IN VITRO ANTIDIABETIC PROPERTIES OF CRUDE ETHANOL AND SOLVENT FRACTIONS OF *Tetracera alnifolia*

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NOVEMBER, 2025.

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

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CERTIFICATION

We the undersigned hereby certify that **OMAGBEMI OMAJUWA PROSPER** with Matriculation Number **BMS2101442** Carried out this work titled; "***IN VITRO* ANTIDIABETIC PROPERTIES OF CRUDE ETHANOL AND SOLVENT FRACTIONS OF *Tetracera alnifolia***", in the Department of Medical Biochemistry, University of Benin, Benin city and we approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

PROF. F. E. OLUMESE	**DATE**
(Project supervisor)	
Dr Bobby .N. Aguebor Ogie	**DATE**
(Head of Department)	
(External Examiner)	**DATE**

DEDICATION

This project work is dedicated to God, for his grace and strength to bring this work to completion.

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The writing of an undergraduate project is in many cases the results of the efforts of many people, and this project is not an exception. My deepest gratitude goes to God, who has provided all that was needed to complete my project work.

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ABSTRACT

Diabetes mellitus represents a major global health challenge, with over 800 million adults affected worldwide and limited therapeutic options, particularly in resource-constrained settings. This study investigated the *in vitro* antidiabetic properties of crude ethanol extract and solvent fractions of roots of *Tetracera alnifolia,* a medicinal plant traditionally used for managing diabetes in West Africa particularly Nigeria. The aim was to evaluate the alpha-amylase and alpha-glucosidase inhibitory activities of the plant extracts and fractions, and to determine their potential as natural antidiabetic agents. The roots of *T. alnifolia* were cut, dried, pulverized and extracted using ethanol. The crude extract was subsequently fractionated using solvents of increasing polarity (n-hexane, chloroform, dichloromethane, n-butanol, and water). Enzyme inhibition assays were performed using standard methods, with acarbose as the control. The IC_{50} values were calculated to determine inhibitory potency. Results showed that the crude extract exhibited remarkable alpha-glucosidase inhibitory activity (IC_{50} = 0.10 mg/mL), approximately 9-fold more than the IC_{50} of acarbose (IC_{50} = 0.93 mg/mL). For alpha-amylase inhibition, the crude extract (IC_{50} = 0.68 mg/mL) was less than that of acarbose (IC_{50} = 0.46 mg/mL). All solvent fractions similarly outperformed acarbose in alpha-glucosidase inhibition, with the n-hexane fraction showing the strongest activity (IC_{50} = 0.19 mg/mL). The superior performance of the crude extract over individual fractions provided compelling evidence for synergistic interactions among multiple phytochemical constituents. The differential selectivity potent alpha-glucosidase inhibition with moderate alpha-amylase inhibition represents an ideal therapeutic profile that may offer a better postprandial glucose control. The study concludes that *T. alnifolia* possesses antidiabetic potential in vitro and this may be responsible for its hypoglycemic property in treatment of diabetes in traditional medicine. Therefore, further investigation through in vivo studies, phytochemical characterization, and determination of bioactive agent for potential development as a natural antidiabetic therapeutic.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Diabetes mellitus has emerged as a major global health crisis, with the number of adults affected increasing by almost four times since 1990 to over 800 million in 2022 (World Health Organization, 2024). This epidemic disproportionately affects low and middle income countries, where more than 90% of the 450 million untreated cases are concentrated, highlighting a significant treatment gap and widening health inequalities (British Medical Journal, 2024). The economic and social burden of this disease is immense, straining healthcare systems and economies worldwide (American Diabetes Association, 2023). In the United States alone, the annual cost of diabetes was estimated at $412.9 billion in 2022, with a significant portion attributed to direct medical costs and lost productivity (ADA, 2023; ADA, 2022). The disease itself is a chronic metabolic disorder characterized by high blood glucose levels, primarily caused by insulin resistance and inadequate insulin production (WHO, 2024). Uncontrolled hyperglycemia leads to severe long-term complications, including blindness, kidney failure, heart attacks, stroke, and amputation. Current pharmacological treatments for diabetes include various drug classes such as insulin, biguanides (such as metformin), sulfonylureas, and alpha-glucosidase inhibitors including acarbose (London Diabetes Centre, 2025). However, these conventional therapies have notable limitations, including side effects like gastrointestinal discomfort with metformin and the risk of hypoglycemia and weight gain with sulfonylureas (LDC, 2025).

A critical challenge also exists in a lack of complete understanding of the precise mechanisms of some modern drugs, with a recent study highlighting a potential knowledge gap regarding GLP-1 agonists that may, in certain circumstances, activate glucagon receptors (Mayor, 2022). These limitations, coupled with the high cost of many modern drugs, underscore the need for new, effective, and accessible therapeutic alternatives. Patients with diabetes often face significant psychological, physical, and social problems, and some adopt a fatalistic view of their condition (Yilmaz *et al.,* 2023). For many, the diagnosis brings a negative perception of their life, highlighting the need for a holistic approach to care that addresses both physical and mental health (Yilmaz *et al.,* 2023; Farid *et al.,* 2023). Patient experiences also highlight significant barriers to medication adherence. Many patients, particularly younger individuals with a short duration of the disease, may stop taking their medication when they "feel good," perceiving their symptoms have eased and that they are young enough to handle the situation without treatment (Guo *et al.,* 2022). This noncompliance is often compounded by fear or pain from the medication itself (Guo *et al.,* 2022). For example, one patient reported discontinuing metformin and acarbose due to stomach ache, while another with rising A1c and weight after 10 years of oral medication eventually required insulin (Guo *et al.,* 2022). These real-world challenges demonstrate that the limitations of conventional therapy are not just pharmacological but are deeply personal and behavioural.

These limitations, particularly in low-income settings, have prompted a reliance on traditional herbal medicine. A survey of diabetic patients in Guinea found that 33% use herbal medicine for treatment (Balde *et al.,* 2020). The motivations for this choice are rooted in practicality and belief: 70% of patients cited easy access to medicinal plants, 48% pointed to lower cost compared to conventional drugs, and 78% were convinced by hearing a positive experience from

others (Balde *et al.,* 2020). In another study in Cameroon, 74.14% of patients using herbal medicine reported feeling a sense of relief (Kamdem, 2016). This widespread use and perceived efficacy of traditional remedies provide a powerful rationale for the scientific investigation of indigenous plants, such as *Tetracera alnifolia*.

1.2 JUSTIFICATION OF THE STUDY

The urgent need for new antidiabetic therapies, particularly those that are affordable and accessible, has made ethnobotanical research a vital avenue for drug discovery. Traditional medicinal practices, refined over generations, provide a rich source of plant-based remedies with a high probability of having relevant biological activity (Ogunlakin and Sonibare, 2022). The genus *Tetracera*, a family of plants with a history of traditional use in treating various ailments, including diabetes, is a compelling candidate for such research (Ogunlakin and Sonibare, 2022). This study will focus on the plant's ability to inhibit alpha-amylase and alpha-glucosidase, two key enzymes responsible for breaking down dietary carbohydrates into absorbable glucose (Sahu and Sahu, 2021). By inhibiting these enzymes, as conventional drugs like acarbose do, the rate of glucose absorption is delayed, which helps to control post-meal blood sugar spikes (Sahu and Sahu, 2021; Al-Othman, 2021). This provide research opportunity to identify a natural, plant-based inhibitor that may offer a more favourable side effect profile than synthetic alternatives.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of this study was to determine the antidiabetic properties of *Tetracera alnifolia* based on its *In Vitro* alpha-amylase, and alpha-glucosidase inhibitory activities. The specific objectives were:

1. to fractionate crude ethanol extract into different fraction in order of increasing polarity
2. to determine the alpha-amylase inhibitory activity of crude ethanol extract and solvent

fractions of *Tetracera alnifolia*.

3. to determine the alpha-glucosidase inhibitory activity of crude ethanol extract and solvent fractions of *Tetracera alnifolia*.
4. to determine the fraction with the best antidiabetic properties.
5. to propose a probable mechanism of action for the antidiabetic property of *Tetracera alnifolia*.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF DIABETES MELLITUS

Diabetes mellitus is a chronic, multifactorial metabolic disorder characterized by persistently elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action, or, most commonly, both (ADA, 2024; Khan *et al.,* 2022). The chronic hyperglycemia of diabetes is inextricably linked to the long-term dysfunction, damage, and eventual failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Zheng *et al.,* 2022). The pathogenesis of Type 1 diabetes (T1D) is primarily autoimmune, marked by the T-cell-mediated destruction of pancreatic β-cells in the islets of Langerhans, leading to an absolute deficiency of insulin (DiMeglio *et al.,* 2018). On the other hand, Type 2 diabetes (T2D), which accounts for over 90% of all cases, is characterized by a combination of insulin resistance in peripheral tissues (muscle, liver, and adipose) and a progressive inability of pancreatic β-cells to compensate by secreting sufficient insulin (DeFronzo *et al.,* 2022). This insulin resistance is often preceded by a state of compensatory hyperinsulinemia, which eventually fails as β-cell function declines (Galicia-Garcia *et al.,* 2020). The diagnosis of diabetes is confirmed by any one of the following criteria: a fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L), a 2-hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT), a hemoglobin A1c (HbA1c) level ≥ 6.5%, or a random plasma glucose ≥ 200 mg/dL in a patient with classic symptoms of hyperglycemia (American Diabetes Association, 2024).

The global prevalence of diabetes has reached pandemic proportions, posing one of the most significant public health challenges of the 21st century. According to the International Diabetes Federation (IDF) Diabetes Atlas (2025), approximately 643 million adults aged 20-79 were

living with diabetes in 2024, and this number is projected to surge to 783 million by 2045. This rising prevalence is driven by complex interactions between genetic, epigenetic, environmental, and socio-economic factors, with urbanization, aging populations, and increasing rates of obesity and physical inactivity being major contributors (Lin *et al.,* 2020). Diabetes is a leading cause of blindness, kidney failure, heart attacks, strokes, and lower limb amputations. In 2022, an estimated 6.7 million deaths were attributable to diabetes and its complications, underscoring its severe mortality impact (WHO, 2023). The economic cost is equally staggering, with global health expenditures on diabetes exceeding $966 billion in 2021, a figure expected to rise (IDF, 2025).

The cornerstone of diabetes management is the achievement of optimal glycemic control (HbA1c < 7% for most adults) to prevent or delay complications, while simultaneously managing other cardiovascular risk factors like hypertension and dyslipidemia (American Diabetes Association, 2024). Lifestyle modifications are the first-line intervention for T2D and are essential for all types of diabetes. This includes medical nutrition therapy focused on carbohydrate counting and portion control, regular physical activity (at least 150 minutes of moderate-intensity exercise per week), and weight management (Evert *et al.,* 2019). Pharmacological therapy is diverse. For T1D, it is synonymous with exogenous insulin administration, delivered via multiple daily injections or continuous subcutaneous insulin infusion (insulin pumps), often integrated with continuous glucose monitoring (CGM) systems (Holt *et al.,* 2021). The drug for T2D management is broad and include:

1. Biguanides (e.g., metformin): They are as first-line treatment drugs and they decrease hepatic gluconeogenesis and improves insulin sensitivity.
2. Sulfonylureas (e.g. glimepiride): They stimulate insulin secretion from pancreatic β-cells.

3. Thiazolidinediones (e.g. pioglitazone): They improve insulin sensitivity in peripheral tissues.
4. SGLT2 inhibitors (e.g., empagliflozin): They promote urinary glucose excretion by inhibiting reabsorption in the kidneys.
5. GLP-1 Receptor Agonists (e.g., liraglutide): They mimic incretin effects, promoting satiety, weight loss, and glucose-dependent insulin secretion.
6. Insulin: Used when other agents fail to maintain glycemic control.

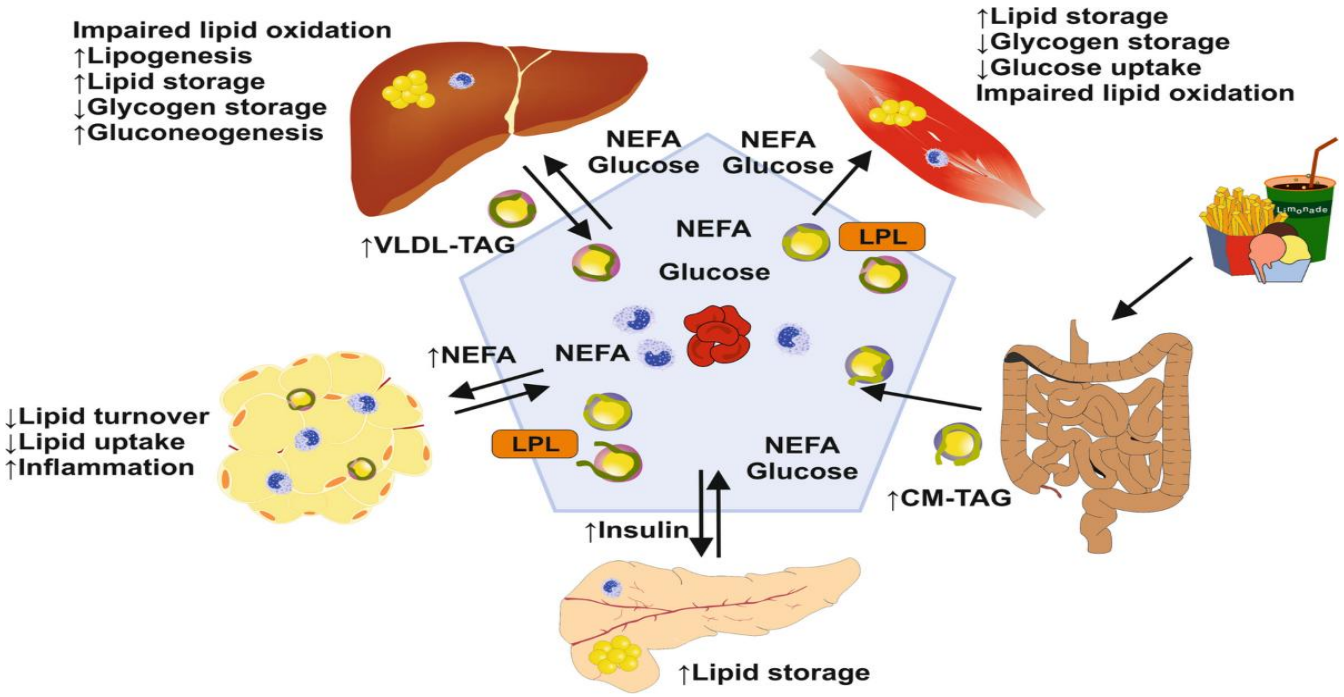

Figure 1: Pathophysiology of Type 2 Diabetes Mellitus

Source: Link.springer.com

2.2 TYPES OF DIABETES MELLITUS

Diabetes mellitus is not a single disease entity but rather a heterogeneous group of metabolic disorders unified by the common feature of chronic hyperglycemia. The classification of diabetes has evolved considerably over the years, driven by advances in understanding of disease pathogenesis, genetic mechanisms, and clinical presentations. The current classification system recognizes several distinct types of diabetes, each with unique etiological, pathophysiological, and clinical characteristics (American Diabetes Association [ADA], 2024; Galicia-Garcia et al., 2020).

Type 1 Diabetes Mellitus (T1D)

Type 1 diabetes mellitus is an autoimmune disorder characterized by the progressive and ultimately complete destruction of insulin-producing pancreatic β-cells within the islets of Langerhans (DiMeglio et al., 2018). This destruction results in an absolute deficiency of insulin, rendering patients dependent on exogenous insulin administration for survival. T1D accounts for approximately 5-10% of all diabetes cases globally and typically manifests in childhood or adolescence, although it can occur at any age (Khan et al., 2022). The pathogenesis of T1D is multifactorial, involving a complex interplay between genetic susceptibility and environmental triggers. The genetic component is strongly associated with specific human leukocyte antigen (HLA) class II alleles, particularly HLA-DR3, HLA-DR4, and HLA-DQ, which are present in more than 90% of children with T1D (DiMeglio et al., 2018). However, genetic predisposition alone is insufficient to cause disease, as evidenced by the fact that only 10-15% of genetically susceptible individuals develop T1D, suggesting that environmental factors play a critical role in disease initiation (Zheng et al., 2022). Environmental triggers implicated in T1D pathogenesis include viral infections (particularly enteroviruses such as coxsackievirus B), early exposure to

cow's milk proteins, vitamin D deficiency, and early introduction of cereals in infancy (DiMeglio et al., 2018). The autoimmune destruction of β-cells is mediated primarily by autoreactive CD8+ T lymphocytes, which infiltrate the pancreatic islets in a process termed insulitis, accompanied by the production of autoantibodies against various β-cell antigens, including insulin, glutamic acid decarboxylase (GAD65), insulinoma-associated antigen-2 (IA-2), and zinc transporter 8 (ZnT8), which serve as valuable biomarkers for disease prediction and diagnosis (DiMeglio et al., 2018; Khan et al., 2022). The clinical presentation of T1D is typically acute and dramatic, with patients presenting with the classic triad of polyuria, polydipsia, and polyphagia, accompanied by unexplained weight loss and fatigue, and in severe cases, diabetic ketoacidosis (DKA), a life-threatening acute complication (DiMeglio et al., 2018; ADA, 2024). Management of T1D requires lifelong insulin replacement therapy, delivered through multiple daily injections or continuous subcutaneous insulin infusion via insulin pumps, with modern management strategies emphasizing intensive insulin therapy integrated with continuous glucose monitoring (CGM) systems and hybrid closed-loop insulin delivery systems (Holt et al., 2021).

Type 2 Diabetes Mellitus (T2D)

Type 2 diabetes mellitus represents the predominant form of diabetes, accounting for more than 90-95% of all diagnosed cases worldwide (Khan et al., 2022). Unlike T1D, T2D is characterized by a dual pathophysiological defect: peripheral insulin resistance and progressive β-cell dysfunction leading to inadequate compensatory insulin secretion (DeFronzo et al., 2022). This type of diabetes typically develops in adults over the age of 40, although there has been an alarming increase in T2D prevalence among children and adolescents in recent decades, paralleling the global obesity epidemic (Zheng et al., 2022). The pathogenesis of T2D is complex and multifactorial, involving intricate interactions between genetic predisposition, epigenetic

modifications, and environmental factors (Galicia-Garcia et al., 2020). While T2D has a strong hereditary component, with first-degree relatives of affected individuals having a 2-6 fold increased risk, it is fundamentally a lifestyle-related disease, with modifiable risk factors playing a predominant role in its development (Lin et al., 2020). The primary metabolic defect in T2D is insulin resistance, a condition in which target tissues (skeletal muscle, adipose tissue, and liver) exhibit diminished responsiveness to the physiological effects of insulin, manifesting as impaired glucose uptake in skeletal muscle, unrestrained hepatic glucose production in the liver despite elevated insulin levels, and increased lipolysis in adipose tissue leading to elevated circulating free fatty acids (DeFronzo et al., 2022; Galicia-Garcia et al., 2020). Initially, pancreatic β-cells compensate for peripheral insulin resistance by increasing insulin secretion, leading to a state of hyperinsulinemia; however, over time, β-cells become unable to sustain this compensatory hypersecretion, resulting in relative insulin deficiency driven by glucotoxicity, lipotoxicity, islet amyloid deposition, oxidative stress, and chronic inflammation (DeFronzo et al., 2022; Galicia-Garcia et al., 2020). The development of T2D is strongly associated with modifiable risk factors including obesity (particularly central adiposity), physical inactivity, unhealthy diet, smoking, and excessive alcohol consumption, as well as non-modifiable risk factors such as increasing age, family history, ethnicity, and history of gestational diabetes (Lin et al., 2020; Khan et al., 2022; Zheng et al., 2022). Unlike T1D, T2D typically has an insidious onset, with many patients remaining asymptomatic for years before diagnosis, and when symptoms occur, they are often mild, including gradual onset of polyuria and polydipsia, fatigue, blurred vision, and slow wound healing, with approximately 20-30% of individuals with T2D remaining undiagnosed (DeFronzo et al., 2022; ADA, 2024; Khan et al., 2022).

Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus is defined as glucose intolerance with onset or first recognition during pregnancy, typically in the second or third trimester, affecting approximately 6-15% of pregnancies globally (ADA, 2024; Zheng et al., 2022). The condition arises due to the physiological insulin resistance that normally accompanies pregnancy, driven by placental hormones such as human placental lactogen, progesterone, cortisol, and prolactin; in healthy pregnancies, pancreatic β-cells compensate for this insulin resistance by increasing insulin secretion 2-3 fold, but in women with pre-existing β-cell dysfunction or predisposition to diabetes, this compensatory mechanism fails, resulting in hyperglycemia (Galicia-Garcia et al., 2020). Risk factors for GDM include obesity, advanced maternal age (>35 years), family history of diabetes, previous history of GDM, previous delivery of a macrosomic infant (>4 kg), polycystic ovary syndrome, and certain ethnicities (South Asian, African American, Hispanic) (ADA, 2024). GDM poses significant risks to both mother and fetus, with maternal complications including increased risk of preeclampsia, cesarean delivery, and future development of T2D (50-70% within 10-20 years post-pregnancy), while fetal and neonatal complications include macrosomia, birth trauma, neonatal hypoglycemia, respiratory distress syndrome, and long-term increased risk of obesity and T2D in offspring (Zheng et al., 2022; Galicia-Garcia et al., 2020). Screening for GDM is typically performed between 24-28 weeks of gestation using an oral glucose tolerance test (OGTT), with management focusing on achieving euglycemia through lifestyle modifications (diet and exercise) in most cases, and insulin therapy or oral agents (metformin, glyburide) reserved for cases where glycemic targets are not met (ADA, 2024). Most women with GDM return to normal glucose tolerance postpartum, but they require lifelong monitoring given their elevated risk of developing T2D (Zheng et al., 2022).

Other Specific Types of Diabetes

Beyond the major categories of T1D, T2D, and GDM, several less common but clinically important forms of diabetes exist, collectively termed "other specific types" (ADA, 2024). Maturity-Onset Diabetes of the Young (MODY) represents a group of monogenic disorders characterized by autosomal dominant inheritance, early onset (typically before age 25), and primary defects in β-cell function without significant insulin resistance, with at least 14 different MODY subtypes identified, each caused by mutations in specific genes (e.g., HNF1A, HNF4A, GCK, HNF1B), accounting for 1-2% of all diabetes cases but frequently misdiagnosed as T1D or T2D (Khan et al., 2022; Galicia-Garcia et al., 2020; ADA, 2024). Neonatal Diabetes Mellitus (NDM) occurs within the first six months of life and can be either transient or permanent, typically caused by genetic mutations affecting β-cell development or insulin secretion, with mutations in the KCNJ11 and ABCC8 genes being most common (DiMeglio et al., 2018; Khan et al., 2022). Drug or chemical-induced diabetes results from medications and chemical agents that impair insulin secretion or action, with common culprits including glucocorticoids, atypical antipsychotics, protease inhibitors, thiazide diuretics, beta-blockers, statins, and immunosuppressive agents (ADA, 2024; Galicia-Garcia et al., 2020). Diseases of the exocrine pancreas, including chronic pancreatitis, hemochromatosis, pancreatectomy, cystic fibrosis-related diabetes, and pancreatic cancer, can lead to diabetes by destroying both exocrine and endocrine tissue (Khan et al., 2022; ADA, 2024). Endocrinopathies such as Cushing's syndrome, acromegaly, pheochromocytoma, hyperthyroidism, and glucagonoma can cause secondary diabetes by antagonizing insulin action or impairing insulin secretion (Galicia-Garcia et al., 2020; ADA, 2024).

Prediabetes

Prediabetes is an intermediate metabolic state between normal glucose homeostasis and overt diabetes, characterized by blood glucose levels that are elevated but not yet in the diabetic range, encompassing two conditions: impaired fasting glucose (IFG; fasting plasma glucose 100-125 mg/dL) and impaired glucose tolerance (IGT; 2-hour OGTT glucose 140-199 mg/dL) (ADA, 2024). Individuals with prediabetes, particularly those with IGT, have a substantially increased risk of progression to T2D (5-10% annual risk) and are already at elevated risk for cardiovascular complications (Zheng et al., 2022). Prediabetes affects approximately 374 million people globally, and its prevalence is rising in parallel with the diabetes epidemic (Khan et al., 2022). Importantly, prediabetes is not an inevitable precursor to diabetes; lifestyle interventions focused on weight loss (7-10% of body weight), increased physical activity (150 minutes/week of moderate-intensity exercise), and dietary modification can reduce progression to T2D by 58% (Zheng et al., 2022). The identification and management of prediabetes represent a critical window of opportunity for diabetes prevention at both individual and population levels (ADA, 2024).

2.3 PREVALENCE OF DIABETES MELLITUS

The global prevalence of diabetes mellitus has reached alarming proportions, constituting one of the most pressing public health challenges of the 21st century. The International Diabetes Federation (IDF) Diabetes Atlas (2025) reports that approximately 643 million adults aged 20-79 years were living with diabetes in 2024, representing a global prevalence of 10.5%, with projections indicating this figure will surge to 783 million by 2045. The epidemic is characterized by substantial geographic variations, with the Western Pacific region harboring the highest absolute number of people with diabetes (206 million), while the Middle East and North

Africa demonstrate the highest age-adjusted prevalence at 16.2% (IDF, 2025). Critically, the burden has shifted dramatically toward low- and middle-income countries (LMICs), which now account for nearly 80% of all diabetes cases worldwide, reflecting rapid urbanization, adoption of Western dietary patterns, increasing sedentary lifestyles, and rising obesity rates in developing economies (WHO, 2024; Lin et al., 2020). The economic toll is staggering, with global health expenditure on diabetes exceeding $966 billion in 2021, representing 9% of total global health spending, while an estimated 6.7 million deaths were attributable to diabetes and its complications in 2022 (IDF, 2025; WHO, 2023). This exponential rise in diabetes prevalence across all regions underscores the urgent need for effective prevention strategies and accessible therapeutic interventions, particularly in resource-limited settings where the burden is growing most rapidly (Khan et al., 2022; Zheng et al., 2022).

Africa presents a unique and increasingly concerning epidemiological landscape for diabetes mellitus, with approximately 24 million adults living with diabetes in 2021, representing a prevalence of 4.5%, the lowest regional prevalence globally yet concealing a more troubling reality (IDF, 2025). The continent faces the highest proportion of undiagnosed diabetes worldwide, with an estimated 54% of people with diabetes remaining undiagnosed due to inadequate disease surveillance systems, limited healthcare infrastructure, insufficient screening programs, and competing healthcare priorities (IDF, 2025; Atun et al., 2017). Diabetes prevalence across Africa varies remarkably, ranging from less than 2% in rural areas of Ethiopia and Uganda to over 12% in urban centers of South Africa and Mauritius, with urban residents demonstrating 2-4 times higher diabetes rates than rural counterparts (Hall et al., 2011). The rapid urbanization transforming African societies, with urban populations expected to triple by 2050, drives profound lifestyle changes including adoption of energy-dense, nutrient-poor diets,

reduced physical activity, and increased psychosocial stress (Atun et al., 2017; Mbanya et al., 2010). The continent's diabetes epidemic is compounded by severe healthcare system challenges, including critical shortages of healthcare professionals, limited access to essential medications (particularly insulin), catastrophic out-of-pocket costs consuming 10-50% of household income, and complex interactions with infectious diseases such as HIV/AIDS, tuberculosis, and malaria (Mbanya et al., 2010; Atun et al., 2017). These factors collectively result in complication rates and diabetes-related mortality substantially exceeding those observed in high-income countries, with diabetic ketoacidosis remaining a major cause of death, particularly among young people with Type 1 diabetes who lack consistent access to insulin (Hall et al., 2011).

Nigeria, one of Africa's most populous nation with over 220 million inhabitants, faces a rapidly escalating diabetes crisis with approximately 5.8 million Nigerian adults living with diabetes in 2021, representing a national prevalence of 5.0-5.5%, projected to reach 8.7 million by 2045 (IDF, 2025; Uloko et al., 2012). Geographic variations within Nigeria are substantial, with urban areas consistently demonstrating higher prevalence (6-12% in major cities like Lagos, Abuja, and Port Harcourt) compared to rural communities (2-4%), reflecting the country's rapid urbanization which increased from 23% in 1990 to over 52% in 2020 (Uloko et al., 2012; Federal Ministry of Health Nigeria, 2015). The nutrition transition has been particularly dramatic, with traditional diets based on yams, cassava, and vegetables increasingly replaced by refined carbohydrates, processed foods, and sugar-sweetened beverages, while obesity rates among urban adults have risen to 20-30%, exceeding 40% among urban women (Uloko et al., 2012; Mbanya et al., 2010). Nigeria's healthcare system faces formidable challenges in diabetes management, allocating less than 5% of its national budget to healthcare, resulting in severe shortages of diabetes specialists, inadequate diagnostic equipment in primary healthcare facilities, and catastrophic medication

costs that can exceed 30-50% of minimum wage, forcing many patients to abandon treatment (Uloko et al., 2012; Ekpenyong et al., 2012). These barriers lead to high rates of undiagnosed diabetes (50-60% of cases), poor glycemic control among diagnosed individuals, and complication rates substantially higher than in high-income countries, establishing diabetes as a major public health and economic challenge for Africa's most populous nation (Uloko et al., 2012; Federal Ministry of Health Nigeria, 2015).

Southern Nigeria, encompassing the South-West, South-South, and South-East geopolitical zones and representing approximately 40% of Nigeria's population, has emerged as the epicenter of the country's diabetes epidemic with prevalence rates substantially exceeding national averages (Chinenye & Young, 2011). Community-based studies consistently demonstrate diabetes prevalence of 8-12% in major urban centers such as Lagos, 10-14% in Port Harcourt (Rivers State), and 6-8% in Enugu (South-East), compared to the national average of 5.0-5.5%, with the urban-rural gradient being particularly pronounced as Lagos demonstrates 3-5 fold higher prevalence than rural South-West communities (Uloko et al., 2012; Chinenye & Young, 2011; Erasmus et al., 1989). This region's higher diabetes burden reflects greater urbanization, economic development, and more pronounced lifestyle transitions, including rapid adoption of Western dietary patterns, higher obesity rates (particularly among women), increased sedentary behaviors, and greater exposure to psychosocial stressors associated with urban living (Chinenye & Young, 2011). Edo State, located in the South-South zone with Benin City as its capital (population exceeding 1.5 million), exemplifies the diabetes challenge facing southern Nigerian urban centers, combining historical significance with modern urbanization and serving as a major commercial hub for the region (Omagbemi et al., 2025). The concentration of diabetes burden in southern Nigeria, particularly in rapidly growing urban areas like Benin City,

underscores the critical need for targeted interventions, improved screening programs, and accessible therapeutic options, making the investigation of locally available medicinal plants such as *Tetracera alnifolia*, traditionally used in this region for managing diabetes, both timely and scientifically justified (Chinenye & Young, 2011; Uloko et al., 2012).

2.4 DRUG USE FOR TREATING DIABETES

The management of T2D often requires a sequential and combinatorial approach to pharmacotherapy. Metformin remains the foundational agent due to its efficacy, safety profile, and low cost (ADA, 2024). After metformin, the choice of a second-line agent is personalized, based on the presence of comorbidities such as atherosclerotic cardiovascular disease (ASCVD), heart failure, or chronic kidney disease (CKD). SGLT2 inhibitors and GLP-1 RAs are now preferred in patients with these conditions due to their proven cardiorenal benefits (Das *et al.,* 2023). Despite these advances, controlling postprandial hyperglycemia (PPG) remains a specific challenge. PPG is a significant contributor to overall glycemic exposure (as measured by HbA1c) and is an independent risk factor for cardiovascular complications (Blaabjerg *et al.,* 2018).

Acarbose is a pseudo-tetrasaccharide of microbial origin and is the most prominent member of the alpha-glucosidase inhibitor class. It acts as a competitive, reversible inhibitor of membrane-bound α-glucosidase enzymes (e.g., sucrase, maltase, glucoamylase) in the brush border of the small intestine (Joshi *et al.,* 2022). Its primary mechanism is to delay the hydrolysis of complex carbohydrates and disaccharides into absorbable monosaccharides (like glucose), thereby blunting the sharp rise in blood glucose following a meal (Tundis *et al.,* 2021). Clinically, acarbose is effective as monotherapy or in combination with other antidiabetic drugs. It can reduce HbA1c by 0.5–0.8% and is particularly effective at lowering postprandial glucose spikes (Balfour & McTavish, 2022). A significant landmark study, the STOP-NIDDM trial,

demonstrated that acarbose could reduce the risk of progressing from impaired glucose tolerance to T2D by 25% over 3.3 years (Chiasson *et al.,* 2022).

However, its use is limited by its prominent gastrointestinal side effects, including flatulence, diarrhea, and abdominal pain, which result from the bacterial fermentation of undigested carbohydrates in the colon. These effects often lead to poor long-term adherence (Balfour & McTavish, 2022). This drawback has fuelled the search for alternative alpha-glucosidase inhibitors, particularly from natural sources, which may offer similar efficacy with a more tolerable side-effect profile.

Table 1: Common Classes of Oral Antidiabetic Drugs and Their Mechanisms

Drug Class	Example(s)	Primary Mechanism of Action	Key Advantages	Key Limitations
Biguanides	Metformin	Decreases hepatic glucose output	First-line, weight-neutral, inexpensive	GI upset, contraindicated in renal impairment
Sulfonylureas	Glibenclamide, Glimepiride	Stimulates insulin secretion from pancreas	Potent, inexpensive	Hypoglycemia risk, weight gain
Thiazolidinediones	Pioglitazone	Improves insulin sensitivity in periphery	Durable effect	Weight gain, edema, heart failure risk
DPP-4 Inhibitors	Sitagliptin, Saxagliptin	Prolongs action of incretin hormones	Weight-neutral, well-tolerated	Possible link to heart failure
SGLT2 Inhibitors	Empagliflozin, Dapagliflozin	Increases urinary glucose excretion	Weight loss, cardiorenal benefits	Genital mycotic infections, volume depletion
Alpha-Glucosidase Inhibitors	*Acarbose*, Miglitol	Delays carbohydrate digestion in intestine	Targets PPG, no systemic absorption	Frequent GI side effects (flatulence, diarrhea)

2.5 ROLE OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE IN CARBOHYDRATE DIGESTION

Dietary carbohydrate digestion is a sequential process initiated in the mouth and completed in the small intestine. It relies on the synergistic action of several enzymes. Salivary α-amylase, secreted in the mouth, begins the hydrolysis of starch (amylose and amylopectin) by breaking internal α-1,4-glycosidic bonds, producing smaller oligosaccharides like maltose and maltotriose (Tundis *et al.,* 2021). Upon entering the duodenum, pancreatic α-amylase continues this process, further breaking down starch into disaccharides (maltose), trisaccharides (maltotriose), and branched oligosaccharides called α-limit dextrins (Drozdowski *et al.,* 2020). Brush border α-glucosidases, located on the enterocytes lining the small intestine and membrane-bound enzymes, complete the digestion: sucrase-isomaltase complex hydrolyzes sucrose, isomaltose, and the α-1,6 bonds in α-limit dextrins while maltase-glucoamylase hydrolyses the α-1,4 bonds in maltose, maltotriose, and other linear oligosaccharides. The final products of this enzymatic cascade: glucose, fructose, and galactose, are then rapidly absorbed into the bloodstream, causing a sharp rise in postprandial blood glucose levels (Tundis *et al.,* 2021).

2.5.1 Enzyme Inhibition

Inhibiting the key enzymes responsible for carbohydrate digestion presents a logical and targeted strategy for managing postprandial hyperglycemia. By slowing the rate of glucose absorption, these inhibitors "flatten" the post-meal glucose curve without requiring systemic absorption or directly affecting insulin secretion (Drozdowski *et al.,* 2020).

This approach offers several advantages. It targets the source of postprandial glucose directly (mechanistic specificity), it can be effectively combined with drugs that address fasting

hyperglycemia or insulin resistance (complement action) and has low risk of hypoglycaemia: when used as monotherapy. It does not typically cause dangerous low blood glucose because its action is contingent on food intake.

The search for effective enzyme inhibitors has extended beyond drugs like acarbose to encompass a wide array of natural compounds, particularly plant-derived phenolics, flavonoids, and terpenoids, which often exhibit potent inhibitory activity against both α-amylase and α-glucosidase (Tundis *et al.,* 2021; Oboh *et al.,* 2022).

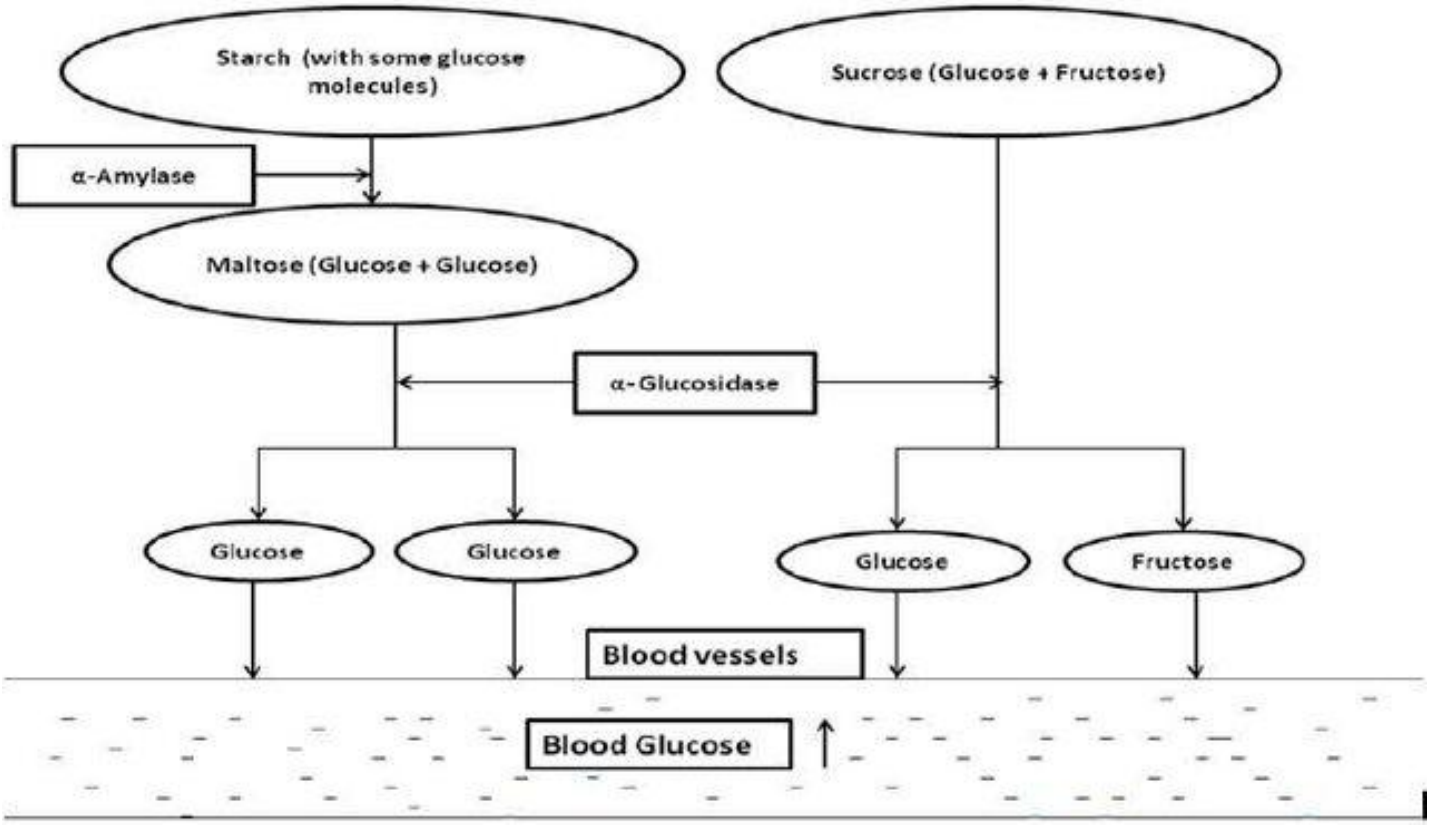

Figure 2: Mechanism of Alpha-Glucosidase and Alpha-Amylase Inhibitors

2.6 *Tetracera alnifolia*

Tetracera alnifolia popularly known ‘Opon in Yoruba, is a perennial shrub or small tree widely distributed in the tropical rainforests of West and Central Africa. It is known by various local names across different regions, underscoring its traditional importance (Adebayo *et al.,* 2020). Plants from the *Tetracera* genus have a long history of use in folk medicine for treating a diverse range of ailments, including diabetes, inflammatory conditions, gastrointestinal disorders, wounds, and pain (Odukoya *et al.,* 2021). The ethnobotanical use of *T. alnifolia* and related species for managing hyperglycemia provides the fundamental rationale for its scientific investigation. This traditional knowledge is a valuable starting point for bioprospecting and drug discovery (Sofowora *et al.,* 2022). Establishing the definitive taxonomic identity of *Tetracera alnifolia* (Willd.) is essential for scientific validation and for comparative phytochemical studies with related medicinal species. The species belongs to the family *Dilleniaceae*, a grouping known to harbor species with documented bioactivity, such as *Dillenia indica*, which exhibits alpha-glucosidase inhibitory properties. This shared phylogenetic lineage provides a strong *a priori* expectation of antidiabetic activity within *T. alnifolia*.

The botanical classification of the plant is presented as follows, establishing its hierarchical position within the plant kingdom

2.6.1 Botanical Classification of *Tetracera alnifolia*

Kingdom	*Plantae*
Division	*Tracheophyta*
Class	*Magnoliopsida*
Order	*Dilleniales*
Family	*Dilleniaceae*
Genus	*Tetracera*
Species	*Tetracera alnifolia*.

Source: Ogunlakin, A.D., & Sonibare, M. A. 2022

Figure 3: *Tetracera alnifolia*

T. alnifolia is geographically widespread across West and Central Africa, thriving in diverse ecological niches including swamp forests, firm ground, thickets, and forest margins from Senegal to Angola. Morphologically, it is a robust, perennial, woody creeper or liana, capable of reaching significant lengths and diameters (up to 20 meters long and 10 cm in diameter). Its large leaves (4–16 cm long) and terminal panicles of white flowers, culminating in dull red, woody fruits containing black seeds with a noticeable orange aril, define its identity.

Although literature on *T. alnifolia* specifically is limited, preliminary studies and research suggest a wide range of biological activities that support its traditional use. Extracts of *Tetracera alnifolia* have demonstrated strong free radical scavenging abilities *IN VITRO* (e.g., against DPPH and ABTS radicals), which is crucial for mitigating oxidative stress, a key pathophysiological feature of diabetes (Afolayan *et al.,* 2022). Traditional use for inflammatory conditions (anti-inflammatory property) is supported by scientific evidence showing inhibition of pro-inflammatory mediators like TNF-α and IL-6 (Odukoya *et al.,* 2021). Given the established link between oxidative stress, inflammation, and insulin resistance, its anti-oxidant, and anti-inflammatory activities provide an indirect but strong basis for its antidiabetic use. The presence of flavonoids and tannin may also suggest potential alpha-amylase and alpha-glucosidase inhibitory activity (Oboh *et al.,* 2022).

2.6.2 Phytochemical Composition of *Tetracera alnifolia*

The therapeutic prowess of *Tetracera alnifolia* is undeniably rooted in its complex and diverse phytochemical profile. This arsenal of bioactive compounds, which the plant produces as part of its secondary metabolism, is responsible for its wide-ranging pharmacological applications.

Phytochemical screenings have consistently confirmed the presence of several fundamental classes of compounds, including tannins, flavonoids, leucoanthocyans, carotenoids, and

saponosides in the leaf extracts, while the root bark has been found to contain alkaloids, steroids, terpenoids, and cardiac glycosides (Balde et al., 2024; Ogunlakin & Sonibare, 2022). This rich consortium of phytochemicals acts synergistically, contributing to the plant's broad-spectrum biological activities. Beyond these general classes, the isolation and characterization of specific, potent molecules have significantly advanced the understanding of its mechanism of action.

1. **Flavonoids**: Flavonoids are a major class of polyphenols widely recognized for their antioxidant, anti-inflammatory, and antimicrobial properties (Harborne, 2023). A landmark discovery from T. alnifolia was the isolation of rhamnocitrin 3-sulphate, a sulphated flavonol, from the leaves and stem (Ogunlakin & Sonibare, 2022). This compound is particularly significant as it was obtained in an exceptionally high yield of 1% through a simple aqueous decoction, suggesting it is a major constituent rather than a minor component (Balde et al., 2024). Furthermore, the presence of the well-studied flavonoid kaempferol adds to the plant's antioxidant and anti-inflammatory potential, as this compound is known to neutralize free radicals and modulate inflammatory pathways (Ogunlakin & Sonibare, 2022).
2. **Tannins**: This class of polyphenolic compounds is known for their astringent and antimicrobial properties (Harborne, 2023). Their ability to precipitate proteins makes them effective in treating wounds and infections, which aligns perfectly with the traditional use of T. alnifolia for skin diseases and infections (Balde et al., 2024).
3. **Saponins**: Saponins are glycosides known for their immune-modulating effects and ability to form foamy solutions (Harborne, 2023). Their presence contributes to the plant's antimicrobial and anti-inflammatory activities (Ogunlakin & Sonibare, 2022). The hemolytic activity of saponins is also a key area of interest in pharmacological research.

4. **Alkaloids and Terpenoids:** These are two large classes of nitrogen-containing (alkaloids) and non-nitrogenous (terpenoids) secondary metabolites often associated with diverse and potent pharmacological activities (Harborne, 2023). Their presence in T. alnifolia broadens its therapeutic potential, as alkaloids are frequently linked to analgesic and antimalarial effects, while terpenoids are known for their anti-inflammatory and anticancer properties (Ogunlakin & Sonibare, 2022). Research on other Tetracera species has identified pentacyclic lupane-type triterpene derivatives, with betulinic acid being the most extensively investigated for its notable pharmacological activities (Balde et al., 2024).

2.6.3 Medicinal Potentials of *Tetracera alnifolia*

The medicinal potentials of *Tetracera alnifolia* are vast, stretching from well-documented traditional uses to robust validations through modern pharmacological testing. This convergence of ethnobotanical knowledge and scientific evidence paints a compelling picture of its therapeutic value.

1. **Anti-inflammatory and Analgesic Properties:** This is one of the most rigorously studied areas of the plant's pharmacology. A hydroethanolic leaf extract of T. alnifolia (HeTA) has demonstrated significant dose-dependent antinociceptive effects in multiple standardized animal models, including acetic acid-induced writhing, formalin-induced paw licking, and the hot plate test (Balde et al., 2024). Importantly, the mechanistic studies revealed that this pain-blocking activity is not singular; it involves a complex interplay of peripheral opioid receptors, the l-arginine-nitric oxide pathway, and ATP-sensitive potassium channels (Balde et al., 2024). Simultaneously, the extract showed potent anti-inflammatory effects against carrageenan-induced paw edema, xylene-induced ear edema, and even chronic models like complete Freund's adjuvant-induced arthritis in rats (Ogunlakin & Sonibare, 2022). This

provides a strong scientific basis for its traditional use in treating headaches, abdominal pain, and rheumatism (Adebayo et al., 2020).

2. **Antimicrobial and Antiparasitic Activities:** Scientific investigations have substantiated the traditional use of T. alnifolia against infections. The plant's extracts have shown broad-spectrum antibacterial activity against clinically relevant pathogens such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (Ogunlakin & Sonibare, 2022). Beyond bacteria, its potential extends to combating tropical parasitic diseases. Methanol and chloroform extracts have demonstrated promising antiprotozoal effects against Trypanosoma and Leishmania species, highlighting its potential as a source of novel antiparasitic agents (Balde et al., 2024).
3. **Safety and Toxicological Profile**: For any medicinal plant, demonstrating safety is paramount. Acute toxicity studies have established that the oral LD_{50} for both aqueous and methanol root extracts in albino rats is greater than 5000 mg/kg body weight, indicating very low acute toxicity (Balde et al., 2024). Sub-acute studies over 28 days further confirmed that the extracts are non-toxic to the liver, kidney, heart, and blood at doses up to 3000 mg/kg (Ogunlakin & Sonibare, 2022). This high safety profile, established through rigorous toxicological assessment, strongly supports the safe traditional use of this plant and de-risks its consideration for further drug development (Adebayo et al., 2020).

2.6.4 Comparison of Plant's Antidiabetic Properties to Drug Acarbose

The comparative analysis of a natural extract like *T. alnifolia* with a standard drug like acarbose is a critical component of ethnopharmacological research. This comparison should be framed around efficacy, mechanism, and safety/tolerability.

The primary basis for comparison is the *In Vitro* inhibitory potency against α-amylase and α-glucosidase enzymes, typically measured by the half-maximal inhibitory concentration (IC_{50} value). A lower IC_{50} indicates a more potent inhibitor. Acarbose is a potent inhibitor of α-glucosidase (IC_{50} values in the nanomolar to low micromolar range) but a relatively weaker inhibitor of α-amylase (IC_{50} in the micromolar range) (Tundis *et al.,* 2021). Many plant extracts and isolated compounds have demonstrated IC_{50} values comparable to or even better than acarbose for α-glucosidase inhibition, while often showing weaker inhibition of α-amylase (Oboh *et al.,* 2022). This is actually a desirable trait. Strong α-amylase inhibition can lead to the undigested starch reaching the large intestine and causing severe distress, while more selective α-glucosidase inhibition primarily affects disaccharide digestion, resulting in a milder side-effect profile.

2.6.5 Safety and Tolerability Profile

This is potentially the most significant area of advantage for plant-derived inhibitors. The use of acarbose is severely limited by gastrointestinal side effects (flatulence, diarrhoea, abdominal pain) in up to 75% of users, leading to poor compliance (Balfour & McTavish, 2022). Natural inhibitor complexes often exhibit a more favourable tolerability profile. This is hypothesized to be due to several factors including a more selective inhibition profile favouring α-glucosidase over α-amylase, the presence of other compounds in the extract that may mitigate gastrointestinal irritation, and lastly, a synergistic action that allows for efficacy at lower, better-tolerated doses of individual constituents (Tundis *et al.,* 2021; Oboh *et al.,* 2022).

CHAPTER THREE

MATERIALS AND METHOD

3.1 CHEMICALS

Chemicals used were: absolute ethanol, n-hexane, chloroform, dichloroform, butanol, acarbose, and starch. All chemicals were of analytical grade.

3.2 EQUIPMENT

Equipment used were water bath, pH meter, spectrophotometer, electronic weighing balance, freeze dryer, separating funnel (1000 mL), measuring cylinders and test tubes.

3.3 COLLECTION OF PLANT

The roots and leaves of *Tetracera alnifolia* were obtained locally from Oyingbo Market in Lagos State. The leaves were identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, where it was assigned a herbarium number, UBH-T_{405}. The roots were thoroughly washed, cut into bits and subsequently spread out on a clean surface in the laboratory for air-drying for three weeks. The dried roots were ground into a fine powder.

3.4 PREPARATION OF PLANT EXTRACT

A total of 4kg of the pulverized plant material was soaked in 10L of ethanol for 72hr and the mixture was stirred intermittently. Afterwards, the mixture was filtered using a muslin cloth; the process was repeated twice to remove all residue. The filtrate was freeze-dried and the ethanol extract was obtained. This extract was kept in a tightly sealed container in a refrigerator until it was needed.

3.5 SOLVENT PARTITIONING OF ETHANOL EXTRACT OF *Tetracera Alnifolia*

The solvent partitioning was done according to the procedure described by Jamil et al., 2012, with few modifications. The crude ethanol plant extract (50g) was dissolved in 20ml of ethanol and the mixture was partitioned with 1.5 L each of n-hexane, chloroform, dichloromethane, n-butanol and water in the increasing order of polarity using a separating funnel. All fractions were freeze dried and stored in a refrigerator.

3.6 DETERMINATION OF ANTI-DIABETIC PROPERTY

3.6.1 Determination of alpha-amylase inhibitory activity

Alpha-amylase inhibition activity of each fraction was determined by the method of Worthington, 1993. An aliquot of 500 µl of the standard (acarbose), crude extract and various fractions (0.2-1.0 mg/ml) and 500 µl (0.02 M) of sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5mg/ml of alpha-amylase was mixed together and incubated for 10 minutes at room temperature. Afterwards, 500 µL of 1% starch solution prepared with 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added and incubated in a water bath at 25°C for 10 minutes. The reaction mixture was stopped by adding 1.0 ml of dinitrosalicylic acid. The mixtures in the test tubes were incubated in boiling water in a water bath for 5 minutes and then cooled. Absorbance of samples, standard and reference control (reaction mixture without sample) was read at 540 nm and percentage inhibition was calculated as:

Alpha amylase inhibitory activity (%) = [(Abs control - Abs sample)/(Abs control)] x 100

3.6.2 Determination of alpha-glucosidase inhibitory activity

Alpha-glucosidase activity of each fraction was determined by the method of Apostolidis et al., 2007. Varied concentrations (0.2-1.0 mg/mL prepared with distilled water) of the standard

(acarbose), crude extract and the various fractions were prepared. Exactly 500 µl of the standard (acarbose), crude extract was mixed with 1 ml of alpha glucosidase solution (prepared in 0.1 M phosphate buffer with pH 6.9) in well labelled separate test tubes and pre-incubated in a water bath at 25°C for 10 minutes. An aliquot of 500 µl of freshly prepared phosphate buffered nitrophenyl-glucopyranoside (5mM) solution was added. The reaction mixture was incubated at 25°C for 5 minutes and the absorbance was read at 405nm. The percentage inhibition was calculated as follows:

Alpha glucosidase inhibitory activity (%) = [(Abs control - Abs sample)/(Abs control)] x 100

3.7 STATISTICAL ANALYSIS

The data from the assays were expressed as mean. Microsoft Excel 2016 version was used to plot the graph.

CHAPTER FOUR

RESULTS

4.1 *In Vitro* α-Amylase Inhibition Assay

Table 4.1: Result of *in vitro* α-Amylase Inhibition of Extracts and Fractions

Concentration (mg/mL)	Ascorbic acid (Standard)	Crude extract	n-Hexane fraction	Chloroform fraction	Dichloromethane fraction	Butanol fraction	Water fraction
0.2	90.90	88.40	87.66	87.04	74.37	78.19	79.17
0.4	90.98	88.11	82.86	85.36	67.57	66.95	71.63
0.6	95.24	85.77	79.21	83.44	58.22	55.02	63.10
0.8	94.46	82.98	75.24	80.69	48.38	42.76	56.33
1.0	95.49	81.55	69.70	74.17	36.00	32.27	43.42

Table 4.1 shows that α-amylase inhibitory activity exhibited clear concentration-dependent patterns across all samples. Acarbose demonstrated consistently high inhibition at various concentration. The crude extract and the various fractions showed strong inhibitory activity, however percentage inhibition decreased with increased concentration with butanol having the least inhibition at 1.0 mg/mL

Non-polar fractions (n-hexane: 87.66-69.70%; chloroform: 87.04-74.17%) maintained moderate to strong activity, while polar fractions showed significantly weaker inhibition. The dichloromethane, butanol, and water fractions exhibited sharp concentration-dependent declines, with butanol showing the steepest drop (78.19-32.27%).

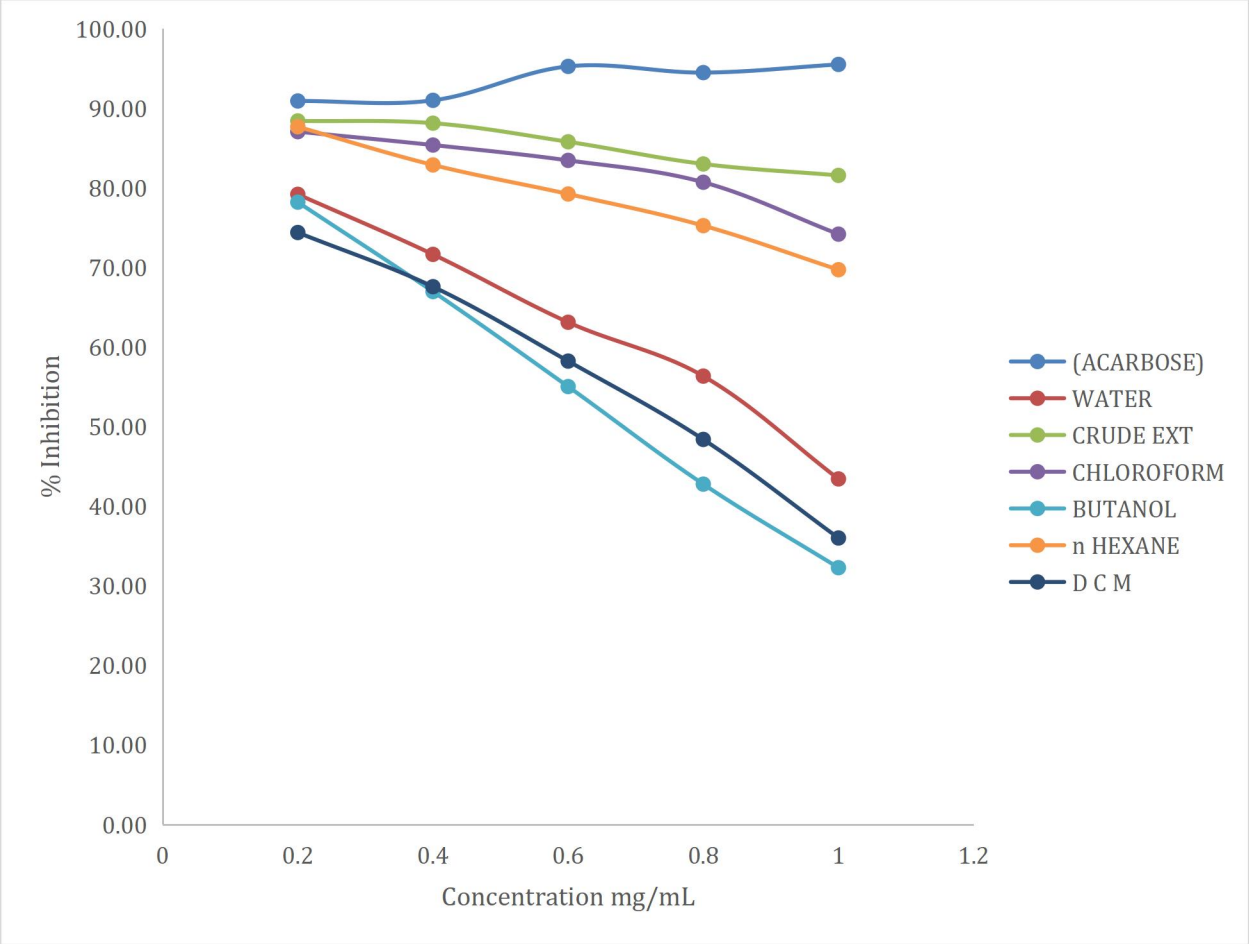

Figure 4: Alpha-Amylase Inhibition by *Tetracera alnifolia* Ethanol Extract and the various fractions

Table 4.2: IC_{50} Values for α-Amylase Inhibition

Sample	IC_{50} (mg/mL)
Acarbose	0.46
Crude Extract	0.68
n-Hexane Fraction	2.91
Chloroform Fraction	2.67
DCM Fraction	2.36
Butanol Fraction	1.27
Water Fraction	3.23

Table 4.2 shows that the crude extract demonstrated substantial activity (IC_{50} = 0.68 mg/mL), Among fractions, butanol showed the strongest activity (IC_{50} = 1.27 mg/mL), followed by chloroform mg/mL, dichloromethane, while n-hexane (2.91 mg/mL) and water. Water fraction had the least inhibition activity least: approximately 4-5 times less inhibition than the crude extract.

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4.2 *In Vitro* α-Glucosidase Inhibition Assay

Table 4.3: Percentage Inhibition of α-Glucosidase by crude ethanol extract and the fractions

Concentration (mg/mL)	Ascorbic acid (Standard)	Crude extract	n-Hexane fraction	Chloroform fraction	Dichloromethane fraction	Butanol fraction	Water fraction
0.2	92.22	49.46	52.32	59.96	51.36	57.53	67.31
0.4	91.93	38.94	35.05	46.11	40.40	44.97	56.75
0.6	92.22	34.05	30.34	37.22	33.58	35.38	48.07
0.8	91.90	31.98	29.59	33.62	30.76	33.40	41.72
1.0	91.43	28.84	28.19	30.69	29.41	29.26	37.26

Table 4.3 revealed the crude extract and fractions showed moderate activity that declined progressively with increasing concentration.

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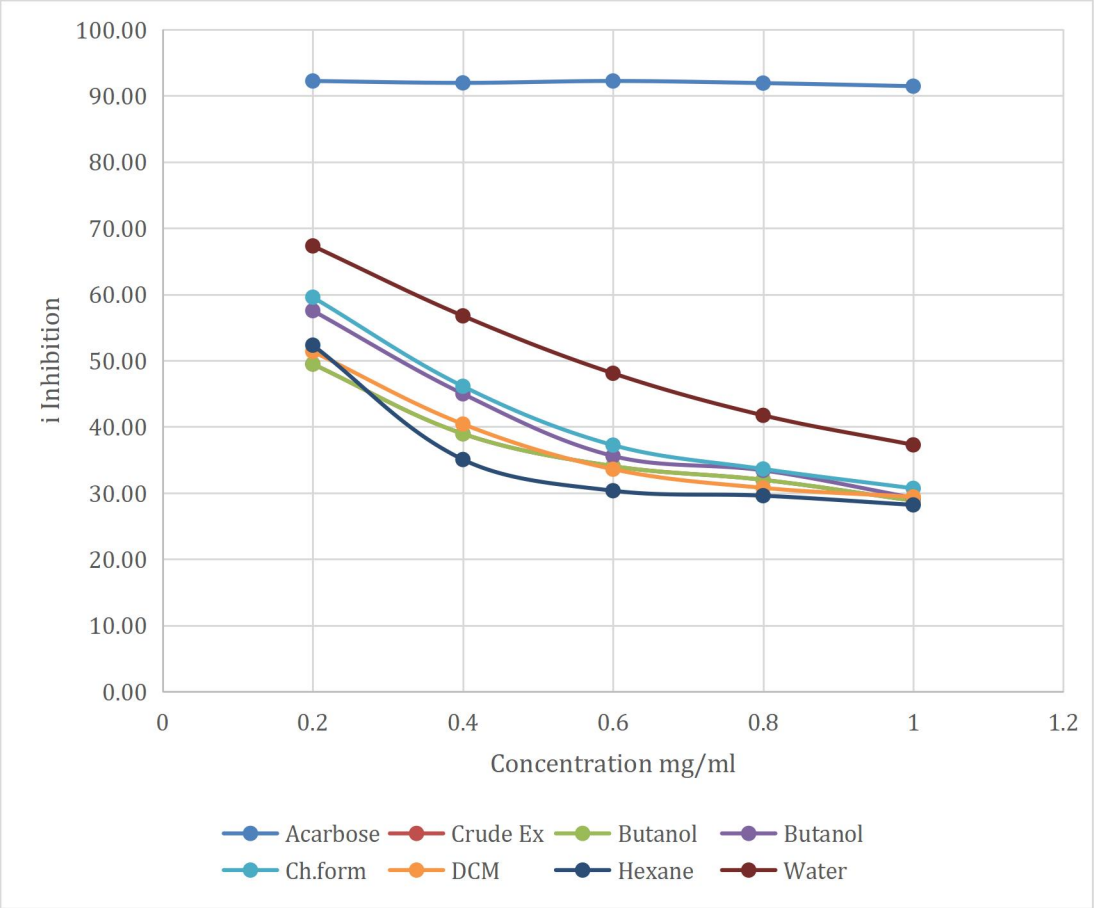

Figure 5: Alpha-glucosidase inhibition by *Tetracera alnifolia* crude ethanol extracts and the various fractions

Table 4.4: IC_{50} Values for α-Glucosidase Inhibition

Sample	IC_{50} (mg/mL)
Acarbose	0.93
Crude Extract	0.10
n-Hexane Fraction	0.19
Chloroform Fraction	0.39
DCM Fraction	0.38
Butanol Fraction	0.39
Water Fraction	0.61

Table 4.4 shows that the crude extract demonstrated exceptional α-glucosidase inhibitory potency (IC_{50} = 0.10 mg/mL), approximately 9-fold that of acarbose (IC_{50} = 0.93 mg/mL). The n-hexane fraction also showed impressive activity (IC_{50} = 0.19 mg/mL), nearly 5-fold more han acarbose. All other fractions had IC_{50} that that indicate higher in vitro inhibitory activity than acarbose.

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CHAPTER FIVE

5.1 DISCUSSION

The study demonstrated that *Tetracera alnifolia* possesses significant alpha-amylase inhibitory activity. The crude ethanol extract exhibited remarkable inhibitory potency with an IC_{50} of 0.68 mg/mL (680 µg/mL), only 1.5-fold less potent than the standard drug acarbose (IC_{50} = 0.46 mg/mL; 460 µg/mL). The IC_{50} value represents the concentration required to achieve 50% enzyme inhibition, with lower values indicating higher potency (R). This finding is particularly noteworthy as it indicates near-pharmaceutical-grade enzyme inhibition activity (R).

Among the solvent fractions, the butanol fraction demonstrated the strongest alpha-amylase inhibitory activity (IC_{50} = 1.27 mg/mL), followed by dichloromethane (IC_{50} = 2.36 mg/mL) and chloroform (IC_{50} = 2.67 mg/mL) fractions. The IC_{50} value of the ethanol crude extract was comparably lower that all fraction indicating the inhibitory activity outperformed all individual fractions. This may suggest evidence for synergistic mechanisms where multiple bioactive compounds cooperate to achieve enhanced enzyme inhibition (Saleh et al., 2025). Also, this supports the traditional medicinal practice of using whole plant extracts in the treatment of various diseases locally (R).

Comparative analysis with other medicinal plants reveals favorable performance of *T. alnifolia.* For instance, *Senna alata* acetone extract showed alpha-amylase inhibitory activity with an IC_{50} of 6.41 mg/mL (Kazeem et al., 2015), approximately 9-fold less inhibition than crude ethanol extract of *Tetracera alnifolia*. Similarly, *Acacia catechu, Dioscorea bulbifera,* and *Swertia chirata* exhibited alpha-amylase inhibition with IC_{50} values of 49.9, 296.1, and 413.5 µg/mL respectively (Shrestha et al., 2020), with *T. alnifolia* crude extract showing comparable activity

to *Acacia catechu.* Studies on *Adiantum caudatum* and *Celosia argentea* extracts reported IC_{50} values of 0.241 and 0.294 mg/mL respectively for their most active fractions (Chandran et al., 2017), better than the inhibitory activity *T. alnifolia* fractions, however comparable to the crude extract. Further optimization through bioassay-guided fractionation may yield fractions with better enzyme inhibition. Terpenoid-rich compounds, particularly 3-oxolupenal and katononic acid isolated from *Nuxia oppositifolia,* demonstrated IC_{50} values of 46.2 and 52.4 µg/mL against alpha-amylase (Alqahtani et al., 2020), approximately 10-15 fold more potent than *T. alnifolia* fractions. .

The alpha-glucosidase inhibition assay revealed exceptional results in this study. The crude extract of *T. alnifolia* demonstrated remarkable inhibitory potency with an IC_{50} of 0.10 mg/mL (100 µg/mL), approximately 9-fold more potent than acarbose (IC_{50} = 0.93 mg/mL; 930 µg/mL). All solvent fractions similarly outperformed acarbose, with the n-hexane fraction showing particularly impressive activity (IC_{50} = 0.19 mg/mL)—nearly 5-fold more potent than the standard drug. The chloroform, dichloromethane, and butanol fractions exhibited comparable moderate activities (IC_{50} values: 0.39, 0.38, and 0.39 mg/mL respectively), while even the water fraction (IC_{50} = 0.61 mg/mL) demonstrated 1.5-fold greater potency than acarbose.

This finding is consistent with those obtained from other medicinal plants. *Myrcia* species inhibited alpha-glucosidase 90-500 times more potently than acarbose, with IC_{50} values ranging from 0.7 to 4.1 µg/mL (Rodrigues et al., 2015). Similarly, *Zataria multiflora* ethyl acetate fraction (IC_{50} = 0.35 mg/mL) and *Salvia mirzayanii* petroleum ether fraction (IC_{50} = 0.4 mg/mL) demonstrated potent alpha-glucosidase inhibition better than acarbose (IC_{50} = 7 mg/mL) (Rasouli et al., 2017), though *T. alnifolia* crude extract (IC_{50} = 0.10 mg/mL) exhibited even better inhibition activity, indicating a more potent natural inhibitors.. *Tamarix nilotica* extracts showed

alpha-glucosidase inhibition with IC_{50} values of 12.5 and 24.8 µg/mL, considerably more potent than acarbose (IC_{50} = 151.1 µg/mL) (Elokely et al., 2022), demonstrating that some medicinal plants can exhibit better inhibitory activity than acarbose, an antidiabetic agent in clinical use. A comprehensive screening of plants from *Apocynaceae, Clusiaceae, Euphorbiaceae,* and *Rubiaceae* families revealed that thirty-seven out of forty-five samples showed more potent alpha-glucosidase inhibition than acarbose (IC_{50} = 117.20 µg/mL), with IC_{50} values ranging from 2.33-112.02 µg/mL (Widyowati & Agil, 2018; Benalla et al., 2021), further validating that some plant-derived inhibitors frequently surpass pharmaceutical standards.

Flavonoid-rich extracts from *Scutellaria baicalensis* demonstrated alpha-glucosidase inhibition with an IC_{50} of 421.54 µg/mL (Yin et al., 2018), with specific flavonoids showing a defined structure-activity relationship where hydroxyl patterns and glycosylation significantly influenced inhibitory potency.

The phytochemical diversity of *T. alnifolia*, documented to include flavonoids (kaempferol, rhamnocitrin 3-sulphate), tannins, steroids, and terpenoids (Ogunlakin & Sonibare, 2022; Balde et al., 2024), aligns with compounds known to exhibit potent alpha-glucosidase inhibition (R). The enhanced inhibitory activity of the crude ethanol extract (IC_{50} = 0.10 mg/mL) compared to vriou individual fractions (IC_{50} = 0.19-0.61 mg/mL) suggests evidence for synergistic interactions between compounds of different chemical classes and polarities (Saleh et al., 2025), interactions i that may be reduced or completely lost upon fractionation.

The results from both enzyme inhibition assays reveal that *T. alnifolia* exhibits differential inhibition activity, with the crude extract demonstrating potent alpha-glucosidase inhibition (IC_{50} = 0.10 mg/mL; 9-fold superior to acarbose) while maintaining moderate alpha-amylase inhibition (IC_{50} = 0.68 mg/mL; 1.5-fold less potent than acarbose). This inhibition profile suggests a

potential therapeutic characteristic as an antidiabetic agents. Excessive alpha-amylase inhibition can lead to maldigestion of starch, resulting in severe gastrointestinal side effects including flatulence, diarrhea, and abdominal discomfort—the primary limitation of acarbose therapy that affects up to 75% of users and leads to poor medication compliance (Kazeem et al., 2015; Balfour & McTavish, 2022). Plant extracts containing selective alpha-glucosidase inhibitors have demonstrated the ability to achieve effective glucose management while displaying mild alpha-amylase inhibition (Rodrigues et al., 2015), thereby minimizing gastrointestinal complications. Comparative studies on sorghum phenolic extracts revealed that some fractions showed far stronger alpha-glucosidase inhibition (about 2000 times) but weaker alpha-amylase inhibition (about 180 times) than acarbose (Zhang et al., 2020), demonstrating that selective inhibition profiles are achievable and therapeutically advantageous. By preferentially targeting alpha-glucosidase while moderately affecting alpha-amylase, *T. alnifolia* may offer better postprandial glucose control with significantly reduced adverse effects, addressing one of the major compliance issues associated with current alpha-glucosidase inhibitor therapy.

5.2 CONCLUSION

This study has demonstrated that *Tetracera alnifolia* possesses potent *in vitro* antidiabetic properties by having a better inhibition of alpha-glucosidase and moderate inhibition of alpha-amylase. The crude ethanol extract exhibited better alpha-glucosidase inhibitory activity with an IC_{50} of 0.10 mg/mL, approximately 9-fold more potent than the standard drug acarbose (IC_{50} = 0.93 mg/mL).

These findings, coupled with the documented safety profile from previous toxicological studies (oral LD_{50} > 5000 mg/kg), support the ethnobotanical use of *T. alnifolia* in diabetes management in traditional medicine in Nigeria and other African countries Also, it suggest alpha amylase and

alpha glucosidase inhibition as one of its probable mechanism of action as an antidiabetic remedy in traditional medicine. Furthermore, it also suggests the plant extract as prospective candidate subject to further investigations for development of natural antidiabetic therapeutics.

RECOMMENDATIONS

Based on these findings, the following recommendations are proposed:

1. Bioassay-guided fractionation and isolation should be conducted to identify, purify, and structurally characterize the specific bioactive compound(s) responsible for the potent enzyme inhibitory activities, particularly from the crude extract and n-hexane fraction.
2. Mechanistic studies including enzyme kinetics, molecular docking studies to predict binding modes, and in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions should be undertaken to understand the precise mechanisms of action.
3. Comprehensive phytochemical profiling using advanced techniques such as HPLC, LC-MS/MS, and GC-MS should be performed to quantify major bioactive constituents and establish quality control parameters for standardization.
4. In vivo validation studies using appropriate animal models of diabetes are essential to confirm the antidiabetic efficacy, establish optimal dosing regimens, and elucidate pharmacokinetic and pharmacodynamic parameters.

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