DETERMINATION OF THE INVITRO ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITY OF BITTERLEAF

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MARCH, 2025

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

MARCH, 2025

CERTIFICATION

We the undersigned hereby certify that **NELSON EHIAGWINA IKHUALOGHE (BMS2001375)** carried out this research in the Department of Medical biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical biochemistry.

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CERTIFICATION OF PROJECT ON PLAGIARISM

We the undersigned attest and declare that the project undertaken by **NELSON EHIAGWINA IKHUALOGHE**

Titled:

DETERMINATION OF THE INVITRO ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITY OF BITTERLEAF

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DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B. Sc) program in the Department of Medical biochemistry.

ACKNOWLEDGEMENTS

I am deeply grateful for the unwavering support and guidance I received throughout this project. First and foremost, I thank God Almighty for His unrelenting support and blessings, which sustained me throughout this journey. I extend my heartfelt appreciation to my supervisor Mrs. Ukwuonwo Ediale A.C for her invaluable guidance, expertise, and encouragement. Your support was instrumental in shaping this work. Also, to my parents Mr. and Mrs. Ikhualoghe, my siblings and friends God bless you all, I am indeed grateful.

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ABSTRACT

Diabetes mellitus is a long-lasting metabolic condition that is marked by high blood sugar levels due to problems with insulin release, its effectiveness, or both. Blocking carbohydrate-breaking enzymes like alpha-amylase and alpha-glucosidase has been recognized as a useful technique for controlling high blood sugar after meals. This research was conducted to assess the effects of Vernonia amygdalina (bitter leaf) extract on the activities of alpha-amylase and alpha-glucosidase in a lab setting and to determine its potential as a natural treatment for diabetes. The leaves of the plant were gathered, dried in the air, and then soaked in ethanol to create the extract. Tests for enzyme inhibition were performed, and the IC_{50} values were calculated to see how the extract's effectiveness compared to the standard medication acarbose. The outcomes showed that Vernonia amygdalina effectively inhibited both enzymes in a way that depended on the concentration used. The IC_{50} for blocking alpha-amylase was 0. 036 ± 0. 005 mg/mL, which is similar to acarbose (0. 031 ± 0. 005 mg/mL), indicating strong blocking ability. On the other hand, the extract showed a weaker effect against alpha-glucosidase, with an IC_{50} of 0. 122 ± 0. 05 mg/mL compared to 0. 081 ± 0. 005 mg/mL for acarbose. These results imply that Vernonia amygdalina could slow down the digestion and absorption of carbohydrates, thus helping to control blood sugar levels after meals. The findings support the traditional uses of Vernonia amygdalina for managing diabetes and emphasize its potential as a plant-based treatment option. Additional studies in living organisms and clinical research are suggested to confirm its effectiveness and safety in treating diabetes.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion, insulin action, or both (Mukhtar *et al*., 2021). In diabetes mellitus, the balance of how the body uses sugars and fats is changed because of problems with making or using insulin. It is a major non-communicable metabolic disease involving huge healthcare cost and high mortality rate (Jiang *et al*.,2020). The number of adults with diabetes was estimated to be 387 million, and diabetes alone caused 4.9 million deaths in the year 2014 (Mela *et al*., 2020). Postprandial hyperglycemia (PPHG) is a condition in which blood glucose level remains high after consuming meal (Jarvis *et al*., 2023), and it is an important factor to be considered in the management of diabetes mellitus and diabetes related secondary complications such as diabetic retinopathy, diabetic neuropathy, cardiovascular diseases, etc. Glycosidic linkages of α-D-(1,4) in carbohydrates are cleaved by α-amylase to produce oligosaccharides, which are further cleaved to monosaccharide glucose by α-glucosidase (Mukhtar *et al*., 2021). Therefore, inhibitors of these enzymes can delay the increase in blood glucose level in people who consume carbohydrate-rich food, and keep the PPHG under control (Mela *et al*., 2020). Acarbose, Miglitol, and Voglibose are medications that help manage postprandial hyperglycemia (PPHG) by blocking certain enzymes. Acarbose works against both α-amylase and α-glucosidase, while Miglitol and Voglibose specifically target only α-glucosidase (Khan et al., 2024). Although these medications are useful for controlling PPHG, they are not recommended for long-term use because they can cause stomach-related issues. Since around 80% of people with diabetes live in

countries with lower and middle incomes, these medications can also be costly. As a result, various research teams have been trying to discover natural inhibitors for α-amylase and α-glucosidase from sources like plants, bacteria, seaweed, and fungi. Consequently, there is growing interest in identifying safer and more effective natural inhibitors derived from plants. Presence of antidiabetic activity in Bitter leaf (*Vernonia amygdalina*), a widely consumed vegetable and medicinal plant in Africa, has attracted considerable attention for its therapeutic potential in managing diabetes (Ogwu and Ikhajiagbe, 2023). The plant is rich in bioactive compounds such as sesquiterpene lactones, flavonoids, alkaloids, saponins, and phenolic acids, many of which exhibit antioxidant, anti-inflammatory, and anti-diabetic properties (Maurya *et al*., 2021). studies have highlighted the ability of bitter leaf extracts to lower blood glucose levels and modulate oxidative stress markers. Despite these findings, the precise biochemical mechanisms underlying its anti-diabetic effects, particularly its inhibitory action on alpha-amylase and alpha-glucosidase, remain inadequately characterized.

1.2AIM OF STUDY

The aim of this study was to investigate the in vitro alpha-amylase and alpha-glucosidase inhibitory activity of *Vernonia amygdalina* and establish a possible mechanism of its potential as a natural therapeutic agent for diabetes management

1.3 OBJECTIVE OF STUDY

The specific objective of this study is

1. To determine the alpha amylase inhibitory activity of V.A
2. To determine the alpha glucosidase inhibitory activity of V.A
3. To determine the possible mechanisms of action of the antidiabetic property of V.A

CHAPTER TWO

LITERATURE REVIEW

2.1. DIABETES MELLITUS

Diabetes mellitus is one of most prevalent and significant metabolic disease that has a deleterious impact on the patients' quality of life (Garg and Duggal, 2022), productivity and involves enormous health costs for virtually every society. The World Health Organization (WHO; 2005) reports that the rising population may cause diabetes rates to increase from 4% in 1995 to 5. 4% by 2025. By 2030, it is predicted that there will be around 6 million individuals suffering from diabetes, and the figure for type 2 diabetes will keep growing in Africa (Arokiasamy et al., 2021). According to the International Diabetes Federation, over 371 million people worldwide were living with diabetes in 2012, with a rate of 8. 3%. Many of these individuals do not even realize they have diabetes. In poorer nations, one out of every twenty adult deaths is linked to diabetes, and Africa experiences the highest death rates from this disease. Diabetes can lead to disabilities, higher healthcare costs, a lower quality of life, and even death (Zhang et al., 2020). Nevertheless, most of these health issues can be avoided with proper self-care, as highlighted in Nigeria's standard treatment guidelines. Various factors complicate diabetes patients' understanding of their condition, medications, and self-management practices. Research indicates that illiterate patients are at a significant risk of not following medical advice due to difficulties in reading and comprehending basic health information. Each year, the worsening of diabetes has been connected to the lack of knowledge and proper self-care practices among patients. Consequently, poor knowledge of self-care can cause poor long-term metabolic control which may lead to the development of diabetic complications such as retinopathy, nephropathy, neuropathy, and atherosclerotic changes (Ishmael, 2022). The cost and affordability of drugs are compelling

problems since many of the anti-diabetic medications cost are high, and they are not readily available too. This according to Kalyango *et al*., (2024), has been considered as one of the major barriers to adherence, it has been minimized by provision of free drugs to patients when in the hospital. Evidence now shows that adjusting what we eat and how much we exercise can help stop or slow down diabetes and its related issues. Managing one's diet involves creating a personalized eating plan that takes into account the person's daily calorie needs, weight, age, physical activity, and past eating habits (Cummings et al. , 2022). Sticking to a daily eating schedule that has the right amount of carbohydrates (50-60%), protein (10-20%), fats (20-30%), less than 300mg of dietary cholesterol each day, and adjusting calories to reach and keep a slight weight loss is crucial for managing type 2 diabetes (Smeltzer et al. 2010). Diabetes mellitus, commonly called diabetes, includes a group of common hormonal disorders that result in consistently high blood sugar levels. This condition happens because the pancreas either does not produce enough insulin or the body's cells do not respond properly to insulin's effects (AlSuhaimi et al. , 2022). Common signs of diabetes are excessive thirst, frequent urination, weight reduction, and blurred vision. If not treated, it can cause serious health problems affecting the heart, eyes, kidneys, and nerves. Each year, around 4. 2 million deaths are tied to diabetes, with about 1. 5 million occurring due to untreated or poorly managed diabetes. The two main kinds of diabetes are type 1 and type 2. The usual treatment for type 1 is insulin therapy (insulin shots), while type 2 can be managed with anti-diabetic drugs (like metformin and semaglutide) along with lifestyle changes (Tegegne et al. , 2024). Gestational diabetes develops during pregnancy for some women but usually goes away soon after giving birth. As of 2021, about 537 million people globally had diabetes, making up 10. 5% of adults, with type 2 diabetes being about 90% of those cases. It is projected that by 2045, roughly 783 million adults, or 1 in 8, will

have diabetes, which would be a 46% increase from current estimates. The rate of diabetes is rising, especially in countries with lower and middle incomes (Abegunde et al. , 2007). Both men and women have similar rates of diabetes, which is the seventh leading cause of death worldwide. The annual cost of diabetes-related medical care is about 760 billion dollars. Key symptoms to be aware of include retinopathy, nephropathy, and neuropathy, which can arise from diabetes. Common signs of untreated diabetes include frequent urination, intense thirst, and weight loss. Other less specific symptoms may show up, like tiredness, blurry vision, and itching in the genital area caused by Candida infections (Maji et al. , 2023). Around half of those affected may not have any symptoms. Type 1 diabetes usually appears suddenly after a period without symptoms, while type 2 develops more gradually; some individuals may not show symptoms for many years. Diabetic ketoacidosis is a serious medical condition that commonly occurs in type 1 diabetes but can also happen in long-standing type 2 diabetes or when there's significant dysfunction of beta cells. This condition results from an excess of ketone bodies, causing symptoms such as nausea, vomiting, stomach pain, a fruity smell on the breath, rapid breathing referred to as Kussmaul breathing, and in severe instances, reduced awareness (Diggle, 2020). Hyperosmolar hyperglycemic state is another crisis that happens due to extreme high blood sugar levels causing dehydration, leading to high sodium levels which can change mental status and cause coma. Hypoglycemia is a known side effect of insulin treatment for diabetes. Immediate symptoms can range from mild ones like sweating, shaking, and heart palpitations to more severe effects like confusion, seizures, loss of consciousness, and, in rare cases, death (Yeager-Cordial et al. , 2022). Repeated hypoglycemic incidents can lower the threshold for when symptoms appear, meaning that mild symptoms may not be noticed until cognitive decline starts to occur.

Diabetes, also known as diabetes mellitus, is a group of common endocrine diseases characterized by sustained high blood sugar levels (Mukhtar *et al*., 2020). Diabetes occurs when the pancreas either doesn't make enough insulin or when the body's cells stop responding properly to this hormone. Common signs of diabetes are extreme thirst, frequent urination, losing weight, and blurred eyesight. If it is not treated, diabetes can result in serious health issues such as problems with the heart, vision, kidneys, and nerves. Every year, diabetes causes around 4. 2 million deaths, with about 1. 5 million of these due to diabetes that is either not treated or poorly managed (Mohajan and Mohajan, 2023). The main forms of diabetes are type 1 and type 2.

2.2 OVERVIEW OF THE MOST SIGNIFICANT SYMPTOMS OF DIABETES

2.2.1 Signs and symptoms

Figure 2.1: Overview of the symptoms of diabetes (Mukhtar *et al*., 2020).

Figure 2.2: Showing Retinopathy, nephropathy, and neuropathy as complications associated with diabetes.

Retinopathy, nephropathy, and neuropathy are potential complications of diabetes, Common symptoms of diabetes include increased thirst, frequent urination, extreme hunger, and unintended weight loss (Dwivedi *et al*., 2020). Various other general signs and indicators might also appear, such as tiredness, unclear eyesight, urine or semen smelling sweet, and itching in the genital area caused by a Candida infection. Around fifty percent of people who are affected might not show any symptoms. Type 1 shows up suddenly after a period without symptoms, whereas type 2 develops more slowly; patients can be without symptoms for many years.

Diabetic ketoacidosis is a medical emergency that occurs most commonly in type 1, but may also occur in type 2 if it has been longstanding or if the individual has significant β-cell dysfunction (Newton and Raskin, 2024). Excessive creation of ketone bodies results in various signs and symptoms, which can include feeling sick, throwing up, stomach pain, a fruity smell like acetone in the breath, heavy breathing called Kussmaul breathing, and in serious situations, a lower awareness level. Hyperosmolar hyperglycemic state is another emergency characterized by dehydration secondary to severe hyperglycemia, with resultant hypernatremia leading to an altered mental state and possibly coma (Alghamdi *et al*., 2021).

Low blood sugar is a known problem that can occur with insulin therapy for diabetes. A sudden onset can show light signs like sweating, shaking, and feeling your heart race, as well as more severe reactions such as trouble thinking, confusion, seizures, being unconscious, and in very rare cases, even death. Recurrent hypoglycemic episodes may lower the glycemic threshold at which symptoms occur, meaning mild symptoms may not appear before cognitive deterioration begins to occur (Orłowska *et al*., 2024).

2.2.2 Complications of diabetes

The main long-term issues caused by diabetes are related to harm done to blood vessels, which can be seen on both large and small scales. Having diabetes increases the chance of getting heart disease by two times, and around 75% of deaths among diabetics happen because of heart artery problems. Other big issues related to blood vessels include strokes and problems with arteries in the limbs. On a smaller scale, diabetes can lead to complications in the eyes, kidneys, and nerves. Damage to the retina, called diabetic retinopathy, is the leading reason for blindness in people

who are of working age. Additionally, the eyes can suffer in other ways, such as developing cataracts or glaucoma. It is advised that diabetics see an eye doctor every year.

Diabetic nephropathy is a significant factor in long-term kidney issues, making up more than half of those needing dialysis in the U. S. Diabetic neuropathy, which affects the nerves, appears in different forms, including loss of feeling, nerve pain, and problems with automatic body functions (like low blood pressure when standing, diarrhea, and issues with sexual function). The decrease in pain feeling makes individuals more vulnerable to injuries that can cause diabetic foot complications (like sores), which is the leading reason for non-injury-related amputations of the lower limbs (Trikta, 2024).

Hearing issues are another lasting problem that comes with diabetes. From a lot of research and many examples of gallstone issues, it seems there might be a connection between type 2 diabetes and gallstones. Individuals who have diabetes face a greater chance of getting gallstones than those who do not have diabetes. There is a relationship between mental sharpness and diabetes; research has indicated that people with diabetes are more likely to experience memory problems and that their decline happens faster than for those without the condition (McCrimmon et al., 2024). This illness also increases the likelihood of falls among older adults, particularly for those who are on insulin treatment.

2.3 THE VARIOUS TYPES OF DIABETES

Table 2.1

Comparison of type 1 and 2 diabetes

Feature	Type 1 diabetes	Type 2 diabetes
Onset	Sudden	Gradual
Age at onset	Any age; average age at diagnosis being 24.	Mostly in adults
Body size	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually, present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Heritability	0.69 to 0.88	0.47 to 0.77
Prevalence (age standardized)	<2 per 1,000	~6% (men), ~5% (women)

Diabetes is divided by the World Health Organization into six different types: type 1 diabetes, type 2 diabetes, mixed types of diabetes (which consist of slowly progressing, immune-related diabetes in adults and ketosis-prone type 2 diabetes), high blood sugar found for the first time during pregnancy, "other specific kinds," and "unclassified diabetes. " Diabetes shows more variety than previously believed, and people can experience a mix of different types.

2.3.1 Type 1

Type 1 accounts for 5 to 10% of diabetes cases and is the most common type diagnosed in patients under 20 years; however, the older term "juvenile-onset diabetes" is no longer used as

onset in adulthood is not unusual (Gregory *et al*., 2022). The illness is marked by the destruction of the beta cells in the pancreatic islets that produce insulin, resulting in a significant lack of insulin. It can be divided into two types: immune-mediated, which is when the body's immune system attacks itself, and idiopathic, which means the cause is unknown. Most instances fall under the immune-mediated category, where an autoimmune response led by T cells results in the destruction of beta cells and subsequently a shortage of insulin. As a result, patients frequently experience unstable and erratic blood sugar levels due to the extremely low amount of insulin and a weakened reaction to low blood sugar situations (Thorp et al., 2023).

Figure 2.3: Autoimmune attack in type 1 diabetes (Awuchi, 2022).

Type 1 diabetes runs in families and is influenced by several genes, including certain HLA types that can increase the chance of getting diabetes. For those who are genetically at risk, the disease might start due to environmental elements like a viral infection or diet (Houeiss et al. , 2022). While many viruses have been linked to this issue, there hasn’t been strong proof to back this

idea for humans so far. Type 1 diabetes can be diagnosed at any age, with many adults receiving this diagnosis. The term latent autoimmune diabetes of adults (LADA) is used when type 1 diabetes appears in adults; it typically develops more slowly than in children. Because of this slower progression, some people refer to it informally as "type 1. 5 diabetes. " Adults with LADA are often wrongly diagnosed as having type 2 diabetes because of their age rather than the underlying cause. LADA leaves adults with higher levels of insulin production than type 1 diabetes, but not enough insulin production for healthy blood sugar levels (Awuchi, 2022).

2.3.2 Type 2

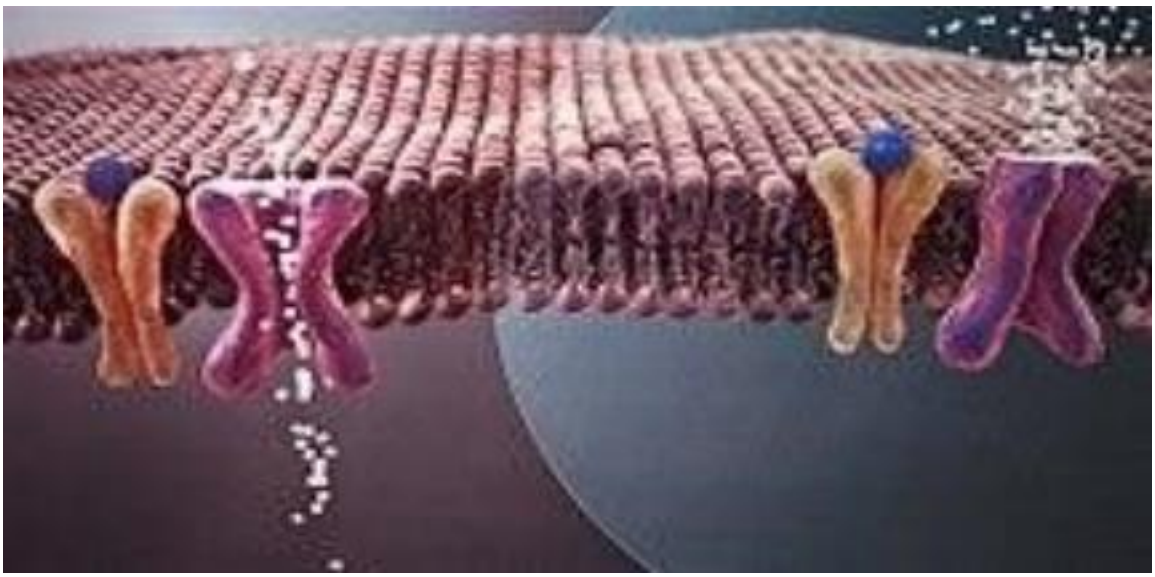

Figure 2.4: Effects of insulin on type diabetes (Awuchi, 2022).

Decreased production of insulin or a weaker response of insulin to its receptor results in elevated blood sugar levels. Type 2 diabetes is marked by insulin resistance, often paired with a somewhat lower insulin production (Goldstein, 2024). The failure of body tissues to properly respond to insulin is thought to involve issues with the insulin receptor, although the exact problems are still

unknown. Cases of diabetes mellitus caused by a specific issue are categorized separately. Type 2 diabetes is the most prevalent form of diabetes mellitus, making up 95% of all diabetes cases (DeFronzo, 2024). Many individuals with type 2 diabetes show signs of prediabetes (such as impaired fasting glucose and/or impaired glucose tolerance) before officially being diagnosed with type 2 diabetes. By making lifestyle adjustments or taking medications that enhance insulin sensitivity or lower the liver's glucose production, the shift from prediabetes to full-blown type 2 diabetes can be delayed or even reversed. Type 2 diabetes primarily arises from both lifestyle choices and genetic factors. Various lifestyle aspects significantly contribute to developing type 2 diabetes, such as obesity (which is defined as having a body mass index over 30), insufficient physical activity, unhealthy eating patterns like the Western Pattern Diet, stress, and living in urban areas (Wu, 2024). Excess body weight is linked to 30% of cases in individuals of Chinese and Japanese heritage, 60-80% in those of European and African descent, and is present in 100% of Pima Indians and Pacific Islanders. Even individuals who are not classified as obese can have a high waist-to-hip ratio. Consuming sugary drinks is linked to a higher risk of developing diabetes. The type of dietary fats also plays a role (Micha et al. , 2020), with saturated and trans fats increasing the risk while polyunsaturated and monounsaturated fats reduce it. Eating too much white rice may heighten the risk of diabetes, particularly among Chinese and Japanese individuals. Insufficient physical activity might elevate diabetes risk for some people. Negative experiences in childhood, such as abuse, neglect, and family troubles, can raise the chances of developing type 2 diabetes in later life by 32%, with neglect having the greatest impact (Huang et al. , 2022). Side effects from antipsychotic medications, particularly metabolic issues, abnormal lipid levels, and weight gain, can also be considered potential risk factors.

2.3.3 Gestational diabetes

Gestational diabetes has similarities to type 2 diabetes in that it involves not enough insulin being produced and the body not reacting well to it. It happens in around 2–10% of pregnancies and might get better or go away after giving birth (Dube et al. , 2021). It is advised that all expecting mothers undergo tests starting from about 24–28 weeks into their pregnancy. Most cases are found during the second or third trimester because that is when hormone levels that block insulin rise. However, after having a baby, around 5–10% of women who had gestational diabetes are discovered to have a different kind of diabetes, usually type 2 (Kim et al. , 2022). Gestational diabetes can be fully managed, but it needs close medical attention all through the pregnancy. Treatment might involve changing diet, monitoring blood sugar levels, and sometimes, using insulin. While it can go away, if gestational diabetes is not managed, it can harm the health of both the mother and the baby. Potential issues for the baby include being larger than average at birth, heart and brain defects, and issues with muscle development. High insulin levels in the baby's blood can reduce production of lung fluid and lead to breathing problems after birth. A rise in bilirubin levels might happen because of the breakdown of red blood cells (Lin et al. , 2023). In serious cases, the baby might not survive, often due to insufficient blood flow from the placenta caused by blood vessel issues. It may be necessary to induce labor if the placenta is not working well. A c-section might be needed if the baby is in distress or if there's a greater chance of injury due to being larger than normal, like in cases of shoulder delivery problems.

The following is a list of disorders that may increase the risk of diabetes:

1. Genetic defects of β-cell function
    i. Maturity onset diabetes of the young
    ii. Mitochondrial DNA mutations
2. Genetic defects in insulin processing or insulin action
    i. Defects in proinsulin conversion
    ii. Insulin gene mutations
    iii. Insulin receptor mutations
3. Exocrine pancreatic defects
    i. Chronic pancreatitis
    ii. Pancreatectomy
    iii. Pancreatic neoplasia
    iv. Cystic fibrosis
    v. Hemochromatosis
    vi. Fibrocalculous pancreatopathy
4. Endocrinopathies
    i. Growth hormone excess (acromegaly)
    ii. Cushing syndrome
    iii. Hyperthyroidism
    iv. Hypothyroidism
    v. Pheochromocytoma
    vi. Glucagonoma

5. Infections
    i. Cytomegalovirus infection
    ii. Coxsackievirus B

6. Drugs
    i. Glucocorticoids
    ii. Thyroid hormone
    iii. β-adrenergic agonists
    iv. Statins

2.3.4 Pathophysiology

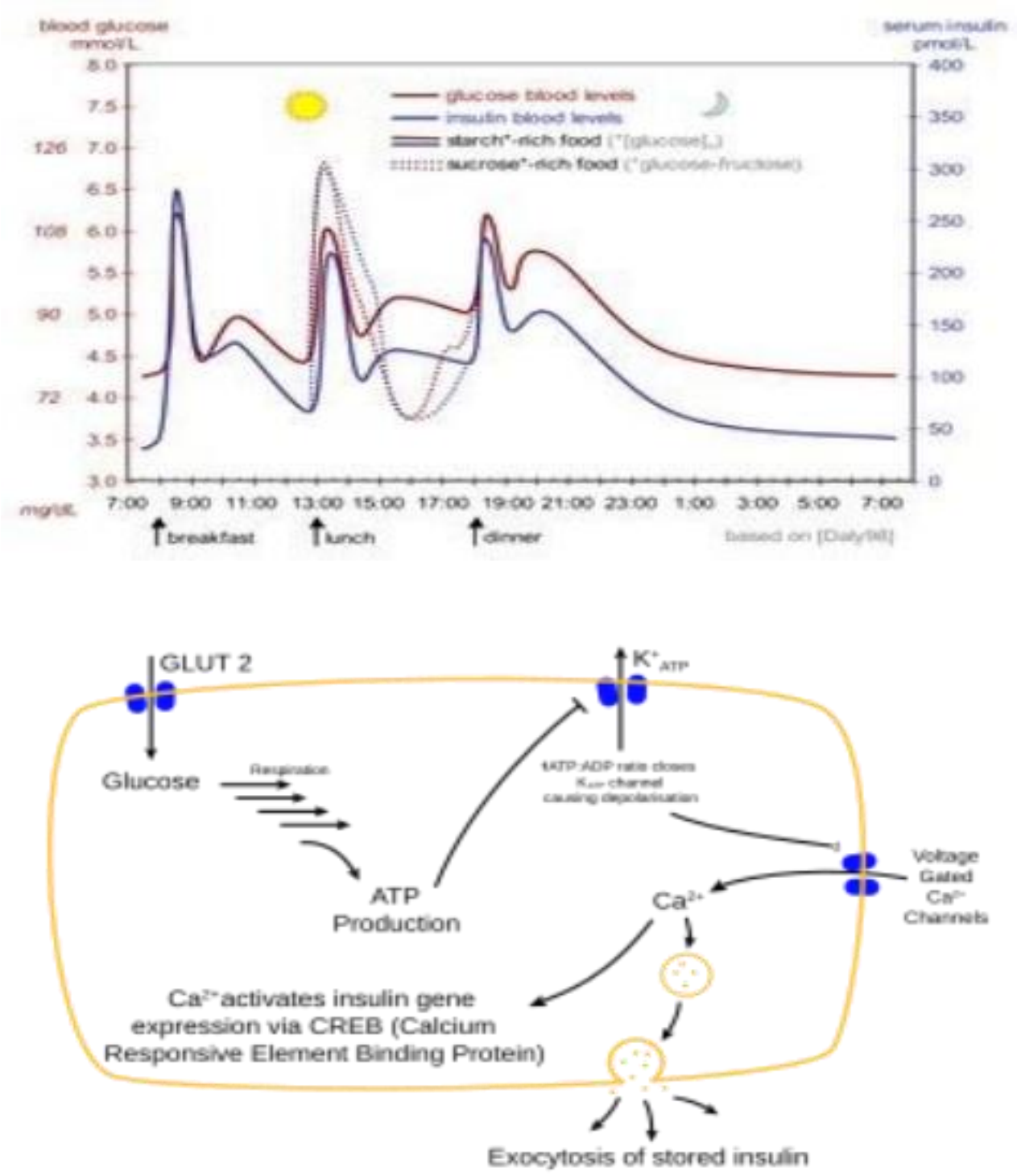

Figure 2.5: Pathophysiology of a diabetic patient (Dimitriadis *et al*., 2021).

The changes in blood sugar levels (red) and the hormone insulin that helps lower sugar (blue) in people throughout a day with three meals are shown. One impact of eating a meal high in sugar compared to one high in starch is emphasized. The process of how insulin is released from healthy pancreatic beta cells is explained. The creation of insulin stays fairly steady in the beta cells. Its release is activated by eating, especially foods that have glucose that can be absorbed (Mann et al., 2020).

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, adipose tissue and muscle, except smooth muscle, in which insulin acts via the IGF-1 (Dimitriadis *et al*., 2021). As a result, a lack of insulin or the unresponsiveness of its receptors is crucial in all types of diabetes mellitus.

The body obtains glucose from three main sources: the intestinal absorption of food; the breakdown of glycogen (glycogenolysis), the storage form of glucose found in the liver; and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body (Dube *et al*., 2021). Insulin plays a critical role in regulating glucose levels in the body. Insulin can stop the process that breaks down glycogen and the formation of new glucose. It helps move glucose into fat and muscle cells, and it encourages the storage of glucose as glycogen.

Insulin is produced and released into the bloodstream by beta cells, which are located in the islets of Langerhans within the pancreas. This release happens when blood sugar levels rise, usually after eating. Around two-thirds of the body's cells use insulin to take in glucose from the blood, which can then be used for energy, converted into other necessary substances, or stored for later (Norton et al., 2022). When blood sugar levels drop, the beta cells release less insulin and start breaking down glycogen into glucose. The hormone glucagon mainly regulates this process,

acting in opposition to insulin. If there's not enough insulin available, if cells don’t react well to insulin (insulin resistance), or if the insulin itself isn't functioning right, the body cells that need glucose don’t absorb it properly, leading to inadequate storage in the liver and muscles. This situation causes consistently high blood sugar levels, poor protein production, and various metabolic issues, such as metabolic acidosis when there is a complete lack of insulin. When blood has too much glucose for an extended period, the kidneys can't absorb all of it and some gets excreted in the urine (glycosuria). This causes the urine to have higher osmotic pressure, preventing the kidneys from reabsorbing water, which results in increased urine output (polyuria) and a higher loss of fluids (Rahman et al., 2021). The body compensates for the loss of blood volume by drawing water from body cells and other areas, causing dehydration and increased thirst (polydipsia). Additionally, not having enough glucose inside cells triggers hunger, leading to overeating (polyphagia).

2.3.5 Diagnosis

Glycated hemoglobin and Glucose tolerance test

Diabetes mellitus is identified through a test that measures the sugar levels in the blood, and it is confirmed by showing at least one of the following:

- Blood sugar level in the blood after fasting is equal to or more than 7. 0 mmol/L (126 mg/dL). For this examination, blood is drawn after not eating for a while, which means in the morning before having breakfast, following a night of fasting or a minimum of 8 hours prior to the examination.

- Plasma sugar levels that are 11. 1 mmol/L (200 mg/dL) or higher, measured two hours after consuming a 75-gram oral glucose load during a glucose tolerance test (OGTT).
- Signs of elevated blood sugar and plasma glucose levels at or above 11. 1 mmol/L (200 mg/dL) whether during fasting or not.
- Glycated hemoglobin (HbA_{1C}) ≥ 48 mmol/mol (≥ 6.5 DCCT

Table 2.2

WHO diabetes diagnostic criteria

Condition	2-hour glucose		Fasting glucose		HbA_{1c}	
Unit	*mmol/L*	*mg/dL*	*mmol/L*	*mg/dL*	*mmol/mol*	*DCCT %*
Normal	< 7.8	< 140	< 6.1	< 110	< 42	< 6.0
Impaired fasting glycaemia	< 7.8	< 140	6.1–7.0	110–125	42–46	6.0–6.4
Impaired glucose tolerance	≥ 7.8	≥ 140	< 7.0	< 126	42–46	6.0–6.4
Diabetes mellitus	≥ 11.1	≥ 200	≥ 7.0	≥ 126	≥ 48	≥ 6.5

A good result, without clear signs of high blood sugar, should be checked again using any of the earlier mentioned methods on a different day. It is better to check fasting glucose levels because it is easier to do, and formal glucose tolerance tests, which take two hours, do not provide any additional benefit over the fasting test. As per current guidelines, two fasting glucose tests showing 7. 0 mmol/L (126 mg/dL) or higher are indicative of diabetes mellitus. The World Health Organization states that fasting glucose levels between 6. 1 and 6. 9 mmol/L (110 to 125 mg/dL) indicate impaired fasting glucose. Individuals who have plasma glucose levels at or above 7. 8 mmol/L (140 mg/dL) but below 11. 1 mmol/L (200 mg/dL) two hours after

consuming a 75-gram oral glucose load are classified as having impaired glucose tolerance. Among these two prediabetic conditions, the latter is particularly a significant risk factor for developing full diabetes mellitus and cardiovascular disease. Since 2003, the American Diabetes Association has defined impaired fasting glucose as a range of 5. 6 to 6. 9 mmol/L (100 to 125 mg/dL). Measuring glycated hemoglobin is more effective than checking fasting glucose for assessing risks of cardiovascular disease and mortality from any reason.

2.3.6 Prevention

There is no known preventive measure for type 1 diabetes. However, islet autoimmunity and multiple antibodies can be a strong predictor of the onset of type 1 diabetes (Primavera *et al*., 2020).

Type 2 diabetes, which makes up about 85 to 90 percent of all diabetes cases globally, can usually be avoided or postponed. This can be achieved by keeping a healthy weight, being active, and consuming nutritious foods. Engaging in more than an hour and a half of physical activity each day can lower the chance of getting diabetes by 28 percent. Effective dietary adjustments that can help in diabetes prevention include eating plenty of whole grains and fiber and selecting healthy fats, like the polyunsaturated fats found in nuts, vegetable oils, and fish. Reducing the intake of sugary drinks and decreasing the consumption of red meat and other foods high in saturated fat can also aid in preventing diabetes. Smoking tobacco is linked to a higher risk of developing diabetes and related issues, so quitting smoking is an important step in prevention. The connection between type 2 diabetes and major risk factors that can be changed, such as being overweight, unhealthy eating, lack of exercise, and smoking, is consistent across different

parts of the world. There is increasing evidence that the root causes of diabetes are influenced by significant factors driving social, economic, and cultural changes, including globalization, urban growth, an aging population, and overall health policy (Hill-Briggs et al., 2020).

A systematic review by Cochrane in 2020 looked at different non-caloric sweeteners alongside sugar, a dummy treatment, and a low-calorie sweetener known as tagatose. However, the findings regarding their impact on HbA1c, weight gain, and negative side effects were not clear. The research considered in the review generally had low reliability and failed to provide information on aspects like quality of life related to health, complications from diabetes, overall death rates, or economic impacts.

2.3.7 Treatment of type 2 diabetes

Type 1 diabetes treatments often involve using a mix of standard or NPH insulin and/or man-made insulin types. For managing type 2 diabetes, patients are generally given pills. The types of oral medications consist of sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, and meglitinides.

- Sulfonylureas Drugs: Tolbutamide and chlorpropamide belong to the first group of medications. The second group includes drugs such as glibenclamide, glipizide, gliclazide, and glimepiride, among others.

Side Effects: low blood sugar, an upset stomach, skin rash or itching, Weight gain.

- Biguanides Drugs: Metformin

Side Effects: Abdominal pain, Nausea, Metallic taste, Mild diarrhea, Anorexia

- Alpha- glycosidase inhibitors: Drugs Acarbose and miglitol

Side Effects: may cause stomach problems such as gas, bloating and diarrhea.

2.4 PLANTS USED IN THE TREATMENT OF DIABETES

Vegetation has been crucial in managing diabetes for a long time, as they have fewer side effects when set against conventional medicines. Lately, many individuals have shifted back to using natural remedies because of their cost, availability, and the adverse reactions associated with standard medications. The biochemical properties of medicinal plants offer promising alternatives for diabetes management. Their active compounds work through multiple mechanisms, including insulin secretion, enzyme inhibition, glucose uptake enhancement, and antioxidative effects. Traditional medicine has long been an essential alternative to modern pharmaceutical treatments. As per the World Health Organization (WHO), traditional medicine that comes from plants is still the main healthcare choice for about 75–80% of people worldwide, especially in developing nations that have a lot of different plants and animals. Many people in these areas prefer traditional medicine because it is a big part of their culture, tends to work well with the human body, and usually has fewer side effects than standard medical treatments.

In recent years, numerous medicinal plants have been recognized for their potential in managing diabetes. Many of these plants have been traditionally used as natural remedies for their antidiabetic and antihyperlipidemic properties. Research has documented over 400 plant species with hypoglycemic effects, yet the search for new antidiabetic compounds from natural sources continues to be of great interest. These plants contain various bioactive compounds, including phenolics, glycosides, alkaloids, terpenoids, flavonoids, and carotenoids, which have demonstrated promising antidiabetic properties. Some of the notable plants used in diabetes management include:

2.5 BITTER LEAF (*VERNONIA AMYGDALINA*)

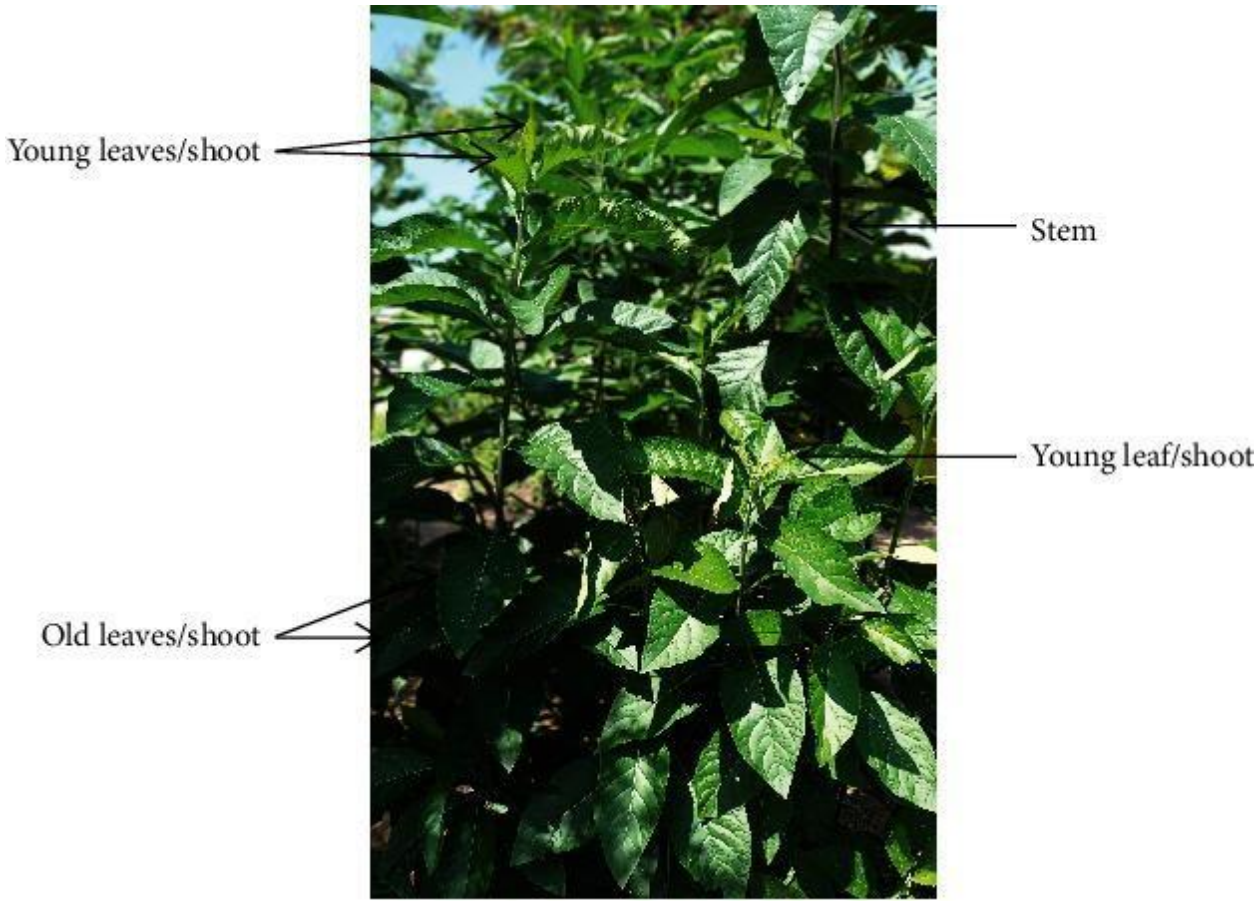

Figure 2.6: *Vernonia amygdalina* plant showing leaves and stem (Konyeme and Ukpene, 2024).

2.5.1 Description of Plant and Its Traditional Use

Vernonia amygdalina, which people often call bitter leaf because of its unpleasant flavor, is a small bush that can reach heights of 3 to 7 meters. This plant is primarily found in tropical regions of Africa but can also be seen in some Asian countries (Dare, 2022). Additional names of this plant and its classification details can be found in Tables 3 and 4. The leaves are a medium to dark green color and have a pointed tip with a narrowing base. They typically measure about 10 to 15 cm in length and 4 to 5 cm in width. The edges of the leaves are smooth, and their shape

ranges from lance-like to oval. Traditionally, the leaves of VA (figure 3) are often used to boost appetite as well as being cooked as a vegetable in various stews and soups. It is normally washed with clean water to reduce the bitterness before use in food preparation in West Africa. (Ogwu, and Ikhajiagbe, 2023).

Table 2.3

Other names of *Vernonia amygdalina*.

Country	**Vernacular names**
Common name	Bitter leaf; toothbrush tree
Ghana	Awonwono
Nigeria	Onugbu
Cameroon	Muop; ndole
Uganda	Mululuza
Tanzania	Tuntwano
Rwanda	Umubilizi
Eritrea	Grava
Malaysia	Pokok bismillah

Table 2.4

Scientific classification.

Kingdom	**Plantae**
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledoneae
Order	Asterales
Family	Asteraceae
Genus	*Vernonia*
Species	*Vernonia amygdalina*

Extracts from the roots and leaves are also utilized in traditional methods to treat various illnesses, including malaria and diabetes. They are known for their anticancer, antimicrobial, antileishmanial, antifertility, anti-inflammatory, antipyretic, pain-relieving, appetite-stimulating, laxative, oxytocin-producing, and wound-healing properties. Among these traditional uses, the effectiveness of these extracts as a treatment for diabetes is particularly well recognized. The ability of VA to help manage diabetes has been tested and validated in many research studies using different types of extraction methods. Because VA is readily available and commonly used in cooking, it offers a cost-effective option for diabetes treatment. However, the potential risks associated with excessive doses of these extracts are a significant barrier to their widespread use in local populations.

2.5.2 Therapeutic Effects of *Vernonia amygdalina*

There are several effects of *Vernonia amygdalina* and they are

i. Hypoglycemic Effect

The leaves of VA have been traditionally used for treating diabetes. Multiple studies with experimental models have shown that VA extracts can significantly reduce blood sugar levels. In a recent experiment, an 80% methanol extract of VA was separated using increasingly polar solvents (n-hexane, chloroform, ethyl acetate, n-butanol, and water). Nine different components (C1-C9) from the chloroform fraction were tested for their ability to lower blood sugar in Wistar Albino rats weighing between 150-200 grams. Rats that had non-fasting blood sugar levels above 300 mg/dl after being made diabetic through a special diet and streptozotocin treatment were considered to have type 2 diabetes and included in this study. The components were purified and given to the rats by mouth at a dosage of 10 mg/kg body weight. After treating the diabetic rats with these components, component 5 (C5) showed the strongest blood sugar-lowering effect of 12. 55%, compared to 18. 07% in rats treated with metformin. A spectroscopic analysis indicated that C5 is 11β,13-dihydrovernolide. All these findings consistently highlight the strong blood sugar-reducing potential of VA, suggesting that it could be further developed for use in treating diabetes.

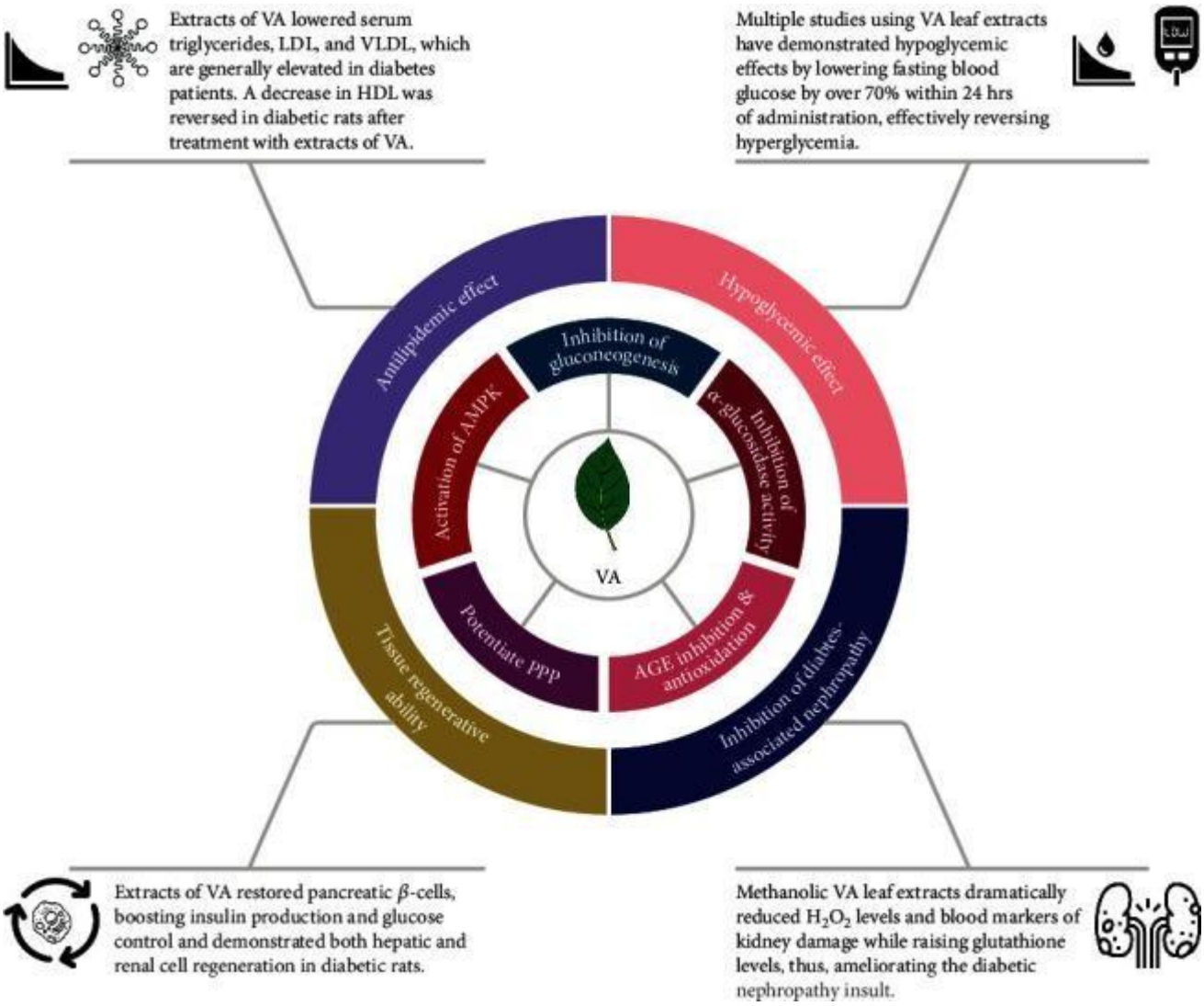

Figure 2.7: Summary of the therapeutic potential of *Vernonia amygdalina* (VA) in diabetes and its associated complications.

ii. Antilipidemic Effect in Diabetes-Associated Dyslipidemia

Diabetic dyslipidemia refers to the disruption in the levels of serum lipids, i.e., low high-density lipoprotein cholesterol (HDL-C), elevated low-density lipoprotein cholesterol (LDL-C), increased VLDL-triglycerides, and the prevalence of small dense LDL (Eraga *et al*., 2022). This represents a significant risk factor for heart problems. Nwanjo explored how water extracts from

VA impact lipid levels in diabetic adult Wistar Albino rats induced by streptozotocin. The diabetic rats received a daily dose of 200 mg/kg of the water extract of VA two times a day for a period of 14 days. After examination, it was found that serum triglycerides and LDL levels were notably reduced in both normal and treated rats in comparison to the diabetic control group. Asante and colleagues conducted another study that showed VA's ability to lower lipid levels. Diabetic rats that were induced by STZ were given ethanolic extracts from young and old VA leaves in dosages of 10, 30, and 300 mg/kg b. w. Clear signs of lipid abnormalities related to diabetes were seen in the untreated control rats. Conversely, the treated rats showed a notable rise in HDL-C levels, often referred to as good cholesterol, which can help lower the risk of heart diseases (Bello et al., 2024). There was also a marked reduction in both LDL-C and VLDL-C levels. Another study reported similar findings, highlighting VA's antlipid properties. In an additional study, treatment for 28 days with the chloroform extract of VA brought back normal levels of serum total cholesterol, triacylglycerol, LDL-C, and the ratio of total cholesterol/HDL-C, which were initially elevated. All these research efforts further confirm VA's potential to combat lipid issues related to diabetes (Konyeme and Ukpene, 2024).

2.5.3 Molecular Mechanisms of *Vernonia amygdalina* as an Antidiabetic Agent

i. Inhibition of *α*-Glucosidase Activity

Alpha-glucosidase is a type of enzyme that helps break down complex carbohydrates into simple sugars, such as glucose. This process results in higher blood sugar levels after meals. Because of how it works, this enzyme is a key target for diabetes treatments. Inhibitors of alpha-glucosidase are very important for this purpose. Researchers looked into the roots and leaves of VA, using different methods of extraction (including water, Soxhlet, and ethanol), to see how they might reduce the activity of alpha-glucosidase (Figure 2. 4) in a lab setting (Konyeme and Ukpene,

2024). Various extracts were made at different strengths of 5, 10, 50, and 100 µg/ml and mixed with α-glucosidase (taken from Saccharomyces cerevisiae) at a concentration of 0. 35 U/ml in 0. 1 M PBS (pH 6. 8) for 10 minutes at 37°C, along with 4 mM p-nitrophenylα-D-glucopyranoside (p-NPG). After measuring the absorbance at 405 nm for 45 minutes, the aqueous and Soxhlet extracts from the roots showed the strongest ability to inhibit the enzyme, with an IC50 of 5. 6 µg/ml and 39. 8 µg/ml, respectively. Using 5 µg/ml of the aqueous root extract led to a 48% reduction in the enzyme's activity and a complete 100% reduction at 100 µg/ml, indicating strong inhibition. The aqueous leaf extract also showed inhibitory effects that depended on the concentration (Dare, 2022). In a more recent study, the bioactive compound luteolin found in VA effectively decreased α-glucosidase activity in a way that depended on its concentration (5, 10, 25, 40, and 50 µM). Likewise, an infusion of VA leaves at concentrations of 15, 30, 60, 120, and 240 µg/ml significantly reduced α-glucosidase activity in a dose-dependent manner, suggesting that VA has properties that can help with diabetes. The findings from these studies consistently demonstrate that both the root and leaf extracts of VA exhibit strong anti-alpha-glucosidase activity, supporting their potential as a diabetes treatment.

ii. Inhibition of α-Amylase activity

α-Amylase is an important enzyme involved in digestion that helps break down starch from food into smaller sugar units known as oligosaccharides and disaccharides. Blocking this enzyme slows down the process of breaking down carbohydrates and lowers blood sugar levels after meals, which is crucial for controlling type 2 diabetes. Vernonia amygdalina, commonly known as bitter leaf, contains many beneficial substances like phenolics, flavonoids, and sesquiterpene lactones. Studies have shown that extracts, particularly those obtained with ethyl acetate, possess significant α-amylase inhibitory activity. For example, in vitro assays report IC_{50} values as low as

3.0 μg/mL, which in some cases compare favorably to standard inhibitors like acarbose. Advanced analytical techniques including LC-HRMS have been used to identify several unique compounds within the active extracts. Among these, one compound (referred to as compound 3) demonstrated robust interaction with α-amylase. Molecular docking studies reveal that compound 3 binds competitively to the enzyme's active site—interacting with key catalytic residues such as Asp197, Glu233, and Asp300. It showed an interaction energy of –8.59 kcal/mol and an inhibition constant (Ki) of 0.503 μM, suggesting that its binding disrupts the normal catalytic mechanism of the enzyme Kinetic analyses indicate that the inhibition of α-amylase by V. amygdalina's bioactive compounds is predominantly competitive. This means that the inhibitors directly occupy the active site, thereby preventing starch from binding. Such a mode of inhibition is advantageous because it allows for a controlled reduction in starch digestion, potentially minimizing adverse gastrointestinal effects seen with stronger synthetic inhibitors. The antidiabetic action of Vernonia amygdalina, through the inhibition of α-amylase, is a multifactorial process. The plant's rich phytochemical content acts synergistically to bind and inhibit the enzyme's active site. These findings not only support its traditional use in diabetes management but also highlight its potential as a source for developing safer, plant-based antidiabetic therapeutics.

iii. Advanced Glycation End Product Inhibition and Antioxidation

The creation of advanced glycation end products, known as AGEs, in the complications linked to diabetes is associated with high blood sugar and oxidative stress (Dare, 2022). Other studies have shown that when AGEs build up, they lead to the generation of reactive oxygen species, or ROS, which causes stress related to glycation in diabetes. Because of this, it is better to prevent complications from lifestyle illnesses like diabetes by blocking the production of AGEs using

natural foods we eat (Konyeme and Ukpene, 2024). In line with this, a study found impressive outcomes from stopping the glycation of BSA caused by ribose when using water, Soxhlet, and alcohol extracts from the roots and leaves of VA after observing the glycation process for five days.

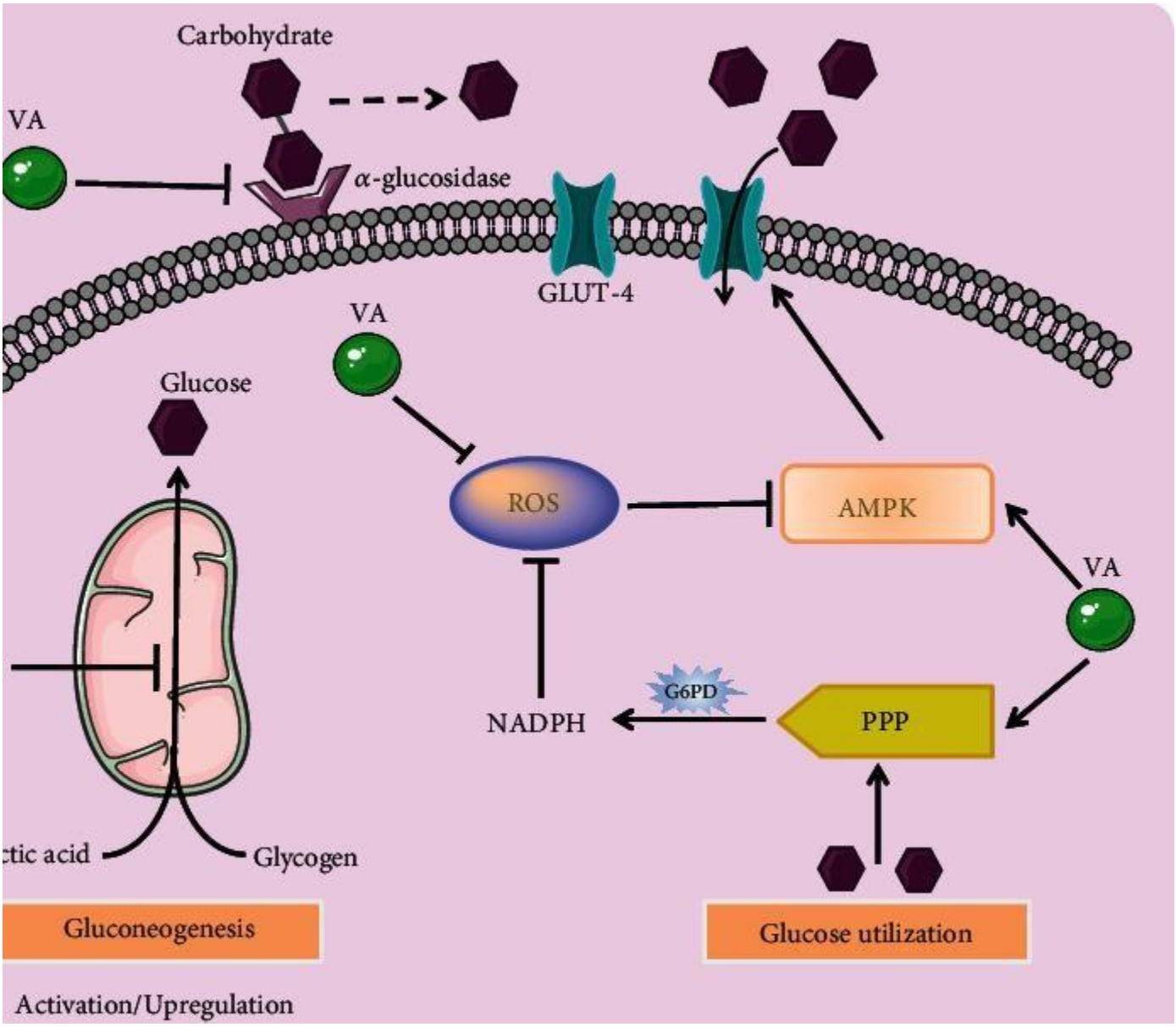

Figure 2.8: Molecular pathways for the antidiabetic activity of *Vernonia amygdalina* (VA). This figure highlights the various mechanistic pathways by which VA exerts its antidiabetic effect.

2.6 CURRENT MANAGEMENT STRATEGIES OF DIABETES

Managing diabetes focuses on maintaining blood sugar levels as close to normal as possible without causing them to drop too low. This is typically achieved through changes in diet, exercising more, losing weight, and taking the right medications, such as insulin or pills. Understanding the condition and being actively involved in treatment is crucial because people who keep their blood sugar levels in check tend to experience fewer and less serious complications (Houeiss et al. , 2022). The target for treatment is to reach an A1C level under 7%. Other health issues that can worsen diabetes are also taken into account. These include smoking, high blood pressure, metabolic syndrome, being overweight, and not getting enough exercise. Special shoes are commonly used to lower the chance of diabetic foot sores by easing the pressure on the feet. Diabetic patients should have their feet checked once a year, which involves testing for feeling, examining foot mechanics, checking blood flow, and looking at foot shape (Newton and Raskin, 2024). For individuals with severe mental health conditions, the effectiveness of self-management programs for type 2 diabetes has not been studied enough, and there is not enough reliable evidence to indicate whether these programs yield the same results as those seen in the broader population.

Individuals diagnosed with diabetes can gain from learning about the illness, treatment options, making changes to their diet, and incorporating exercise. These actions help maintain both short-term and long-term blood sugar levels in a healthy range. Moreover, since there is a greater

chance of heart-related issues, it is advised to make lifestyle changes to manage blood pressure (Cummings et al. , 2022). Losing weight may stop the shift from prediabetes to type 2 diabetes, lower the chances of heart disease, or even lead to a partial improvement in those already with diabetes. There isn't one specific eating plan that fits everyone with diabetes. Healthy eating styles like the Mediterranean diet, low-carb diet, or DASH diet are frequently suggested, but there's no strong evidence showing that one is better than the others (Cummings et al., 2022). The American Diabetes Association states that "lowering total carbohydrate intake for those with diabetes has shown the strongest evidence for enhancing blood sugar levels," and for those with type 2 diabetes who struggle to meet blood sugar goals or wish to cut back on diabetes medications, low or very low carbohydrate diets can be an effective option. For people with type 2 diabetes who are overweight, any eating plan that leads to weight loss works well.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and Reagents

All laboratory equipment was obtained from accredited suppliers and rigorously checked to meet experimental standards. Essential items for sample handling and processing included basic consumables such as masking tape, test tube holders, disposable tissues, gloves, and face masks to ensure a clean and safe laboratory environment. The study also utilized a range of chemicals and reagents, including distilled water and ethanol as solvents; dinitrosalicylic acid (DNSA), phosphate buffer, sulfuric acid, sodium phosphate, and acetonitrile were used to stabilize the samples and facilitate specific chemical reactions.

3.1.2 Equipment

The Vernonia amygdalina leaves were collected from a local market located at Uselu, Benin city Nigeria and managed using aluminum foil, Pyrex conical flasks, and universal containers to safeguard sample integrity. Additionally, beakers (500 mL and 1000 mL), a glass stirring rod, and a handkerchief were used during sample preparation and mixing, while a micropipette ensured precise liquid measurements. For analytical measurements, high-quality instruments were employed. A pH meter from Sigma (Germany) was used to determine the pH of the samples, which is crucial for enzyme activity assays, and a UV-Visible spectrophotometer (also from Sigma) measured light absorption to quantify compound concentrations. A centrifuge from Sigma enabled the separation of sample components by density, and an oven provided controlled heating and drying conditions necessary for sample processing prior to analysis.

3.2 METHODS

3.2.1 Plant Collection and Identification

New leaves of Vernonia amygdalina were bought from nearby shops in Uselu, a place in Benin City, Edo State, Nigeria. The leaves were carefully washed with distilled water to get rid of any dirt and unwanted substances, and then they were allowed to air dry at room temperature to remove extra moisture and avoid any contamination. After they were fully dried, the leaves were processed using a machine to grind them into a fine powder, achieving the best particle size for effective extraction of beneficial compounds.

Figure 3:1: *Vernonia amygdalina* being dried (Source: personal)

3.2.2 Extraction

The powdered Vernonia amygdalina was subjected to ethanol extraction. A measured portion of the powder was placed in a clean 1000 mL beaker, and sufficient ethanol was added to fully submerge the material. The container was then sealed with aluminum foil to minimize

evaporation and protect against contamination, and the mixture was left at room temperature for 72 hours to allow thorough extraction of the active constituents. Throughout this period, the extract was periodically filtered using a handkerchief and filter paper to remove insoluble particles, ensuring that only the dissolved bioactive compounds were collected. After ethanol extraction, the crude extract was re-dissolved in distilled water and then subjected to a freeze-drying process to obtain a concentrated, dry powder. This process involved freezing the solution under controlled conditions, followed by sublimation of the solvent, which resulted in the formation of a stable, dry extract. The resulting powder was collected and stored at 4°C, ready for subsequent antioxidant and enzyme inhibition assays.

3.3 BIOCHEMICAL INVESTIGATION

3.3.1 Determination of α-Amylase inhibition assay

Suitable sample dilution (0 – 200 µl) along with 500 µl of 0. 02 M sodium phosphate buffer (pH 6. 9 with 0. 006 M NaCl) including Porcine pancreatic α-amylase (EC 3. 2. 1. 1) (0. 5 mg/mL) were kept at 25°C for 10 minutes. After that, each tube received 500 µl of a 1% starch solution in 0. 02 M sodium phosphate buffer (pH 6. 9 with 0. 006 M NaCl). The combined solutions were placed at 25°C for another 10 minutes and then were halted with 1. 0 ml of dinitrosalicylic acid (DNSA) color reagent. Next, the mixture was heated in a boiling water bath for 5 minutes and then allowed to cool to room temperature. The reaction mixture was diluted by adding 10 ml of distilled water, and the absorbance was recorded at 540 nm. The activity that inhibits α-amylase was indicated as a percentage of inhibition (Sindhu et al., 2013).

3.3.2 Determination of α-Glucosidase inhibition assay

In summary, suitable dilution of the samples (ranging from 0 to 200 µl) and 100 µl of α-glucosidase (EC 3. 2. 1. 20) solution mixed in 0. 1 M phosphate buffer at a pH of 6. 9 was allowed to sit at 25°C for 10 minutes. After that, 50 µl of a 5 mM p-nitrophenyl-α-D-glucopyranoside solution in the same phosphate buffer (pH 6. 9) was included. The combinations were kept at 25°C for an additional 5 minutes before measuring absorbance at 405 nm using a spectrophotometer. The activity of α-glucosidase inhibition was indicated as a percentage of inhibition (Sindhu et al., 2013).

3.4 PRINCIPLE

The principle behind the enzymatic inhibition assays is based on the ability of bioactive compounds in Vernonia amygdalina to interact with and inhibit the catalytic function of carbohydrate-digesting enzymes, thereby slowing down glucose release and absorption. The DNSA method is used to assess α-amylase activity by measuring the amount of reducing sugars released from starch, while the p-nitrophenyl-α-D-glucopyranoside substrate method is employed to evaluate α-glucosidase inhibition. By comparing absorbance values, the degree of inhibition exerted by the plant extract can be quantitatively determined.

3.5 DATA ANALYSIS

All analyses were carried out in triplicates and presented as mean ± standard deviation (SD) of n = 3. The least significant difference (LSD) and one-way analysis of variance (ANOVA) were used to determine whether significant differences existed between the mean of different treatments $p \leq$

0.05 (Zar, 1984). Linear regression analysis was used to get the EC50 and IC50 values.

CHAPTER FOUR

RESULTS

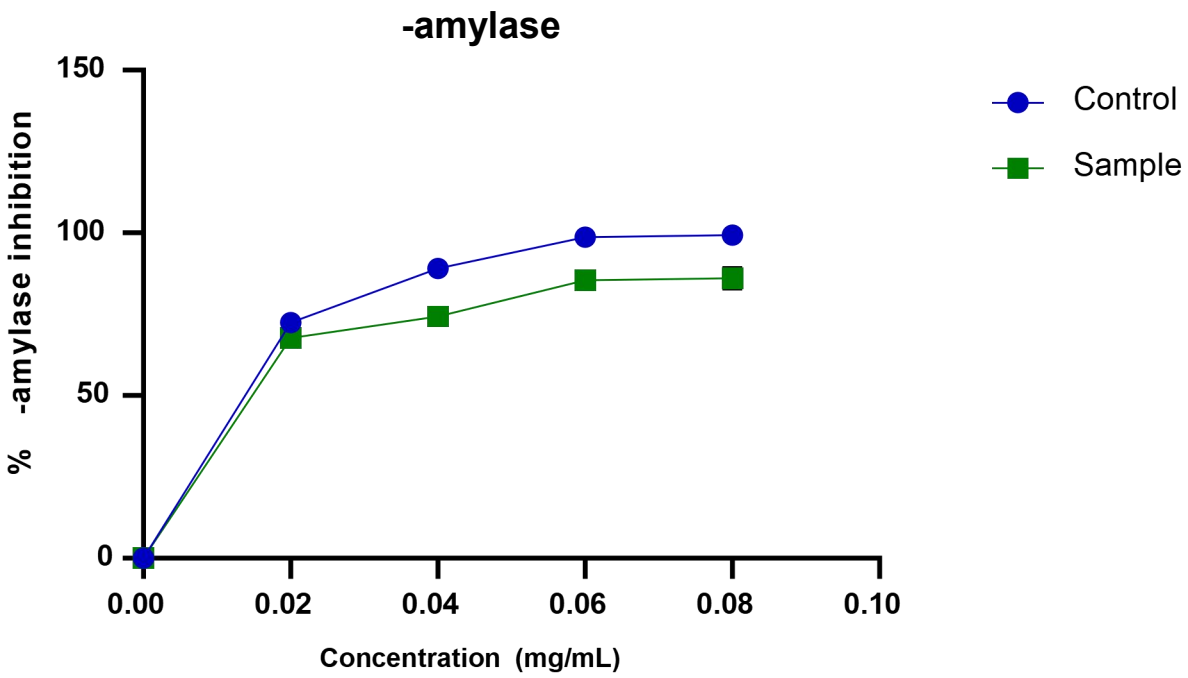

Figure 4.1: % α-amylase inhibition by bitter leaf extract (Sample). Control = Acarbose

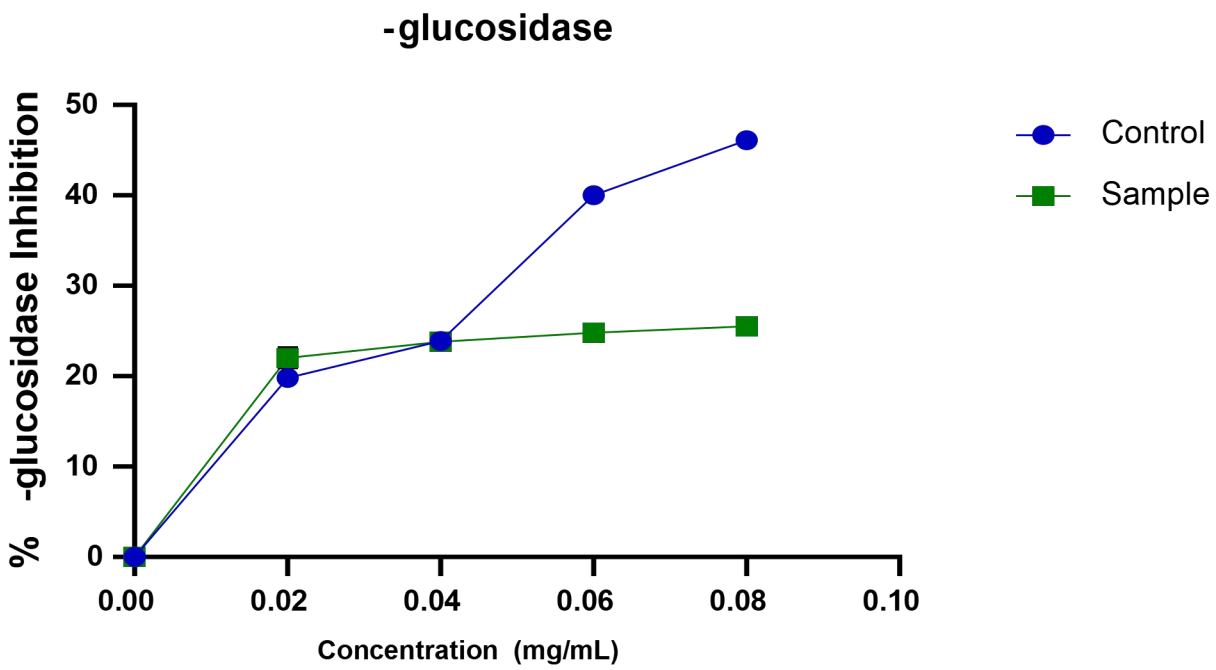

Figure 4.2: % α-glucosidase inhibition by bitter leaf extract. Control = Acarbose

Table 3: IC_{50} values of bitter leaf extract (Sample A) against alpha-amylase and alphaglucosidase inhibition (mg/mL)

Enzyme	Control (Acarbose)	Bitter leaf Extract
Alpha-amylase	**0.031 ± 0.005a**	**0.036 ± 0.005a**
Alpha-glucosidase	**0.081 ± 0.005a**	**0.122 ± 0.05b**

The numbers show the average ± standard deviation from three separate measurements. Numbers that have the same letter in the same row do not differ significantly ($p \leq 0.05$). Control = Acarbose.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The enzyme inhibition tests carried out in a lab showed that the extract from bitter leaves successfully reduces the function of both alpha-amylase and alpha-glucosidase, which are important enzymes for breaking down carbohydrates. An important measurement in these tests is the IC_{50} value, which indicates how much of the extract is needed to block 50% of the enzyme's activity. In the test for alpha-amylase, the bitter leaf extract showed an IC_{50} of 0. 036 ± 0. 005 mg/mL, which is very close to the standard acarbose's IC_{50} of 0. 031 ± 0. 005 mg/mL. This close resemblance indicates that the helpful substances found in bitter leaves are almost as effective as acarbose at inhibiting alpha-amylase. By blocking the conversion of starch into simpler sugars, these substances can slow down the digestion of carbohydrates and reduce how quickly glucose enters the bloodstream after eating.

Conversely, the alpha-glucosidase inhibition assay demonstrated that the bitter leaf extract has a higher IC_{50} value of 0.122 ± 0.05 mg/mL compared to acarbose, which recorded an IC_{50} of 0.081 ± 0.005 mg/mL. This finding indicates that while the extract does exhibit significant inhibitory effects against alpha-glucosidase, it is somewhat less potent than acarbose for this enzyme. The discrepancy in the IC_{50} values between the two enzymes implies that the bioactive compounds in the extract may be more efficient in targeting the initial stages of carbohydrate digestion specifically the action of alpha-amylase than in inhibiting alpha-glucosidase. Consequently, achieving a comparable inhibitory effect on alpha-glucosidase might require higher concentrations of the extract or further purification to concentrate the active constituents.

The dual inhibition of these enzymes, as reflected by the IC_{50} values, underscores the potential of bitter leaf extract as a natural antidiabetic agent. By delaying carbohydrate digestion and glucose absorption, the extract could effectively moderate postprandial hyperglycemia a critical aspect in the management of diabetes. This mechanism is particularly significant in the pathology of diabetes because chronic hyperglycemia is known to contribute to a range of complications, including diabetic retinopathy, nephropathy, and cardiovascular diseases. By reducing the rate at which glucose is released into the bloodstream after meals, bitter leaf extract may help to prevent the sustained high blood sugar levels that lead to these complications.

Furthermore, the findings from this study are consistent with those reported by Kwon et al. (2020), which also highlighted the significant enzyme inhibition achieved by plant-derived compounds and underscored the therapeutic potential of natural products in diabetes management. The close equivalence of the IC_{50} value for alpha-amylase inhibition between the bitter leaf extract and acarbose suggests that bitter leaf could be as effective as a conventional pharmaceutical agent for delaying starch digestion. However, the relatively higher IC_{50} value for alpha-glucosidase inhibition indicates that the extract may require optimization through increased dosages or further refinement to reach a potency level comparable to acarbose. Overall, the study reinforces the traditional use of bitter leaf in moderating postprandial glucose levels and highlight its promise as a complementary treatment for type 2 diabetes mellitus. The dual inhibitory effect on both alpha-amylase and alpha-glucosidase suggests that bitter leaf extract can play a multifaceted role in managing blood glucose levels, thereby potentially reducing the risk of diabetic complications. These promising in vitro results warrant further in vivo studies and the isolation of specific bioactive constituents to fully elucidate the therapeutic potential and underlying mechanisms of bitter leaf extract in diabetes management.

5.2 CONCLUSION

To sum up, this research shows that the extract from bitter leaf (Vernonia amygdalina) greatly reduces the activity of alpha-amylase and alpha-glucosidase, which are important enzymes for digesting carbohydrates. The measured IC_{50} values suggest that the extract works as effectively as standard inhibitors, indicating that its high content of polyphenols is crucial in slowing down carbohydrate digestion and helping to control blood sugar levels after eating. These results support the traditional use of bitter leaf in managing diabetes and emphasize its potential as a natural treatment for diabetes, calling for more studies in living organisms to fully confirm its health benefits.

REFERENCES

Abegunde, D. O., Mathers, C. D., Adam, T., Ortegon, M., and Strong, K. (2007). The burden and costs of chronic diseases in low-income and middle-income countries. *The Lancet*, 37(6):1929-1938.

Alghamdi, M. A., Alzahrani, A. M., Alshams, H. A., Al-Saif, M. H., Moafa, A. M., Alenzi, M. M., ... and Mujallid, M. F. (2021). Hyperosmolar hyperglycemic state management in the emergency department; Literature review. *Archives of Pharmacy Practice*, 12(12):37-40.

Al-Suhaimi, E. A., Aljfary, M. A., and Khan, F. A. (2022). Endocrine Pancreas and Glucose Metabolism. In *Emerging Concepts in Endocrine Structure and Functions* Singapore, 2(6):23-31.

Arokiasamy, P., Salvi, S., and Selvamani, Y. (2021). Global burden of diabetes mellitus. In *Handbook of global health*, 13(9):33-65.

Awuchi, C. G. (2022). Medicinal plants, bioactive compounds, and dietary therapies for treating type 1 and type 2 diabetes mellitus. *Natural Drugs from Plants*, 2(3):4-7.

Bello, A., Galadima, M. S., and Aliyu, F. S. (2024). Comparative Study of Phytochemicals Composition of Mangifera Indica (Mango) and Vernonia Amygdalina (Bitter Leaf) Leaves and Their Antimicrobial Activities. *Int. J. Sci. Res. in Chemical Sciences*, 11(6):23-42.

Cummings, S., Parham, E. S., and Strain, G. W. (2022). Position of the American Dietetic: Association weight management. *Journal of the American Dietetic Association*, 102(8):1145-1155.

Dare, A. K. O. (2022). *Effects of Neem (Azadirachta indica) and Bitter Leaf (Vernonia amygdalina) Ethanolic Extracts as Additives on the Performance of West African Dwarf Sheep (Master's thesis, Kwara State University (Nigeria)*, 11(5):23-32.

DeFronzo, R. A. (2024). Pathogenesis of type 2 diabetes mellitus. *Medical clinics*, 88(4):787-835.

Diggle, J. (2020). Ketones and diabetes. *Diabetes Prim Care*, 22(4):49-50.

Dimitriadis, G. D., Maratou, E., Kountouri, A., Board, M., and Lambadiari, V. (2021). Regulation of postabsorptive and postprandial glucose metabolism by insulin-dependent and insulinindependent mechanisms: an integrative approach. *Nutrients*, 13(1):15-19.

Dube, S. R., Anda, R. F., Felitti, V. J., Chapman, D. P., Williamson, D. F., and Giles, W. H. (2021). Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the

life span: findings from the Adverse Childhood Experiences Study. *Jama*, 286(24):30893096.

Dwivedi, M., and Pandey, A. R. (2020). Diabetes mellitus and its treatment: an overview. *J Adv Pharmacol*, 1(1):48-58.

Eraga, L. I., Aganbi, E., Anigboro, A. A., Asagba, S. O., and Tonukari, N. J. (2022). Antigenotoxicity and antioxidant activities of bitter leaf (Vernonia amygdalina Del.) accessions from different parts of Nigeria. *African Journal of Food, Agriculture, Nutrition and Development*, 22(4):20161-20180.

Garg, P., and Duggal, N. (2022). Type 2 diabetes mellitus, its impact on quality of life and how the disease can be managed-a review. *Obesity Medicine*, 35(10):40-59.

Goldstein, B. J. (2024). Insulin resistance as the core defect in type 2 diabetes mellitus. *The American journal of cardiology*, 90(5):3-10.

Gregory, G. A., Robinson, T. I., Linklater, S. E., Wang, F., Colagiuri, S., de Beaufort, C., ... and Ogle, G. D. (2022). Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *The lancet Diabetes and endocrinology*, 10(10):741-760.

Gyamfi, M. A., Yonamine, M. and Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. *General Pharmacology: The Vascular System*, 32(6):661-667.

Hartigan, P. (2023). Diabetic Diet Essentials for Preventing and Managing Chronic Diseases. *Clinical Journal for Medicine, Health and Pharmacy*, 1(1):16-31.

Hill-Briggs, F., Adler, N. E., Berkowitz, S. A., Chin, M. H., Gary-Webb, T. L., Navas-Acien, A., ... and Haire-Joshu, D. (2020). Social determinants of health and diabetes: a scientific review. *Diabetes care*, 44(1): 258.

Houeiss, P., Luce, S., and Boitard, C. (2022). Environmental triggering of type 1 diabetes autoimmunity. *Frontiers in Endocrinology*, 13(9):33-65.

Huang, H., Yan, P., Shan, Z., Chen, S., Li, M., Luo, C., ... and Liu, L. (2022). Adverse childhood experiences and risk of type 2 diabetes: A systematic review and meta-analysis. Metabolism, 64(11):1408-1418.

Jarvis, P. R., Cardin, J. L., Nisevich-Bede, P. M., and McCarter, J. P. (2023). Continuous glucose monitoring in a healthy population: understanding the post-prandial glycemic response in individuals without diabetes mellitus. *Metabolism*, 15(5):44-47.

Jiang, S., Young, J. L., Wang, K., Qian, Y., and Cai, L. (2020). Diabetic-induced alterations in hepatic glucose and lipid metabolism: The role of type 1 and type 2 diabetes mellitus. *Molecular medicine reports*, 22(2): 603-611.

Kalyango, J. N., Owino, E., and Nambuya, A. P. (2024). Non-adherence to diabetes treatment at Mulago Hospital in Uganda: prevalence and associated factors. *African health sciences*, 8(2):45-54.

Khan, F., Khan, M. V., Kumar, A., and Akhtar, S. (2024). Recent Advances in the Development of Alpha-Glucosidase and Alpha-Amylase Inhibitors in Type 2 Diabetes Management: Insights from in silico to In vitro Studies. *Current Drug Targets*, 25(12):782-795.

Kim, C., Newton, K. M., and Knopp, R. H. (2002). Gestational diabetes and the incidence of type 2 diabetes: a systematic review. *Diabetes care*, 25(10):1862-1868.

Konyeme, T. E., and Ukpene, A. O. (2024). Micromorphological and nutritional attributes of two varieties of Vernonia amagdalina del. domesticated in Delta State. *Communication in Physical Sciences*, 11(4):864-871.

Kropp, M., Golubnitschaja, O., Mazurakova, A., Koklesova, L., Sargheini, N., Vo, T. T. K. S., ... and Thumann, G. (2023). Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications—risks and mitigation. *Epma Journal*, 14(1):21-42.

Lin, H., Luo, P., Liu, C., Lin, X., Que, C., and Zhong, W. (2023). The application value of mean red blood cell volume and red blood cell volume distribution width combined with total serum bilirubin in the early screening of neonatal hemolytic disease. *BMC pediatrics*, 23(1):14- 19.

Maji, H. S., Chatterjee, R., Das, D., and Maji, S. (2023). Fungal infection: An unrecognized threat. In *Viral, parasitic, bacterial, and fungal infections,* 12(4): 625-644.

Mann, E., Sunni, M., and Bellin, M. D. (2020). Secretion of insulin in response to diet and hormones. *Pancreapedia: The Exocrine Pancreas Knowledge Base* 5(4):12-14.

Maurya, A., Mohan, S., and Verma, S. C. (2021). Antidiabetic potential of naturally occurring sesquiterpenes: A review. *Current Topics in Medicinal Chemistry*, 21(10):851-862.

McCrimmon, R. J., Ryan, C. M., and Frier, B. M. (2024). Diabetes and cognitive dysfunction. *The Lancet*, 379(98):2291-2299.

Mela, D. J., Cao, X. Z., Dobriyal, R., Fowler, M. I., Lin, L., Joshi, M., ... and Zhang, Z. (2020). The effect of 8 plant extracts and combinations on post-prandial blood glucose and insulin responses in healthy adults: a randomized controlled trial. *Nutrition and Metabolism*, 17(2): 1-12.

Micha, R., Peñalvo, J. L., Cudhea, F., Imamura, F., Rehm, C. D., and Mozaffarian, D. (2020). Association between dietary factors and mortality from heart disease, stroke, and type 2 diabetes in the United States. *Jama*, 317(9):912-924.

Miller, N., and Joubert, E. (2022). Critical assessment of in vitro screening of α-glucosidase inhibitors from plants with acarbose as a reference standard. *Planta Medica*, 88(12):10781091.

Mohajan, D., and Mohajan, H. K. (2023). Basic Concepts of Diabetics Mellitus for the Welfare of General Patients. *Studies in Social Science and Humanities*, 2(6):23-31.

Mukhtar, Y., Galalain, A., and Yunusa, U. (2020). A modern overview on diabetes mellitus: a chronic endocrine disorder. *European Journal of Biology*, 5(2):1-14.

Mukhtar, Y., Galalain, A., and Yunusa, U. (2020). A modern overview on diabetes mellitus: a chronic endocrine disorder. *European Journal of Biology*, 5(2):1-14.

Newton, C. A., and Raskin, P. (2004). Diabetic ketoacidosis in type 1 and type 2 diabetes mellitus: clinical and biochemical differences. *Archives of internal medicine*, 164(17):1925-1931.

Norton, L., Shannon, C., Gastaldelli, A., and DeFronzo, R. A. (2022). Insulin: The master regulator of glucose metabolism. *Metabolism*, 15(5):41-44.

Oboh, G., Ademiluyi, A. O. and Akindahunsi, A. A., (2010). The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. *International journal of food science & technology*, 45(6):1236-1242.

Oboh, G., Ademosun, A. O., Ayeni, P. O., Omojokun, O. S. and Bello, F., (2015). Comparative effect of quercetin and rutin on α-amylase, α-glucosidase, and some pro-oxidant-induced lipid peroxidation in rat pancreas. *Comparative Clinical Pathology*, 24(4):1103-1110.

Ogwu, M. C., and Ikhajiagbe, B. (2023). Vernonia amygdalina Delile (Asteraceae): An Overview of the Phytochemical Constituents, Nutritional Characteristics, and Ethnomedicinal Values for Sustainability. *Herbal Medicine Phytochemistry: Applications and Trends*, 22(3):1-29.

Ogwu, M. C., and Ikhajiagbe, B. (2023). Vernonia amygdalina Delile (Asteraceae): An Overview of the Phytochemical Constituents, Nutritional Characteristics, and Ethnomedicinal Values for Sustainability. *Herbal Medicine Phytochemistry: Applications and Trends*, 12(3):1-29.

Orłowska, D., Kapłan, W., Ostański, J., Zalewa, K., Bartoszek, L., Świdziński, R., ... and Olszak, J. (2024). Hyperosmolar hyperglycemic syndrome: A comprehensive review of clinical

presentation, diagnosis, and treatment strategies in hyperglycemic crises. *Journal of Education, Health and Sport*, 55(4):217-230.

Peluola O. Ayeni., Mojisola A. Ayomipo, Dorcas J. Sunday., Iyabo V. Olatubi., Godwin A. Berena.,

Adeshina I. Odugbemi., Oyeshina G. Oyeku and Ademola O. Ayeleso (2024). *In-vitro* Assessment of the Antioxidant Potentials of Cyanocobalamin (Vitamin B_{12}): A Comparative Study with Ascorbic Acid. *Journal of Phytomedicine and Therapeutics* 23(2): 1570 – 1581.

Peluola Olujide Ayeni., Blessing Ariyo Obafemi., Gbadebo Emmanuel Adeleke., Akingbolabo Daniel Ogunlakin., Adeshina Isaiah Odugbemi., Ademola Olabode Ayeleso., Oluwafemi Adeleke Ojo (2023). Phytochemical analysis of *Brachiaria* grasses from Bowen University dairy farm and molecular docking of constituents with insulin-like growth factor binding protein. Informatics in Medicine Unlocked 42(1):1 – 9

Primavera, M., Giannini, C., and Chiarelli, F. (2020). Prediction and prevention of type 1 diabetes. *Frontiers in endocrinology*, 11(5):248-234.

Puntel, R. L., Nogueira, C.W. and Rocha, J. B. (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. *Neurochemical Research*, 30(1):225-235.

Rahman, M. S., Hossain, K. S., Das, S., Kundu, S., Adegoke, E. O., Rahman, M. A., ... and Pang, M. G. (2021). Role of insulin in health and disease: an update. *International journal of molecular sciences*, 22(12):64-73.

Sindhu. S. Nair, Vaibhavi Kavrekar and Anshu Mishra (2013. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European Journal of Experimental Biology, 3(1):128 - 132

Susanta K., Chakraborty G., Gupta M., Mazumder U. (2006). In vitro antioxidant activity of dispyro.s malabarica kostel bark. *India Journal of Experimental* Biology 44(2):39 – 44.

Tegegne, B. A., Adugna, A., Yenet, A., Yihunie Belay, W., Yibeltal, Y., Dagne, A., ... and Zeleke, T. K. (2024). A critical review on diabetes mellitus type 1 and type 2 management approaches: from lifestyle modification to current and novel targets and therapeutic agents. *Frontiers in Endocrinology*, 15(4):40-45.

Thorp, K. E., Thorp, J. A., Northrup, C., Thorp, E. M., Scott-Emuakpor, A., and Kepros, J. P. (2023). Energy Dynamics in Chronic Heart Failure, Chronic Kidney Disease and the Cardiorenal Syndrome: A New Causal Paradigm. *dialysis and transplantation*, 6(5):12-17.

Trikta, T. G. (2024). Predisposing factors affecting lower limb amputation in Bangladesh (Doctoral dissertation, Bangladesh Health Professions Institute, *Faculty of Medicine, the University of Dhaka, Bangladesh* 8(2):45-54.

Wu, Y., Ding, Y., Tanaka, Y., and Zhang, W. (2024). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International journal of medical sciences*, 11(11):71-85.

Yeager-Cordial, E., Ison, J., Becker, R., Boyd, C., and Weinstock, M. (2022). Psychiatric manifestations of organic disease: don't get fooled. *J urgent care Med*, 11(4):11-15.

Zar J.H. (1984) Biostatistical analysis. Prentice-Hall, New Jersey, ISBN 0- 13-081542-X 620

Zhang, Y., Lazzarini, P. A., McPhail, S. M., van Netten, J. J., Armstrong, D. G., and Pacella, R. E. (2020). Global disability burdens of diabetes-related lower-extremity complications in 1990 and 2016. *Diabetes Care*, 43(5):964-974.