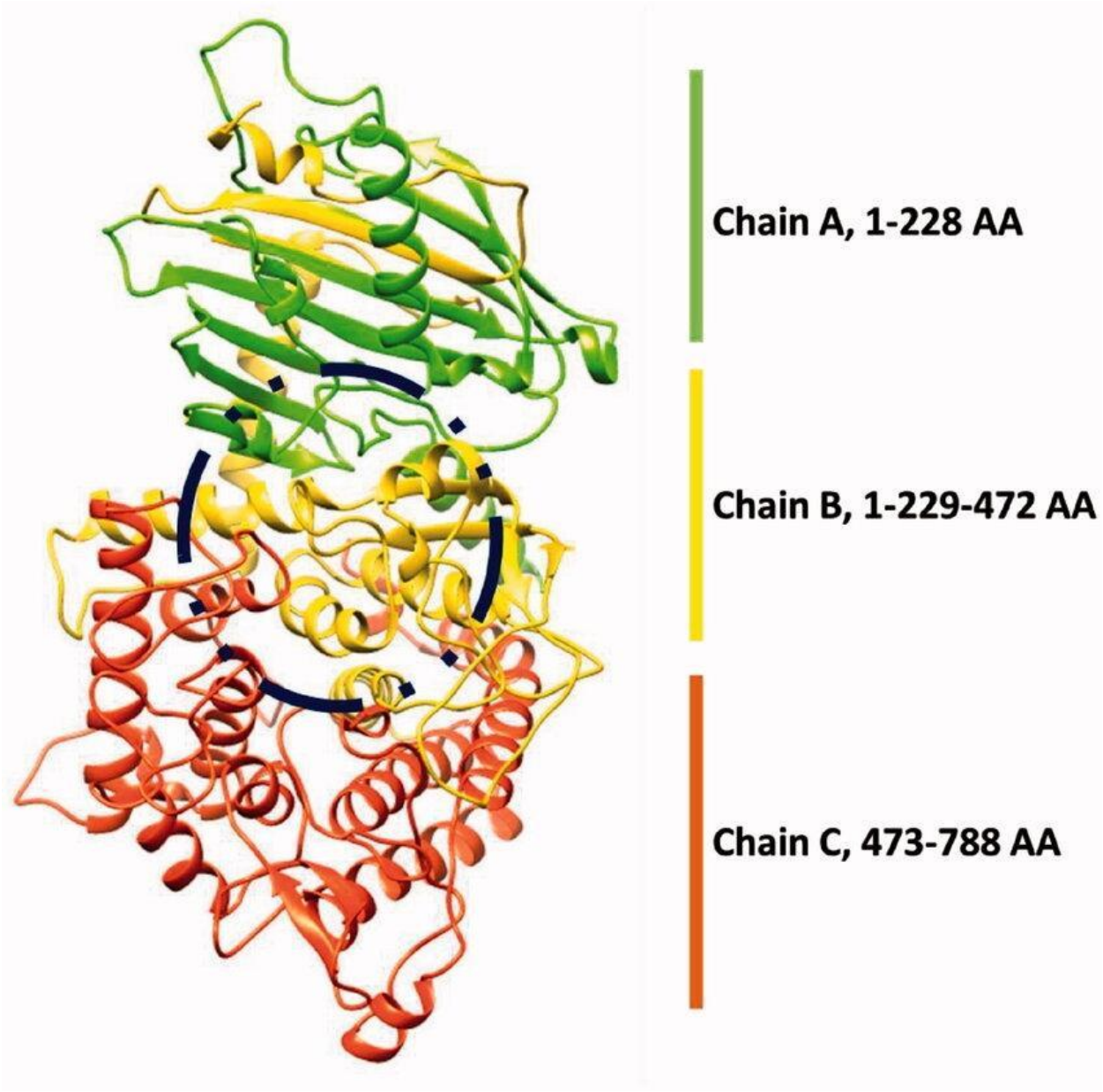
Chain A, 1-228 AA
Chain B, 1-229-472 AA
Chain C, 473-788 AA

Fig. 1.4 showing 3D structure of α-Glucosidase Enzyme

Source: Researchgate

2.1.2 Structure and Function of α-Glucosidase Enzyme

The intestinal brush border contains the membrane-bound glycoprotein α-glucosidase, which hydrolyzes terminal α-1,4-glycosidic bonds to liberate glucose (Bischoff, 1994). Maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI), its two catalytic subunits, play complementary roles in the breakdown of carbohydrates (Quezada-Calvillo et al., 2008).

Aspartate and glutamate, two conserved acidic residues necessary for substrate interaction, are found in its catalytic sites (Sim et al., 2008). The enzyme needs metal ions for structural stability and operates best at pH 6–7 (Yamamoto et al., 2010). It promotes the absorption of glucose into the bloodstream and completes the last stage of carbohydrate digestion by exo-acting hydrolysis of oligosaccharides and disaccharides (van de Laar, 2008; Lebovitz, 2001).

Since rapid glucose absorption and postprandial hyperglycemia are caused by excessive α-glucosidase activity, inhibiting it is a useful treatment strategy (Chiasson et al., 2002). The design of both synthetic and natural inhibitors has been influenced by structural investigations that reveal α-glucosidase has a (β/α)8-barrel fold characteristic of GH31 enzymes (Sim et al., 2008; Kim et al., 2005; Liu et al., 2018).

2.2.3 Role of α-Glucosidase in Carbohydrate Digestion and Glucose Regulation

By transforming oligosaccharides and disaccharides into absorbable glucose, α-glucosidase catalyzes the last phase of carbohydrate digestion (Bischoff, 1994; Lebovitz, 2001). Amylase

breaks down starch into smaller units, which are then hydrolyzed into glucose by α-glucosidase and absorbed by intestinal transporters such GLUT2 and SGLT1 (Quezada-Calvillo et al., 2008). This enzyme is essential to postprandial glucose management because it significantly affects the pace at which glucose enters the bloodstream following meals (van de Laar, 2008). Rapid glycaemic spikes brought on by excessive exertion contribute to oxidative stress and early metabolic dysfunction (Chiasson et al., 2002).

Inhibiting α-glucosidase reduces postprandial hyperglycemia and decreases the digestion of carbohydrates. Competitive inhibition is how medications like voglibose, miglitol, and acarbose function (Campbell, 2009; Kim et al., 2005). Flavonoids, alkaloids, terpenoids, and phenolics are examples of natural substances that exhibit inhibitory actions with less gastrointestinal adverse effects (Tadera et al., 2006; Liu et al., 2018).

2.2.4 Mechanism of α-Glucosidase Inhibition as a Therapeutic Target

One important treatment approach for controlling postprandial hyperglycemia is α-glucosidase inhibition. Inhibitors provide a slower and more regulated rise in blood glucose following meals by delaying the digestion of carbohydrates (Lebovitz, 1997; Chiasson et al., 2002).

Natural substrates like maltose and sucrose are prevented from entering the enzyme's active site by these inhibitors' competitive and reversible binding (Van de Laar et al., 2005). This mechanism is useful for managing type 2 diabetes since it functions without the need for insulin (Casirola and Ferraris, 2006).

Acarbose, miglitol, and voglibose are examples of synthetic inhibitors that successfully lower glucose spikes by imitating carbohydrate transition states (Bischoff, 1994; Van de Laar, 2008). However, gastrointestinal distress brought on by undigested carbohydrates may restrict their use (Hanefeld, 1998).

With their competitive and non-competitive inhibitory actions and additional antioxidant benefits, plant-derived chemicals offer intriguing options (Tadera et al., 2006; Kumar et al., 2011). Because of this, α-glucosidase is a promising target for additional therapeutic development.

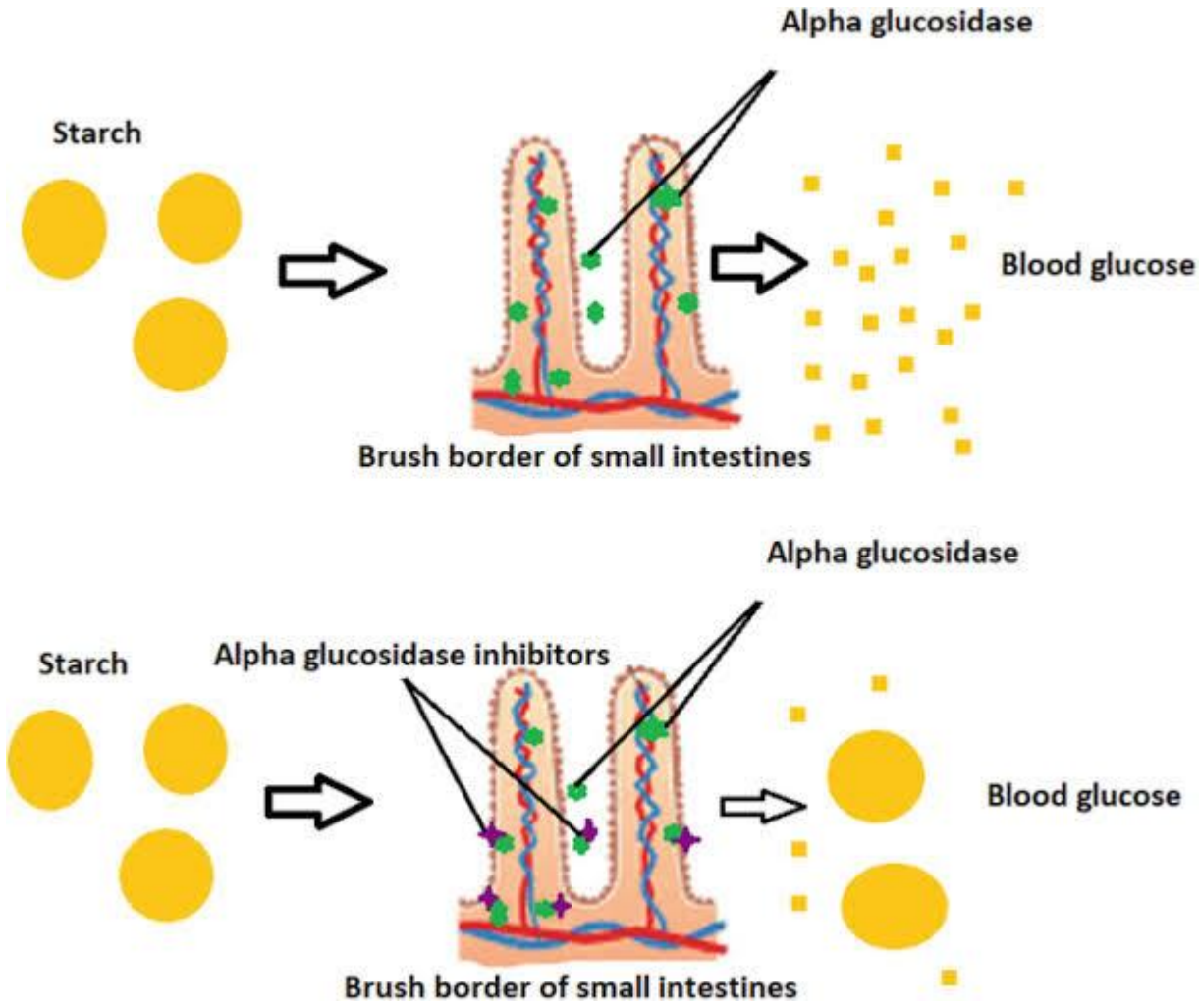

Fig. 1.5 showing the mechanism of action of α-Glucosidase enzyme and its inhibition

2.2.5 Existing α-Glucosidase Inhibitors and Their Limitations

By competitively inhibiting intestinal α-glucosidase and reducing the digestion of carbohydrates, α-glucosidase inhibitors like acarbose, miglitol, and voglibose are used to manage postprandial hyperglycemia in Type 2 diabetes (Van de Laar, 2008).

Acarbose is a microbial pseudotetrasaccharide that successfully lowers postprandial glucose and inhibits both α-amylase and α-glucosidase. However, because undigested carbs enter the colon, it induces dose-dependent gastrointestinal adverse effects (Lebovitz, 1997; Hanefeld, 1998; Chiasson et al., 2002).

Although renal excretion restricts its usage in patients with compromised kidney function, miglitol, a desoxynojirimycin derivative, is systemically absorbed and lowers both fasting and postprandial glucose levels (Campbell et al., 2010; Standl and Schnell, 2012).

Voglibose must be taken before meals and acts locally in the intestine with less systemic absorption, resulting in fewer gastrointestinal side effects, however some discomfort may still occur (Kaku, 2011; Saito et al., 1998).

These inhibitors have limited glucose-lowering effects and may decrease patient compliance despite their advantages. This has prompted studies on substitutes made from plants that would have fewer adverse effects (Van de Laar et al., 2005; Kumar et al., 2011).

2.3. The Picralima nitida Plant (Family: Apocynaceae)

In traditional African medicine, Picralima nitida is a common medicinal herb. Its various phytochemical components support its significance in the creation of natural product drugs by contributing to documented actions such as antidiabetic, analgesic, and antibacterial properties.

2.3.1 Botanical Description and Geographical Distribution of Picralima nitida

Picralima nitida is a 35-meter-tall evergreen tree with paired hard fruits that contain numerous bitter seeds, small white to yellow blooms, and opposite glossy leaves (Burkill, 1997; Iwu, 2014). Locals refer to it as Osu or Osun in Igbo, Abere in Yoruba, and Akuamma in Ghana (Adewunmi and Ogundaini, 1987).

Because of alkaloids like akuammine and akuammidine, the seeds are frequently used in traditional medicine to treat ailments like malaria, fever, gastrointestinal issues, and diabetes (Tona et al., 2004; Oliver-Bever, 1986; Otimenyin et al., 2008).

The plant thrives in humid lowland areas and rainforests throughout West and Central Africa, including Nigeria, Ghana, Ivory Coast, Cameroon, and the Democratic Republic of the Congo (Dalziel, 1937; Iwu, 2014; Burkill, 1997).

Despite its traditional importance, research on its mechanisms and standardization is still being conducted to promote its safe use in contemporary medicine (Otimenyin et al., 2008; Tona et al., 2004).

Fig. 1.6a showing unripe nitida

Source: Tela Botanica.

Fig. 11.6b showing Picraluma nitida ripe Picralima

Source: Lifestyle Seeds

2.3.2 Ethnomedicinal Uses of Picralima nitida

African traditional medicine makes extensive use of Picralima nitida, also called the Akuamma tree, to treat diabetes, fever, malaria, gastrointestinal problems, and discomfort (Iwu, 2014; Tona et al., 2004; Burkill, 1997). Because of its antipyretic and antibacterial qualities, the seeds, which are particularly prized for their bitterness, are frequently used to treat fevers and infections (Adewunmi and Ogundaini, 1987).

The powdered or dried seeds are consumed or infused in water to treat stomach problems and malaria in places like Cameroon, Ghana, and Nigeria (Oliver-Bever, 1986). Alkaloids with

antimalarial, antipyretic, and analgesic properties, such as akuammine and akuammidine, are associated with these effects (Otimenyin et al., 2008).

Additionally, diarrhea, dysentery, and stomach ache have historically been treated using the bark and root preparations (Iwu, 2014; Burkill, 1997). The plant is also used to treat diabetes because its seeds and fruit extracts are thought to lower blood sugar, perhaps by inhibiting enzymes that break down carbohydrates such α-glucosidase (Adewunmi and Ogundaini, 1987; Tona et al., 2004).

P. nitida is also used to treat menstruation problems, discomfort, inflammation, arthritis, jaundice, and hypertension (Burkill, 1997; Iwu, 2014). Despite its widespread use, research on its pharmacological mechanisms and safety is still underway (Tona et al., 2004; Otimenyin et al., 2008).

2.3.3 Phytochemical Constituents of Picralima nitida

Alkaloids are the main substances that give Picralima nitida its antimalarial, analgesic, antidiabetic, and antibacterial properties (Adewunmi and Ogundaini, 1987; Iwu, 2014). Picralima nitida contains a variety of bioactive phytochemicals. Key indole alkaloids include akuammine, akuammidine, akuammigine, pseudoakuammigine, picraline, and akuammicine, many of which show central nervous system effects and opioid receptor activity that account for analgesic qualities (Otimenyin et al., 2008; Kam et al., 2004).

Flavonoids, tannins, saponins, and glycosides are other components (Burkill, 1997; Tona et al., 2004). Tannins have antibacterial and anti-inflammatory properties related to gastrointestinal

illnesses, whereas flavonoids offer antioxidant protection (Iwu, 2014; Adewunmi and Ogundaini, 1987).

By enhancing insulin sensitivity and glucose metabolism, saponins may promote hypoglycemic action (Adebayo et al., 2017). Additional cardioprotective and antioxidant benefits are provided by glycosides (Otimenyin et al., 2008).

Additionally, sterols, phenolics, and terpenoids associated with antibacterial and anti-inflammatory properties are found in seed extracts (Tona et al., 2004; Burkill, 1997). The plant's overall pharmacological potential is increased by the combination of these phytochemicals which encourages further investigation into its possible medicinal uses (Iwu, 2014; Kam et al., 2004).

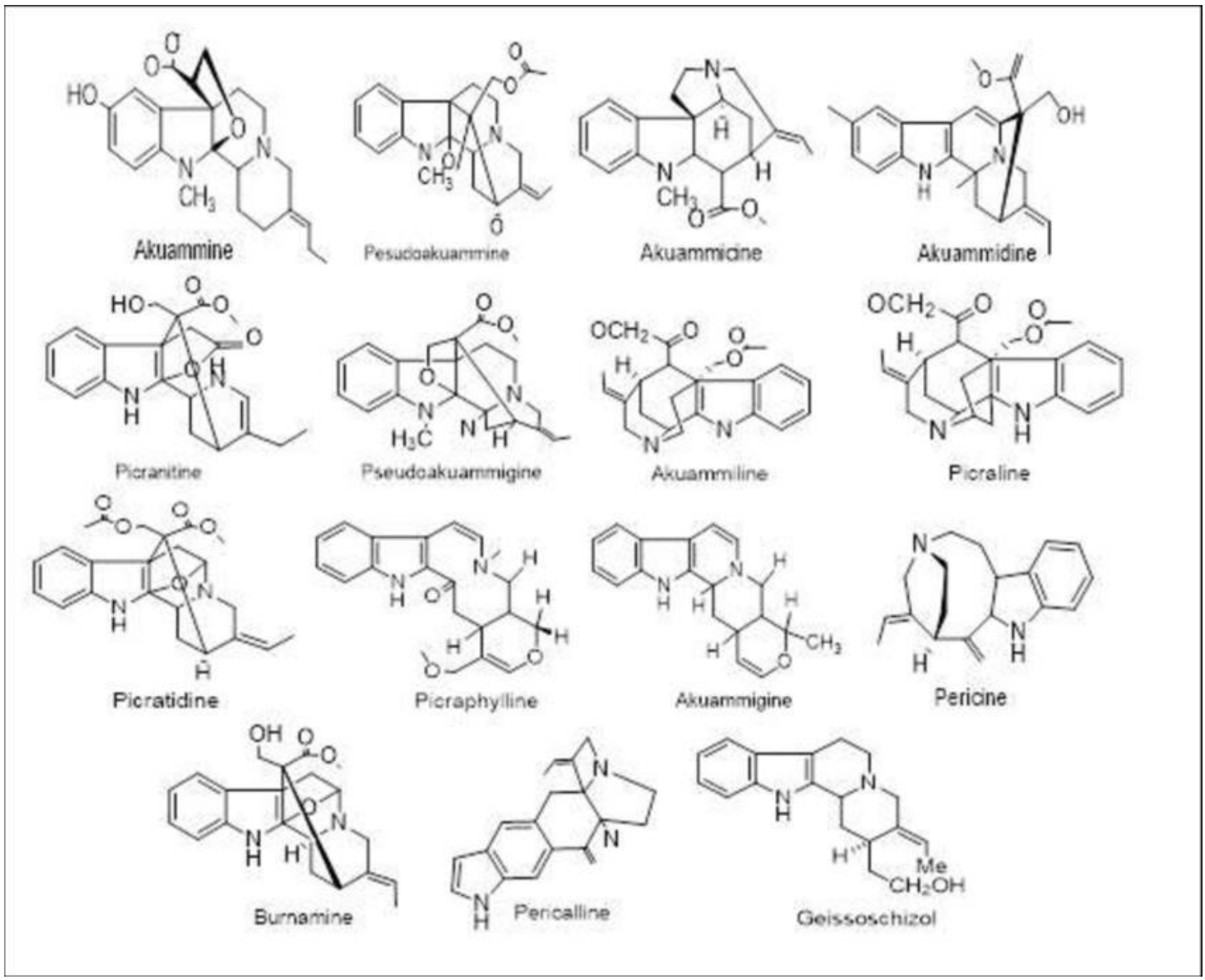

Fig. 1.7 showing the structures of various phytochemicals present in plants including Picralima nitida.

Source: ResearchGate

2.4.1 Pharmacological Activities of Picralima nitida

Because of its alkaloids, flavonoids, tannins, saponins, and glycosides, Picralima nitida has a variety of pharmacological properties, including antioxidant, antibacterial, anti-inflammatory, analgesic, and antidiabetic effects (Iwu, 2014; Kam et al., 2004).

Antioxidant Activity

Flavonoids and phenolic compounds, which scavenge free radicals and boost enzymes like SOD and CAT, are associated to the plant's antioxidant activity (Adebayo et al., 2017; Adedapo et al., 2009). Its application against oxidative and metabolic illnesses is supported by these characteristics (Iwu, 2014).

Antimicrobial Activity

Because of its tannins and alkaloids, P. nitida preparations suppress infections such Pseudomonas aeruginosa, Escherichia coli, Candida albicans, and Staphylococcus aureus (Otimenyin et al., 2008; Tona et al., 2004). Its historic usage for wound care and gastrointestinal illnesses is supported by these effects (Burkill, 1997).#

Anti-inflammatory and Analgesic Activity

While alkaloids like akuammine and akuammidine that act on opioid receptors produce analgesic effects, the anti-inflammatory action requires suppression of mediators including prostaglandins and cytokines (Kam et al., 2004; Otimenyin et al., 2008).

Antidiabetic Activity

Through processes including enzyme inhibition, increased insulin production, and improved glucose absorption, P. nitida extracts lower blood glucose levels and improve lipid profiles in

diabetic animal models (Adebayo et al., 2017; Iwu, 2014). β-cell protection is further supported by antioxidant activity.

Additional Pharmacological Properties

Additionally, the plant exhibits hepatoprotective and antimalarial properties, with alkaloids exhibiting efficacy against Plasmodium falciparum and shielding hepatic cells from oxidative damage (Tona et al., 2004; Adedapo et al., 2009).

2.4.2 Toxicological Profile and Safety Assessment of Picralima nitida

Picralima nitida has a dose-dependent safety profile, according to toxicological research. While high or extended doses may result in biochemical and organ-level changes, low to moderate doses exhibit reasonable tolerability (Adebayo et al., 2017; Adedapo et al., 2009). At modest doses, acute toxicity tests reveal little behavioral alterations (Tona et al., 2004; Otimenyin et al., 2008). Hepatotoxic or nephrotoxic consequences could result from repeated high doses, though.

Its alkaloids may interact with opioids or CNS-acting medications due to their ability to interact with CNS receptors (Kam et al., 2004; Posadzki et al., 2013).

The requirement for standardized extracts and regulated dosing is highlighted by the paucity of data on long-term toxicity, genotoxicity, and reproductive safety (Iwu, 2014; Tona et al., 2004).

2.4.3 Antidiabetic Potential of Picralima nitida

Alkaloids, flavonoids, and saponins are thought to be responsible for the antidiabetic effects of Picralima nitida, which has long been used to treat diabetes (Ezeigbo et al., 2016). Extracts decrease oxidative stress, increase insulin secretion, improve glucose absorption, and inhibit α-amylase and α-glucosidase (Ota and Uloko, 2019). Significant improvements in β-cell function, blood glucose reduction, and antioxidant status restoration have been shown in animal experiments (Ezeigbo et al., 2016; Mbatchou et al., 2021). There is limited clinical validation in people despite robust preclinical findings, necessitating additional research (Mbatchou et al., 2021).

2.4.4 Previous Studies on Picralima nitida and Blood Glucose Regulation

Extracts from seeds, fruits, and leaves have been repeatedly shown to have hypoglycemic effects in animal models of diabetes (Ezeigbo et al., 2016; Olubomehin et al., 2013). Methanol seed extract was shown by Ezeigbo et al. (2016) to reduce blood glucose in a dose-dependent manner. Improvements in antioxidant enzyme activity and fasting blood glucose were observed by Olubomehin et al. (2013). While Ota and Uloko (2019) reported suppression of α-glucosidase and α-amylase, Mbatchou et al. (2021) reported β-cell repair and metabolic enzyme modulation. These results suggest several synergistic pathways, but human trials are needed to prove them.

2.4.5 Reported Mechanisms of Picralima nitida Antidiabetic Effect

Antioxidant activity, β-cell protection, and inhibition of α-glucosidase and α-amylase are the sources of P. nitida's antidiabetic actions (Mbatchou et al., 2021).
By delaying the digestion of carbohydrates, enzyme inhibition lowers postprandial hyperglycemia (Ota and Uloko, 2019; Ezeigbo et al., 2016).
By boosting endogenous defense mechanisms including SOD, CAT, and GPx, antioxidant substances reduce oxidative stress and increase insulin sensitivity (Olubomehin et al., 2013). Additionally, extracts increase insulin secretion and encourage β-cell regeneration (Ezeigbo et al., 2016).

P. nitida is a promising natural antidiabetic drug due to these combination actions (Mbatchou et al., 2021).

2.4.6 Comparative Studies of Picralima nitida Extracts

Phytochemical content and biological activity vary depending on the extraction solvent used (Ezeigbo et al., 2016).
Alkaloids, flavonoids, tannins, and saponins are found in aqueous extracts, which have antioxidant and enzyme-inhibiting properties (Olubomehin et al., 2013; Ota and Uloko, 2019). Higher concentrations of lipophilic alkaloids, including akuammidine, are produced by ethanolic extracts, which improve antioxidant and antidiabetic properties (Mbatchou et al., 2021; Umeokoli et al., 2019).
Because of their wider phytochemical extraction, methanolic extracts frequently exhibit the highest α-glucosidase inhibition and antioxidant capacity (Ezeigbo et al., 2016).

2.4.7 Dose-Dependent and Duration-Dependent Effects of Picralima nitida

P. nitida's medicinal effects intensify with dosage and time. According to Umeokoli et al. (2019), low to moderate doses (100–400 mg/kg) considerably improve metabolic indicators and lower fasting blood glucose levels.

Although higher doses improve insulin stimulation and enzyme inhibition, they may also raise the risk of toxicity (Ota and Uloko, 2019).

Compared to short-term exposure, longer treatment periods (14–28 days) result in better β-cell regeneration and antioxidant repair (Olubomehin et al., 2013; Mbatchou et al., 2021). larger doses also result in increased analgesic and anti-inflammatory effects because of larger alkaloid concentrations (Umeokoli et al., 2019).

2.5.1 Mechanistic Link between Picralima nitida and α-Glucosidase Inhibition

Picralima nitida contains alkaloids, flavonoids, tannins and saponins that influence glucose metabolism and may inhibit α-glucosidase activity (Olowokudejo et al., 2008). Since α-glucosidase hydrolyses complex carbohydrates into glucose (Bischoff, 1994), its inhibition slows digestion and reduces postprandial blood glucose (Van de Laar, 2008).

The bioactive constituents of P. nitida may bind to the enzyme's active or allosteric sites, reducing its activity (Nwaehujor et al., 2011). Flavonoids and phenolics interact through hydrogen bonding and hydrophobic forces to inhibit enzyme function (Kwon et al., 2008). Antioxidant activity of the plant may protect β-cells from oxidative stress (Adesokan et al., 2013). Picralima nitida may also enhance peripheral glucose utilisation and insulin sensitivity (Feyisayo et al., 2019). Water-soluble alkaloids such as akuammine and akuammidine contribute to α-glucosidase inhibition (Iwu, 2014; Ugwu et al., 2013). Overall, its phytochemical interactions likely delay glucose absorption and moderate hyperglycemia.

2.5.2 Possible Interaction of Picralima nitida Phytochemicals with α-Glucosidase

Leaf extracts of P. nitida have shown α-glucosidase inhibition with IC50 values around 3 mg/mL, displaying both competitive and non-competitive mechanisms (Kazeem et al., 2013). Fractionation and docking studies identified active compounds in seed extracts with strong inhibitory affinity, including sub-fractions exhibiting IC50 values near 25 µg/mL and docking scores around −8.84 kcal/mol (Lawal et al., 2025).

The plant contains flavonoids, tannins, saponins, alkaloids and terpenoids (Mutiu et al., 2013; Akinwunmi and Amadi, 2019), many of which form hydrogen bonds or hydrophobic interactions with enzyme residues (Tadera et al., 2006). These interactions reduce substrate access or alter enzyme conformation. The combined antioxidant effects may support β-cell protection and glucose regulation.

2.5.3 Molecular Docking or Biochemical Evidence

Docking studies demonstrate that compounds such as genistein, cirantin and lupeol show strong affinity for α-glucosidase, forming hydrogen bonds and hydrophobic interactions similar to or stronger than acarbose (Lawal, 2025).

Biochemical assays support these findings, showing dose-dependent inhibition by P. nitida extracts, with mixed competitive and non-competitive effects (Kazeem et al., 2013). Additional studies report low IC50 values for α-glucosidase inhibition in plant fractions (Akinwunmi and Amadi, 2019).

Together, molecular and biochemical data confirm that P. nitida phytochemicals effectively bind to α-glucosidase and reduce carbohydrate breakdown, supporting their antidiabetic potential.

2.5.4 Role of Alkaloids and Flavonoids in Enzyme Inhibition

Flavonoids inhibit α-glucosidase by binding to active sites through hydrogen bonding and hydrophobic interactions (Kim et al., 2000). Compounds such as quercetin and luteolin delay intestinal glucose absorption (Ono et al., 2011). Flavonoid-rich fractions of P. nitida show strong enzyme inhibitory activity (Akinwunmi and Amadi, 2019).

Alkaloids act through ionic and covalent interactions that disrupt catalytic residues (Pandey et al., 2013). Alkaloids such as berberine and strictosidine are known α-glucosidase inhibitors (Kazeem et al., 2013). Similar indole alkaloids in P. nitida, including akuammine and akuammidine, contribute to its inhibitory effects (Iwu et al., 1990).

Their combined antioxidant and anti-inflammatory actions further support β-cell protection and glycemic regulation (Kwon et al., 2008).

2.5.5 Correlation Between Enzyme Inhibition and Antidiabetic Effects In Vivo

Inhibition of α-glucosidase slows carbohydrate digestion and reduces postprandial glucose levels (Tadera et al., 2006; Kim et al., 2000). Extracts that inhibit α-glucosidase in vitro often correspondingly reduce blood glucose and improve glucose tolerance in diabetic animals (Kwon et al., 2008).

Picralima nitida leaf extracts similarly reduced fasting glucose in STZ-induced diabetic rats, consistent with their enzyme inhibition (Kazeem et al., 2013). Flavonoids and alkaloids also enhance insulin sensitivity and protect β-cells through antioxidant effects (Iwu et al., 1990).

Thus, α-glucosidase inhibition is a strong predictor of the plant's in vivo antidiabetic efficacy (Ono et al., 2011).

2.6.1 Summary of Research Gaps

Despite evidence of antidiabetic activity, the precise biochemical pathways of P. nitida, including α-glucosidase inhibition, remain insufficiently defined (Akinwunmi and Amadi, 2019; Kazeem et al., 2013). Few studies compare aqueous, ethanolic and methanolic extracts, even though solvent choice affects phytochemical yield and activity (Kazeem et al., 2013).

There is also limited molecular evidence confirming exact binding interactions with α-glucosidase (Ono et al., 2011). Most studies rely on in vitro assays with few in vivo or long-term evaluations (Tadera et al., 2006).

Toxicological assessments are incomplete, with limited chronic or dose-dependent studies (Nworgu et al., 2008). Additionally, variations in plant source and phytochemical consistency remain unaddressed (Osei-Safo et al., 2010).

2.6.2 Limitations in Previous Studies on Picralima nitida

Many studies lack molecular pathway analysis and do not link individual phytochemicals to specific antidiabetic mechanisms (Kazeem et al., 2013; Osei-Safo et al., 2010). Extraction methods vary widely, creating inconsistencies in phytochemical content and biological outcomes (Iwu et al., 1990).

Most research relies on crude extracts rather than isolated compounds (Akinwunmi and Amadi, 2019). Few studies extend findings to in vivo or clinical testing, limiting translation from laboratory to real-life applications (Tadera et al., 2006).

Toxicity assessments are limited to acute studies (Nworgu et al., 2008). Differences in geographical source and harvesting conditions are also under-explored (Osei-Safo et al., 2010).

Computational studies that could clarify enzyme binding interactions are notably scarce (Ono et al., 2011).

2.6.3 Lack of Detailed Enzyme-Targeted Investigations

Current research provides limited enzyme-specific data, with most studies focusing on general antidiabetic or antioxidant properties rather than mechanisms involving α-glucosidase or α-amylase (Olowokudejo et al., 2020). Few studies include biochemical or docking investigations that confirm enzyme interactions (Adebayo et al., 2022).

Crude extracts are typically used, making it difficult to attribute effects to specific compounds (Iwu et al., 1999). There is also a lack of structure–activity relationship analysis (Sofidiya et al., 2021).

Therefore, more enzyme-targeted research using kinetic analysis and molecular modelling is needed to clarify mechanisms of action.

2.6.4 Need for Evaluating α-Glucosidase Inhibition In Vivo Using Aqueous Extract in Male Wistar Rats

Most studies on P. nitida focus on in vitro assays or general hypoglycemic effects rather than in vivo α-glucosidase inhibition (Adebayo et al., 2022). Since α-glucosidase is essential for carbohydrate digestion, in vivo assessment is necessary to confirm physiological relevance (Tundis et al., 2010).

Traditional use of P. nitida relies on aqueous preparation, yet aqueous extracts have not been extensively studied in animals (Olowokudejo et al., 2020). Male Wistar rats provide a reliable model for studying glucose metabolism and enzyme interaction (Szkudelski, 2001).

Investigating α-glucosidase inhibition in vivo can help link enzyme activity with glucose regulation and tissue responses, supporting its potential therapeutic value (Sofidiya et al., 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Equipment and Apparatus

The following materials and equipment were used during the study:

Glassware and Consumables:

Cotton wool

Chloroform

Dissecting set

Methylated Spirit

Distilled water

Plain, EDTA and lithium heparin sample containers

Syringes (5 mL and 10 mL)

Insulin syringe (1mL)

16–18 gauge stainless-steel gavage needle

Nose mask

Lancets

Hand gloves

Machines and Instruments:

1. Glucometer (NEWSPRING Glucose Meter, China; Model KF-B12)
2. Weighing scale (NEWSPRING Digital Scale, China; Model NS- 790)
3. Storage system (HAIER THERMOCOOL, China; Model HRF- 3500X)
4. Grinder (Dry Herb Industrial Grinder, Model SYB-18B)
5. Freeze Dryer (BIOBASE Freeze Dryer, China; Model BK- FD10S)
6. Oven (WINCOM Thermostat Oven, Model OV-A25SF)
7. pH Meter (PHS-25 pH meter, China; Model PHS-25)

3.2 Animal Experimental Study

The purpose of this biomedical experimental investigation was to examine the effects of Picralima nitida fruit aqueous extract on α-glucosidase levels in male Wistar rats that had been given streptozotocin to develop diabetes. The twenty-four (24) male Wistar rats that were physiologically normal weighing between 107.6g and 169.3g that were obtained from the Department of Anatomy, University of Benin, Nigeria The animals were kept in regular laboratory conditions at the Department of Anatomy, University of Benin, Nigeria, in clean, well-ventilated plastic cages with soft wood shavings(saw-dust) for bedding. Before the experiment started, the rats were acclimated to a controlled environment for four(4) weeks, which included a temperature of 22 ± 2°C, a relative humidity of 50–60%, and a 12-hour light/dark cycle.

The rats were provided water ad libitum (freely) and conventional rat feed(Chicken Grower Feed, Top Feeds Ltd, Nigeria) with an approximate nutrient composition of 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus, during this acclimation period.

The Departmental Animal Ethics Committee granted ethical approval for the study, and all methods complied with worldwide guidelines for the humane treatment of laboratory animals. After acclimation, the twenty-four(34) rats were divided into seven (7) groups of five (5) rats each except for Group 0 that has only four (4) rats based on their initial body weight ranges: Group 0 (155g- 169.3g; Group 1 (107.6g – 120.3g); Group 2 (143.2g – 148.2g); Group 3 (129.0g – 136.9g); Group 4 (138.7g – 142.2g); Group 5 (123.5g – 128.8g) and Group 6 (148.3g – 154.5g). To facilitate easy identification, the rats were individually labelled using coloured markers (Genital Violet) on specific body parts, including the hand, leg, back, head, and tail while some were plain (unmarked). Each rat was assigned a unique identification number based on the part of the body where the colour was applied.

During the experiment, the rats were closely monitored for any sign of distress, behavioural changes, or alterations in their physical conditions. Their weights were recorded weekly to observe any significant changes that might indicate the effects of experimental procedures. Also, their fasting blood glucose was recorded weekly (between the hours of 7:30am and 8;30am). The rats were kept under optimal care and all handling procedures were designed to minimize stress and maintain their well being.

At the end of the experimental period (7 days), the rats were sacrificed humanely using chloroform anaesthesia, following ethical guidelines for animal research. Blood samples were collected via cardiac puncture where necessary in labeled sample containers (lithium heparin, plain) and kept in a refrigerator to preserve their integrity until analysis.

This study was conducted following the ethical guidelines outlined by the relevant ethics board of University of Benin. Throughout the study, efforts were made to ensure the humane treatment of the animals and to adhere strictly to ethical practices in biomedical research.

3.3 Collection and Identification of Plant Material

Fresh fruits of Picralima nitida (about 300 in numbers, weighing between 0.2kg-0.3kg of which many were unripe and few ripe ones) were purchased from Oliha market, along Siloko Road, Benin City, Edo state, Nigeria. The plant material was authenticated and identified by a botanist in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, where a voucher specimen was deposited for reference. The Voucher Number given was UBH-P424.

3.4 Preparation of Picralima nitida Extract

The fruits were initially sorted out in order to remove soil, stones, insects, rotten or mouldy ones and only fresh, healthy, clean, and mature fruits were used. The fresh fruits were washed thoroughly with clean water to remove dirt and debris. The rind (outer part) was manually peeled off, and the seeds and the pulp were cut into smaller sizes and divided into six(6) batches for easy drying Thereafter, they were air-dried under shade for 7–10 days to prevent photo-degradation of bioactive compounds. The dried material was further oven-dried at 45°C for about four(4) weeks to ensure complete moisture removal. The oven-dried material was weighed to be 2,202.19g and then ground into fine powder using an electric grinder (Dry Herb Industrial Grinder, Model SYB-18B). The grinding process was thorough to ensure that a smooth fine powder was obtained, which would facilitate efficient extraction of the bioactive compounds.

The powdered material was weighed, and was soaked in distilled water at a ratio of 1:10 w/v (100 g of powder in 1000 mL of water) for 72 hours with intermittent stirring to facilitate extraction of the bioactive compounds. The mixture was filtered using a muslin cloth, separating the filtrate (solvent + bioactive compounds) away from the moist grounded matter. The filtrate was freeze-dried using a Freeze Dryer (BIOBASE Freeze Dryer, China; Model BK- FD10S) to obtain a solid residue. This process involves freezing and sublimation of the water content under

low temperature and pressure resulting in a stable dried extract. The resulting dried extract was weighed to determine the percentage yield and stored in an airtight container at 40°C until use. At the end of the freeze drying process, a total yield of 1,271.98g (57.78%) of the extract was obtained.

Phytochemical analysis of the freeze dried aqueous extract revealed the presence of significant bioactive compounds. The extract was found to contain 39.24 mg GAE/g extract of total phenols and ~24.39 mg QE/g extract of flavonoids ~24.4 mg QE/g, as reported by Ilenowa et al. (2024). The bioactive compounds are known for their antioxidant properties and contribute to the therapeutic potential of the extract.

The preparation method ensured that the extract retained its bioactive properties while providing a safe and effective preparation for experimental use.

3.5 Experimental Design

The study used twenty-four(24) male Wistar rats that were physiologically normal weighing between 107.6g and 169.3g that were obtained from the Department of Anatomy, University of Benin, Nigeria The animals were kept in regular laboratory conditions at the Department of Anatomy, University of Benin, Nigeria, in clean, well-ventilated plastic cages with soft wood shavings(saw-dust) for bedding. Before the experiment started, the rats were acclimated to a controlled environment for four(4) weeks, which included a temperature of 22 ± 2°C, a relative humidity of 50–60%, and a 12-hour light/dark cycle. One out of the thirty-five rats died during acclimatisation process possibly because of stress from transportation to new environment or inability to adapt to new environment, hence, the number used.

The remaining rats were provided water ad libitum (freely) and conventional rat feed(Chicken Grower Feed, Top Feeds Ltd, Nigeria) with an approximate nutrient composition of 16% crude protein, 8% crude fibre, 5% crude fat, 1% calcium, and 0.4% phosphorus, during this acclimation period. The Departmental Animal Ethics Committee granted ethical approval for the study, and all methods complied with worldwide guidelines for the humane treatment of laboratory animals.

Experimental Grouping

Twenty-four(24) rats were divided into five (5) groups of five (5) rats each except for Group 0 that has only four (4) rats, shown as follows:

Group 0 (Normal/Negative Control): Received normal feed and water only.

Group 1: (Diabetic/Positive Control): Received Streptozotocin (STZ) only, no treatment.

Group 2: Diabetic rats treated with Glibenclamide (glanil)(5mg/kg)

Group 3: Diabetic rats treated with Picralima nitida extract (100 mg/kg).

Group 4: Diabetic rats treated with Picralima nitida extract (500 mg/kg).

The grouping was based on the mean body weight of the animals, which ranged between 107.69 g and 183.59 g. To facilitate easy identification, the rats were individually labelled using coloured markers (Genital Violet) on specific body parts, including the hand, leg, back, head, and tail while some were plain (unmarked). Each rat was assigned a unique identification number based on the part of the body where the colour was applied.

After diabetes was confirmed, the rats were regrouped as stated above but only two(2) rats in each group except for the positive control having four(4) rats.

3.7 Administration of Picralima nitida

The aqueous extract of Picralima nitida fruit (pulp and seeds) as previously described was administered to the experimental groups (G3-G6) . Group 0 (G0) served as the control (negative control) which received only water and feed alongside citrate buffer based on the volume given to rats in other groups with similar weights, providing a baseline for comparison to determine the effects of the extract and Group 2 (G2) which served as positive control received glibenclamide, a standard antidiabetic medicine in order to compare the extract's activity with the standard antidiabetic medicine.

Weights were used to allocate the rats into seven(7) groups of five(5) rats each. The oral gavage technique (Turner et al., 2011; Diehl et al. 2001) was used to introduce the aqueous extract of Picralima nitida into the rats.. Rats in the various Groups that were confirmed diabetes were isolated from those that were non- diabetic and regrouped randomly into six(6) experimental groups of two(2) rats each, except the positive control group, having four(4) rats. Diabetic rats in G3-G6 received the extract via oral gavage in addition to feed and water in doses of 100mg/kg, 200mg/kg, 400mg/kg and 500mg/kg respectively. Throughout the treatment period no signs of poisoning and animal deaths were observed.

3.8 Dosage Calculation of Picralima nitida

The required concentrations of the Picralima nitida extract were prepared based on the individual body weight of the rats.The dosage was calculated as follows :

i) For a standard dose of 200mg/kg;

200mg of extract is required for 1kg body weight of rat

ii) For a rat weighing 136.6 g, the exact dose to be given is ;

$$\frac{200 \text{ mg} \times 136.6 \text{ g}}{1000 \text{ g}}$$

$$= 27.32 \text{ mg}$$

Hence, the volume of aqueous extract solution required for a rat weighing 136.6 g was calculated as follows, with the concentration of 1g/10mL,

1g = 10mL

1000mg = 10mL

$$\text{Volume} = \frac{27.32 \text{ mg} \times 10\text{mL}}{1000 \text{ mg}}$$

Volume = 0.27mL

iii) 1g (1000 mg) of the extract was dissolved in 10mL of distilled water to make the stock solution.

This method was used for all the rats in G3-G6 that was confirmed diabetes, ensuring that each rat received the appropriate extract dosage according to their body weight. The extract was administered orally once daily using a calibrated syringe and the stainless-steel oral gavage needle for a treatment period of (7) days.

3.8 Induction of Diabetes Using Streptozotocin (STZ); Administration of Streptozotocin (STZ) (Glanil)

i) Preparation of Streptozotocin Solution

Streptozotocin (STZ) was used to induce diabetes mellitus in experimental rats. The streptozotocin was freshly prepared by dissolving 0.30g of streptozotocin in 10 mL of 0.1 M citrate buffer (pH 4.5). The buffer solution was mixed thoroughly until the streptozotocin completely dissolved. The preparation was done under low light conditions to prevent degradation, and the resulting solution was stored in a refrigerator until use.

ii)Dosage Calculation for Streptozotocin

The dosage of Streptozotocin used for induction was 60 mg/kg body weight. The dose was calculated as follows:

i) For a standard dose of 60mg/kg; 60mg of streptozotocin is required for 1kg body weight of rat

ii) For a rat weighing 154g, the exact dose to be given is ;

60 mg ×154 g/1000 g

= 9.24 mg

Hence, the volume of streptozotocin solution required for a rat weighing 154g was calculated as follows, with the concentration of 0.3g (300mg) of Streptozotocin in 10mL

300mg = 10mL

Volume = 9.24mg×10mL/300mg

= 0.31mL

Each rat's dose and volume was determined individually using the same formula to ensure accuracy. The calculated volume of streptozotocin solution was then administered intraperitoneally to each volume

iii) Diabetes was induced in overnight-fasted rats using a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 60 mg/kg body weight, freshly dissolved in 0.1 M citrate buffer (pH 4.5). After induction, the rats were allowed to drink 0.4% glucose solution for 24 hours to prevent initial hypoglycemia. Blood glucose levels were measured 72 hours post-induction using a glucometer. Rats with fasting blood glucose levels above 200mg/dL were considered diabetic. However, after six(6) days awaiting the others to be induced in order to have a larger number of rats induced with diabetes, all their fasting blood glucose dropped possibly due to reduced food intake due to sickness/anorexia resulting in hypoglycemia. The rats that were remaining were re-induced as four died after the first inducing due to hypoglycemia.

iii)Preparation of Citrate Buffer

The citrate buffer was freshly prepared by 0.1L(100mL) of 0.1M citrate buffer, pH 4.50, by dissolving 20.80g/L citric acid monohydrate and 0.29g trisodium citrate dihydrate in ≈80 mL of water. Then, the pH.was checked and adjusted to 4.50 and then volume was brought to to 0.1L(100mL).

pH and Weight Calculations

The pH was calculated using the nderson–Hasselbalch equation as follows:

pH= pKa + log [B]/[A]

pH = 4.50; pKa3 = 6.50

4.5 = 6.5 + log [B]/[A]

10^-2 = [B]/[A]

10^{-2} [A] = [B]

10^{-2} [A]= [B]

[B] = 0.01[A]

Let A + B = Molar concentration M) --------- (1)

[B] = 0.01[A] ---------- (11)

[A] + 0.01[A] = M

Molar concentration of citrate buffer to be prepared is 0.1M

[A]+ 0.01[A) = 0.1M

1.01[A] = 0.1

[A]= 0.1/1.01

[A] = 0.099M (mol/L)

By substitution,

[B] = 0.01[A] = 0.01× 0.099M

[B] = 0.00099M ~ 0.001 M(mol/L)

But,

Molar Concentration (Molarity) = Mass Concentration/ Molar mass

Mass Concentration = Molar Concentration × Molar mass

Mass concentration of A = 0.099M × 210.14g

= 20.80g/L

Mass concentration of B = 0.001M × 294.10g

= 0.29 g/L

20.80g citric acid monohydrate (A) and 0.29g trisodium citrate dihydrate (B) in ≈800 mL of water. Then, the pH.was checked and adjusted to 4.50 and then volume was brought to to 1L(1000mL).

0.1M citrate buffer (pH 4.5) were prepared by mixing appropriate amounts of citric acid and sodium citrate in distilled water. The buffer was mixed thoroughly and ensured to be free from light and moisture exposure. The prepared buffer was stored under refrigeration to prevent degradation over time.

iv)Re-inducing of Diabetes using Streptozotocin (STZ)

The streptozotocin was freshly prepared by dissolving 0.20g of streptozotocin 0.05M citrate buffer (pH 4.5). The buffer solution was mixed thoroughly until the streptozotocin completely dissolved. The preparation was done under low light conditions to prevent degradation, and the resulting solution was stored in a refrigerator until use.

v)Dosage Calculation for Streptozotocin

The dosage of Streptozotocin used for induction was 60 mg/kg body weight. The dose was calculated as follows:

i) For a standard dose of 40mg/kg; 40mg of streptozotocin is required for 1kg body weight of rat

ii) For a rat weighing 110g, the exact dose to be given is ;

40mg ×110g/1000 g

= 4.40mg

Hence, the volume of streptozotocin solution required for a rat weighing 110g was calculated as follows, with the concentration of 0.2g (200mg) of Streptozotocin in 10mL

200mg = 10mL

Volume = 4.40mg×10mL/200mg

= 0.25mL

Each rat's dose and volume was determined individually using the same formula to ensure accuracy. The calculated volume of streptozotocin solution was then administered intraperitoneally to each rat. Diabetes was induced in overnight-fasted rats using a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 40 mg/kg body weight, freshly dissolved in 0.05M citrate buffer (pH 4.5). After induction, the rats were allowed to drink 0.4% glucose solution for 24 hours to prevent initial hypoglycemia. Blood glucose levels were measured 72 hours post-induction using a glucometer. Rats with fasting blood glucose levels above 110 mg/dL were considered diabetic.

vi)Preparation of Citrate Buffer

The citrate buffer was freshly prepared by 0.1L(100mL) of 0.05 M citrate buffer, pH 4.50, by dissolving 1.04g citric acid monohydrate and 0.0147g trisodium citrate dihydrate in ≈80 mL of water. Then, the pH was checked and adjusted to 4.50 and then volume was brought to to 0.1L(100mL).

pH and Weight Calculations

The pH was calculated using the nderson–Hasselbalch equation as follows:

pH= pKa + log [B]/[A]

pH = 4.50; pKa3 = 6.50

4.5 = 6.5 + log [B]/[A]

10^-2 = [B]/[A]

10^-2 [A] = [B]

10^-2 [A]= [B]

[B] = 0.01[A]

Let A + B = Molar concentration M) --------- (1)

 [B] = 0.01[A] ---------- (11)

[A] + 0.01[A] = M

Molar concentration of citrate buffer to be prepared is 0.05M

[A]+ 0.01[A) = 0.05M

1.01[A] = 0.05

[A]= 0.05/1.01

[A] = 0.0495M (mol/L)

By substitution,

[B] = 0.01[A] = 0.01× 0.0495M

[B] = 0.000495M ~ 0.0005 M(mol/L)

But,

Molar Concentration (Molarity) = Mass Concentration/ Molar mass

Mass Concentration = Molar Concentration × Molar mass

Mass concentration of A = 0.0495M × 210.14g

= 10.40g/L

Mass concentration of B = 0.0005M × 294.10g

= 0.147 g/L

Hence, for 0.1L (100mL) of citrate buffer

A = 10.40 g/L × 0.1L = 1.04g

B = 0.147 g/L × 0.1L = 0.0147g

1.04g citric acid monohydrate (A) and 0.0147g trisodium citrate dihydrate (B) in ≈80 mL of water. Then, the pH.was checked and adjusted to 4.50 and then volume was brought to to 0.1L(100mL).

0.05M citrate buffer (pH 4.5) were prepared by mixing appropriate amounts of citric acid and sodium citrate in distilled water. The buffer was mixed thoroughly and ensured to be free from light and moisture exposure. The prepared buffer was stored under refrigeration to prevent degradation over time.

vii)Post-Induction Care

After the administration of Streptozotocin, the rats were given 0.4% glucose solution orally for 24 hours to prevent hypoglycemic shock. Subsequently, the rats were maintained on a standard diet and water ad libitum.

viii)Confirmation of Diabetes

Seventy-two (72) hours after STZ injection, fasting blood glucose levels were determined using a glucometer. Rats showing fasting blood glucose levels above 110mg/dL were considered diabetic and selected for further studies.

3.10 Weight and Blood Glucose Evaluation

The rats received their respective doses of the extract daily for seven(7) days. Throughout this period, body weight and blood glucose levels were monitored at the beginning and end of the study. Blood glucose measurements were taken after an overnight fast using a glucometer (Newspring Glucose Meter, Model KF-B12, China). Blood samples were obtained from the tail tip using a sterile lancet, and the corresponding glucose values were recorded.

Body weights were measured using a high-precision weighing scale (NEWSPRING Digital Scale, China; Model NS- 790).

Note: The results presented below include the initial and final body weights of each rat, the changes in weight, as well as the initial and final glucose levels and the corresponding differences in glucose values.

Group	Label	Treatment (Dose, route)	Rat ID	Baseline Weight (g)	Baseline FBG (mg/dL)	Weight (g) before Induction	FBG(mg/dL) Before Induction	FBG(mg/dL) 4 days after induction
G0	Normal Control	Distilled water(oral)	C1	169.3	81	197.5	83	70
			C2	163.5	79	176.0	81	67
			C3	155.0	68	189.4	79	72
			C4	167.7	77	192.0	90	59
G1	Diabetic Control	Streptozotocin (60mg)	S1	143.2	52	169.1	76	72
			S2	145.6	68	160.8	83	77
			S3	144.0	56	182.1	86	47
			S4	148.2	68	178.2	94	125
			S5	144.9	70	154.7	95	34
G2	Glibenclamide	Glibenclamide drug(5mg, oral)	M1	123.5	76	136.1	104	232
			M2	124.1	76	158.8	81	144
			M3	126.6	90	139.4	88	88

			M4	127.5	76	145.6	99	133
			M5	128.7	68	157.3	83	83
G3	P.nitida Low	P.nitida	D1	138.7	72	141.6	74	52
		extract(200mg, oral)	D2	140.5	70	152.6	104	135
			D3	140.7 141.8	58	156.2	72	43
			D4	142.2	85	161.0	77	279
			D5		72	161.1	79	130
G4	P.nitida High	P.nitida extract	H1	151.8	77	175.6	90	194
		(500mg, oral)	H2	154.3	72	166.0	97	250
			H3	152.1	65	166.2	76	263
			H4	154.5	50	182.4	67	90
			H5	148.3	85	149.4	94	238

3.11 Samples Collection

The rats were fasted overnight at the conclusion of the treatment period. Tail vein pricks were used to collect blood samples for the measurement of fasting blood glucose. The vein was punctured with a sterile lancet, and a drop of blood was taken right away to measure the glucose using a glucometer (NEWSPRING Glucose Meter, China; Model KF-B12).

Additional blood samples were obtained by heart puncture and placed in simple tubes for the biochemical measurement of α-glucosidase activity. To extract serum, the tubes were centrifuged for ten minutes at 3000 rpm after being allowed to clot. Before analysis, every sample was appropriately labeled and kept at -4°C.

3.8 Blood Collection

Good hygiene was maintained in the animal house through regular cleaning of cages and replacement of sawdust bedding. The health and general well-being of the rats were closely monitored, and food and water were provided daily.

On the seventh (7^{th}) day, the rats were fasted overnight, re-weighed, and subsequently sacrificed. Anesthesia was induced using chloroform to minimize pain and distress. The animals were euthanized in a chloroform chamber, after which a lateral incision was made in the abdominal cavity. Blood samples were then collected by cardiac puncture and dispensed into specific containers depending on the intended analysis:

i. Plain bottles: For biochemical assays
ii. Heparin bottles: To preserve plasma integrity

The blood samples were stored at 4°C until analysis. Prior to biochemical evaluation, the samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The resulting serum was

used to determine levels of C-reactive protein and magnesium using standard biochemical procedures.

3.12 Biochemocal Analysis

3.12.Serum Analysis of α-Glucosidase Activity Assay

Method

The standard colorimetric approach based on the hydrolysis of p-nitrophenyl-α-D-glucopyranoside (pNPG) was used to measure the α-glucosidase activity in serum. Using spectrophotometry, the amount of p-nitrophenol (pNP) released was measured.

The synthetic substrate p-nitrophenyl-α-D-glucopyranoside (pNPG) is hydrolyzed by Principle α-glucosidase to produce p-nitrophenol (pNP), a yellow chromophore.

A spectrophotometer is used to detect the intensity of the yellow color at 405 nm, which is directly proportional to enzyme activity.

The unit of measurement for enzyme activity is μmol of pNP released per minute per milliliter.

Procedures

1. Samples of serum were thawed on ice.
2. Clean test tubes or microplates were used to prepare a reaction mixture that included phosphate buffer and pNPG substrate.
3. After adding a defined volume of the sample to the reaction mixture, it was incubated at 37°C for a predetermined amount of time (usually 10–20 minutes).

4. The addition of sodium carbonate solution halted the process.
5. A reagent blank was used to measure the absorbance at 405 nm.
6. The molar extinction coefficient of p-nitrophenol was used to calculate the enzyme activity.

3.13 Statistical Analysis

3.13.1 α-glucosidase activity (serum)

The mean ± standard error of the mean (SEM) was used to express all data. One-way Analysis of Variance (ANOVA) and Tukey's post hoc test were used to examine group differences in α-glucosidase activity via serum. The threshold for statistical significance was fixed at $p < 0.05$. Tables and figures were used to summarize the results as needed.

CHAPTER FOUR

4.1 Results

This chapter presents the effects of aqueous Picralima nitida extract administered via gavage on alpha-glucosidase concentrations in experimental rats. The animals were assigned into five (5) groups (G0, G1, G2, G3, and G4), each originally consisting of five(5) rats. However, due to diabetes not induced in all the rats, only two(2) rats were present in each group after regrouping. Blood samples collected from the rats were analyzed in the laboratory for alpha glucosidase concentrations. Statistical analysis was performed. Statistical analysis was performed The data were expressed as means ± S.E.M. The differences among Groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done by the Duncan's post hoc test. A value of $P< 0.05$ was accepted as significant. Graphpad Prism version 7.04 was used to plot the graph

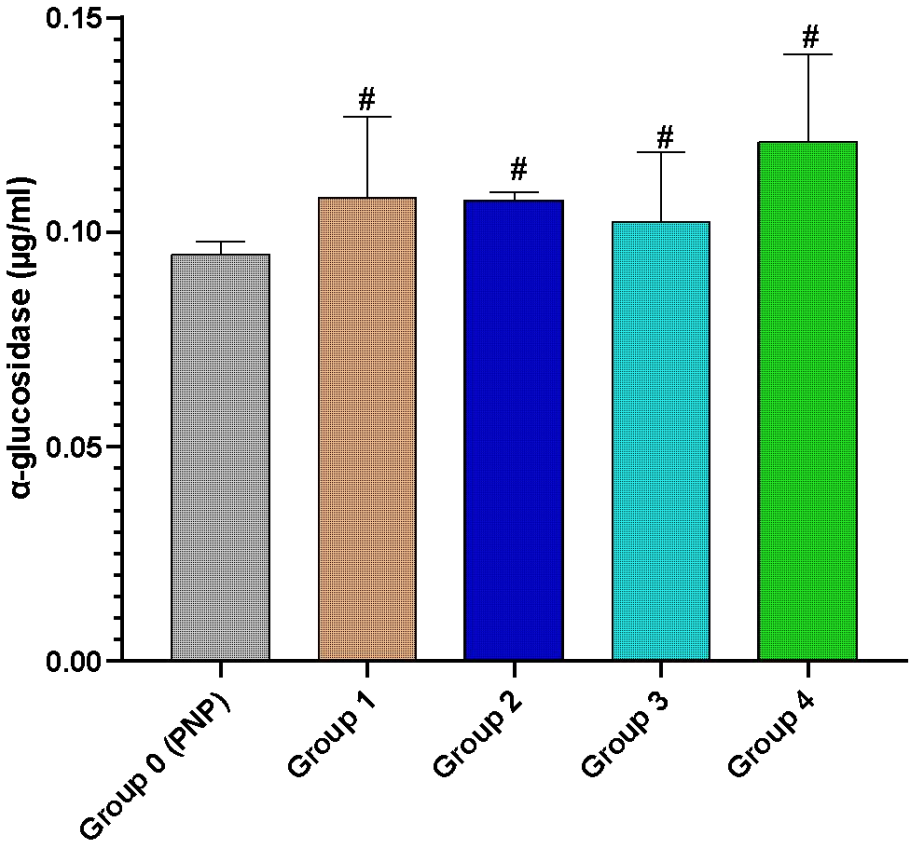

Figure 4.1: Effect of *Picralima nitida* on α-glucosidase concentrations of Male wistar rats. Values are expressed as mean ± SEM. # represent statistical significant at P < 0.05 when compared to group 0.

The study above shows that when compared to group 0 (PNP), groups 1-4 significantly increased α-glucosidase concentrations.

CHAPTER FIVE

Discussion of Results

This chapter discusses the effects of streptozotocin (STZ) induction, glibenclamide treatment, and graded doses of *Picralima nitida* fruit extract on fasting blood glucose and α-glucosidase activity in male Wistar rats. The interpretation focuses on the metabolic response of each treatment group relative to the normal control.

5.1 α-Glucosidase Activity in Experimental Groups

Alpha-glucosidase activity, expressed as p-nitrophenol (pNP) concentration, is presented in Fig. 4.1. The results showed a significant increase ($p < 0.05$) in enzyme activity across all treatment groups (G1–G4) when compared with the normal control (G0).

Figure 4.1: Showing Values of α-Glucosidase concentrations expressed as Mean ± SEM

Group Mean ± SEM

G0 0.09 ± 0.0, G1 0.11 ± 0.0#, G2 0.11 ± 0.0#, G30.10 ± 0.0#, G4 0.12 ± 0.0#

Values expressed as mean ± SEM.

# indicates significant difference at $p < 0.05$ compared to G0.

5.2 Interpretation of α-Glucosidase Activity

When compared with the control group (G0), all treatment groups demonstrated elevated α-glucosidase activity:

G1 (Diabetic Group):

Showed a marked rise (0.11 ± 0.0), confirming that diabetes enhances intestinal α-glucosidase activity and increases carbohydrate breakdown.

G2 (Glibenclamide):

Displayed a similar elevation (0.11 ± 0.0), indicating that glibenclamide's hypoglycaemic effect does not involve α-glucosidase inhibition.

G3 (Low Dose Extract):

Showed a mild increase (0.10 ± 0.0), suggesting a modest stimulatory effect of the extract at low concentration.

G4 (High Dose Extract):

Recorded the highest enzyme activity (0.12 ± 0.0), indicating a dose-dependent enhancement of α-glucosidase.

The collective outcome shows that neither diabetes, glibenclamide, nor the extract suppressed α-glucosidase; instead, all conditions stimulated the enzyme to varying degrees.

5.3Biological Implications

α-Glucosidase is responsible for converting complex carbohydrates into absorbable glucose in the small intestine. Increased activity leads to:

Faster carbohydrate digestion.

Increased glucose release into the bloodstream.

A potential rise in post-prandial glucose availability.

The elevated enzyme levels observed across all treated groups suggest enhanced carbohydrate hydrolysis. In diabetes (G1), this aligns with known metabolic disturbances where intestinal enzymes become upregulated. Glibenclamide (G2) did not reduce α-glucosidase activity, reinforcing that its glucose-lowering effect acts through insulin stimulation rather than digestive enzyme control.

The *Picralima nitida* extract groups (G3 and G4) also showed elevated enzyme activity, with G4

presenting a clear dose-dependent rise. This implies that the extract may not inhibit α-glucosidase; instead, it enhances enzyme activity while still contributing to glucose reduction through other metabolic pathways.

5.4 Discussion in Relation to Treatment Effects

Although α-glucosidase activity increased in all treatment groups, earlier fasting blood glucose results indicated that the extract produced glucose-lowering effects, particularly at certain doses. The coexistence of:

Increased α-glucosidase activity

Reduced fasting glucose levels

suggests that the antihyperglycaemic action of *Picralima nitida* is independent of enzyme inhibition.

Possible mechanisms include:

Improved insulin sensitivity

Enhanced peripheral glucose uptake

Partial β-cell protection or regeneration

Reduction of oxidative stress

Modulation of hepatic glucose output

These pathways can lower fasting glucose even when carbohydrate-digesting enzyme activity remains elevated.

5.5 Summary of Key Findings

α-Glucosidase activity significantly increased in all treated groups when compared with the control.

Diabetes itself (G1) caused elevated enzyme activity consistent with metabolic dysregulation.

Glibenclamide (G2) did not inhibit α-glucosidase, aligning with its known insulin-secretagogue mechanism.

Picralima nitida extract produced dose-dependent increases in α-glucosidase, highest in G4. Despite elevated enzyme activity, the extract previously showed glucose-lowering effects, indicating alternative antihyperglycaemic mechanisms.

Conclusion

This study assessed the effects of aqueous Picralima nitida fruit extract on fasting blood glucose and α-glucosidase activity in streptozotocin-induced diabetic male Wistar rats. The results showed that the diabetic group (G1) exhibited markedly elevated glucose levels compared with the normal control (G0), confirming successful induction of diabetes. Administration of P. nitida fruit extract produced notable antihyperglycaemic effects, particularly in the low-dose (G3) and high-dose (G4) groups, where fasting blood glucose levels were substantially reduced. The glibenclamide group (G2) also showed strong glucose-lowering activity as expected.

In contrast, α-glucosidase activity did not decrease in response to treatment. All treated groups—including G1, G2, G3, and G4—showed significantly higher enzyme concentrations than the control (G0), indicating that neither glibenclamide nor P. nitida extract acted as an α-glucosidase inhibitor. The highest α-glucosidase activity was observed in the high-dose extract group (G4), suggesting a dose-related stimulatory effect on carbohydrate-digesting enzyme activity.

These findings demonstrate that the glucose-lowering ability of P. nitida fruit extract is not mediated through α-glucosidase inhibition. Instead, its antidiabetic activity may involve alternative mechanisms such as improved insulin sensitivity, protection or regeneration of pancreatic β-cells, enhanced peripheral glucose utilization, or modulation of hepatic glucose

output. The extract's ability to reduce blood glucose despite elevated α-glucosidase levels further supports this conclusion.

Overall, aqueous Picralima nitida fruit extract exhibits promising antidiabetic potential independent of α-glucosidase suppression. Further research is recommended to clarify its molecular mechanisms, determine optimal dosing, and evaluate long-term efficacy and safety for potential therapeutic application.

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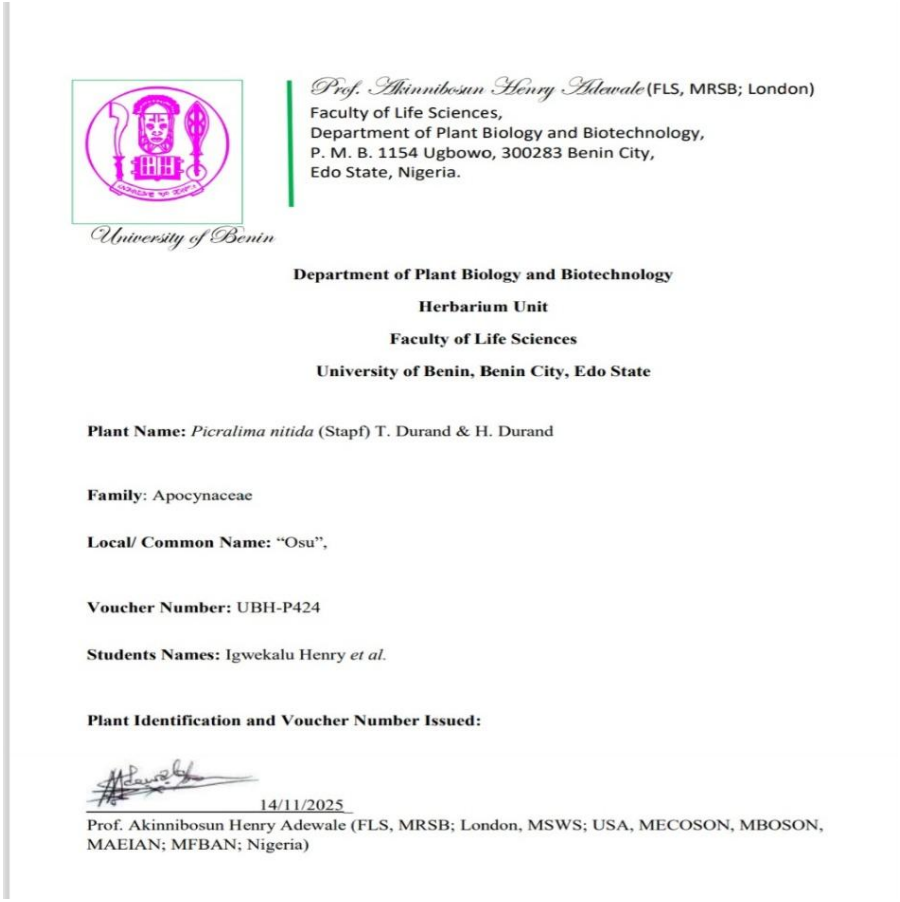
APPENDICES

Appendix I: Grouping and Identification of Rats

Groups	**No of Rats**	**Weight Range (g)**	**Identification (specific body parts labelled with Genital Violet (GV)**
G0	4	155.0 – 169.3	C1- Head C2- Head & Back C3- Leg & Back C4- Leg
G1	5	107.6 – 120.3	A1- Back & Tail A2- Tail A3- Plain A4- Two Legs A5- Head
G2	5	143.2 – 148.2	M1- Plain M2- Tail M3- Two Legs M4- Head M5- Leg
G3	5	129.0 – 136.9	L1- Two legs L2- Tail & Back L3- Head & Tail L4- Two legs & Back L5- Back
G4	5	138.7 – 142.2	D1- Legs & Back D2- Tail D3- Leg D4- Hand D5- Plain

G5	5	123.5 – 128.8	S1- Back & Hand S2- Hand & Tail S3- Hand S4- Back S5- Head
G6	5	148.3 – 154.5	H1- Head & Back H2- Plain & Leg H3- Back H4- Plain H5- Hand & Back

Appendix II: Plant Classification By Hebariuum Unit of the Department of Plant Biology



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Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Picralima nitida* (Stapf) T. Durand & H. Durand

Family: Apocynaceae

Local/ Common Name: "Osu",

Voucher Number: UBH-P424

Students Names: Igwekalu Henry *et al.*

Plant Identification and Voucher Number Issued:

14/11/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MECOSON, MBOSON, MAEIAN; MFBAN; Nigeria)

and Biotechnology

Appendix III: Anima Ethical Clearance from the Department of Anatomy, University of Benin.

Appendix IV: Photographs of Experimental Activities

Figure IV a: Picralima nitida fruit bought at Oliha market, along siloko road, Benin City, Edo state, alongside the chopping, drying , extraction and freeze drying process.

Fresh Picralima nitida fruits

Chopped Picralima nitida frut.

Droed Picralima nitida

Grounded Picralima nitida

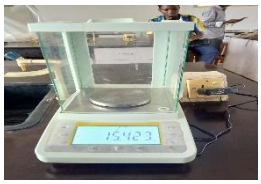
Freeze-dried Picralima. nitida

Extraction of Picralima nitida

Freeze-drying machine

Extract of Picralima nitida

Oven



Weighing Scale

Figure IV b: Acclimation of rats

Figure IV c: **Effect of *Picralima nitida* on α-glucosidase concentrations in STZ induced diabetic Male Wistar rats.**

Parameters (ng/mL)	Group 0 (PNP)	Group 1	Group 2	Group 3	Group 4
α-glucosidase (µg/ml)	0.09 ± 0.0	0.11 ± 0.0#	0.11 ± 0.0#	0.10 ± 0.0#	0.12± 0.0#

Values are expressed as mean ± SEM. # represent statistical significant at $P < 0.05$ when compared to group 0.

Figure IV d : Newspring glucometer

Figure IV e: Centrifugation of Blood samples to obtain serum

Centrifuge machine and. Blood samples

Centrifuged Blood samples

Figure IV f: Streptozotocin obtained from Bridge Biotech Limited

Place bought

Producer.

Streptozotocin powder