CHAPTER ONE

INTRODUCTION

1.1 Background of Study

One of the most common metabolic diseases in the world, diabetes mellitus is characterized by persistently high blood sugar levels brought on by deficiencies in either the action or production of insulin, or both. It is linked to chronic harm to a number of organs, most notably the kidneys, heart, blood vessels, nerves, and eyes. Over 422 million people worldwide have diabetes, according to the World Health Organization (2023), and this figure is expected to increase dramatically in emerging nations as a result of dietary changes, sedentary lifestyles, and urbanization. For instance, according to the International Diabetes Federation (2022), there are presently over 3 million persons with diabetes in Nigeria, most of them are ignorant of their illness. Although there are recent therapies like insulin and oral hypoglycemic medications, also have negative side effects and are difficult to get, having problems with patient adherence. As a result, alternative therapies especially those derived from natural and traditional medicinal plants have gained popularity (Luliana *et al*., 2023).

Phyllanthus niruri, sometimes referred to as "stone breaker," or "gale of the wind," is one such plants that has drawn interest from all around the world due to its potential for medicinal use (Musa *et al*., 2023). Native to tropical parts of the globe, including West Africa, South America, and Southeast Asia, *P. niruri* has long been used to cure a variety of illnesses, including kidney stones, hepatitis, malaria, and most importantly, diabetes. Its complex phytochemical content, which contains flavonoids, alkaloids, lignans, tannins, saponins, and terpenoids, makes it a member of the Euphorbiaceae family. It has been shown that these substances have antidiabetic, hepatoprotective, nephroprotective, anti-inflammatory, and antioxidant qualities (Musa *et al*., 2021; Gupta and Bose, 2024).

A growing number of recent scientific investigations have examined *P. niruri's* potential as an

antidiabetic. Significant glucose-lowering effects of its extracts have been shown in preclinical studies using in vitro enzyme inhibition tests and in vivo models of diabetes (such as streptozotocin and alloxan-induced diabetic rats). Flavonoids extracted from *P. niruri* shown strong inhibition of the enzymes alpha-amylase and alpha-glucosidase, which are important for the digestion of carbohydrates, according to a research by Luliana *et al*. (2023). By slowing down the digestion of carbohydrates, inhibition of these enzymes lowers postprandial blood glucose levels. Additionally, in diabetic animal models, aqueous extracts have been shown to decrease oxidative stress, improve peripheral glucose absorption, and stimulate insulin secretion (Trinh *et al*., 2020).

Although preclinical results are encouraging, there has been little clinical use of these discoveries. There are few human clinical studies examining *P. niruri's* effectiveness in managing diabetes, and they often lack methodological rigor. The majority of the information that is currently available comes from observational or ethnobotanical research, which cannot definitively prove effectiveness or safety. However, the plant is still extensively used in traditional medicine, often in the form of capsules or decoctions, and when taken in the right dosages, it has a high safety margin (Smith and Lee, 2023).

Alternative, affordable, and culturally acceptable treatments are desperately needed, especially in light of the growing prevalence of diabetes worldwide and the shortcomings of traditional therapy. *P. niruri* and other plant-based medications provide a possible path for the creation of novel antidiabetic drugs. However, more thorough pharmacological, toxicological, and clinical research is needed to support its medicinal claims and make it easier to integrate it into official healthcare systems (Pabbathi *et al*. 2023)

By conducting scientific experiments to assess the antidiabetic potential of *P.niruri* extract, this study seeks to add to the expanding body of knowledge. This research aims to give empirical evidence in favor of its use in the treatment of diabetes by examining its phytochemical

composition, biological activity, and mechanism of action. It is believed that the results would support the creation of innovative, plant-based treatments for diabetes mellitus in addition to validating conventional claims (Trinh *et al*., 2020).

1.2 Justification of the Study

Diabetes mellitus remains one of the most prevalent non-communicable diseases globally, with the International Diabetes Federation estimating over 537 million adults living with the condition worldwide. In sub-Saharan Africa, particularly in Nigeria, the prevalence of type 2 diabetes is on the rise due to changing lifestyles, urbanization, and poor dietary habits (Chinenye and Ogbera, 2021). Conventional antidiabetic drugs, although effective, are often accompanied by high costs, limited accessibility in rural areas, and adverse side effects, including gastrointestinal discomfort, and liver toxicity (Olokoba *et al*., 2020). These challenges highlight the urgent need for alternative and complementary therapies that are both effective and affordable.

P. niruri has been used in medicine for centuries for managing various ailments, including diabetes. Recent pharmacological studies have revealed the presence of potent bioactive compounds such as phyllanthin, hypophyllanthin, quercetin, and gallic acid, which exhibit antioxidant, and anti-inflammatory activities (Kumar *et al*., 2020; Abdulazeez *et al*., 2022). However, despite these findings, there remains a research gap in the comprehensive evaluation and standardization of the plant's antidiabetic potential, especially within the Nigerian context.

This study is, therefore, justified on several grounds. Firstly, it aims to contribute to the growing body of scientific knowledge validating traditional medicine using rigorous, evidence-based methodologies. Secondly, it explores a readily available and naturally occurring plant with high therapeutic potential, thereby providing a basis for developing cost-effective and accessible herbal alternatives for diabetes management. Thirdly, by analyzing both the antidiabetic

efficacy of *P. niruri*, the research could pave the way for future drug development and pharmacological standardization, especially within low-income and resource-limited settings.

1.3 Statement of Problem

Despite the increasing global burden of type 2 diabetes mellitus, effective long-term management remains challenging due to side effects, high cost, and diminishing efficacy of conventional antidiabetic medications (Gupta and Bose, 2024). This challenge has led to a growing interest in plant-based therapeutics with fewer side effects and broader accessibility.

P. niruri traditionally used in various cultures for diabetes and kidney ailments, contains diverse bioactive compounds such as flavonoids, alkaloids, lignans, tannins, saponins, and terpenoids, which exhibit antioxidant and glucose-lowering actions. In vitro investigations have demonstrated potent α-glucosidase inhibition by water extracts of *P. niruri*, with IC_{50} values as low as 0.9 μM for compounds like corilagin and repandusinic acid (Trinh *et al*., 2020).

Similarly, in vivo animal studies have consistently shown hypoglycemic effects in diabetic models: aqueous and ethanol extracts significantly reduce fasting blood glucose, improve lipid profiles, and protect pancreatic tissue in alloxan- or STZ-induced diabetic rodents (Okoli *et al*., 2022). For example, Pabbathi *et al*. (2023) observed dose-dependent glucose lowering in alloxan-induced mice using methanolic extracts of seeds and leaves, comparable to glibenclamide treatment. Okoli *et al*. (2022) reported that methanol extracts suppressed postprandial glucose, reduced hemoglobin glycation, increased liver glycogen, and inhibited alpha-amylase and alpha-glucosidase activities in diabetic rats

However, despite this robust preclinical evidence, *P. niruri* suffers from critical gaps:

(i) Scarcity of well-designed clinical trials in human diabetic populations, with most evidence derived from animal or in vitro studies and ethnobotanical observations (Smith and Lee, 2023)

(ii) Lack of dosage standardization, pharmacokinetic data, and safety evaluation in prolonged use.

(iii) Limited mechanistic understanding in vivo, beyond enzyme inhibition few studies address biomarkers of insulin sensitivity, oxidative stress, or β-cell preservation.

Thus, the central problem is the lack of translational research bridging the promising preclinical antidiabetic effects of *P. niruri* with clinical relevance. Without standardized extracts, dose guidance, or human efficacy and safety data, the therapeutic potential remains largely theoretical.

This study seeks to address this translational gap by evaluating *P. niruri* extract in a controlled antidiabetic experimental model, focusing on standardized phytochemical profiling, dose-response relationships, mechanistic biomarkers (e.g., enzyme inhibition, glucose uptake, insulin sensitivity, oxidative markers), and safety/tolerability thus providing the rigorous evidence needed to support future clinical investigation.

1.4 Aim of the Study

The aim of this study was to perform a comparative analysis of *P.niruri* extracts using polar and non-polar solvent extractions. And also to evaluate the impact of solvent polarity on the extraction efficiency and antidiabetic properties of the plant.

1.5 Objectives of the Study

1. To evaluate the antidiabetic activity of *P. niruri* extracts obtained using polar and non-polar solvents.

2. To compare the antidiabetic activities of *P. niruri* extracts obtained using polar and non-polar solvents.

3. To assess the overall effectiveness of *P. niruri* extracts in diabetes managements.

1.6 Research Questions

1. What phytochemical constituents are present in the extract of *P. niruri* that may contribute to its antidiabetic activity?

2. Does *P. niruri* extract significantly reduce blood glucose levels in diabetic experimental models compared to untreated controls?

3. What are the differences in antidiabetic activity between polar and non-polar extracts of *P. niruri*?

1.7 Significance of the Study

With an increasing incidence in both industrialized and developing nations, diabetes mellitus especially type 2 diabetes is a chronic metabolic illness that is becoming a greater danger to world health. The high cost of traditional drugs, inadequate healthcare infrastructure, and restricted access to routine medical care all contribute to the burden of diabetes in Nigeria and other low-resource environments. Although synthetic medications are available, they often have unfavorable side effects, poor patient compliance, and decreased long-term effectiveness. These difficulties show how urgently effective, safe, accessible, and culturally acceptable alternative treatments are needed.

The results of this study will add to our understanding of plant-based antidiabetic agents and could pave the way for the creation of reasonably priced herbal diabetes treatments. Additionally, it will help integrate scientifically validated herbal therapies into mainstream healthcare, which will benefit researchers, healthcare providers, pharmaceutical companies, and policymakers. Most significantly, this study may result in better treatment choices for people with diabetes, especially in underprivileged areas.

1.8 Research Hypothesis

1.8.1 Null Hypothesis

There is no significant difference in the antidiabetic activity of *P. nirur*i extracts obtained using polar and non-polar solvents.

1.8.2 Alternate Hypothesis

There is a significant difference in the antidiabetic activity of *P. niruri* extracts obtained using polar and non-polar solvents.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of *P.niruri*

P. niruri is a member of the family Phyllanthaceae (formerly Euphorbiaceae) a plant found in tropical and subtropical areas. This plant is known by the names dukong anak in Malay, zhuzicao in China, in Brazil, and Bhumyamalaki in southern India (Lee *et al*., 2023). This plant may be found on land up to 1000 meters above sea level, in rocky areas, in moist areas such beside rivers, or amid grasses and bushes. With light or dark green stems, *P. niruri* is a wild herb plant that originated in Asia and has since spread to the African, Australian, and American continents. There are eight to twenty-five leaves on each branch or twig. With dimensions of 0.5-2 x 0.25-0.5 cm, the leaves are green. Both the male and female blooms are white. Female flowers are found above the leaf axils, whereas male flowers are found beneath them. Longitudinal rupture of the mature meniran anthers is expected (Rosidah, 2023). The *P. niruri* plant is generally extremely good for your health since it includes a number of full chemical compounds. Alkaloids, flavonoids, tannins, saponins, steroids, phenols, and terpenoids are all found in *P. niruri* . Betasitosterol and lupeol acetate are components of triterpenoid compounds. Furthermore, *P. niruri* contains molecules of lignans, phyllanthin, hypophyllanthin, glycosides, ellagitannins, triterpenes, phenyl propanoids, ricinolic acid, niruriside, phyltetralin, essential oils, anthraquinones, and arbutin in addition to bioactive compounds of flavonoids, tannins, alkaloids, and steroids (Ramandeep *et al*., 2020). While rutin, niruritenin, and other niruri components are found in the stem, flavonoid chemicals like quercetin are mostly found in the leaves. Every section of the plant contains lignin compounds, including phyllanthin and hypophyllanthin. In tropical nations, this plant has long been used to treat a wide range of illnesses, such as intestinal infections, kidney stone, diabetes, chronic liver disease, hepatitis B virus infection prevention syphilis, bronchitis, gonorrhea, and asthma, as well as immune

system stimulation (Rosidah, 2023). *P. niruri's* pharmacological qualities, which include immunomodulation, antiviral, antibacterial, diuretic, anti-hyperglycemic, and hepatoprotector, enable it to cure a wide range of illnesses (Ramandeep *et al*., 2020) This plant is utilized to strengthen the immune system in Indonesia. It has been shown that this plant extract functions as an immunomodulator, enhancing macrophage phagocytic activity and blood peripheral proliferative activity (Rosidah, 2023). According to Vasquez *et al*. (2018), plants that have been used historically to cure illnesses have long been a source of support for therapeutic requirements, and their applications and harmful effects need scientific proof. Studies on medicinal plants are now a global scientific truth that extends beyond their positive effects on health to include production and a nation's economic structure (Muñoz *et al*., 2020). Additionally, indigenous people, who often lack access to traditional health care systems because of their seclusion or economic situation, rely heavily on medicinal herbs. Numerous medicinal plants have been used experimentally as antidiabetic treatments and have been shown to be helpful in the treatment of diabetes (Malviya *et al*., 2020; Mirhoseini *et al*., 2020; Sekar *et al*., 2020). Preethi (2020) claims that approximately 800 plant species worldwide have antidiabetic properties. The significance and interest in proving the antidiabetic effects of plants and separating bioactive compounds from medicinal plants worldwide have been demonstrated by scientist (Hasani-Ranjbar *et al*., 2021; Kavishankar *et al*., 2020; Patel *et al*., 2021; Arumugam *et al*., 2021; Grover *et al*., 2021). Natural medicines mostly derived from plants have been investigated in the majority of investigations using chemically induced diabetes models. Although some research are still conducted on bigger animals, the majority of diabetes investigations are conducted on rodents (Fröde and Medeiros, 2022). Among this example of such promising medicinal plants includes *P. niruri.* It is a member of the family Euphorbiaceae and is mainly found in the tropical and subtropical regions of the world such as Asia, South America, and Africa. Modern medicine has been utilized since time in memorial to treat the

plant as a remedy to several diseases such as liver diseases, kidney stones and urinary tract infections. Scientific research developments in the recent past have authenticated its traditional use by naming it as a possible key to diabetes control (Trinh *et al*., 2021).

2.2 Scientific Classification of *P. niruri*

According to Rosidah (2023) *P. niruri* is classified as follows:

Kingdom: Plantae

Division: Spermatophyta

Sub Division: Tracheophyta

Class : Magnoliopsida

Order: Malpighiales

Family: Phyllanthaceae

Genus: Phyllanthus

Species: Phyllanthus niruri L.

Fig 1.1: The natural habitat of *P. nirur*

Source: (Rosidash,2023)

2.3 Medicinal Plants in the Managements of Diabetes Mellitus

A metabolic condition known as diabetes mellitus is a prevalent chronic illness that is posing a major risk to human health. The frequency of by 2030, it is predicted that up to 10.2% of people worldwide (578 million) would have diabetes mellitus (Saeedi *et al*., 2019). About 90% of all cases of diabetes are reported to have type 2 diabetes mellitus (T2DM), which is also the most common clinical form of the disease and its primary consequence (Furrianca *et al*., 2020; Patel *et al*., 2021). Furthermore, due to its growing severity and influence on cardiovascular disease, which is now regarded as the main cause of mortality in industrialized nations, diabetes mellitus has been categorized as the pandemic of the twenty-first century (Valdés *et al*., 2020). Diabetes imposes a substantial and growing economic burden on society and health systems, requiring continuous care and treatment to prevent complications and adverse outcomes (Elmusharaf *et al*., 2025). Particularly in developing nations where resources are limited and medical plants are crucial to the management of this disease, the high prevalence and long-term complications of diabetes mellitus, along with the adverse effects of antidiabetic medications, have sparked a vigorous search for new oral hypoglycemic agents derived from plants used in traditional medicine for the treatment of this pathology.

According to population surveys conducted in Peru, the incidence of diabetes has risen, with around two new cases reported for every 100 individuals.

2.4 Pharmacological Activities of *P. niruri*

P. niruri is an herbaceous plant with a rich history of traditional medicine dosing practice in tropical parts of the globe such as Asia, Africa, and South America, among others. Its use has

recently increased scientific interest into its therapeutic potential in the past few years (2012-2025), with a wide range of pharmacological effects established interestingly, anti-diabetic, hepatoprotective, antimicrobial, anti-inflammatory, antioxidant, and nephroprotective properties (Zhang and Li, 2024).

2.4.1 Antidiabetic activity

Numerous studies have examined P. *niruri's* antidiabetic properties in both in vitro and in vivo experimental settings. It has been shown that the plant may have a beneficial effect on glucose metabolism. According to a Beidokhti et al. (2021) it interferes with the digestion and absorption of carbohydrates, causing hypoglycemia consequences. Alpha-glucosidase, an enzyme that converts complex carbohydrate into glucose, is inhibited in one suggested mechanism. This lowers postprandial blood glucose levels by delaying the intestinal absorption of glucose (Akinmoladun *et al.,* 2021). It has been shown that *P. niruri* aqueous and ethanolic extracts affect glucose absorption in peripheral tissues, either boosting the effects of insulin or simulating them in muscle and fat cells (Kalailingam *et al*., 2020)

Along with the impact on glucose metabolism, *P. niruri* also seems to have insulin-sensitizing qualities. Given that insulin resistance is a key pathogenic element in type 2 diabetes, this is very pertinent (Venkateswaran *et al*., 2023). It has been shown that several of the plant's bioactive constituents, including quercetin, ellagic acid, corilagin, phyllanthin, and gallic acid, activate pathways linked to increased insulin sensitivity (Jaiswal *et al*., 2023). The nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ), which controls the metabolism of fats and carbohydrates, may be impacted by these substances. *P. niruri* may improve glucose absorption and restore insulin responsiveness via this mechanism (Kalailingam *et al*., 2020). The antioxidant activity of *P. niruri* is another important way that it aids in glycemic control. Because of its detrimental effects on pancreatic β-cells and insulin signaling pathways, oxidative stress is a known factor in the development and progression of

diabetes. By scavenging free radicals, the antioxidant chemicals found in *P. niruri* protect cells from oxidative harm (Yasir *et al*., 2022). Thereby maintaining pancreatic function, this protective action may lower the chance of acquiring diabetes such retinopathy, neuropathy, and nephropathy. Another medicinal aspect of *P. niruri* that contributes to its antidiabetic impact is its anti-inflammatory action. People with type 2 diabetes and insulin resistance often have chronic low-grade inflammation. It has been discovered that the plant's anti-inflammatory components inhibit pro-inflammatory cytokines and alter inflammatory pathways, which may enhance metabolic performance. Lowering inflammation may improve insulin signaling and stop the advancement of tissue damage brought on by diabetes (Ifeoma and Chika, 2020).

The antidiabetic potential of *P. niruri* is further supported by animal studies, which demonstrate improvements in serum insulin levels, fasting blood glucose, and glycosylated hemoglobin (HbA1c) after treatment with the plant extract. Histopathological improvements in pancreatic tissues are often reported in these research, indicating that *P. niruri* may also have a protective or regenerative impact on β-cells (Rai *et al*., 2020). Both acute and sub-chronic toxicity investigations have shown that *P. niruri* has a positive safety profile (Yasir *et al*., 2022). When given at therapeutic dosages, no significant side effects have been seen in animal studies. This enhances its suitability for next clinical studies and possible drug creation. Although there are currently few human investigations, preliminary clinical findings point to possible advantages in controlling blood sugar levels; nonetheless, bigger, controlled trials are required to verify safety and effectiveness in a range of populations. *P. niruri* has attracted attention as a multi-target agent in the treatment of diabetes because of these therapeutic qualities (Muthukumaran *et al*., 2021). Because of its capacity to alter many pathways, including insulin sensitivity, oxidative stress, inflammation, and carbohydrate metabolism, it is a viable option for adjunct therapy in the treatment of diabetes. However, for its incorporation into traditional treatment

frameworks, extract standardization, effective dose determination, and thorough human studies are necessary (Ifeoma and Chika, 2020).

2.4.2 Invitro Antidiabetic Action

The interest in examining this plant's potential as an in vitro antidiabetic has grown in recent years. Inhibiting important enzymes that metabolize carbohydrates, such α-amylase and α-glucosidase, which are essential for postprandial glucose rise, is usually the focus of these investigations (Ifeoma and Chika, 2020)

P. niruri has been shown in several in vitro investigations to have strong inhibitory effect against the enzymes α-glucosidase and α-amylase. Muthukumaran et al. (2021), for instance, found that the plant's methanolic extracts inhibited both enzymes in a dose-dependent way, with an inhibitory profile similar to that of the common synthetic antidiabetic medication acarbose. A key component of managing diabetes is reducing post-meal blood glucose increases, which is achieved by delaying the digestion and absorption of carbohydrates.

In vitro experiments using enzyme-linked assays, these substances efficiently attach to the active sites of digestive enzymes, interfering with their function and delaying the digestion of carbohydrates (Rai *et al*., 2020). Research using 3T3-L1 adipocyte cell lines has shown that *P. niruri* extracts could improve glucose absorption via the activation of insulin signalling pathways. In one such study, Ifeoma and Chika (2020) found that adipocyte cultures treated with *P. niruri* aqueous fractions significantly increased their absorption of glucose.

Glucose absorption and enzyme inhibition, *P. niruri's* in vitro antioxidant capacity significantly enhances its antidiabetic effectiveness. The pathophysiology of diabetes is directly associated with oxidative stress, especially when it comes to pancreatic β-cell failure. It has been shown that P. niruri extract's high total phenolic and flavonoid content scavenges free radicals and shields β-cells from oxidative damage, which indirectly improves glycaemic control (Yasir *et al*., 2022). In vitro research provides strong proof that *P. niruri* has important antidiabetic

effects. It is a potential option for supplemental diabetes treatment because of its capacity to prevent oxidative damage to pancreatic cells, improve glucose absorption, and block enzymes that break down carbohydrates (Rai *et al*., 2020).

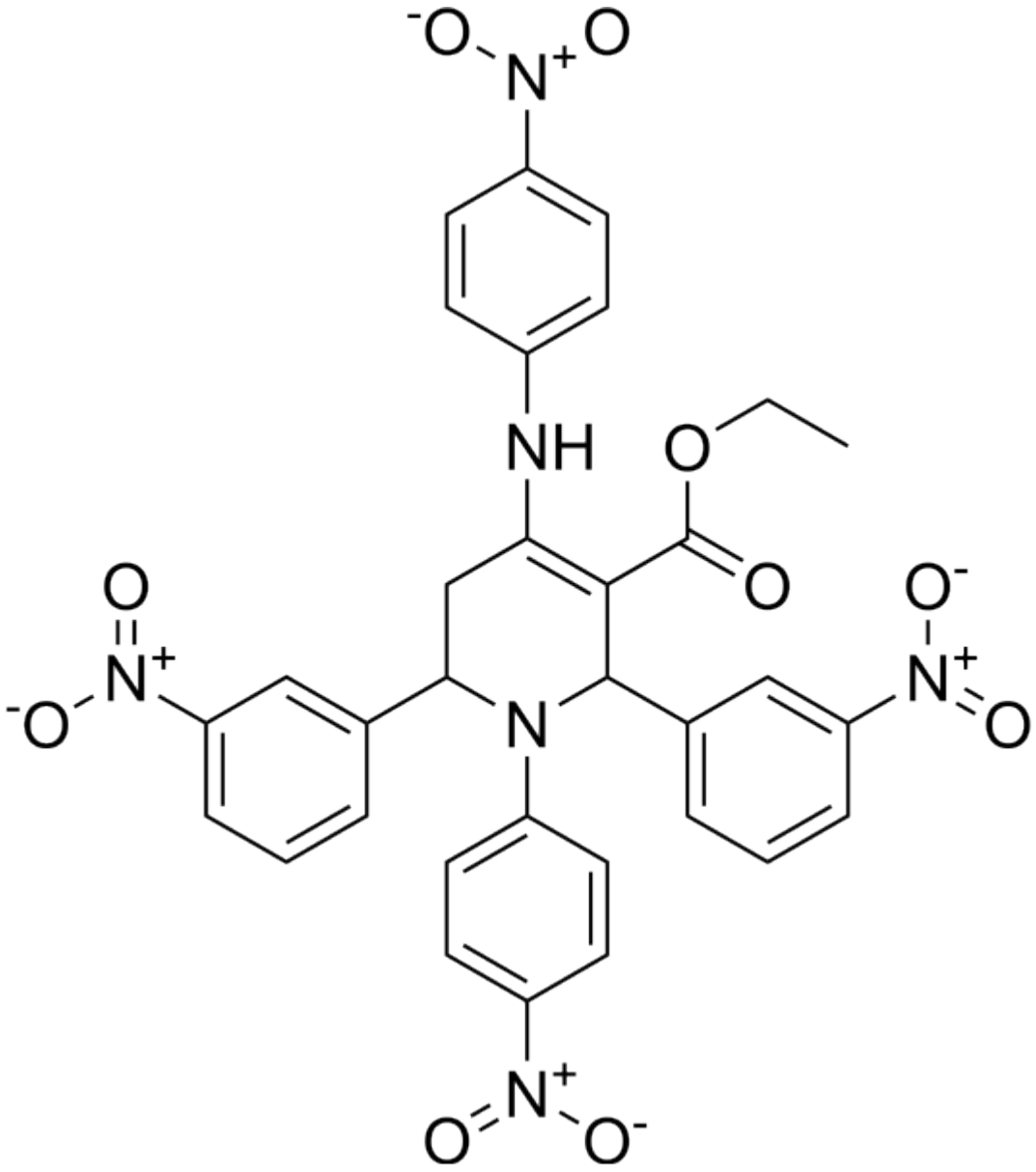

Figure 2.2: Structure of alpha-glucosidase and alpha-amylase

(Minoo *et al*., 2024)

2.4.3 Antioxidant Activity

Modern research has shown that the plant's therapeutic efficacy is strongly linked to its ability to counteract oxidative stress, a biological imbalance that underlies many chronic diseases, including diabetes, cardiovascular disease, and cancer. The plant has long been used in Ayurvedic and folk medicine for liver ailments, urinary disorders, and diabetes (Gajalakshmi *et al*., 2022). When the body's natural antioxidant defences are overpowered by reactive oxygen species (ROS), oxidative stress results. These reactive chemicals have the ability to harm DNA, proteins, and biological structures, impairing cell function (Kumar *et al*., 2023). By scavenging free radicals and boosting the activity of endogenous antioxidant enzymes, plants high in natural antioxidants provide protective mechanisms. Because of its high concentration of secondary metabolites, including as phenolics, flavonoids, tannins, and lignans, which are known to contribute to its antioxidant profile, *P. niruri* is especially well-known (Rai *et al*., 2020). The capacity of *P. niruri* to scavenge free radicals, including hydroxyl and superoxide anions, has been validated by several in vitro and in vivo studies. In order to avoid lipid peroxidation and maintain the structural integrity of cellular membranes, its phytoconstituents work in concert to neutralize these dangerous species (Yasir *et al*., 2022). The presence of polyphenolic substances such as gallic acid, ellagic acid, quercetin, corilagin, and phyllanthin is mostly responsible for the antioxidant capacity. It has been shown that these substances reduce inflammation and cellular stress by inhibiting oxidative enzymes and modifying oxidative pathways (Srirama *et al*., 2022). The antioxidant properties of the plant extend beyond scavenging direct radicals. By increasing the activity of intrinsic antioxidant enzymes such glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), *P. niruri* also demonstrates indirect antioxidant benefits (Iqbal *et al*., 2020). The overexpression of these enzymes promotes systemic protection against oxidative damage and serves as a crucial line of defense in cellular redox equilibrium. Antibacterial, antioxidant, anti-inflammatory and anti diarrhea potentially of the whole plant extract of *P. niruri*. (Islam *et al*., 2024).

P. niruri's antioxidant qualities also support its therapeutic benefits in the treatment of metabolic diseases like diabetes. Insulin resistance and β-cell dysfunction are known to be influenced by oxidative stress. In order to prevent and treat diabetes and its consequences, *P. niruri's* ability to fend off oxidative stress may assist maintain pancreatic function and lower systemic inflammation. This molecular underpinning is consistent with contemporary pharmacological discoveries and supports the traditional usage of *P. niruri* in glycaemic management (Yasir *et al*., 2022). *P. niruri's* antioxidative activity bolsters its anti-inflammatory, nephroprotective, and hepatoprotective properties. Oxidative damage may seriously harm the liver and kidneys, particularly when there is medication toxicity, infection, or long-term metabolic stress. Improved histological profiles of these organs have been repeatedly linked to the administration of *P. niruri* extracts in animal models, indicating a cytoprotective effect fueled by its antioxidant mechanism. Since inflammation and oxidative stress are connected pathological processes, the plant's anti-inflammatory properties are intimately tied to its antioxidant capability, even though they are often addressed separately (Rai *et al*., 2020). The antioxidant activity of *P. niruri* highlights its potential as a multipurpose agent in natural health care from a phytotherapeutic standpoint. Its pharmacological breadth is improved and its application in integrative medicine is supported by the presence of a variety of antioxidant substances. The consistent evidence of antioxidant properties across several experimental models supports the importance of *P. niruri* in situations linked to oxidative stress, even if a large portion of the study is preclinical. *P. niruri* exhibits compelling antioxidant attributes that substantiate in both traditional and modern medici8ne. By scavenging free radicals, enhancing endogenous antioxidant enzymes and safe guarding tissues from oxidative damage, the plant emerge as a viable natural strategy for addressing oxidative stress driven disorder. These properties underpin its inclusion pharmacological researched

focused on metabolic syndromes, degenerative conditions and chronic illnesses where redox imbalance is a key pathogenic factor (Hassan *et al*., 2024; Conde de la Rosa *et al.*, 2025).

2.5 Alpha Amylase and Antidiabetic Management

A vital digestive enzyme called alpha amylase is released by the pancreas and salivary glands. It catalyzes the degradation of ingested starch into smaller oligosaccharides and maltose, which are then transformed into glucose in the small intestine by α-glucosidase. This mechanism directly affects the postprandial (after-meal) increase in blood glucose levels and is essential to the digestion of carbohydrates. α-amylase's quick breakdown of starch in people with diabetes mellitus, particularly type 2 diabetes, is a major cause of postprandial hyperglycemia, a condition associated with long-term consequences such neuropathy, retinopathy, and cardiovascular disease (Dirir *et al*., 2021).

As a result, blocking α-amylase has emerged as a crucial treatment approach for diabetes. α-amylase inhibitors slow down the enzymatic digestion of starch, which delays the absorption of glucose and causes a steady rise in blood sugar instead of abrupt surges. Although synthetic α-amylase inhibitors like acarbose and miglitol are currently used in clinical settings, prolonged usage of these medications is often linked to adverse effects such flatulence and pain in the abdomen. Finding natural plant-derived alpha amylase inhibitors that may provide efficient glycaemic management with fewer side effects has thus attracted increasing attention (Uti, 2025).

2.6 Alpha Glucosidase and Antidiabetic Management

The last stage in the digestion of carbohydrates is catalyzed by the crucial enzyme α-glucosidase, which is found in the brush border of the small intestine. It converts oligosaccharides and disaccharides into glucose, which is then taken up by the circulation. This quick breakdown and absorption of glucose leads to severe postprandial hyperglycemia in those with diabetes mellitus, particularly type 2 diabetes. One of the main risk factors for

diabetes complications, such as kidney failure, nerve damage, and cardiovascular disease, is frequent blood sugar increases (Kashtoh and Baek, 2022).

Inhibiting α-glucosidase has become an essential treatment approach for managing diabetes. α-glucosidase inhibitors lower the rate of glucose absorption by delaying the enzymatic conversion of carbohydrates to glucose, which results in more stable postprandial blood glucose levels. Clinically, synthetic inhibitors like acarbose, voglibose, and miglitol are employed for this purpose; however, their adverse effects, which include bloating, diarrhea, and gastrointestinal distress, have prompted the quest for safer, plant-derived substitutes (Dirir *et al*., 2021).

2.7 Lipase Activity in Antidiabetic

The digestive enzyme lipase, which is mostly released by the pancreas, hydrolyzes dietary lipids into glycerol and free fatty acids so that the small intestine can absorb them. Because lipid metabolism and glucose homeostasis are intimately related, lipase activity has significant implications for managing diabetes even though its main function is tied to fat metabolism. Elevated triglycerides, decreased HDL cholesterol, and increased LDL cholesterol are the hallmarks of dyslipidemia, a frequent metabolic disorder in type 2 diabetes that exacerbates hyperglycemia and leads to insulin resistance (Kashtoh and Baek, 2022).

Thus, it is thought that controlling lipase activity is important for antidiabetic treatment. By lowering fat absorption, pancreatic lipase inhibition may help manage obesity, a significant risk factor for type 2 diabetes. In clinical settings, medications like the well-known lipase inhibitor orlistat are used to treat diabetes associated with obesity. But too much lipase inhibition might cause gastrointestinal distress, thus safer substitutes are required (Babu, 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of Study

The study was carried out at the University of Benin, Benin City, Edo State, Nigeria.

3.2 Study Location

The study was conducted at the University of Benin, Benin City, Edo State, Nigeria. Extraction procedures were carried out at the Department of Chemistry and the Department of Pharmacy (Pharmacognosy), both within the University of Benin, which are equipped with the necessary laboratory facilities for solvent extraction and related analyses. Subsequent antidiabetic analyses were performed at Docchy Laboratories, Awka, Anambra State, Nigeria a specialized laboratory facility furnished with modern equipment for biochemical and phytochemical investigations.

3.3 Materials

3.3.1 Chemical and Reagents

Polar solvent (analytical grade) was obtained from Sigma-Aldrich, Germany, while non-polar solvent (LR grade, stabilized) was procured from Molychem, India. All solvents and reagents used were of high analytical grade.

3.3.2 Equipment

The major equipment used in this study included an electronic weighing balance (Model: TS200, OHAUS), rotary evaporator (Model: Julabo F10), UV-Visible spectrophotometer (Model: 752N, Hinotek), hot air oven (Model: DHG-9023A, Memmert), muffle furnace (Model: HT-MF1400-6.75S/G), and gas chromatography–mass spectrometry (GC–MS) system (Agilent 7890 GC coupled with 5975 Mass Selective Detector).

3.4 Method

3.4.1 Collection and Identification of *P. niruri*

Fresh samples of *P. niruri* were collected within the University of Benin premises around the Faculty of Basic Medical Sciences, Benin City, Edo State, Nigeria. The plant was identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, and its identity was confirmed under the voucher number UBH-p406 at the Herbarium Unit.

3.4.2 Preparation of plant extract

The plant extract was prepared at the Department of Chemistry and the Department of Pharmacy (Pharmacognosy), University of Benin. The leaves of *P. niruri* were separated from the stems, cleaned to remove debris, and thoroughly washed with clean tap water. They were air-dried at room temperature under laboratory conditions for two weeks and then pulverized into fine powder using a commercial blender. The total weight of the dried pulverized sample was 536.3 g.

3.4.3 Polar Extraction

The extraction was carried out using a modified method of Onyeukwu *et al*. (2024). A total of 225 g of the powdered leaf was macerated in 1.2 L of ethanol in a brown glass jar, properly sealed with aluminum foil, and left to stand for 72 hours at room temperature. The mixture was first filtered with cheesecloth to remove coarse debris and then passed through Whatman Grade 1 filter paper, yielding 675 ml of filtrate. The filtrate was concentrated using a rotary evaporator and further air-dried, producing 3.9 g of dry extract. The extract was subsequently stored at 4^{o}C in a refrigerator required for use.

3.4.4 Non-Polar Extraction

The extraction was carried out using a modified method of Njideaka *et al*. (2024). A total of 225 g of the powdered leaf was macerated in 1.2 L of diethyl ether in a brown glass jar, sealed with aluminum foil, and allowed to stand for 72 hours at room temperature. The mixture was filtered initially with cheesecloth and subsequently with Whatman Grade 1 filter paper, producing 560 ml of filtrate. The filtrate was concentrated using a rotary evaporator and air-dried, yielding 3.5 g of dry extract. The extract was subsequently stored at 4ºC in a refrigerator required for use.

3.4.5 Percentage Yield

The percentage yield for each extraction was determined using the formula:

Yield (%) = (Dry weight of extract / Dry weight of plant material) ×100

Polar extraction: Yield = (3.9g / 225g) ×100 = 1.73 %

Non- polar extraction: Yield = (3.5g / 225g) ×100 = 1.56 %

The polar extraction gave a slightly higher yield (1.73%) compared to the diethyl ether extraction (1.56%). This difference may be attributed to the polarity of ethanol, which, as a polar solvent, is more efficient in extracting polar phytochemicals from *P. niruri*. In contrast, diethyl ether, a non-polar solvent, primarily extracts non-polar constituents, resulting in a relatively lower yield.

3.4.6 Alpha Glucosidase Inhibitory Test.

Method: p-Nitrophenyl- α-D-glucopyranoside (pNPG) (Kim *et al*.,2005)

Principle: α- Glucosidase hydrolyses p-nitrophenyl- α-D-glucopyranoside (pNPG) to release p-nitrophenol, released p-nitrophenol is measured spectrophotometrically at 405nm.

Procedure:

α-glucosidase (1 U/ml) from Saccharomyces cerevisiae was preincubated with 250 µl extracts for 10 minutes. p-Nitrophenyl glucopyranoside substrate solution (pNPG, 3 mM) prepared in 20 mM phosphate buffer (pH 6.9) containing 2 mg/ml BSA was added to start the reaction. The reaction mixture was incubated at 37° C for 20 minutes and stopped with 1 ml of Na2CO3 (1M). Alpha glucosidase activity was determined by measuring paranitrophenol released from pNPG at 405 nm.

Calculations

The percent inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{ODcontrol - ODsample \times 100}{OD\ control}$$

3.4.7 Alpha amylase Inhibitory Test.

Method: Dinitrosalicylic Acid (DNS) assay (McCue and Shetty, 2004).

Principle: Alpha-Amylase hydrolyzes starch to release reducing sugars, which react with Dinitrosalicylic Acid reagent to form a colored complex measurable at 540 nm. Inhibitors reduce sugar formation, leading to lower absorbance, which reflects the percentage inhibition of enzyme activity.

Procedure:

A volume of 250 µl of extract or acarbose (1-300 mg/ml) was mixed with 250 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase at a concentration of 0.5 mg/ml. The mixture was preincubated at for 10 minutes.Then, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and incubated at 25° C for another 10 minutes. The reaction was stopped by adding 500 µl of dinitrosalicylic acid (DNS). The tubes were then incubated in a water bath at 95° C for 5 minutes and cooled at room temperature followed by dilution with 5 ml distilled water. The optical density was measured at 540 nm.

Calculation:

The inhibitory activity on alpha amylase was calculated as percent inhibition using the following formula:

$$\% \text{ inhibition} = \frac{ODcontrol - ODextracts\ OD\ \times 100}{control}$$

3.4.8 Determination of the Activity of Lipase

Method: p-Nitrophenyl Butyrate (pNPB) colorimetric assay (Schinner *et al.,* 1996).

Principle: Lipase catalyzes the hydrolysis of p-nitrophenyl butyrate to release p-nitrophenol, which produces a yellow color measurable at 400 nm. The intensity of the color is directly proportional to lipase activity.

Procedure:

The substrate, p-nitrophenyl butyrate was incubated with the soil samples at 27ºC. Soil inoculation was carried out by weighing 0.5g of sieved soil sample into a beaker.50ml of p-nitrophenyl butyric acid was added After 45 minutes incubation at pH 5.4, 27^0c and centrifugation the resulting supernatant was carefully transferred into clean test tubes and the absorbance of the released p-nitrophenol was determined colorimetrically at 400nm with the molar extinction coefficient of 1.48 x 10-2M-1$cm^{-1.}$

Calculations:

$$\text{Lipase activity mol/min} = \frac{total\ reaction\ volume \times absorbance}{Extinction\ coefficient \times sample\ volume}$$

3.4.9 Glucose adsorption capacity

Method: Glucose adsorption capacity assay (Ou *et al*., 2001)

Principle: The assay was based on the ability of a sample (such as plant extract) to bind glucose molecules from solution under physiological conditions. When the sample is incubated with a known glucose solution at 37 °C, part of the glucose becomes adsorbed onto the surface or matrix of the sample. After centrifugation, the unbound glucose remains in the supernatant, and its concentration is measured.

Procedure: Glucose adsorption capacity of the sample was determined in vitro. The extracts (1%) were added to 25 ml of glucose solution (50 mM). The mixture was well mixed and incubated at 37^0C for 6 hours, centrifuged at 4000g for 20min and the glucose content in the supernatant was determined.

Calculations:

The bound glucose was calculated using:

$$\text{The glucose adsorption (mM glucose/g extract)} = \frac{[(G1-G2 \times Volume\ of\ solution)]}{Weight\ of\ sample}$$

G1 = glucose concentration of original solution

G2 = glucose concentration after 6 h incubation)

3.5 Statistical Analysis

All experimental results were carried out in triplicates and expressed as Mean ± Standard Deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) to assess differences between the different concentrations of extracts and the standard drug (Acarbose). Post-hoc multiple comparison tests were applied, and significance was indicated using superscripts (a, b, c), where each letter denotes a significant difference ($p < 0.05$) relative to specific concentrations within the same extract. The level of significance was set at $p < 0.05$

for all analyses. F-values and corresponding p-values are provided in the results tables to demonstrate the strength of differences across groups.

CHAPTER 4

RESULTS

Table 4.1 presents the percentage inhibition of alpha-amylase activity by the polar extract, non-polar extract of *P. niruri*, and the standard drug acarbose at concentrations ranging from 5mg/mL to 100mg/mL. The results are expressed as Mean ± SD, with statistical significance indicated relative to lower concentrations. For the polar extract, the mean inhibition was 39.29 ± 0.17 at 5mg/mL, which increased to 42.73 ± 0.30 at 10mg/mL. This value was statistically significant ($p < 0.0001$) when compared with 5mg/mL. At 50 mg/mL, the inhibition then increased to 52.74 ± 0.21, showing significance relative to both 5 mg/mL and 10 mg/mL. The highest inhibition was recorded at 100mg/mL (58.30 ± 0.21), which was significant when compared with 5mg/mL, 10mg/mL, and 5mg/mL. For the non-polar extract, inhibition values followed a similar dose-dependent pattern, with 32.30 ± 0.26 at 5mg/mL, 40.30 ± 0.30 at 10mg/mL (significant compared to 5mg/mL), and 48.20 ± 0.11 at 5 mg/mL (significant relative to both 5 mg/mL and 10 mg/mL). At 100 mg/mL, inhibition reached 62.9 ± 0.32, and this value was significant when compared with 5mg/mL, 10mg/mL, and 50mg/mL ($p < 0.0001$). The standard drug acarbose demonstrated the highest inhibitory effect across all concentrations, with 79.02 ± 0.08 at 5 mg/mL, 79.39 ± 0.60 at 10mg/mL, 80.94 ± 0.74 at 50 mg/mL, and 84.18 ± 0.32 at 10 mg/mL. Inhibition at 50 mg/mL was significant compared to 10mg/mL, while 100mg/mL was significant relative to both 10mg/mL and 50mg/mL ($p < 0.0001$).

Table 4.1: Alpha amylase inhibition test of polar and non-polar extracts of *P. niruri* compared with Acarbose at different concentration

	Alpha Amylase Inhibition Test (% Inhibition)					
	5mg/mL	10mg/mL	50 mg/mL	100 mg/mL	F value	P value
Polar Extract	39.29±0.17	42.73±0.30a	52.74±0.21ab	58.3±0.21abc	4368	<0.0001
Non-polar Extract	32.3±0.26	40.3±0.30a	48.2±0.11ab	62.9±0.32abc	7353	<0.0001
Acarbose	79.02±0.08	79.39±0.60	80.94±0.74ab	84.18±0.32abc	64.89	<0.0001

Table represented as Mean±SD. a represents significance with 5mg/mL, b represents significance with 10mg/mL, c represents significance with 50mg/mL.

P<0.05 indicates statistical significance

Table 4.2 presents the effects of the polar extract, non-polar extract of *P. niruri,* and the standard drug acarbose on glucose adsorption (expressed as mM glucose/g) at concentrations of 5, 10, 50, and 100 mg/mL. The results are expressed as Mean ± SD, with statistical significance indicated relative to lower concentrations. For the polar extract, glucose adsorption at 5 mg/mL was 0.35 ± 0.11, which significantly increased to 1.08 ± 0.27 at 10 mg/mL ($p < 0.05$ compared to 5 mg/mL). At 50 mg/mL, adsorption decreased to 0.47 ± 0.11, which was statistically significant compared to 10 mg/mL. A further reduction was observed at 100 mg/mL (0.31 ± 0.11), also significant relative to 10 mg/mL. Thus, while there was an initial rise in absorption at 10 mg/mL, higher concentrations (50 and 100 mg/mL) significantly suppressed glucose absorption. For the non-polar extract, adsorption was 0.35 ± 0.04 at 5 mg/mL and 0.39 ± 0.06 at 10 mg/mL, showing no significant difference. At 50 mg/mL, absorption increased markedly to 0.84 ± 0.31, which was significant compared to both 5 mg/mL and 10 mg/mL. However, at 100 mg/mL, absorption declined to 0.29 ± 0.05, showing a significant reduction relative to 50 mg/mL. This indicates a biphasic response with peak absorption at 50 mg/mL before inhibition at higher concentration.

Table 4.2: Glucose Adsorption Capacity of polar and non-polar extracts of *P. niruri* compared with Acarbose at different concentration*s.*

	Glucose adsorption (mMglucose/g)					
	5mg/mL	10mg/mL	50 mg/mL	100 mg/mL	F value	P value
Polar Extract	0.35±0.11	1.08±0.27a	0.47±0.11b	0.31±0.11b	13.37	0.0017
Non-polar extract	0.35±0.04	0.39±0.06	0.84±0.31ab	0.29±0.05c	6.882	0.0132
Acarbose	4.8±0.15	5.63±0.63	7.76±2.71	9.03±3.12	2.578	0.1264

a represents significance with 5mg/mL, b represents significance with 10mg/mL, c represents significance with 50mg/mL

P<0.05 indicates statistical significance

Table 4.3 presents the effect of polar and non-polar extract of *P. niruri* on lipase activity at concentrations of 40 mg/mL, 80 mg/mL, 120 mg/mL and 160 mg/mL, with results expressed as mean ± SD. For the polar extract, lipase activity began at 65.47 ± 0.689 µmol/g at 40 mg/mL. This activity increased markedly at 80 mg/mL (168.9 ± 1.38 µmol/g) and continued to increase reaching 267.1 ± 0.34 µmol/g at 120 mg/mL. The highest activity was recorded at 160 mg/mL with 328.1 ± 0.69 µmol/g, which was significantly greater than all preceding concentrations. For the non-polar, lipase activity started at 78.57 ± 1.38 µmol/g at 40 mg/mL, increased to 169.9 ± 0.34 µmol/g at 80 mg/mL, and then rose further to 283.6 ± 1.03 µmol/g at 120 mg/mL. The peak was observed at 160 mg/mL with 489.7 ± 1.03 µmol/g, representing a nearly six-fold increase compared to the baseline (40 mg/mL). Each concentration was significantly higher than the one before it, confirming a consistent dose-dependent effect.

Table 4.3: Lipase Activity of *P. niruri* extracts at different concentrations

	Lipase(µmol/g)					
	40mg/mL	80mg/mL	120 mg/mL	160 mg/mL	Fvalue	p value
Polar Extract	65.47±0.689	168.9±1.378a	267.1±0.3445ab	328.1±0.689abc	53584	<0.0001
Non-polar Extract	78.57±1.379	169.9±0.344	283.6±1.034	489.7±1.034	90724	<0.0001

a represents significance with 40mg/mL, b represents significance with 80mg/mL, c represents significance with 120mg/mL

P<0.05 indicates statistical significance

Table 4.4 presents the inhibitory activity of *P. niruri* extracts on alpha glucosidase, expressed as mean ± SD. Both polar and non-polar were tested at different concentrations (40, 80, 120, and 160 mg/mL). Superscripts (a, b, c) indicate statistical significance between concentrations within each extract ($p < 0.05$). For polar extract, inhibition started at 63.58 ± 0.12% at 40 mg/mL, and this increased steadily to 67.28 ± 0.25% at 80 mg/mL, which was significantly higher than the initial concentration. At 120 mg/mL, inhibition then rose to 71.15 ± 0.19%, showing another significant increase, and the highest activity was observed at 160 mg/mL with 73.87 ± 0.19%, which was significantly higher than all previous concentrations. In contrast, non-polar extract showed a slightly different pattern. At 40 mg/mL, the inhibition was 61.54 ± 0.19%, which increased significantly to 65.22 ± 0.16% at 80 mg/mL. A marked rise was observed at 120 mg/mL, where inhibition reached 75.62 ± 0.19%, the peak value for this extract. However, unlike the polar extract, inhibition dropped at 160 mg/mL to 60.00 ± 0.07%, despite being statistically different from earlier concentrations.

Table 4.4: Alpha-Glucosidase Inhibitory activity of *P. niruri* extracts at different concentrations.

Alpha-Glucosidase Inhibitory Test						
	40mg/mL	80mg/mL	120 mg/mL	160 mg/mL	Fvalue	p value
Polar Extract	63.58±0.1235	67.28±0.247a	71.15±0.1884ab	73.87±0.1884abc	1647	<0.0001
Non-polar Extract	61.54±0.185	65.22±0.158a	75.62±0.185ab	60±0.07abc	5465	<0.0001

a represents significance with 40mg/mL, b represents significance with 80mg/mL, c represents significance with 120mg/mL . P<0.05 indicates statistical significance

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Table 4.1 shows that *P. niruri* extracts inhibit alpha amylase in a concentration-dependent manner, as shown by the alpha amylase inhibition experiment from lowest concentration (5 mg/mL) to the highest (100 mg/mL), the inhibition of both polar and non-polar extracts increased gradually. This pattern suggests that *P. niruri's* bioactive components, particularly its flavonoids, tannins, phenolics, and lignans, have the ability to inhibit the enzyme activity that breaks down complex carbohydrates. Because it delays the digestion of carbohydrates, this inhibition has pharmacological significance as it lowers postprandial blood glucose from rising, which are essential for the treatment of type 2 diabetes.

In comparison to the polar extract, the non-polar extract exhibited a somewhat greater level of inhibition at 100 mg/mL, indicating that less polar chemicals that are soluble in diethyl ether could be more effective in inhibiting alpha amylase. Acarbose, the common antidiabetic medication employed as a control, showed much more inhibitory efficacy at all tested dosages, however. Although medicinal plants like *P. niruri* exhibit moderate enzyme inhibition, their activity levels are typically lower than synthetic inhibitors. Despite this, they are still useful as safer and more accessible substitutes.

These results are consistent with earlier studies. The tendency seen in this investigation was confirmed by Muthukumaran *et al*. (2021), who found that polar extracts of *P. niruri* showed dose-dependent inhibition of alpha amylase and alpha glucosidase in vitro. Similarly, Rai *et al*. (2020) supported the importance of phytochemicals in enzyme suppression by highlighting the role of *P. niruri's* phenolic components, namely quercetin and gallic acid, in its mild alpha amylase inhibitory effect. Yasir *et al*.'s comprehensive review and meta-analysis from 2022

also found that *P. niruri* extracts consistently inhibited various in vitro tests, with action rising with concentration and being mechanistically associated with delayed glucose absorption. When taken as a whole, these investigations demonstrate that *P. niruri's* enzyme-inhibitory action is repeatable and supported by science.

Table 4.2 show that *P. niruri* extracts has the capacity to bind glucose molecules in vitro glucose adsorption test. Although the activities of the polar and non-polar extracts were somewhat mild in comparison to the common medication, acarbose, they both demonstrated detectable glucose adsorption. It's interesting to note that adsorption was not quite linear with concentration; the non-polar extract showed its maximum activity at 50 mg/mL, whilst the polar extract peaked at 10 mg/mL before declining at higher doses. This variation could indicate that interactions between glucose and other components in the extract or the saturation of active phytochemical sites affect glucose-binding. Acarbose, on the other hand, demonstrated consistently high glucose adsorption at all doses, confirming its well-established function as a strong inhibitor of the breakdown and absorption of carbohydrates.

In the context of managing diabetes, *P. niruri's* ability to bind glucose is important. The extracts may decrease the quantity of free glucose accessible for intestinal absorption by adsorbing glucose molecules, which lowers the glycemic effect of meals high in carbohydrates. This technique provides a second avenue to control postprandial hyperglycemia, complementing the plant's recognized alpha -amylase and alpha-glucosidase inhibitory actions.

Trinh *et al*. (2017) found that *P. niruri* extracts improved glucose transport in muscle cells and inhibited enzymes that break down carbohydrates, hence modulating glucose metabolism in vitro. The results of their study lend to the theory that the plant works via a variety of complimentary processes to lower glucose and enhance cellular use, even though they did not assess glucose adsorption directly. In a separate study, Elfahmi *et al*. (2020) showed *that P. niruri* containing herbal formulations dramatically lowered postprandial glucose levels in

animal models. These effects are related to the plant's ability to adsorb glucose, which perhaps helps explain its antidiabetic potential.

Table 4.3 shows that *P. niruri* extracts activate lipase activity in a concentration-dependent manner. As the concentration was increased from 40 mg/mL to 160 mg/mL, lipase activity significantly increased in both polar and non-polar extracts. Lipase activity was consistently greater in the non-polar extract than in the polar extract; the greatest impact was seen at 160 mg/mL. This implies that whereas polar components in the ploar extract also have a significant but less role in lipase-related activity, less polar chemicals isolated with diethyl ether may contribute more effectively.

Because of the cumulative effects of phytochemicals including lignans, tannins, and flavonoids, the observed dose-dependent response is consistent with the general pharmacological premise that increased stimulation of enzyme activity occurs at higher concentrations of bioactive plant extracts. Crucially, this action reveals an additional possible mechanism by which *P. niruri* might support metabolic control, namely by affecting lipid metabolism, which is often dysregulated in type 2 diabetes.

These results are consistent with those of Adewole *et al*. (2023), who found that *P. niruri* extracts enhanced lipid management by modulating the activity of digestive enzymes, including lipase, in experimental diabetes mice. Guessan *et al*. (2022) conducted a similar finding, observing that polar extracts of *Phyllanthus* species changed lipase activity in vitro. This suggests that the genus has broader metabolic effects than only glycemic control.

The effects of concentration and extract potency, however, are where the differences lie.

The current data demonstrate increased lipase activity, despite previous research (Shakya and Gupta, 2021) reporting lipase suppression as a strategy of lowering fat absorption and treating obesity-linked diabetes. This might be a result of differences in the extraction techniques, plant sections used, or phytochemical makeup, which can either activate or inhibit the enzyme. The

discrepancy highlights the intricacy of phytochemical interactions, since depending on the polarity of the solvent and the test conditions, certain chemicals may increase enzyme activity while others repress it.

Table 4.4 shows how *P. niruri* polar and non-polar extracts, at varying doses, inhibit α-glucosidase. With the polar extract exhibiting a consistent, concentration-dependent rise in activity from 63.58% at 40 mg/mL to 73.87% at 160 mg/mL, both extracts showed strong inhibitory capability. Strong inhibition was also shown by the non-polar extract, which peaked at 75.62% at 120 mg/mL but shown a decline in activity at 160 mg/mL. This might be a sign of enzyme saturation or antagonistic interactions with phytochemicals at higher doses.

The findings have pharmacological significance as postprandial hyperglycemia is decreased by inhibiting alpha-glucosidase, which slows the conversion of complex carbs to glucose. This is a crucial tactic in the treatment of diabetes. Recent studies showing *P. niruri's* substantial phytochemical composition, especially flavonoids, lignans, and phenolic acids, which function as natural alpha-glucosidase inhibitors, are consistent with the significant inhibition shown in its extracts (Khan *et al*., 2021; Paul *et al*., 2023). Other medicinal plants have also shown similar patterns of concentration-dependent inhibition, demonstrating that phytoconstituents might serve as a natural supplement or substitute for synthetic inhibitors such as acarbose, perhaps with fewer adverse effects.

5.2 Conclusion

The study confirms that *P. niruri* has notable antidiabetic potential through multiple mechanisms. Both polar and non-polar extracts inhibited alpha-amylase and alpha - glucosidase, suggesting their role in reducing postprandial hyperglycaemia. They also showed glucose adsorption capacity and influenced lipase activity, highlighting additional pathways relevant to type 2 diabetes management. These effects are likely due to bioactive compounds such as flavonoids, tannins, and phenolic acids, even though the extracts were less potent than

standard drugs like acarbose. Overall, the findings provide strong support for the traditional use of *P. niruri* and suggest its promise as a natural complementary therapy in diabetes care.

5.3 Recommendations

1. In order to validate the antidiabetic benefits shown in vitro, future research should include in vivo test with animal models.

2. The extracts of *P. niruri* should be used as supportive therapy for antidiabetic management

3. Isolation and measurement of the active substances causing the biological activities and phytochemical profiling.

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APPENDIX I

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)

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University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Phyllanthus niruri* var. *amarus* (Schum. & Thonn.) Learndri

Family: Phyllanthaceae

Local Name: Stone breaker, Shatterstone, Earth Gooseberry, Gulf leafflower, Chanca piedra

Voucher Number: UBH-P406

Student Name: Theophilus Illuebbey *et al.*

Plant Identification and Voucher Number Issued by:

28/05/2025

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

APPEENDIX II

Weighing of pulverized plant

APPENDIX III