

**EVALUATION OF ANTIOXIDANT AND ANTI-DIABETIC PROPERTIES OF ARIST
OLOCHIA RINGENS ROOT THROUGH PHYTOCHEMICAL AND MINERAL PROFILING**

BY

MUHYIDEEN HASSANAT ADEOLA

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CERTIFICATION

This is to certify that this research work was carried out and reported by **MUHYIDEEN HASSANAT ADEOLA** (Matriculation Number: **HND/23/SLT/FT/0492**) under my supervision in the Department of Science and Laboratory Technology (Biochemistry Unit), Institute Of Applied Sciences, Kwara State Polytechnic Ilorin, Kwara State, Nigeria.

Mr. Oseni Adio
(Project Supervisor)

Date

Mrs. Salaudeen K.A
(Head of Unit)

Date

Dr. Usman Abdulkareem
(Head of Department)

Date

DEDICATION

This project is dedicated to the Almighty God, whose boundless wisdom and guidance have helped me throughout my studies and this project work.

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ABSTRACT

Aristolochia ringens is a medicinal plant that has been used traditionally in the management of several diseases. Aim: This study is focused on investigating the phytochemical contents, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of *Aristolochia ringens* (Vahl.) root. Materials and Methods: The plant materials were collected, dried, coarsely grounded, and extracted using methanol. The methanol extract was then partitioned into n-hexane and ethyl acetate to obtain the respective extracts. The qualitative phytochemical screening of the extracts was carried out using standard methods. Selected elements were determined from the plant material using Atomic Absorption Spectroscopy (AAS). The antioxidant assays were carried out using the reducing power and 2,2-Diphenyl-1-picrylhydrazyl assay methods. The alpha-amylase inhibitory activities were determined preliminarily using the starch-iodide assay. Results: The extraction gave the methanol extract (ArMe) which on partitioning gave the n-Hexane (ArMH), ethyl acetate extract (ArEa), and the residual methanol extract (ArRMe), qualitative phytochemical screening shows the presence of flavonoids, steroids, cardiac glycosides, and phlobatannin in all the extracts with tannins and alkaloids found in only ArRMe, saponins is found in ArRMe and ArEa. Elemental analysis shows a significant level of the selected elements Ca, Mg, K, Fe, Zn, Na, Cu, Co, and Se in ppm. Antioxidant results show that all extracts exhibit dose-dependent reducing properties and an increase in DPPH scavenging activity. Conclusion: These results further confirmed some of the traditional uses of *A. ringens* in the management of high blood pressure, diabetes, and inflammatory conditions.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Diabetes is a collection of metabolic disorders marked by high blood sugar levels, caused by impaired insulin production, insulin function, or a combination of both. The occurrence of this disorder is on the rise globally and is likely to hit 300 million by 2025 (Gupta and Phatak, 2003). Two major classes of diabetes have been identified. Type 1 diabetes (formerly known as insulin dependent) occurs when the pancreas fails to produce the insulin which is essential for survival. This form develops most frequently in children and adolescents, but is being increasingly noted later in life. Type 2 diabetes, also known as non-insulin dependent results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide (Inzuchi *et al.*, 2015). It occurs most frequently in adults, but is being noted increasingly in adolescents as well. Gestational diabetes is situational and occurs only in pregnancy but it is a risk factor for the development of type 2 mellitus later in life (Zhu and Zhang, 2016). Insulin-dependent diabetes is treated with exogenous insulin administration (Gbolade, 2009).

Available management options for type 2 diabetes mellitus include stimulation of endogenous insulin secretion, improvement of the action of insulin at the receptor sites, oral antidiabetic agents, such as biguanides and sulfonylureas and the inhibition of degradation of dietary starch by glycosidases such as α -amylase and α -glucosidase (Grover *et al.*, 2002; Sulyman *et al.*, 2016). Many useful herbs introduced in pharm

acological and clinical trials have confirmed their blood sugar lowering effect, repair of β -cells of islets of Langerhans (Gupta *et al.*, 2007). The final step in the digestion of polysaccharides and disaccharides is the hydrolysis of α -d-glucose residues from the non-reducing end of α -glucoside by α -glucosidase (Gupta *et al.*, 2007). α -Glucosidase (E.C. 3.2.1.20) activity has been linked with increased levels of plasma glucose, and its inhibition is often exploited in down regulating glucose absorption in type 2 diabetes mellitus sufferers (Wang *et al.*, 2013).

Most of the α -glucosidase inhibitors earlier reported are sugars or derivatives of sugar moieties such as acarbose which is the first member of α -glucosidase inhibitors approved for the management of type 2 diabetes. Pancreatic α -amylase (E.C. 3.2.1.1) is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α -(1-6) and α -(1-4) oligoglucans. Hence, retardation of starch digestion by inhibition of enzymes such as α -amylase plays a key role in the control of diabetes (Sabiou *et al.*, 2016). Inhibitors of pancreatic α -amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels (Sabiou *et al.*, 2016).

Consequently, exploring the binding behaviour and inhibitory effect of inhibitors with α -glucosidase and α -amylase are of great importance for drug-enzyme interactions and therapeutic applications (Grover *et al.*, 2002). *Trigonella foenum-graecum*, *Allium sativum*, and *Aristolochia ringens* are well known plants reported to possess antidiabetic compounds (Grover *et al.*, 2002; Eidi *et al.*, 2006 and Sulyman *et al.*, 2016). However, detailed studies on the structures, kinetics and inhibitory mechanisms of

these antidiabetic plants are scarce. *Aristolochia ringens* is a bushy climber native of tropical America, introduced to most West African countries as a garden ornamental, and has become naturalized in roadside bush in Sierra Leone, Ghana, Nigeria, (Burkill, 1985) and Democratic Republic of Congo (De Groot *et al.*, 2006). However, the kinetic, structural elucidation and mechanism of inhibition of the plant with α -glucosidase and α -amylase have not received much attention.

1.2 Problem Statement

In recent years, reports of antidiabetic potential of various plant extracts have been documented. However, the lack of their nature of interaction, mechanism of inhibition, and identification of the bioactive principles has retarded the progress ought to have been recorded in the discovery of new lead compounds. These problems were addressed in this work by providing data on the mode of interaction, and chemical profile of *A. ringens*, thereby contributing to existing knowledge in the management of type 2 diabetes mellitus.

1.3 Aim and Objectives

The overall aim of this study is to investigate and establish the enzyme activities and probable mechanism of hypoglycaemic action of ethanolic root extract of *Aristolochia ringens*.

This aim was achieved through the following specific objectives:

1. To elucidate the structures of the bioactive principles from *Aristolochia ringens* root using chromatographic techniques (Liquid chromatography-mass spectrophotometry).
2. To establish the probable mechanism of action of ethanolic root extract of *Aristolochia ringens* root.
3. To investigate the inhibition mechanism of α -glucosidase and α -amylase by ethanolic root extract of *Aristolochia ringens*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Types of Diabetes Mellitus

Two main types of diabetes mellitus have been described. These include the insulin dependent diabetes (IDD) or Type 1 diabetes and non-insulin dependent diabetes (NIDD) or Type 2 diabetes. Gestational diabetes is conditional and often transient. Type 1 diabetes results from absolute insulin deficiency, usually caused by autoimmune destruction of pancreatic islet cells. The initial clinical presentation may be ketoacidosis with an acute illness, or a more gradual presentation with symptoms of hyperglycaemia. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type I diabetes, or juvenile onset diabetes, results from a cellular-mediated autoimmune destruction of the β -cells of the pancreas. On the basis of pathophysiology of the disease, this type of diabetes can be further classified as immune-mediated (Rother, 2007).

Type 2 accounts for 90–95% of those with diabetes. It is previously referred to as non insulin dependent diabetes, or adult onset diabetes, and encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. This form of the disease occurs predominantly in adults, especially in p

ersons older than 30 years of age, but it may occur at any age. In recent years there has been a dramatic upsurge of T2DM in children, some younger than 4 years of age (Guthrie and Guthrie, 2009). The global trend to an increase in Type 2 diabetes in African populations is linked to the increase in obesity (Sobngwi, 2001), 18 longevity and other factors such as: Increase in development, Increase in disposable income, urbanization, mechanization, globalization of food markets, changes in lifestyles and behaviours (Waxman and Norum, 2004). Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (Metzger and Coustan, 1998).

This form of diabetes is connected with pregnancy. The definition applies whether insulin or only diet modification is used for treatment and whether or not the condition persists after pregnancy. Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. The prevalence may range from 1 to 14% of all pregnancies, depending on the population studied and the diagnostic tests employed. This classification does not refer to the woman with Type 1 or Type 2 diabetes who becomes pregnant, but to the individual whose diabetic condition developed due to pregnancy (Guthrie and Guthrie, 2004).

2.2 Complications of Diabetes Mellitus

Microvascular and macrovascular complications are the two categories of diabetic complications. Coronary heart disease and stroke are the greatest cause of morbidity and mortality in diabetes. Preventing these complications in type 2 diabetes which is often associated with other cardiovascular risk factors, are major challenges. In dyslipidaemia, increased levels of low density lipoprotein (LDL) cholesterol, consistin

g mostly of small dense particles, promote atherogenesis. Hypertension promotes the development and progression of vascular disease. The characteristic lipid abnormality in patients with type 2 diabetes is dyslipidaemia occasioned by increased levels of both triglycerides and LDL cholesterol, and a low level of high-density lipoprotein (HDL) cholesterol (Bate and Jeroms, 2003). Microvascular complications result from the prolonged toxic and detrimental effects of hyperglycaemia on body tissues such as the cells of the kidney, nerve and eyes. Data from trials over the past 10 years show that controlling hyperglycaemia and hypertension reduces microvascular complications in both type 1 and type 2 diabetes (Bate and Jeroms, 2003).

These include nephropathy, peripheral neuropathy and retinopathy. Diabetic nephropathy also known as Kimmelstiel–Wilson syndrome, or nodular diabetic glomerulosclerosis (Berkman and Harold, 1973) and intercapillary glomerulonephritis, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. About 20%–30% of patients with diabetes have evidence of overt diabetic nephropathy, defined as persistent clinically detectable proteinuria in association with hypertension and reduced glomerular filtration rate (Marshall, 2003). The earliest sign of diabetic renal disease is the presence of subclinical increases in urinary albumin excretion, termed microalbuminuria (urinary albumin excretion rate, 30–300mg/24 h or 20–200 µg/min; or albumin creatinine ratio > 2.5 mg/mmol in men and > 3.5 mg/mmol in women). Microalbuminuria is also an independent risk factor for cardiovascular disease (Bate and Jeroms, 2003).

Foot ulcers and amputations are a major cause of morbidity for people with diabetes. Risk factors for these complications are the presence of peripheral neuropath

y, altered biomechanics in the foot and peripheral vascular disease. About half of all lower-limb amputations in people with diabetes are preventable. Annual screening for these conditions is recommended. Amitriptyline, carbamazepine and gabapentin are helpful in the symptomatic management of painful peripheral neuropathy (Bate and Jerums, 2003). Diabetic retinopathy is the leading cause of blindness in the adult population (VanNewkrik *et al.*, 2001). In type 1 diabetes, almost all patients develop signs of retinopathy in the first 20 years. In type 2 diabetes, up to third of patients have retinopathy at diagnosis (Fong *et al.*, 2003), increasing to two-thirds within 20 years. The most important treatable risk factors are hyperglycaemia and hypertension. Diabetic retinopathy progresses silently until visual loss occurs.

2.3 Antidiabetic Plants

Ethno-botanical data indicates that more than 800 plants are used in folklore medicine as remedies for the treatment of diabetes due to their effectiveness, less side effects and relatively low cost (Ocvirk *et al.*, 2013). Despite the wide usage of pharmaceutical oral hypoglycaemic drugs and insulin therapies as core of diabetes management, they have prominent side effects and fail to significantly alter or amend the course of diabetic complications (Alhassan *et al.*, 2017). Aside high cost, the common side effects linked with oral antihyperglycaemic agents are hypoglycaemia, weight gain, gastrointestinal disorders, peripheral oedema and impaired liver function (Joseph and Jini, 2013). Because natural remedies are comparatively safer and more efficient than orthodox antidiabetic drugs, the practice and study of traditional medicine have become ubiquitous worldwide (Alhassan *et al.*, 2017).

Insulin-dependent diabetes mellitus is treated with exogenous insulin (Gbolade, 2009) and non insulin dependent diabetes mellitus with synthetic oral hypoglycaemic agents like sulphonylureas and biguanides (Covington, 2001; Sulyman *et al.*, 2016). While exogenous hormone administration fails as a curative agent for complications of diabetes, synthetic oral drugs produce adverse health effects (Raheja, 1997).

Different medicinal systems are using the active plant constituents which were discovered as natural hypoglycaemic medicine. The use of crude extracts of medicinal plants in the management of diabetes mellitus is widely practiced in Nigeria (Oguanobi *et al.*, 2012). Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic ones. Anti-hyperglycaemic effects of some of these traditional plants are attributed to their ability to restore the function

of pancreatic tissues by causing an increase in insulin production or restore the functions of insulin receptors (Malviya *et al.*, 2010). Some inhibit the intestinal absorption of glucose through the inhibition of digestive enzymes of carbohydrates, mainly α -amylase and α -glucosidase thereby affecting the rate of glucose absorption and subsequently postprandial glucose level (Kazeem *et al.*, 2013; Sabiu *et al.*, 2017).

The anti-diabetic properties of these plants could be attributed to their phytochemical constituents which include; glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., all of which are frequently implicated as having anti-diabetic effect (Malviya *et al.*, 2010; Irondi *et al.*, 2016). They are less toxic, relatively cheap and popular (Sabiu *et al.*, 2017). Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world e.g., Native American Indian, Jewish (Covington, 2001), East Indian, Mexican and African (Gbolade, 2009). Likewise in this age, the plants and herbs are still being used as decoctions or in other extracted forms for their blood sugar lowering potential. Many useful herbs introduced in pharmacological and clinical trials have been confirmed to elicit their effects by lowering blood sugar level and repairing of β -cells of islets of Langerhans (Sulyman *et al.*, 2016).

Many modern pharmaceuticals used in conventional medicine today also have natural plant origins. Among them, metformin was derived from the flowering plant, *Galega officinalis* (Goat's Rue or French Lilac), which was a common traditional remedy for diabetes (Akindele *et al.*, 2015). Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines and present day drugs for many rural populations in devel

oping countries. Yeh *et al.* (2003) reported seven most promising diabetes supplements including five herbs *Coccinia indica*, American ginseng, *Momordica charantia*, *Gymnema sylvestre*, and *Aloe vera*. However, detailed studies on the efficacy, mechanism of action and safety including inhibition kinetics of the plant extracts are scanty.

2.3.1 *Aristolochia ringens* (Aristolochiaceae)

Aristolochia ringens is a glabrous bushy climber native of tropical America, introduced to most West African countries as a garden ornamental, and has become naturalized in roadside bush in Sierra Leone, Ghana, Nigeria (Burkill, 1985) and DR Congo (De Groot *et al.*, 2006). The plant is commonly called 'Dutchman's pipe' and 'Snake work' but local names in Nigeria include 'Akoigun' (Yoruba, Southwest Nigeria) and 'Dumandutsee' (Hausa, Northern Nigeria). The plant has been reported for its various medicinal applications. The antidiabetic potential of the ethanolic extract of this plant has been thoroughly investigated and reported (Sulyman *et al.*, 2016). The anti-cancer (Akindele *et al.*, 2015), anti-diarrheal (Dharmalingan *et al.*, 2014), stimulant (Minari and Idris, 2015) and anti-inflammatory (Ruth *et al.*, 2014) potentials of the plant have been reported.



Plate 1: Aristolochia ringens root

2.4 Classes and Mechanisms of action of Antidiabetic Drugs

Different antidiabetic drugs have been described. They include biguanides, sulphonylureas, thiazolidinediones, secretagogues and carbohydrate-linked enzyme inhibitors. The most common biguanide is metformin. Metformin has been available since the 1950s and its historic roots and origin can be traced back to the guanidine-rich *Galega officinalis* (goat's rue or French lilac) which has traditionally been used in Europe to treat diabetes (Rena *et al.*, 2017). Metformin has a variety of clinical actions that extend beyond just the glucose-lowering effects such as weight reduction, improving lipid profiles and vascular effects, which includes improving endothelial function, as well as decreasing PAI-1 levels. With the introduction of thiazolidinediones in 1997, the world watched the peroxisome proliferator activated receptor (PPAR)- γ agonists with

th anticipation.

The net effect of these drugs results from stimulation of a nuclear PPAR- γ that regulates the transcription of genes culminating in an increase in insulin sensitivity. Troglitazone, the forerunner drug, was withdrawn in 2000 following reports of fatal hepatotoxicity, and the future of rosiglitazone currently hangs in the balance, owing to a possible increased risk of myocardial infarction and cardiovascular-related deaths (Rena *et al.*, 2017). Acarbose was the first glucosidase inhibitor and was introduced to the market in the early 1990s. This class of drug has the advantage of reducing postprandial hyperglycaemia without associated weight gain. Its usage is at present hampered by unfortunate gastrointestinal side effects despite a good safety record. The α -glucosidase inhibitors inhibit the activity of the glucosidase enzymes which are present in the brush border of enterocytes in the intestinal villi. Disaccharide and oligosaccharide cleavage is prevented with a net decrease in intestinal carbohydrate absorption. Overall, the α -glucosidase inhibitors reduce postprandial insulin concentrations through the attenuated rise in postprandial glucose levels (Chiasson, 2007).

Inhibition of α -amylase and α -glucosidase has been shown to down regulate the rate glucose absorption (Sabiou *et al.*, 2017). A new class of drug called incretins with novel mechanism of action has also been described (Baggio and Drucker, 2007). The small intestine secretes glucagon-like peptide-1 (GLP-1) as well as glucose-dependent insulintropic polypeptide (GIP, previously called gastric inhibitory peptide) in response to food intake. These hormones stimulate insulin secretion, insulin gene expression and pancreatic beta-cell growth. Furthermore, they mediate the incretin effect which augments insulin secretion following oral administration of glucose. The GLP-1 mol

ecule is subject to rapid degradation by the DPP-IV (dipeptidyl peptidase) enzyme. Patients with type 2 diabetes have greatly impaired or absent incretin-mediated insulin secretion due to a decrease in the level of GLP-1 which leads to a decrease in glucose-dependent secretion of insulin by the pancreatic beta-cells (Drucker and Nauck, 2006; Inzuchi and McGuire, 2008).

2.5 Characterization of Plant Extract

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos *et al.*, 2006). Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds that can be used in the management of different conditions such as ulcer, diabetes mellitus, infertility and anti-cataract (Saheed *et al.*, 2015; Sulyman *et al.*, 2016; Sabiu *et al.*, 2016 and Ajani *et al.*, 2017). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy (Sasidharan and Menon, 2010). According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compounds from plant resources are extraction, pharmacological screening, isolation and characterization, toxicological and clinical evaluation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material and Authentication

The fresh root of *Aristolochia ringens* used in this study was procured from Oja-oba, Ilorin, Kwara State, Nigeria and authenticated at the Botany Department, University of Ilorin, Ilorin, Nigeria.

3.1.2 Chemicals and Reagents

The following are the materials used to carry out this study.

- Glucosidase from baker's yeast and porcine pancreatic alpha-amylase obtained from Tokyo Chemical Industry Co. (Shanghai, China).
- Sodium phosphate buffer of pH 7.0 prepared at the laboratory of Medical Biochemistry and Pharmacology, Kwara State University, Malete, Nigeria.
- Acarbose and p-nitrophenyl-d-glucopyranoside (pNPG) are products purchased from Sigma–Aldrich Co. (St. Louis, MO, USA), starch (Sigma–Aldrich Co. (St. Louis, MO, USA).
- Dinitrosalicylic acid (DNS) colour reagent (Tokyo Chemical Industry Co. (Shanghai, China)
- Ethanol, distilled water and other reagents used for this study are of high analytical grades