EVALUATION OF ANTIOXIDANT AND ANTI-DIABETIC PROPERTIES OF ARISTOLOCHIA RINGENS ROOT THROUGH PHYTOCHEMICAL AND MINERAL PROFILING

BY

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HND/23/SLT/FT/0490

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY (BIOCHEMISTRY UNIT), INSTITUTE OF APPLIED SCIENCES, KWARA STATE POLYTECHNIC ILORIN, KWARA STATE, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF (HND HONOURS) IN BIOCHEMISTRY

JULY, 2025

CERTIFICATION

This is to certify that this research work was carried out and reported by **ADEYEMO H ABIBAT IYABO** (Matriculation Number: **HND/23/SLT/FT/0490**) under my supervision in the Department of Science and Laboratory Technology (Biochemistry Unit), Institut e Of Applied Sciences, Kwara State Polytechnic Ilorin, Kwara State, Nigeria.

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DEDICATION

This project is dedicated to the Almighty God, whose boundless wisdom and guidanc

e have helped me throughout my studies and this project work.

ACKNOWLEDGEMENTS

I extend my deepest gratitude to Mr. Oseni Adio, my esteemed supervisor and t he Head of the Unit, Science Laboratory Technology (Biochemistry unit), Mrs. Salaud een K.A., whose unwavering guidance, expertise and support have been instrumental i n shaping this project. Her invaluable mentorship and support have truly made a diffe rence, and I am profoundly grateful for her dedication to my academic growth.

I extend my sincere appreciation to all my departmental lecturers, for their assi stance and support throughout this project. Their dedication, diligence and expertise have played a crucial role in facilitating my research work. Whether it was preparing materials, or providing technical guidance, their contributions have been indispensabl e to the success of the project.

To Mr. Oseni Adio, your support, selflessness and dedication have been a beac on of light on my journey. Your willingness to go above and beyond to support me, w hether through lending a listening ear, offering words of wisdom or providing practica l assistance has touched my heart in many ways. Thank you for taking care of me lik e a daughter, it means a lot.

My appreciate goes to my beloved parents Mr and Mrs Adeyemo whose boun dless love, sacrifices, unwavering support, wisdom, counsel and spiritual guidance ha ve been the cornerstone of my journey. Your constant encouragement has been the s ource of my strength and inspiration and I am forever grateful for your endless blessi ngs, prayers and extraordinary support and love. This project is a testament to your u

nwavering love and dedication and I dedicate its success to you with all my heart.

Also to my lovely brothers, Adeyemo Ademola, Adeyemo Adedeji, Adeyemo Abi ola words cannot express the depth of my gratitude and love for you. Your unwaverin g support, camaraderie and brotherly love have been a source of comfort and strengt h throughout this period of time. From late-night study sessions to moments of laugh ter, your presence has been a constant source of joy and inspiration. I am immensely happy to have you as my big brothers and sisters, I cherish the bond that we share m ore than words can convey.

I further express my appreciation to my lovely grandma person of Adebisi Alim ot who stood by me during to program. may Almighty Allah continue to bless you abu ndantly (Amen). Special thanks to my friends, halimon, Hassant, Akeem , beauty for t heir friendship support and laughter throughout this journey.

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-l-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 (AmH), 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 lev els, caused by impaired insulin production, insulin function, or a combination of bot h.. 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 identifi ed. 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 fr equently 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 re spond 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 (I nzuchi *et al.,* 2015). It occurs most frequently in adults, but is being noted increasingl y in adolescents as well. Gestational diabetes is situational and occurs only in pregna ncy but it is a risk factor for the development of type 2 mellitus later in life (Zhu and Z hang, 2016). Insulin-dependent diabetes is treated with exogenous insulin administrat ion (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 inhibitio n of degradation of dietary starch by glycosidases such as α-amylase and α-glucosid ase (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-(l-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 (Sabiu *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 (Sabiu *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). *Trigonellafo enumgraecum, 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 s, 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 inhi bition, and identification of the bioactive principles has retarded the progress ought t o 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 pr ofile of *A. ringens*, thereby contributing to existing knowledge in the management of t ype 2 diabetes mellitus.

1.3 Aim and Objectives

The overall aim of this study is to investigate and establish the enzyme activiti es and probable mechanism of hypoglycaemic action of ethanolic root extract of *Aris tolochia ringens*

This aim was achieved through the following specific objectives:

1. To elucidate the structures of the bioactive principles from *Aristolochia ringens* roo t using chromatographic techniques (Liquid chromatography-mass spectrophotometr y).

2. To establish the probable mechanism of action of ethanolic root extract of *Aristolo chiaringens* root.

3. To investigate the inhibition mechanism of α-glucosidase and α-amylase by ethan olic 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 in sulin dependent diabetes (IDD) or Type 1 diabetes and non-insulin dependent diabete s (NIDD) or Type 2 diabetes. Gestational diabetes is conditional and often transient. T ype 1 diabetes results from absolute insulin deficiency, usually caused by autoimmun e destruction of pancreatic islet cells. The initial clinical presentation may be ketoacid osis with an acute illness, or a more gradual presentation with symptoms of hypergly caemia. This form of diabetes, which accounts for only 5–10% of those with diabete s, previously encompassed by the terms insulin dependent diabetes, type I diabetes, o r 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 individ uals who have insulin resistance and usually have relative (rather than absolute) insu lin 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 h as 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 Afric an populations is linked to the increase in obesity (Sobngwi, 2001), 18 longevity and other factors such as: Increase in development, Increase in disposable income, urbani zation, mechanization, globalization of food markets, changes in lifestyles and behav iours (Waxman and Norum, 2004). Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (M etzger and Coustan, 1998).

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

2.2 Complications of Diabetes Mellitus

Microvascular and macrovascular complications are the two categories of dia betic complications. Coronary heart disease and stroke are the greatest cause of mor bidity and mortality in diabetes. Preventing these complications in type 2 diabetes wh ich 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 abnormalit y 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 (HD L) cholesterol (Bate and Jerums, 2003). Microvascular complications result from the prolonged toxic and detrimental effects of hyperglycaemia on body tissues such as t he cells of the kidney, nerve and eyes. Data from trials over the past 10 years show th at 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 ne phropathy also known as Kimmelstiel–Wilson syndrome, or nodular diabetic glomeru losclerosis (Berkman and Harold, 1973) and intercapillary glomerulonephritis, is a pro gressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. A bout 20%–30% of patients with diabetes have evidence of overt diabetic nephropath y, defined as persistent clinically detectable proteinuria in association with hypertensi on and reduced glomerular filtration rate (Marshall, 2003). The earliest sign of diabeti c renal disease is the presence of subclinical increases in urinary albumin excretion, t ermed microalbuminuria (urinary albumin excretion rate, 30–300mg/24 h or 20–200 μg/min; or albumi creatinine ratio > 2.5 mg/mmol in men and > 3.5 mg/mmol in wom en). 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 di abetes. 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 l ower-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 pop ulation (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 retin opathy at diagnosis (Fong *et al.,* 2003), increasing to two-thirds within 20 years. The most important treatable risk factors are hyperglycaemia and hypertension. Diabetic r etinopathy 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 sid e effects and relatively low cost (Ocvirk *et al.,* 2013). Despite the wide usage of phar maceutical oral hypoglycaemic drugs and insulin therapies as core of diabetes mana gement, they have prominent side effects and fail to significantly alter or amend the c ourse of diabetic complications (Alhassan *et al.,* 2017). Aside high cost, the common side effects linked with oral antihyperglycaemic agents are hypoglycaemia, weight ga in, 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 (Gbolad e, 2009) and non insulin dependent diabetes mellitus with synthetic oral hypoglycemi c 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 hypoglycemic medicine. The use of crude extracts of medicinal plants in the management of diabetes mellitus is widely practiced in Nigeria (Oguano bi *et al.,* 2012). Plant drugs and herbal formulations are frequently considered to be le ss toxic and free from side effects than synthetic ones. Anti-hyperglycaemic effects o f 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 functi ons of insulin receptors (Malviya *et al.,* 2010). Some inhibit the intestinal absorption of glucose through the inhibition of digestive enzymes of carbohydrates, mainly α-am ylase and α-glucosidase thereby affecting the rate of glucose absorption and subsequ ently postprandial glucose level (Kazeem *et al.,* 2013; Sabiu *et al.,* 2017).

The anti-diabetic properties of these plants could be attributed to their phytoch emical constituents which include; glycosides, alkaloids, terpenoids, flavonoids, carot enoids, etc., all of which are frequently implicated as having anti-diabetic effect (Malv iya *et al.,* 2010; Irondi *et al.,* 2016). They are less toxic, relatively cheap and popular (S abiu *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 A merican 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 in troduced in pharmacological and clinical trials have been confirmed to elicit their effe cts by lowering blood sugar level and repairing of β-cells of islets of Langerhans (Suly man *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, *G alega officinalis* (Goat's Rue or French Lilac), which was a common traditional remed y for diabetes (Akindele *et al.,* 2015). Traditional antidiabetic plants might provide ne w oral hypoglycemic compounds, which can counter the high cost and poor availabili ty 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 supplemen ts including five herbs *Coccinia indica,*

American ginseng, *Momordica charantia, Gymnema sylvestre,* and *Aloe vera.* Howev er, detailed studies on the efficacy, mechanism of action and safety including inhibiti on kinetics of the plant extracts are scanty.

2.3.1 *Aristolochia ringens* (Aristolochiaceae)

Aristolochia ringens is a glabrous bushy climber native of tropical America, intr oduced to most West African countries as a garden ornamental, and has become nat uralized in roadside bush in Sierra Leone, Ghana, Nigeria (Burkill, 1985) and DR Cong o (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 'D umandutsee' (Hausa, Northern Nigeria). The plant has been reported for its various m edicinal applications. The antidiabetic potential of the ethanolic extract of this plant h as been thoroughly investigated and reported (Sulyman et al., 2016). The anti-cancer (Akindele et al., 2015), anti-diarrheal (Dharmalingan *et al.,* 2014), stimulant (Minari an d Idris, 2015) and anti-inflammatory (Ruth *et al.,* 2014) potentials of the plant have be en 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, sul phonylureas, thiazolidinediones, secratagogues and carbohydrate-linked enzyme inhi bitors. The most common biguanide is metformin. Metformin has been available sinc e 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 Eur ope to treat diabetes (Rena *et al.,* 2017). Metformin has a variety of clinical actions th at extend beyond just the glucose-lowering effects such as weight reduction, improvin g lipid profiles and vascular effects, which includes improving endothelial function, a s well as decreasing PAI-1 levels. With the introduction of thiazolidinediones in 1997, t he world watched the peroxisome proliferator activated receptor (PPAR)-γ agonists wi

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. T roglitazone, the forerunner drug, was withdrawn in 2000 following reports of fatal hep atotoxicity, and the future of rosiglitazone currently hangs in the balance, owing to a possible increased risk of myocardial infarction and cardiovascular-related deaths (R ena *et al.,* 2017). Acarbose was the first glucosidase inhibitor and was introduced to t he market in the early 1990s. This class of drug has the advantage of reducing postpr andial hyperglycaemia without associated weight gain. Its usage is at present hampe red by unfortunate gastrointestinal side effects despite a good safety record. The α-gl ucosidase inhibitors inhibit the activity of the glucosidase enzymes which are present in the brush border of enterocytes in the intestinal villi. Disaccharide and oligosacchar ide cleavage is prevented with a net decrease in intestinal carbohydrate absorption. O verall, 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 th e rate glucose absorption (Sabiu *et al.,* 2017). A new class of drug called incretins wit h novel mechanism of action has also been described (Baggio and Drucker, 2007). Th e small intestine secretes glucagon-like peptide-1 (GLP-1) as well as glucose-depende nt insulinotropic polypeptide (GIP, previously called gastric inhibitory peptide) in respo nse to food intake. These hormones stimulate insulin secretion, insulin gene expressi on and pancreatic beta-cell growth. Furthermore, they mediate the incretin effect whic h 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 incretinmediated 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 Oj a-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 obt ained 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 purcha sed fromSigma–Aldrich Co. (St. Louis, MO, USA), starch (Sigma–Aldrich C o. (St. Louis, MO,USA).
- Dinitrosalicyclic acid (DNS) colour reagent (Tokyo Chemical Industry Co. (Shanghai, China)
- Ethanol, distilled water and other reagents used for this study are of high a nalytical grades

3.2 Methods

3.2.1 Preparation of Extract

Dried root of *Aristolochia ringens* plant was weighed and pulverized using grin ding machine (LP500/200) before extracting with 1000ml of 70% ethanol for 24h wit h intermittent shaking. The resulting filtrate was concentrated at 60°C using a rotary e vaporator (Model R110, Brinkmann Instruments Inc, United States) (Sulyman *et al.,* 20 16). Finally, the concentrated extract was dried on a water bath at (TSGP20, Thermo s cientific Company, UK) to yield the plant extract.

3.2.2 Phytochemical Analysis

3.2.2.1 Qualitative Phytochemical Screening

Phytochemical analysis extract was carried out using the method described by Odebiyi and Sofowora (1978) for the detection of saponins, tannins, phenolics, alkalo ids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

1. Alkaloids: Exactly 1cm³ of 1%HCl was added to 3cm³ of the extracts in a test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in t he following tests: 2 drops of Wagner's reagent was added to 1cm³ of the extracts. A r eddish-brown precipitate indicates the presence of alkaloids

2. Tannins: Exactly 1cm³ of freshly prepared 10% KOH was added to 1cm³of the extra cts. A dirty white precipitate indicates the presence of tannins.

3. Phenolics: Exactly 2 drops of 5% $FeCl_3$ was added to 1cm³ of the extracts in a test tube. A greenish precipitate indicates the presence of phenolics..

4. Glycosides: Exactly 10cm³ of 50% H_2SO_4 was added to 1cm³ of the extracts, the mi

xture was heated in boiling water for 15 minutes. 10cm³ of Fehling's solution was ad ded and the mixture boiled. A brick red precipitate indicates the presence of glycoside s.

5. Saponins: Exactly 2cm³ of the extract in a test tube was vigorously shaken for 2 mi nutes. Frothing indicates the presence of saponins.

6. Flavonoids: Exactly 1cm³ of 10% NaOH was added to 3cm³ of the extracts. A yello w colouration indicates the presence of flavonoids.

7. Steroids: Exactly 5 drops of concentrated H_2SO_4 was added to 1cm³ of the extract s. Red colouration indicates the presence of steroids

8. Phlobatannins: Exactly 1cm³ of the extracts was added to 1% HCl. A red precipitate indicates the presence of phlobatannins.

9. Triterpenes: Exactly 5 drops of acetic anhydride was added 1cm³ of the extracts. A drop of concentrated H_2SO_4 was then added and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A blue green colou r indicates the presence of triterpenes.

10. Phytosterols: Exactly 50mg is dissolved in 2ml acetic anhydride. To this, one or t wo drops of concentrated H2SO4 is added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols (Finar 1986).

11. Terpenoids: Exactly 5ml of aqueous extract of the sample is mixed with 2ml of C HCl3 in a test tube 3ml of concentrated H_2SO_4 is carefully added to the mixture to for m a layer. An interface with a reddish-brown coloration is formed if terpenoid is prese nt.

12. Amino acid: Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acet

one) are added to 2 ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids (Yasuma and Ichikawa, 1953).

3.2.3 α-Amylase Inhibitory Assay

The method reported by Sabiu *et al.* (2016) was adopted for this study. Briefly, 500 µl of *Aristolochia ringens* extract and 500 µl of 0.02 M phosphate buffer pH 6.9, containing porcine α- amylase (Cat. No. 10080, Sigma Aldrich Chemical Co, Steinhei m, Germany) at a concentration of 0.5 mg/ml was incubated at 25°C for 10 min. After pre incubation, 500 µl of 1% starch (R & M Chemicals, Essex, UK) solution in 0.02 M p hosphate buffer, pH 6.9, was added. The reaction mixture was then incubated at 25°C for 10 min. The reaction was stopped with 1ml 3,5- dinitrosalicylic acid (DNS) (Cat. N o. D 0550, Sigma Aldrich Chemical Co, USA) color reagent. The test tubes were then i ncubated in a boiling water bath for 5 min and cooled to room temp. Absorbance (A) was measured at 540 nm. Percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} \quad \frac{A540 \text{ control} - A540 \text{ extract}}{A540 \text{ control}} \times 100\ \%$$

* A540 control= absorbance of sodium phosphate buffer (0.02 M, pH 6.9)

A540 extract= absorbance of *A.ringens*

3.2.4 α-Glucosidase Inhibitory assay

The method described by Elsnoussi *et al.* (2012) and reported by Sabiu *et al.* (2016) was followed. Different concentration (0.1-1.0 mg/mL) of the extract or acarb ose were prepared and 50 µL from each stock solution was mixed with 100 µL of 0.1 M phosphate buffer (pH 6.9) containing 1.0 M of α-glucosidase solution and incubat

ed at 25ºC for 10 min. Following this, 50 µL of 5 mM *p*NPG solution in 0.1 M phosph ate buffer (pH 6.9) was added and the reaction mixtures were further incubated at 25 ºC for 5 min. The absorbance in each case was read at 405 nm and the values comp ared with a control which contained 50 µL of the buffer instead of the extract. The as say was triplicated and the inhibitory effect of the extract on the activity of α- glucosi dase was calculated. Using standard calibration curve, the concentration of the extrac t causing 50% inhibition (IC50) of α-glucosidase activity was extrapolated

CHAPTER FOUR

RESULT AND DICUSSION

4.1 Chemical profile of *Aristolochia ringens*

Phytochemical screening of ethanolic root extract of *Aristolochia ringens* reve aled the presence of alkaloids, tannins, flavonoids, phenolics, terpenoids, saponins, tri terpenes and phlobatannins (Table 4.1). However, glycosides, anthraquinones, steroid s, coumarins and amino acids were tested for but not detected. The phytochemical s creening revealed the presence of diverse groups of secondary metabolites that have been reported to possess hypoglycaemic qualities. Alkaloids, phenolics, and flavonoi ds have earlier been implicated in anti-hyperglycemic studies (Sulyman *et al.,* 2016, Ir ondi *et al.,* 2016 and Sabiu *et al.,* 2017). *Aristolochia ringens* is rich in saponin, alkalo id, flavonoids and polyphenols. This is consistent with reports of Alali *et al.* (2006) an d Bemaba *et al.,* 2012) on other species of Aristolochiaceae, *Aristolochia maurorum* and *Aristolochia longa* respectively.

In table 3, the quantitative phytochemical screening of ethanolic root extract of