Evaluation of anti-diabetic properties of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* using a streptozotocin-induced diabetes mouse model

Dr. Samuel Nderitu Nyaga, BVM (UON)
Department of Veterinary Anatomy and Physiology
University of Nairobi

A thesis submitted in partial fulfillment ofrequirements for Masters of Science degree

In Comparative Animal Physiology

University of Nairobi

DEDICATION

To my dad **Mr. Harrison Nyaga Karanja**, and mum, **Mrs. Rose Muthon Nyaga.**

Love, support, and encouragement from you kept me going.

DECLARATION

This thesis is my original work and has not been presented in any other institution for examination or any other purposes.

Signature……  …… Date……**24/08/2020**……

Dr. Samuel Nderitu Nyaga, BVM.
Department of Veterinary Anatomy and Physiology,
University of Nairobi

CERTIFICATION

This thesis has been submitted with our approval as University supervisors.

Signature……  …… Date…24th August 2020……

Prof. Peter MbaabuMathiu, BVM, PhD.

Associate Professor,

Department of Veterinary Anatomy and Physiology,

University of Nairobi

Signature……  …… Date……24/08/2020….....

Prof. Cecilia Moraa Onyango, BSc, MSc, PhD.

Associate Professor,

Department of Plant Science and Crop Protection,

University of Nairobi

ACKNOWLEDGMENT

It will not be possible to mention by name everybody who assisted me in one way or the other due to limitation of space, but I am eternally grateful to everybody. I wish to express sincere gratitude to my supervisors Prof Mbaabu Mathiu and Prof. Cecilia Onyango, without whose guidance and mentorship this thesis would not have seen the light of day.

I thank the University of Nairobi, for offering a full scholarship to me. The Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for financial support during the laboratory phase of the project.

Superb technical assistance extended to me by Mr.Kwoba and Mr.Tsuma from the Department of Veterinary Anatomy and Physiology is highly appreciated.

Additionally, I wish to thank my fellow students at the department, Dr.Loise Kamau and Dr. Pauline Mureithi, for their cooperation and support along the course of this work. Words of advice and encouragement from Dr.Ochwang'i, Dr.Kaluwa, Dr.Nasimolo, Dr.Wambugu, and Dr.Kimani are highly appreciated.

Last and not least, I wish to thank all my family both immediate and extended for the moral support at which juncture I, in a unique way, thank my only brother Rufus Karanja and cousin David Gichure for hosting me.

To everybody who assisted me, I pray that God will bless you abundantly.

Above all! ALL GLORY TO GOD.

ABSTRACT

Diabetes mellitus is a non-communicable disease that poses an immense challenge to the health of people worldwide. *Solanum nigrum*, which is a complex of many species in the family *Solanaceae* ,has been recorded to be used by many communities in the management of diabetes. This study aimed to evaluate the phytochemical, antidiabetic activity, and safety of two confirmed species, namely; *Solanum villosum*and *Solanum nigrum* var. *sarrachoides* usingstreptozotocin-induced diabetes mice model.

Solanum species were grown at Kenya Agricultural and Livestock Research Organisation (KALRO) Muguga. Qualitative assessment for phytochemical constituents was carried out. Acute toxicity was conducted based on OECD guideline 423. Diabetes was induced by injection of streptozotocin at a dose of 200mg/kg body weight intraperitoneally after the mice fasted for 8 hours. Aqueous extracts were administered orally using an oral gavage at doses of 150 and 300 mg/kg body weight for each plant daily and monitored weekly for 28 days.

The results showed that both plants contain vital phytochemicals. Flavonoids, alkaloids, tannins, saponins, phenols, and glycosides were present in both plants. However, phytosterolsand coumarins were only present in *Solanum nigrum* var. *sarrachoides*. Both plants did not show toxicity. On the antidiabetic assay, both plants showed efficacy with *Solanum nigrum* var. *sarrachoides* being more potent at both doses.

The differences in the activity can be attributed to differences in phytochemicals composition and concentration. The study validates the use of these plants by herbalists and recommends further studies on the plants to elucidate the active compounds that can be used as novel therapies for diabetes. Additionally, the study recommends the evaluation of other species in this complex for anti-[1]diabetic properties.

1

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AESV: Aqueous extract of *Solanum villosum*

AESS: Aqueous extracts of *Solanum nigrum* var.*sarrachoides*

IDF: International Diabetes Federation

WHO: World Health Organisation

LMIC: Low and Medium Income Countries

GAD: Glutamic Acid carboxylase

HLA: Human leucocyte Antigen

NCD: Non-communicable Disease

DKA: Diabetic Ketoacidosis

LA: Lactic acidosis

STZ: Streptozotocin

KDMIC: Kenya Diabetes Management Information Centre

WDF: World Diabetes Foundation.

KALRO: Kenya Agricultural and Livestock Research Organisation

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus (DM) is a non-communicable disorder that is metabolic in origin with variant causes and presenting with chronic hyperglycemia with aberrations in the metabolism of carbohydrates, fat, and protein. The underlying cause is defects in insulin metabolism and action (WHO, 2016). Sustained hyperglycemia results in both short-term and long-term pathophysiological defects.

DM is on the rise. It is estimated that 463 million adults had diabetes in 2019, compared to 108 million in 1980 (1DF, 2019). The International Diabetes Federation (2019) projected that the number of diabetic people in the world would rise to 642 million by the year 2040.Further, it was estimated that the disease caused 1.5 million deaths directly and 2.2 million deaths indirectly by increasing the risk of cardiovascular disease and other diseases. It is believed that every six seconds, a person dies from a diabetes-related complication (Pasquel & Umpierrez, 2014). The rise in prevalence is now highest in Low and Medium Income Countries (LMICs), with 75% of all adults living with diabetes living in these countries (WHO, 2016). In sub-Saharan Africa, it is projected that there will be a 141% increase in patients suffering from DM diabetes to stand at 34.2 million by 2040.

The symptoms of diabetes are predominantly polydipsia, polyuria, vision blurring, and unexplained weight loss(WHO, 2019). Complications associated with diabetes cause dysfunction and failure of various vital organs in the body. The commonly affected tissues being those in the eyes, in kidneys, neurons, and blood vessels as a result of different pathophysiological processes. Acute complications include ketoacidosis and not-ketotic hyperosmotic state(Patel *et al.,* 2014). The long-term effects depend on the specific organs

affected and may result in blindness, renal failure, or cardiovascular diseases(Chen *et al.*, 2019).

The main types of diabetes are; type 1 and type 2. Type 1 is caused by a lack of insulin production, while Type 2 occurs due to insulin resistance with relative insulin deficiency. In both cases, management aims at lowering the blood sugar levels in the short run and restoring normal carbohydrates, lipids, and fat metabolism in the long term. Type 1 is managed by insulin injections, while Type 2 is controlled by both insulin and some antidiabetic agents such as sulfonylureas, biguanides, α-gluconidase inhibitors, and thiazoliadones. These drugs have different pharmacokinetic and pharmacodynamics. However, these conventional therapies have immense efficacy and safety concerns. Additionally, the cost of these drugs and especially insulin, which is vital in diabetes management, has been limiting to many patients, especially in low-income setups. These shortcomings of conventional therapies have increased reliance on herbal remedies, many of which scientific validation is not available.

The area of medicinal plants continues to be of enormous importance not only for the management of many conditions affecting man and animals but also as a potential area of development of new drug molecules. Over 80% of the world's population still relies on herbal medicines. Researchers have reported that many plants (over 800 species) have antidiabetic properties(Antu *et al.*, 2014).The medicinal properties have been attributed to secondary metabolites found in these plants.

African nightshade (ANS) (*Solanum nigrum L*) genus is a complex of several species. Species from this genus have been utilized as leafy vegetables by many Kenyan communities. Additionally, some have been used to manage different ailments, including stomach aches,

eye infections, and swollen glands. Species within this complex have also been recorded to being used as an anti-diabetic in different communities *(Kamau et al.,* 2017).

Animal models are vital tools in elucidating pathophysiological mechanisms that underlie complex diseases such as diabetes and in screening substances for both efficacy and safety. In diabetes research, rodents (mice and rats) have found extensive use. Chemical ablation of β-cells of the Islets of Langerhans in the pancreases using diabetogenic compounds Alloxan and Streptozotocin is the most commonly used method in research.

This study aimed at assessing phytochemical composition, safety, and anti-diabetic properties of aqueous extracts of two species of the *Solanum nigrum L* complex. Two species, namely *Solanum villosum* and *Solanum nigrum var.sarracoides,*were assessed using Streptozotocin-induced diabetes model in mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology and Types of Diabetes Mellitus

Diabetes mellitus (DM) refers to metabolic disorders of glucose metabolism precipitated by aberrations in insulin function. It occurs as a result of a deficiency in insulin in the body (Alberti & Zimmet, 1998; Irons, 2013;Chen *et al.*,2019). Previously, types of diabetes were classified as either insulin-dependent or insulin-independent. WHO revised this nomenclature as compelling evidence emerged that all kinds of diabetes were associated with insulin (Alberti & Zimmet, 1998; WHO, 1998; WHO, 2019). Majorly, the described types of diabetes are; diabetes type 1, diabetes type 2, and gestational diabetes.

2.1.1 Type I diabetes

Type 1 diabetes mainly results from the destruction of β-cells in the islet of Langerhans in the pancreases hence occasioning lack of insulin.In the earlier classification, it was termed 'Insulin-dependent type' because patients of this condition must be put on insulin therapy for survival.This condition occurs commonly but is not limited to children and adolescents (WHO, 2016).Characteristically, it has been described as a heterogeneous and polygenic disorder and classified into either autoimmune type 1A or idiopathic with B- cell obstruction type 1B (American Diabetes Association, 2015; Tests & Diabetes, 2015).Type 1 diabetes is identified by the presence of markers of islets immune destruction, which includes, among others, autoantibodies to insulin, glutamic acid decarboxylase (GAD65), tyrosine phosphate 1A-2 and 1A- 2B.

The actual cause of this condition has been and remains elusive.Genetic predisposition remains the most incriminated with HLA-DR/DQ alleles having been shown to either protect or predispose individuals. The strongest linkage was found to be with the human leukocyte antigen (HLA)-D genes, which are located on chromosome six. Possible triggers of the autoimmune destruction include molecular mimicry, presence of viral genome in the islets, insulitis, and beta-cell destruction induced by specific cytokines (Jun & Yoon, 2001). Type 1 cause between 5% and 10% of the diabetes cases. Therefore, this makes it the 4th main contributor to deaths caused by NCDs (WHO, 2014). Despite having a minor contribution to the total burden of diabetes, it is the major type of diabetes affecting children, accounting for over 90% of diabetes cases in children. In terms of laboratory studies, this diabetes is relatively easy to induce (Goud *et al.,* 2015).

2.1.2Type 2 diabetes

Type 2 DM results from either insulin resistance and intolerance or both (El-busaidy *et al*., 2014; Irons, 2013; Kanungo *et al*., 2014). The pathophysiology is more complicated because it is a multifactorial disease(Tests & Diabetes, 2015). It is one of the most prevalent diseases in the world, with an enormous burden both socially and economically (WHO, 2016). It was approximated that almost 382 million people globally live with type 2 diabetes, and roughly 175 million cases of the disease remain undiagnosed with most of the countries affected being in the lower and middle-income range. In 2015 it was postulated that the worldwide economic cost of diabetes was $1·31 trillion indirect costs, and since Type 2 cases are the majority, it contributed the highest to the burden (WHO, 2016). In 2011 the estimated cases of type 2 diabetes were 14 million, and it is expected to rise to almost 23.9 million by 2030

due to changes in lifestyle and environment in Africa which are the critical contributors to type 2 diabetes (Victoria *et al.,* 2011).

Insulin resistance refers to the situation whereupon release insulin;there is a lack of appropriate biological response. Resistance to insulin can either be due to genetic factors or environmental factors. The genetic factors affect peripheral cells, especially muscle and liver cells. Environmental factors linked to insulin resistance include aging and obesity. Insulin resistance though unclear manifests itself possibly through down-regulation, deficiencies, or genetic polymorphisms of tyrosine phosphorylase for the insulin receptor, IRS proteins or PIP-3 kinase, or maybe abnormalities of GLUT 4 function. Furthermore, genetic defects affecting any of the enzymes involved in the downstream action of insulin can be a manifestation of insulin resistance (Al-Noaemi & Shalayel, 2011; Siddiqui *et al.,* 2013). Initially, insulin resistance results in increased release of insulin by β cells to cater to the insensitivity, which eventually leads to the obliteration of the β cells as a result of either glucose or fat toxicity, genetic defect, or exhaustion (Zhang *et al.,* 2016).

Insulin deficiency in type 2 DM results from the death of β cells, and this forms the primary difference from that experienced in type 1 diabetes. A combination of glucose and fat toxicity, genetic defect, or exhaustion destroys the cells; hence no insulin is produced. Eventually, glucose regulation in the blood is affected, and thus leading to hyperglycemia (Gan, 2013).

2.1.3 Gestational DM

Gestational diabetes refers to diabetes detected and associated with gestation that is not convectional diabetes (Meek *et al.*, 2015). This type predisposes women to develop diabetes in the future, and so are their children (Booth *et al.*, 2017). It is associated with fetal

metabolic disturbance, macrosomia, and stillbirths (Al-Noaemi & Shalayel, 2011; Coustan, 2013; Joshi *et al.,* 2013). Though the precise pathophysiological mechanisms are unknown, pregnancy hormones such as estrogen, progesterone, and cortisol are implicated for the development of this condition (Booth *et al.,* 2017). Human placental lactogen has also been shown to be diabetogenic(Al-Noaemi & Shalayel, 2011).

2.2 Complications of DM

Diabetes mellitus is a predisposing condition. Patients have higher risks of major infections, cardiovascular and neurological diseases (Alve *et al*., 2012; Ammari*et al.*,2004; Haththotuwa, 2016). These complications are secondary to hyperglycemia and cause the most devastation (Forbes & Cooper, 2013). The exact mechanism of this complication underlies genetic, epigenetic, nutritional factors, and sedentary lifestyles (Papatheodorou, 2016). The complication is classified as either acute or chronic (WHO, 2016).

2.2.1 Acute complications of DM

Acute complication of DM includes diabetic ketoacidosis (DKA), hyperosmolar non-ketotic coma (HNC), Lactic acidosis (LA) and hypoglycemia(Becker & Hux, 2011; Fishbein & Palumbo,1995). DKA and HNC are more common and are related to insulin insufficiency (Fishbein & Palumbo, 1995; Heyne *et al.,* 2015). LA occurs in co-morbidity with cardiovascular disease, while hypoglycemia occurs as a complication of diabetes of hyperglycaemic management, mostly with insulin (Fishbein & Palumbo, 1995).

DKA is the first indicator of diabetes in about 30% of all cases(Becker & Hux, 2011; Silva *et al.,* 2017). If not managed, it can lead to a mortality rate of 9-14% (Fishbein & Palumbo, 1995). Ketosis occurs as a result of increased lipolysis, which generates ketone bodies from

the adipocytes. Patients present with nausea, vomiting, CNS depression and in the extreme a comma (Tests & Diabetes, 2015)

HNC occurs in conditions of relative or absolute insulin deficiency and inadequate fluids. It is more prevalent in patients ailing from type 2 diabetes. Patients present with polyuria, postural hypertension, altered mental state, Lethargy, seizure, and comma (Pasquel & Umpierrez, 2014).

2.2.2 Chronic Complications of DM

The hyperglycemia characteristic of diabetes occasions chronic complications that affect diverse body organs (Liu *et al*., 2010).Chronic complications are responsible for most of the deaths associated with diabetes (Papatheodorou*et al.*, 2016; Stanifer *et al.*, 2016).These complications include; retinopathy, neuropathy, nephropathy, and cardiovascular disorders (Tolonen, 2015; WHO, 2016).

Cardiovascular disorders are the most common of the complication and are the primary cause of mortality in most of the cases. It accounts for 52% of all deaths associated with type 2 diabetes (Haththotuwa, 2016; Liu *et al*., 2010).The cardiovascular disorders associated include congestive heart failure, ischaemic heart disease, and peripheral vascular diseases. Most of these cardiovascular effects occur secondary to arteriosclerosis of the arteries and coronary artery (Haththotuwa, 2016).

Nephropathy is characterized by proteinuria, diabetic glomerular lesions, and loss of glomerular filtration rate (Brennan *et al*., 2013; Lim, 2014).The reduced glomerular filtration occurs either as a result of the thickening of the basement membrane or increased renal vascular endothelial growth factor (VEGF), which is both antigenic and a permeability factor (Pop-Busui *et al.*, 2017).

Retinopathy affects about 75% of all people with diabetes and causes adult blindness (Forbes & Cooper, 2013; WHO, 2016).The vascular lesions can either be proliferative, where blood vessels appear on the retina in response to hypoxia. The non- proliferative form presents with retinal microaneurysms and small hemorrhages on the retina (Heyne et al., 2015). Almost all diabetics have some neuropathic problems (Lim, 2014). The pathophysiology of nerve injury by hyperglycemia is not well elucidated through polyol accumulation, damage from glycosylated end products, and oxidative stress are often incriminated (Gardiner *et al.*, 2007). Up to 50% of the neuropathies are asymptomatic (Pop-Busui et al., 2017)

2.3 Opportunistic infections of DM

It is well known that diabetic has an increased propensity for infections (Alves *et al.*, 2012).Urinary tract, respiratory, and skin infections are the most common of this infection (Dryden *et al.*, 2015; Haththotuwa, 2016).This predisposition to disease has been attributed to the weakened response of T-cells, impairment of neutrophil function, and aberrations of humoral immunity (Muller *et al.*, 2005). This, as a result of the hyperglycaemic environment as well as the microvascular changes in diabetes (Gan, 2013). Infections from *Staphylococcus aureus, Escherichia coli,Streptococcus pneumonia,* and *Mycobacterium tuberculosis* are most prevalent (Alves *et al.*, 2012; Simonsen *et al*., 2015). Rare infections affecting diabetes patients include *Rhinocerebral mucormycosis* and malignant otitis externa(Petrikkos *et al*., 2012).

2.4 Diagnosis of diabetes mellitus

A correct, timely diagnosis is paramount in the management and treatment of diabetes mellitus (Tests & Diabetes, 2015). The criterion employed for confirmation for a person

presenting with signs of diabetes differs from that of an asymptomatic person with blood glucose above the lower limit (Tests & Diabetes, 2015; WHO, 2016).

In most scenarios, patients are prompted to seek medical attention by symptoms such as polyuria, polydipsia, unexplained weight loss, drowsiness, or bouts of comas. For patients with severe symptoms, a singular blood measurement above the cut-off diagnostic value is confirmatory (Brugnara et al., 2016; WH0, 2016). However, a single blood measurement should never be used as the basis for diagnosis in asymptomatic patients (Chamberlain *et al*., 2016).

Oral glucose tolerance test (OGTT) is employed in the identification of people within specific ranges of blood glucose. The criteria applied are those symptomatic patients with randomly sampled blood glucose value above 200mg/dl or 11.1mmol/L, those with a fasting plasma glucose more than 126mg/dl or more than 110mg/dl for whole blood or blood glucose levels above 200mg/dl after two hours in a glucose tolerance test (WHO, 2016). Fasting is set to mean a state of non-calorimetric intake for a minimum of 8 hrs. (WHO, 2016).

Venous blood glucose levels remain the universally accepted biomarker of diabetes mellitus. Despite the strong correlation between elevations in plasma glucose and glycatedhemoglobin that between fasting glucose and hemoglobin in individuals with normal or mildly elevated levels is not very clear, and hence the use of hemoglobin in diagnosis is still not universally accepted.

OGTT is helpful because fasting glucose only misses about 30% of cases and is mainly used to identifying people with IGT (American Diabetes Association, 2018). It is recommended that individuals with fasting plasma glucose of 110-125mg/dl (6.1-6.9mmol/l) should be tested to determine their glucose tolerance status. Blood is collected after fasting and after

2hrs after oral glucose dosage of 75g.In children, 1.75g/kg is used in this test (Chamberlain et al., 2016; WHO, 2016)

2.5 Treatment of DM

The overall aim of management is to ensure restoration and continuous maintenance of blood glucose levels near normal as much as it can be possible to avert the ensuing complications of diabetes(Chaudhury *et al.,* 2017; Schmid, 2007). Conventional management of diabetes involves the use of oral agents and insulin (Abiola *et al*., 2016; Stein *et al*., 2013).

2.5.1 Insulin therapy

Insulin remains the most potent glucose-lowering agent so far (Sartorius *et al*., 2014). Discovered by Banting and Best in 1922, Insulin therapy has provided effective control of hyperglycemia in patients (Shejja *et al*., 2010). However, the risk of life-threatening hypoglycemia associated with insulin has encouraged more research in this area. Insulin reduces blood sugar through many processing, such as reducing hepatic glucose production and increasing glucose uptake, especially by muscles (Home *et al*., 2014; Kanungo *et al*., 2014; Shejja *et al*., 2010).It also reduces insulin resistance by improving abnormal lipoprotein characteristics of insulin insensitivity, especially in type 2 cases (Chaudhury *et al*., 2017).

Patients who have type 1 diabetes have to depend entirely on external insulin sources to prevent the development of ketosis and hyperglycemia, which can result in fatal diabetic ketoacidosis if not managed (Kanungo *et al.,* 2014; Schmid, 2007). This is because most cases have 80-90% destruction of β-cells at the time of diagnosis. Insulin therapy for type 2 diabetes patients is introduced later in the course of the management regime of the disease (Abiola *et al.*, 2016).

Insulin produced is of 3 broad types; Animal insulin that is derived from cow and pigs, the insulin that is genetically engineered and insulin analog which is genetically engineered with similar but the modified amino acid sequence and maybe synthetic (Chaudhury *et al.,* 2017; Silva *et al.,* 2017).The development of genetic engineering facilitated the production of insulin in *E. coli* and yeast (Walsh, 2005).Since 2004 to 2013 insulin is majorly produced from mammalian cell (56%); *E. coli* (24%); *S. cerevisiae* (13%); transgenic animals & plants (3%) and insect cells (4%). Depending on the onset and duration of action, insulin is classified as either short, intermediate, or long-acting (Shejja *et al.,* 2010).

Insulin is provided via subcutaneous injection or continuous subcutaneous insulin infusion using a pump (Kanungo *et al.,* 2014). The initial dose is 0.5-1 unit/kg per day, which can be adjusted (Abiola *et al.,* 2016). Other than hypoglycemia, weight gain is also a significant adverse effect associated with the use of insulin (Golen *et al.,* 2014).

2.5.2 Oral anti-diabetic drugs

Oral medication is essential, especially in type 2 diabetes. This is so because most of the drugs act on the residual β-cell to enhance insulin production (Stein *et al.,* 2013). The progressive nature of the disease renders the oral agents ineffective mostly after 4-5 years, necessitating the use of insulin (Abiola *et al.,* 2016). Oral agents available are classified into five groups; Sulphonylureas, biguanide, α-glucosidase inhibitors, thiazolidones, and meglitinide(Mane *et al*., 2012; Stein *et al.,* 2013).Each of these classes has different pharmacological actions aimed at reducing hyperglycemia. They are used either individually or in combinations.

Metformin is the most common biguanide in use. It has been available since 1950 and is the drug of choice in obese type 2 diabetes because of its weight-reducing effect (Irons, 2013). It

is said to be anti-hyperglycemic and not hypoglycaemic, but the exact mechanism of action is not very clearly elucidated (Chaudhury *et al.,* 2017). It is thought to mediate and improve insulin sensitivity by targeting the protein Adenosine 5'monophosphate.The profound effect is in the liver, where it inhibits gluconeogenesis and glycogenesis and muscles where it induces up-regulation of GLUT-4 and GLUT-1 membrane transport proteins, thence increasing glucose uptake (Zyl *et al.,* 2008). When used in combination with sulphonylureas, metformin improves glycaemic control even in individuals not responsive to sulphonylureas(Cahn & Cefalu, 2016; Davies *et al.,* 2017).

Sulphonylureas were discovered in 1920 and remain important agents of managing diabetes (Peyrot *et al.,* 2005). The mechanism of action is a direct stimulatory effect by closing ATP-sensitive K^+ channels when bound by the transmembrane sulphonylurea (SUR-1) receptor (Invest & Kir, 2010).This occasion influx of Ca^+ which promotes the release of the preformed insulin granules that lie just below the cell membrane (Zyl *et al.,* 2008). Drugs in this class are associated with hypoglycemia because they potentiate insulin production even when the glucose levels are normal(Irons, 2013). The commonly used in this class is the second generation sulphonylurea, which includes gliclazide, glipizide, glibenclamide, and glimepiride (Stein *et al.,* 2013). The side effects include weight gain, skin reactions, and cholestatic hepatitis (Silvia *et al.,* 2016). Sulphonylureas can be used in combination with other drug groups except for other secretagogues(Cahn & Cefalu, 2016).

Thiazolidones are a newer class of drugs introduced in 1977.The most commonly used are pioglitazone and rosiglitazone. Pharmacologically they are peroxisome proliferator-activated receptor (PPAR)-γ agonists(Irons, 2013; Zyl *et al.,* 2008). They mediate their function by binding the PPAR-γ receptor that is mostly expressed in adipocytes muscles and the

liver,which switches on the transcription of genes that important in metabolism (Silvia *et al.,* 2016). Consequently, there is enhanced synthesis and translocation of specific glucose transport proteins. Their use, in combination with insulin, is contraindicated in Europe due to an increase in weight. Thiazolidones use is controversial with troglitazone having been withdrawn due to fatal hepatotoxicity side effects and also rosiglitazone being implicated in causing myocardial infarction (Zyl *et al.,* 2008).

Acarbose, Miglitol, and Voglibose are the commonest α-glucosidase inhibitors in the market (Zhang *et al.,* 2016). Theseclass of drugs is either used in monotherapy or combination but is majorly recommended as a first-line oral agent by both ADA & EASD. These drugs exert the effects by competitively inhibiting α-glucosidase in the small intestines to slow carbohydrate breakdown and prevent post-prandial hyperglycemia(Irons, 2013). This class has been shown to have less toxicity and is as effective as metformin.

Meglidinineanalogs are secretagogues drugs that are similar in the mechanism of action to sulphonylurea but are considered less effective(Mane *et al.,* 2012). They interact with receptors on the β-cells, although the biding is weaker, and hence they are considered to be short-acting (Chaudhury *et al.,* 2017; Stein *et al*., 2013). Unlike sulphonylureas, they require high levels of blood glucose to be effective. The most commonly used in this class are netaglidine and repaglinide(Chaudhury *et al.,* 2017).

2.6 Challenges in current treatment strategies of diabetes

Management of diabetes still face myriads of challenges (Kanungo *et al.,* 2014; Wais *et al.,* 2012). Insulin obtained through purification is associated with clinical problems as a result of impurities (Home *et al.,* 2014). These include; local and systemic insulin allergies, insulin

resistance due to immunological reactions, disrupted time course of action because of antibodies, and lipodystrophy at injection sites (Home *et al.,* 2014).

Chemotherapy drugs used have many documented side effects affecting various body organs and body systems. Above all, therapies for diabetes are both directly and indirectly expensive (Kamau *et al.,* 2016). Amongst the 11 health system factors needed to ensure a favourable diabetes management environment, the two most important are accessibility and affordability of drugs.

2.7 Anti-diabetic plants

Since time immemorial, communities have used plants as a source of the potent antidiabetic drug. There are many plants claimed to possess this property. Ethnobotanical surveys reported show many plants have antidiabetic properties (Kamau *et al.,* 2016; Kamau *et al.,* 2017; Mamun-or-Rashid *et al.,* 2014; Mujesh & Namita, 2013). Equally, there are many plants whose antidiabetic effects have been proved in animal and human studies (Kasali *et al.,* 2013; Mujesh & Namita, 2013). These plants documented to be used across the world and whose effects have been validated especially in animal studies include; *Aralia elata, Azadirachta indicia, Cinnamonum zeglanicum, Coptis chinesis, Allium sativum, Medica gosativum, Biophylum sensitivum, Brassica nigrum, Ficus bengalenesis,Urtica dioica* among others(Mamun-or-Rashid *et al.,* 2014; Mujesh & Namita, 2013).

2.7.1*Solanumnigrum L*

Solanumnigrum commonly referred to as African or black nightshade, belongs to the family Solanaceae (Matasyoh & Bosire, 2016), has over 90 genera, and over 2000 species which are distributed all over the world most. Most occur as weeds, but due to their variable uses, cultivation is also practiced (Onyango *et al.,* 2016). Additionally, some of these continue to

be important sources of pharmaceutical substances; for example, *Solanum dulcamara* is cultivated for corticosteroids. Among these genera, *Solanum nigrum* is the most common and most diverse. It has been described as a complex of many species. There are nine species in this complex with the commonest species found in Kenya being *S. nigrum, S. scabrum, S. nigrum v*ar.*sarrachoides* (Figure 1), *S.villosum*(Figure 2), *S.americanum and S.physalifolium* (Ontita*et al.,* 2016)

African nightshades are annual herbs that grow up to a height of 0.6m (Matasyoh & Bosire, 2016). In temperate regions, most of the flowers around July and September with berries ripening around August and October (Taab & Andersson, 2009). There exist a lot of variations in the morphological appearances of members of this complex (Matasyoh & Bosire, 2016; Ontita *et al.,* 2016; Onyango *et al.,* 2016).This includes variations in shapes and sizes of leaves, the color of ripe berries, color and shapes of flowers, growth heights, and hairiness of leaves (Taab & Andersson, 2009; Wagio, 2016). These characteristics are important in the classification and identification of different species.

Figure 1: **Photograph showing *Solanum nigrum* var.*sarrachoides***

Figure 2: Photograph showing *Solanum villosum*

2.7.1.1 *Uses of Solanum nigrum*

Species within this genera have found extensive use as leafy vegetables and continue to be cultivated in Kenya (Matasyoh & Bosire, 2016; Onyango *et al.,* 2016).Most of this consumption is in rural areas because of the availability (Sw & Bokelmann, 2017). The plants have been shown to have high levels of protein, calcium, carbohydrates, vitamin A and vitamin C (Wagio, 2016). Oxalate, which is an anti-nutrient, has been reported to be in high levels in these plants (Zebish *et al.,* 2016). However, prior preparation by cooking has been found to reduce these anti-nutritive factors significantly (Ejoh, 2017).

2.7.1.2 Medicinal use

These plants have seen diverse folkloric use in the management of ailments (Abu *et al.,* 2017; Atanu *et al.,* 2011; Chauhan *et al.,* 2012). In Kenya, they have been reported to be used in the management of eye infections, stomach aches, ulcers, constipation, swollen glands (Edmonds & Chweya, 1997; Ontita *et al.,* 2016). Leaves have also been used in the management of toothache (Kokwaro, 1993;Matasyoh & Bosire, 2016). Generally, the plant has been reported and shown to possess antioxidant, hepatoprotective, anticancer, anti-ulcerogenic, antidiabetic effects and anti-inflammatory properties by different investigators (Abu *et al.,* 2017; Anandan *et al.,* 2015; Kasali *et al.,* 2013; Lettre & Linn, 2010).

Solanum nigrum has been used for diabetes treatment in many communities and hence a lot of validation studies in animals (Ahir *et al.,* 2013; Lettre & Linn, 2010; Umamageswari, 2017).Different extracts preparations have been assessed for their antidiabetic efficacy. Aqueous extract at a dosage of 400mg/kg body weight per day for 21 days has been reported to have significant ant-hyperglycemia and hyperlipidemia in alloxan-induced diabetes model of diabetes (Sengottaiyan & Nadu, 2012). Umamageswari*et al.* (2012) reported a dose-

dependent blood-glucose-lowering effect of aqueous extract at doses of 200mg/kg body weight per day from the seventh day of treatment in alloxan-induced Wistar rat model. In a glucose tolerance test at 350mg/kg body weight in a mice model, there was no significant reduction. Organic solvent extracts have also been shown to have antidiabetic effects (Atanu *et al.,* 2011). Tiwari and Jain (2012) reported significant anti-hyperglycemic using ethanolic extracts of leaves. The stem extracts have not been shown to have significant effects (Sengottaiyan *et al.,* 2016).

2.7.2 Phytochemical composition

The medicinal value of plants has been attributed to phytochemicals present. These are secondary metabolites in plants that possess physiological activities aimed at ensuring plant survival in harsh conditions and also to discourage predation (Gogoi, 2012). Elucidation of these chemicals is not only relevant for understanding the medicinal benefits but can be useful as industrial compounds such as tannins, oils, and gums (Anandan et al., 2015).

Aqueous extracts of leaves of *Solanum nigrum* have been shown to contain saponins, tannins, alkaloids, terpenoids, flavonoids, glycosides, steroids, phenols and anthraquinones(Anandan *et al.,* 2015; Gogoi, 2012; Karunakar, 2017; Patel *et al.,* 2014). Among the plant's parts, aqueous extracts of the leaves are seen to be more consistent in their phytochemical composition. There are variations in phytochemical compositions even of the same plant parts in the literature. This is explained by the difference in environmental conditions prevailing in different regions, methods of drying (sun drying or shade drying), and method of analysis. Shade drying has been shown to preserve much of the phytochemicals (Tiwari & Jain, 2012).

The antidiabetic activity of phytochemicals is majorly due to the prevention of oxidative damage and control of post-prandial hyperglycemia due to inhibition of digestive enzymes especially α-glucosidase on the luminal surface (Wang *et al.*, 2013). Phenolic, alkaloids, flavonoids, tannins, and glycosides are the most common active compounds in anti-diabetic plants (Abu *et al.*, 2017; Mujesh & Namita, 2013). However, it is challenging to attribute this activity entirely to a single component because of the intricate nature of their association. Anti-diabetic studies using purified components of the components are still at infancy stages.

2.8 Animal Models

Animal models are essential in the elucidation of pathophysiological mechanisms that underlie DM (Katsuda *et al*., 2013; King, 2012).Animal models are also useful tools in screening novel drugs for the management of this condition (O'brien *et al*., 2014). The animal model used depends on the specific type of diabetes mellitus being simulated and occasioned complication of interest (King, 2012). Additionally, it depends on the pharmacological effect that relates to DM under study. Rodents are the most common model employed in diabetes research (Rees & Alcolado, 2015). Animal models are considered cheap and yield valuable information for processing novel substances (Herrath & Nepom, 2009).

2.8.1 Animal models for the study of type 1 DM

Naturally, type 1 diabetes occurs as a result of autoimmune destruction of beta-cell and hence hypoinsulinemia and, consequently, hyperglycemia (Jun & Yoon, 2001). Animal models vary from those that use chemical ablation to using animals bred to spontaneously develop diabetes mellitus (King, 2012; Herrath & Nepom, 2009).The use of two diabetogenic compounds commonly achieves chemical ablation; streptozotocin and alloxan(Katsuda *et al.,* 2013; O'brien *et al.,* 2014). These models have had extensive use in testing new formulations of drugs and testing transplantation interventions(Deeds *et al.,* 2011).

The autoimmune models used include non- obese (NOB) and Biobreeding (BB) rat (Leiter & Schile, 2013).Virus-induced models especially using viruses such as encephalomyocarditis virus, coxsalkie, and kilhan rat virus, have been used with limited successes (Jun & Yoon, 2001). This is because the response of the animals is dependent on the establishment of the virus (Herrath & Nepom, 2009). Many Transgenic models have been developed to study different aspects of this disease, most common is the *AKITA* mice developed in Japan from the introduction of a mutation in the insulin gene two which introduces aberrations in the processing of insulin resulting in misfolded proteins (King, 2012).

2.8.2 Animal models for type 2 DM

Type 2 is characterized by the resistance of tissue to insulin (Abiola *et al.,* 2016). Most models are obese because of their close relationship with type 2 diabetes development. Obesity is either induced by feeding high-fat diets to rodents or as a result of mutations that may occur naturally or induce by genetic manipulation (Katsuda *et al.,* 2013). The most

common models include KK MICE, lead$^{/db}$ mice, OLETF rats, TarryHO/jng mice, among others (Islam *et al.,* 2013; King, 2012).

The streptozotocin-induced diabetes model in rats and mice is still the most common, especially in screening novel therapies for antidiabetic effects (Rees & Alcolado, 2005). This is owed to the fact that they are relatively cheaper and more available especially to the scientist in developing countries and suggested as primary screening methods (Katsuda *et al.,* 2013; King, 2012; O'brien *et al.,* 2014).

2.8.2.1 Streptozotocin

Streptozotocin (STZ) is a commonly used diabetogenic compound used in animal models, especially rodents such as mice, rats, guinea pigs, and hamsters (Leiter & Schile, 2013; Omonkhua *et al.,* 2014). Mammals such as monkeys and rabbits are also susceptible to STZ (Goud*et al.,* 2015; Deed *et al*., 2011). It is an aminoglycoside antibiotic produced by the soil bacterium *Streptomyces achromogenes* and exists in two anomeric forms, α, and β (Goud *et al.,* 2015).

Streptozotocin is a glucose analog that selectively enters the β- cells of the Islets of Langerhans in the pancreases through the low-affinity glucose transport proteins (GLUT2).Within the β-cell, STZ induces cell death through several pathways majorly through DNA alkylation (Goud *et al.,* 2015). The alkylation effect is attributed to its nitrosourea moiety (O'brien *et al.,* 2014). Beyond alkylation, STZ also induces cell death by a generation of free radical species, which leads to DNA fragmentation. Further, STZ is nitric oxide (NO) donor where NO has been incriminated in many pathophysiological processes, and it is believed that it helps in destroying the cells by such as restricting mitochondrial ATP generation.

The destruction of pancreatic β cells results in aberrations in insulin production, and consequently, hyperglycemia ensues. This destruction is permanent, and hence the hyperglycemia is sustained. Many variant schedules, dosages, and administration routes for inducing diabetes in mice using STZ have been reported. However, the most common protocol involves the administration of a single high dose of 150-200mg/kg body weight intraperitoneally(Patel *et al.,* 2014). Multiple single-dose regimes where STZ at 40mg/kg body weight intraperitoneally is administered consecutively for five days can also be used (Goud *et al.,* 2015).

It has been shown that there exist sex differences in sensitivity to STZ, with females being less sensitive (Szkudeski, 2001). This phenomenon is attributed to the protective effects of estradiol on β-cells against apoptosis, which is caused by oxidative stress induced by STZ. Further, STZ is preferred to Alloxan, another diabetogenic compound in inducing type 2 diabetes in animals because it is less toxic, produces more sustained hyperglycemia, and has higher induction successes compared to alloxan (Islas-Andrade *et al*., 2000).

2.8.3 Glucose measurement

The idea of the use of biosensor to measure glucose was proposed by Clark and Lyons in 1962 (Joseph Wang, 2001). Since then, a lot of significant strides have been made in the area of glucose measurement due to improved technology after the first kit was developed in 1975 (Wang, 2001) This has seen the development of different types of biosensor most of which are commercially available (Yoo & Lee, 2010). Blood glucose measurement leverages on the assay of interactions of enzymes, hexokinase, glucose oxidase (GOx) or glucose-1-dehydrogenase (GDH).Most biosensors use either GOx or GDH with GOx being the most

common. Hexokinase activity assayed by spectrophotometry is a standard reference method (Wang, 2008)

Any biosensor is made of three parts namely; a biological recognition element that detects a biological element (such as enzyme, antibodies, nucleic acid), a transducer which converts target molecule to a signal and a signal processing system to generate a readable output (Yoo & Lee, 2010). There are five types of transducers; electrochemical, optical, thermometric, piezoelectric, and magnetic. The electrochemical biosensors are the most common which can either be potentiometric, amperometric or conductometric (Jackiewicz, 2016). An enzymatic amperometric glucose biosensor was employed for this study because of its relatively high selectivity for glucose.

The principle behind amperometric biosensors is that the immobilized glucose oxidase catalyzes the oxidation of glucose by molecular producing glucuronic acid and hydrogen peroxide. GOx requires Flavin adenine dinucleotide (FAD) as a redox cofactor which accepts electrons and is reduced to $FADH_2$.

$$\text{Glucose} + O_2 + 2H_2 \rightarrow \text{Gluconic acid} + H_2O_2$$
$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e$$

The hydrogen is oxidized at the anode which recognizes the number of electron transfers which is proportional to glucose molecules in the blood. Non-invasive methods using infra-red technology are also being developed (Lam *et al*., 2010)

2.9 OBJECTIVES

2.9.1 General objective

To determine anti-diabetic efficacy of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides*

2.9.2 Specific objectives

1. To investigate the qualitative phytochemical composition of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides*
2. To investigate the acute toxicity of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* aqueous extracts in mice.
3. To investigate the anti-hyperglycemic effect of aqueous extract of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* in streptozotocin-induced diabetic mice.
4. To investigate the effects of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* aqueous extracts on liver enzymes of treated mice.
5. To investigate the effects of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* aqueous extracts on body weights of treated mice.

2.9.3 Hypotheses

1. *Solanum nigrum* species,i.e.,*Solanum villosum* and *Solanum nigrum* var. *sarrachoides,* have similar phytochemical composition.
2. *Solanum villosum* and *Solanum nigrum* var.s*arrachoides* aqueous leaf extracts do not have significant toxicity in mice.
3. *Solanum villosum* and *Solanum nigrum* var.s*arrachoides* aqueous leaf extracts have significant anti-hyperglycemic effects in mice.
4. *Solanum villosum* and *Solanum nigrum* var.s*arrachoides* aqueous leaf extracts have a significant effect on levels of liver enzymes.
5. *Solanum villosum* and *Solanum nigrum* var.s*arrachoides* aqueous leaf extracts have significant effects on the body weights of treated mice.

2.9.4 Problem statement and Justification

Despite diabetes being among the leading diseases in terms of morbidity and mortality, its management continues to be stifled by myriad of challenges. The high cost of treatment, lack of efficacy, toxicity, and side effects of commonly used drugs are among the challenges. There is, therefore, a need to enhance the search for novel therapies that can help in the management of this condition.

The use of herbal remedies has continuously extended from the rural folk to become a significant source of therapy even for the urban population. Majorly, this is driven by the relatively lower cost and the relatively low risk of side effects compared to conventional drugs. Despite the exponential increase in usage, herbal remedies have significant issues of concern as far as efficacy and safety are concerned.

Scientific information on the efficacy and safety of herbal medicines is either scanty or completely absent. Additionally, research has shown that validation in different geographical regions of the world does not automatically guarantee similar results in a separate area. This is because of the profound environmental influence on the presence of phytochemical compounds in the plant. It is, therefore, necessary to evaluate local species of plants for their efficacy and safety

Solanum nigrum, which is a complex, has several species within it. However, in literature, very few investigators have given the specific species used within this genus *Solanum nigrum*. This occasion a misleading generalization of its effects. There is, therefore, a need to assess the particular species for their safety and efficacy and within specific environmental zones.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Growing of African nightshade (ANS) genotypes

Solanum villosum and *Solanum nigrum* var.*sarrachoides*plants used were grown at KALRO Muguga experimental site during the long rain season. KALRO Muguga is located 27 km northwest of Nairobi at about 2096 m above the sea level. The area receives typically bimodal mean rainfall of 900mm to 1000 mm annually with long rains of 550 mm falling in mid-March to June and the short rains of 400 mm falling in mid-October to December.

3.2 Harvesting and identification of plant samples

Whole plant samples were handpicked from the field and placed in a sisal basket for transportation to the lab. The plant samples were identified at the University of Nairobi Herbarium in the Department of Botany and sample specimens deposited for future reference. The leaves were then cleaned and air-dried at the Department of Veterinary Anatomy and Physiology in Chiromo.

3.3 Preparation of plants for extraction

The leaves were sorted in the lab to remove defective ones and washed in clean running water. Clean leaves were air-dried in the shade for seven days. Once dry, they were weighed and ground into a fine powder ready for extraction.

3.4 Aqueous extracts preparation

The extract was prepared by decoction method using 100 grams of powder leaves in one liter of distilled water for 30 minutes on a hot plate. The decoction was then filtered using Whatman filtered paper (No.2), and the filtrate centrifuged at 5000rpm for 10 minutes to collect the supernatant. This was followed by freeze-drying (Christ Beta 336, Martin Christ Freeze dryers, Osterode Germany).The freeze-dried samples were placed in airtight containers, Labeled and stored at -4^0C for further analysis.

3.5 Phytochemical analysis

The samples were screened for major phytochemical compounds using standard laboratory procedures according to Trease and Evans (2000) as described below;

3.5.1 Test for Alkaloids

A few drops of Wagner's Reagent (Iodine in Potassium Iodide) was added to 3ml extract solution A Brown, reddish precipitate was confirmation of the presence of alkaloids

3.5.2 Tests for Flavonoids: Alkaline Reagent Test

NaOH (2ml of 2% solution) was added to the plant extract solution (3 ml). The formation of an intense yellow color demonstrated the presence of flavonoids. Upon the addition of a few drops of diluted acid, the solution became colorless, which further confirmed the presence of flavonoids.

3.5.3 Test for Coumarins

Half a gram of the extract was added into a test tube covered with a filter paper moistened with 1N NaOH. The tube was put in a hot water bath and then cooled. Yellow fluorescence color was an indication of coumarin

3.5.4 Test for Tannins

The sample (0.5g) was boiled in 20mls of water and filtered. Ferric Chloride (0.1%) was added to the filtrate. The formation of brownish-green or blue-black color was an indication of the presence of tannins.

3.5.6 Test for Phenols: Ferric Oxide Test

Three to four drops of ferric oxide was added to the extract. The formation of bluish-black color was confirmation of the presence of phenols.

3.5.7 Test for Saponins

The crude extract was dissolved in 5ml of distilled water in a test tube and shaken vigorously. Frothing for 20 minutes was an indication of the presence of saponins. Three drops of olive oil were added to the mixture. Formation of an emulsion was further proof of the presence of saponin.

3.5.8 Test for Glycosides

Fehling's reagent was added to the mixture of 10ml of 50% H_2SO_4 and 1ml extract after boiling in a hot water bath. Brick red precipitate indicated the presence of glycosides.

3.6 Acute toxicity assay

The acute oral test was conducted as stipulated out in OECD guidelines 423 (OECD, 2001).In the experiment, female mice used were procured from the Department of Public Health Pharmacology and Toxicology University of Nairobi. Standard laboratory conditions were maintained, i.e., 12hrs light and 12hrs darkness cycle, room temperature, and humidity. The mice were fed on mice chow procured from 'Unga Group Limited' and allowed free access to water. Before the experiment proper, they were allowed to acclimate with laboratory conditions for one week.Normal saline was used to dissolve the aqueous extracts to allow for oral administration using oral gavage. This was done so that no animal receive volumes above 0.1 ml to avoid overhydrating the animals. As per the guideline, animals were first administered with 300 mg/kg bwt; if no mortality were observed, this would be escalated to 2000 mg/bwt.

Similarly, if deaths occurred, the test would be repeated at 5000mg/kg. To ascertain the effects, animals were continuously observed for the initial 30 minutes, in intervals within the first 24 hours, and after thatdaily for up to 2 weeks. Signs of toxicity and their corresponding time of occurrence were noted. Any dead animal was to undergo necropsy and samples taken for histopathology. The outcome of the acute toxicity test help in estimating LD_{50} aimed at placing the extracts in categories (1, 2, 3, 4, or 5) based on the OECD guidelines. At the close of the experiment, the animals were sacrificed under anesthesia (16mg/kg bwt + 60mg/kg bwt (xylazine +ketamine) (Parasuraman*et al.*, 2010).

3.7 Ethical Approval

These studies were conducted at the University of Nairobi campus in Chiromo. Ethical approval was obtained from the ‘‘Biosafety, Animal use, and Care Committee’’ of the Faculty of Veterinary Medicine, University of Nairobi vide letter reference FVM BAUEC/2016/106

3.8 Anti-diabetic Assay

3.8.1 Experimental Animals and Grouping

Mice were purchased from the Department of Public health, Pharmacology, and Toxicology, University of Nairobi. The animals were placed in plastic and acrylic cages (Figure 3)in respective groups of six and maintained in standard laboratory conditions (temperature of between 20-25^{0}C, Humidity about 60% and a 12/12 light day cycle).They were acclimated for two weeks in the laboratory. They were fed on mice chow (Sigma feeds) and water *ad libitum*. For the experiment, they were randomly assigned to experimental and control groups (n =6), as shown in figure 3 and indicated in table 1.

Figure 3: **Photograph showing mice in acrylic cages during the study**

Table 1: **Experimental designs of anti-diabetic properties of aqueous extracts of *S. villosum* (AESV) and *S. nigrum* var.*sarrachoides* (AESS)**

Group	Description	Treatment
1	Normal control	Vehicle (Normal saline)
2	Diabetic (untreated)	Vehicle (Normal saline)
3	Diabetic treated	Glibenclamide (5 mg/kg bwt)
4	Diabetic treated	AESNS (150mg/kg bwt)
5	Diabetic treated	AESNS (300mg/kg bwt)
6	Diabetic treated	AESS (150mg/kg bwt)
7	Diabetic treated	AESS (300mg/kg bwt)

3.8.2 Induction of diabetes

Streptozotocin-induced diabetic mice model was used. Mice fasted for 8 hours before intraperitoneal injection with Streptozotocin (STZ, Sigma Aldrich, USA) single dose of 200mg/Kg body weight. Streptozotocin was reconstituted using sterile normal saline (0.9% Sodium Chloride) at a concentration of 20mg/ml. Blood was collected from the tail veinafter overnight fast using a glucometer (Softstyle®, Chemlabs, Kenya).Mice with fasting blood glucose levels above 250 mg/dl were considered diabetic.

3.8.3 Determination of Blood glucose levels.

Blood was obtained from a prick on the lateral tail vein and blood glucose determined using a glucometer (Softstyle®, Chemlabs, Kenya).Glucose readings were expressed in g/dl. Fasting blood glucose level was determined on days 7, 14, 21, and day 28 to access changes.

3.8.4 Biochemical parameters

Blood for biochemical evaluation was collected via cardiac puncture at euthanasia. The blood was allowed to clot and left for10 minutes at room temperature for the serum to form. Serum was separated by centrifugation at3000rpm for 10 minutes and stored at−20ºC until required for analysis. Liver enzymes, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, were determined using commercial kits (Humalyzer 2000, Human Diagnostics®,Germany) according to the manufacturers' protocol.

3.9 Data analysis

Data was expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) using statistical package SAS version 9.2 of 2008 was used to determined differences between groups means. GraphPad 7 was used for graphical representation. Significant level was set at $P<0.05$. Qualitative assessment was done for the acute-toxicity assay

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Analysis

Characterization of constituent compounds of aqueous extracts of *S. villosum* and *S. nigrum* var. *sarrachoides*leaves showed that they contain bioactive plant compounds that belong to various multi-component classes of; alkaloids, flavonoids, tannins, saponins, phenols, phytosterols, coumarins and glycosides (Table 2). However, phytosterols and coumarins were absent in *S. villosum* extract, as shown in table 2. The results of the qualitative phytochemical analysis are presented in Table 2 below.

Table 2: **Phytochemical Present in Aqueous Extract of The Leaves of *S. villosum* and *S. nigrum*var.*sarrachoides* Leaves.**

Type of phytochemical	**Aqueous extract of *S. villosum* leaves**	**Aqueous extract of *S. nigrum* var.*sarrachoides* leaves**
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Phenols	+	+
Phytosterols	-	+
Coumarins	-	+
Glycosides	+	+

4.2 Acute Toxicity study

4.2.1 Acute Toxic Effects of Aqueous Extracts of S. *villosum* and *S. nigrum* var.*sarrachoides.*

Aqueous extracts of both plants did not cause any mortality even at the highest dose of 2000mg/kg body weight. Equally, there was no discernible change in the demeanor and behavior of the animals during the observation period. There was no color change on the coat of the test animals, even on day 14. Consequently, the tested plants fell on category five at a dose of 2000mg/kg body weight, which has an estimated LD_{50} of 2500 mg/kgbwt according to the guidelines of OECD (2001).

4.3 Anti-diabetic Assay

4.3.1 Effect of induction of diabetes on mice

Immediately upon administration of Streptozotocin intraperitoneally, the mice were observed to raise fur and also showed restricted movements and aggregation when placed back in the cage, as shown in **Figure 4**. Some of the animals passed urine. Two days after the induction process, the diabetic mice appeared lethargic with little activity as compared to the normal control mice. The diabetic mice also presented themselves with anorexia, polyuria, and polydipsia.

Figure 4: **A photo showing aggregation behavior of the mice after injection with Streptozotocin**

4.3.2 Effect of *Solanum villosum* aqueous leaf extracts (AESV) on the fasting blood glucose levels in mice

At the start of the experiment (day 0), all the diabetic mice groups had significantly (p<0.05) higher blood glucose levels as compared to the non-diabetic ones (Table 3). Administering 300 mg/kg body weight of *S. villosum* aqueous leaves extract produced significant (p<0.05) reduction in fasting blood glucose levels as compared to the untreated diabetic group by day 14 (Table 3). This, however, was significantly (p<0.05) higher as compared to the standard drug glibenclamide at 5mg/kg body weight (Table 3). Notably, on the administration of aqueous extracts of *S. villosum* (at a dose of 150 mg/kg body weight), there was no significant difference (p>0.05) compared to the untreated diabetic group by day 14.

On increased dosage to 300 mg /kg body weight, there was a significant (p<0.05) reduction in fasting blood glucose levels of the mice when compared to the diabetic mice on 21 of the study. At 150 mg/kg body weight, there was no significant reduction in blood glucose between the treated and untreated diabetic mice on day 21 (Table 3).

After 28 days of administration with *S. villosum* extracts (of 150mg and 300mg), the mice showed significant (p<0.05) reduction in levels of fasting blood glucose when compared to both the diabetic untreated and normal mice. Additionally, at 150 mg/kg body weight administration of the extract, the fasting blood glucose levels were significantly (p<0.05) higher as compared to the mice that received 5 mg/kg body weight of glibenclamide. However, there was no significant (p>0.05) difference in levels of fasting blood glucose between the mice treated with 300 mg extract and those treated with glibenclamide drug (Table 3).The percentage change in blood glucose levels per group is shown in table 4 below.

4.3.3 Effect of S*olanum nigrum* var.*sarrachoides* aqueous leaf extracts (AESS) on the fasting blood glucose levels in mice.

Administration of 150 mg and 300 mg/kg body weight S. *nigrum* var.*sarrachoides* leaves aqueous extract to diabetic mice induced a significant ($p<0.05$) reduction in blood glucose level as compared to the untreated diabetic group (Table 3). This was, however, significantly ($p<0.05$) higher than of the mice treated with glibenclamide (5 mg/kg body weight) on day 14. There was no significant ($p<0.05$) difference in the levels of fasting blood glucose between mice treated with the extract at a dose of 300mg per kg body weight and those treated with glibenclamide by day 28 (Table 3). The percentage change in blood glucose levels per group is shown in table 4 below.

Table 3: **Fasting Blood Glucose levels in mice.**

Group		Blood Glucose Level in mg/dl (Lsmean±SEM)				
	Description	Initial	Day 7	Day 14	Day 21	Day 28
G1	Normal	102.52±11.34[b]	99.8±14.35[b]	98.98±12.53[f]	98.1±12.10[d]	102.67±10.97[c]
G2	Diabetic (untreated	305.58±12.35[a]	323.4±12.41[a]	341.75±12.34[a]	381.32±13.41[a]	368.23±13.37[a]
G3	Glibenclamide	337.18±11.82[a]	296.08±13.21a	191.53±13.12[cde]	126.17±14.21[d]	104.60±12.94d[ce]
G4	AESV 150mg/kg bwt	317.37±12.34[a]	322.78±11.62[a]	288.58±11.72[ab]	276.63±13.13[b]	272.10±11.81[b]
G5	AESV 300mg/kg bwt	321.15±13.10[a]	303.72±15.13[a]	247.98±12.81[bc]	214.52±12.68[c]	170.18±13.14[ce]
G6	AESS 150 mg/kg bwt	305.33±12.16[a]	273.25±13.26[a]	230.18± 13.16[be]	203.08±12.34[c]	196.32±12.61[c]
G7	AESS 300mg/kgbwt	333.77±11.76[a]	293.90±12.68[a]	238.45±12.71[bd]	198.13±12.21[c]	162.25± 13.42[ce]

All values are expressed as Mean ±SEM (n=6). One way ANOVA was done, followed by Turkey's test. Means in a column with one or more letter superscript in common are not significantly different ($p<0.05$).

Table 4: Percentage change in blood glucose levels in diabetic mice administered with different treatments for four weeks.

Treatment	**Change in blood glucose levels (%)**
Standard drug (5mg/kg bwt)	68.5
*S. Villosum (*150 mg /kg bwt)	14.2
*S. Villosum (*300mg/kg bwt)	47
S. nigrum var. *sarrachoides (*150 mg /kg bwt)	35.6
S.nigrum var. *sarrachoides (*300mg/kg bwt)	51.3

4.4 Effects of administration aqueous extracts of *Solanum villosum and Solanum nigrum* var. *sarachoides* on the liver function test.

4.4.1 Alanine aminotransferase (ALT) levels

There was no significant (p>0.05) difference in the levels of ALT between the mice groupstreated with aqueous extracts of *S. villosum* and those that were treated with either glibenclamide (5 mg/kg) or aqueous extracts of *S. nigrum* var. *sarrachoides*(Figure 5). This was observed on both dosages of 150 mg and 300 mg/kg body weight (Figure 5). However, there was a significant difference in the levels of ALT between the diabetic control mice and either the normal, glibenclamide, AESV or AESS treated mice (Figure 5)

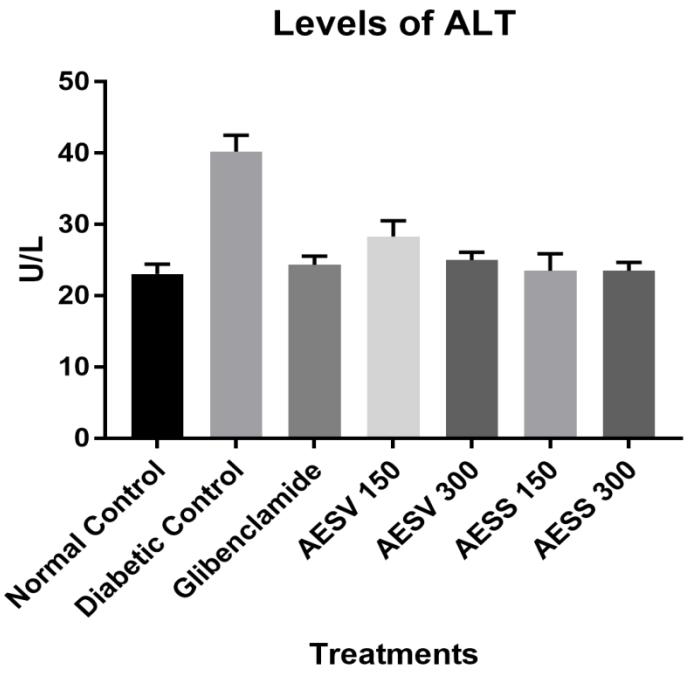

Figure 5: Levels of ALT in normal and diabetic mice groups

4.4.2 Aspartate Aminotransferase (AST) levels

The levels of AST in the serum of the diabetic control mice was significant ($p<0.05$) higher than those of the normal control, glibenclamide treated, S. *villosum* treated, and *S. nigrum* var. *sarrachoides* treated (Figure 6). In addition, the levels of AST in the mice that received glibenclamide (5mg/kg body wt), *S. villosum* (300 mg/kg body wt),and *S. nigrum* ar. *Sarrachoides* (300 mg/kg body wt) were not significantly different ($p>0.05$) from those of the normal control group (Figure 6).

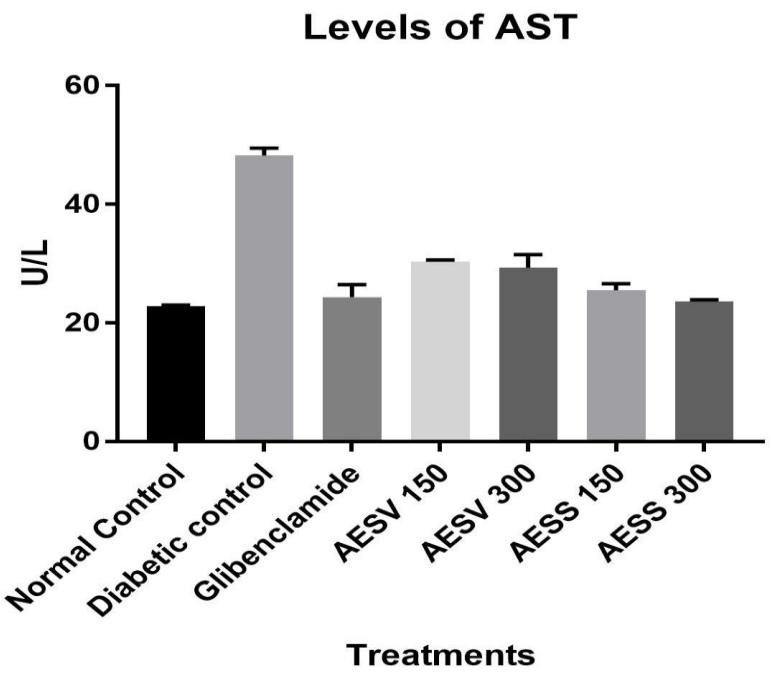

Figure 6: Levels of AST in normal and diabetic mice groups

4.4.3 Alkaline phosphatase levels

Results show no significant ($p<0.05$) differences in the serum ALP levels between mice that were treated Aqueous extracts of *Solanum villosum*150 mg and 300 mg per kg bodyweight, mice that received aqueous extracts of *Solanum nigrum* var. *sarrachoides* 150 mg and mice that received when compared to the diabetic control group (Figure 6). The results also show significant ($p<0.05$) difference in mice that received Glibenclamide at 5 mg/kg/bdwt *Solanun nigrum* var. *sarrachoides*300 mg kg bodyweight; this was, however, not significant when compared to the normal control group (Figure 7).

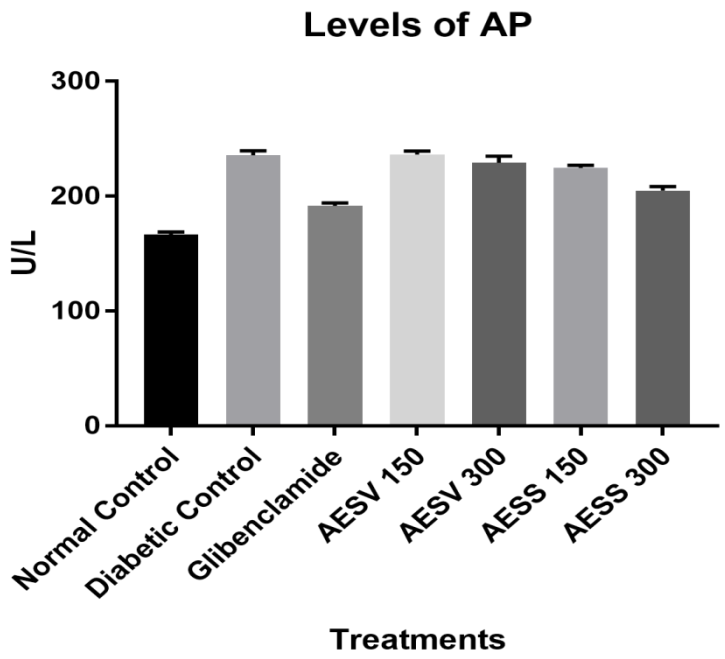

Figure 7: Levels of AST in normal and diabetic mice groups

4.5 Effect of administration of aqueous extract of *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* on body weights.

There was a significant ($p<0.05$) loss of body weight in diabetic mice as compared to the normal mice after seven days (Table 5). After two weeks, the weight loss of the diabetic mice was significantly higher than of the diabetic ones that were treated with either glibenclamide, AESV or AESS (Table 7). The body weights of diabetic mice treated with *S. villosum* (at dosages of 150 and 300mg/kg body wt) were similar ($p>0.05$) to those of glibenclamide treated group(Table 5). There was no significant ($p>0.05$) difference in body weight of mice treated with 150 mg/kg b wt extracts of either *S. villosum*or *S. nigrum* var. *sarrachoides* and those of the diabetic control group (Table 5).

Mice that received aqueous extracts *of S. nigrum* var. *sarrachoides* at both 150 mg and 300 mg exhibited a decrease in body weight that was significantly ($p<0.05$) lower as compared to the untreated diabetic mice (Table 5). The decrease in body weight on treatment with extracts of either *S. villosum*or *S. nigrum* var.*sarrachoides* (for both dosages of 150 and 300mg/kg body wt) was not significantly ($p>0.05$) different to those treated with glibenclamide (Table 5). Weight loss was highest with *Solanum villosum* at 150 mg/kg body weight (Table 5). The percentage of changes in body weights is shown in table 6 below.

Table 5: **Means of body weights of all test mice for 28 days**

Group		Body weight in Grams (Lsmean±SEM)				
	Treatment	Initial	Day 7	Day 14	Day 21	Day 28
G1	Normal Mice	27.11±2.43[a]	26.64±1.43[a]	26.44±2.17[a]	27.79±3.01[a]	32.04±4.81[a]
G2	Diabetic Non-treated	26.24±3.21[a]	21.32±2.69[b]	19.39±1.24[c]	18.59±3.27[b]	15.89±2.17[c]
G3	Glibenclamide	26.86±1.34[a]	22.53±2.18[b]	24.11±3.12[ab]	21.51±2.59[b]	22.79±3.27[b]
G4	AESV 150mg/kg body weight	26.77±4.12[a]	21.72±3.10[b]	21.97±2.87[bc]	20.55±1.83[b]	18.84±1.59[bc]
G5	AESV 300 mg/kg body weight	26.50±4.31[a]	23.17±2.89[ab]	22.30±3.13[abc]	22.18±2.55[b]	20.80±1.97[b]
G6	AESS 150 mg/ kg body weight	26.30±5.32[a]	23.37±1.89[ab]	23.05±2.64[abc]	20.67±3.14[b]	20.92±3.96[b]
G7	AESS 300 mg/ kg bodyweight	26.41± 2.91[a]	24.74±2.69[ab]	21.18±4.26[bc]	22.33±4.71[b]	22.33±3.42[b]

All values are expressed as mean ±SEM (n=6). One way ANOVA was done, followed by Tukey's test. Means in a column with one or more letter superscript in common are not significantly different ($p<0.05$).

Table 6: **Percentage change in the bodyweight of diabetic mice administered with different treatments for four weeks.**

Treatment	**Change in blood glucose levels (%)**
Standard drug (5mg/kg bwt)	68.5
*Solanum villosum (*150 mg /kg bwt)	14.2
*Solanum villosum (*300mg/kg bwt)	47
Solanum nigrum var. *sarrachoides (*150 mg /kg bwt)	35.6
Solanum nigrum var. *sarrachoides (*300mg/kg bwt)	51.3

CHAPTER FIVE

5.0 DISCUSSION

The study aimed at evaluating the antidiabetic properties of two species in the *Solanumnigrum* complex, i.e.,*Solanum villosum* and *Solanum nigrum* var. *sarrachoides*using streptozotocin-induced diabetic mice model. From the results, it shows that aqueous extracts of leaves from both *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* possess significant antidiabetic ameliorating effects in the model used. The findings are discussed therein;

5.1 Phytochemical Evaluation

In the qualitative phytochemical analysis, both extracts showed the presence of important phytochemical compounds that have been shown to impart medicinal properties in plants (Anandan *et al.*, 2015; Gogoi, 2012). Specific secondary metabolites that have been shown to confer anti-diabetic effects include glycosides, alkaloids, terpenoids, and flavonoids (Wang *et al.*, 2013). In addition to their antidiabetic effects, these chemicals also possess other beneficial effects, including antioxidant, anticancer, anti-inflammatory properties that have been demonstrated in many studies (Chauhan *et al.*, 2012; Wais *et al.*, 2012; Wang *et al.*, 2013). In this study, the aqueous extracts from leaves of *Solanum nigrum* var. *sarrachoides* possessed all the phytochemicals tested, i.e., flavonoids, alkaloids, tannins, saponins phenols phytosterols, coumarins, and glycosides. Comparatively, *Solanumvillosum* extract showed the presence of all the phytochemicals,exceptphytosterols and coumarins. This corroborates with the findings of Matasyo*et al.,* (2013) that showed different species in the *Sonalum nigrum* complex have different phytochemical composition. The results agree with those of Bella *et al.* (2016), who reported the presence of tannins, alkaloids, flavonoids, and saponins. The differences in the antidiabetic properties of the two extracts observed in this study can be

attributed to this difference. Primarily the absence of phytosterols, which have been shown to possess anti-diabetic effect (Wang *et al.,* 2017).

Additionally, the finding is significant because it buttresses the need for individual species within the *Solanum nigrum* to be assessed for its anti-diabetic properties. The results agree with other researchers who have shown the presence of important phytochemicals in *Solanum nigrum* (Anandan *et al.,* 2015; Gogoi, 2012). Further, differences in soils and climatic conditions prevalent in different regions occasion the differences in phytochemical composition (Kokwaro, 1993).

5.2 Acute toxicity studies.

The safety of any drug is of paramount importance and can be a serious limiting factor to its utilization. OECD has developed guidelines (OECD 423) for an effective assessment of the safety of herbal remedies (OECD, 2001). The present study revealed that both the extracts have considerably wide safety margins. The guidelines emphasize the following as the significant signs that point to toxicity; respiratory, circulatory, behavioral changes convulsions, lethargy, diarrhea, tremors, salivation coma, or death. None of these signs were observed upon extract administration.

Notably, results from the present study agree with others reported (Jagatheeswari *et al.,* 2013). The present study, therefore, buttresses the evidence already present the safety of *Solanum nigrum* complex species. It can be concluded that both species are safe with an estimated LD_{50} of 2500 mg/kgbwt, which cannot prevent their utilization in the field of herbal medicines.

5.3 Hypoglycaemic effect

In the present study, aqueous extracts of leaves from both plants were observed to have significant antidiabetic effect compared to untreated diabetic mice. Daily administration of the extracts at 150 mg and 300 mg per kg bodyweight for four weeks produced a dose-dependent reduction in fasting blood glucose levels. These results agree with those of other researchers who have reported similar effects (Abu *et al.,* 2017; Ahiret *et al.,* 2013; Atanu *et al.,* 2011; Tiwari & Jain, 2012). The glucose-lowering effects of the extract are postulated to be a result of the potentiating effect of the extract that is exerted on insulin release, which enhances its release from remnants of β-cells of the Islets of Langerhans in the pancreas (Forbes & Cooper, 2013). The extracts may also mediate extra-pancreatic action in glucose metabolism pathways by stimulating an increase in peripheral glucose uptake and a decrease in gluconeogenesis and glycogenesis with the net effect of reducing blood glucose levels. In addition, antioxidant activities help in the amelioration of the impact(Jun & Yoon, 2001).

Further, inhibition of activities of enzymes α-glucosidase and pancreatic lipase has been suggested as a mechanism for antidiabetic effect (Qi *et al.,* 2012).Although the extracts showed a significant reduction in fasting blood glucose, *S. nigrum* var. *sarrachoides* exhibited a higher potency in the hypoglycaemic effect. At the dosage of 150 mg/ kgbwt, it reduced the blood glucose levels significantly from day 14 compared *to Solanum villosum* that showed a significant reduction from day 21 at the same dosage. However, both extracts at a dosage of 300 mg reduced the fasting blood glucose level significantly. This reduction was as good as the reduction exhibited by glibenclamide. The above is in contrast with the 150 mg dosages whose levels were higher compared to the glibenclamide group. Therefore, both extracts showed dose-dependent effects. The above-described phenomenon can be attributed to differences in active phytochemicals and their concentrations, including the

peculiarity of their respective modes of action. The secondary metabolites have different action sites in the body that can influence their action (Gogoi, 2012).

5.4 Liver enzymes

Diabetes mellitus manifests itself with alteration in the metabolism of glucose and lipids. Consequently, this causes the pathology of various organs, including arteriosclerosis, atherosclerosis, nephropathy, and neuropathy (Pasquel&Umpierrez, 2014; Pop-Busui*et al.*, 2017). Transaminases are marker enzymes in the blood that are important in the manifestation of liver or heart damage. Increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) are suggestive of liver and heart damage. It has been observed that the administration of STZ in mice causes hepatotoxicity that causes leakage of these marker enzymes into the blood in a dose-dependent manner (Omonkhua*et al.*, 2014). Streptozotocin at 200mgkg body in mice used in this study induced severe diabetes in mice (Goud*et al.*, 2015). The ability to ameliorate diabetic effects causes a reduction in the levels of these enzymes.

In the present study, all extracts showed a significant reduction in levels of ALT compared to the untreated diabetic group. The reduction was, however, not sufficient to revert the enzymes to the normal levels. Characteristically, this phenomenon can be attributed to the inability of the treatments to restore the normal state of functioning of the hepatocytes damaged by STZ (Omonkhua*et al.*, 2014). Similarly, the extract resulted in a reduction of AST levels when compared to the untreated diabetic control. The resultant reduction was significantly different when compared to the glibenclamide treated mice. However, *S. nigrum var sarrachoides*at 300 mg produced a reduction that was not significantly different compared to glibenclamide. This suggests that the latter was more effective. This can be attributed to the presence of phytochemicals in increased concentration (Aba & Asuzu, 2018). In the AP assay, the extracts from both plants did not produce a significant reduction in levels

apart from *S. nigrum* var. *sarrachoides*, which produced a reduction that was not significantly different when compared to both normal and glibenclamide treated mice. Therefore, these findings suggest an increased potency. These results show that *Solanum villosum* and *Solanum nigrum* var. *sarrachoides* possess some protective effects on the liver.

Further, the results agree with the findings of other researchers (Maniyar, 2012; Razali*et al.,* 2014).This can be attributed to the presence of tannins and flavonoids (Gogoi, 2012). From the study, it can be concluded that *S. nigrum var.sarrachoides* at 300 mg/kgbwt is more potent in reversing the liver damage in this model.

5.5 Effect on Bodyweight

Diabetes is a debilitating condition that is characterized by remarkable weight loss. The weight loss occurs as a result of aberration in the body's metabolic processes (Forbes & Cooper, 2013). Therefore, amelioration of weight loss is an indicator of the improvement of metabolic properties. Diabetic mice showed a significant loss in body weight when compared to the normal non-diabetic mice. This finding agrees with those of Wang *et al.,* 2013 being reflective of the situation in diabetes mellitus, where the patients experience progressive weight loss (Stanifer*et al.,* 2016). In this case, the loss in body weight after diabetes induction using STZ has been attributed to increased catabolic reactions in the body (Goud et al., 2015). Diabetes mellitus occasions aberrations in glucose homeostasis, and hence the body mobilizes protein sources for energy resulting in muscle wasting (Deed *et al.*,2011). In the present study, the administration of aqueous extracts of *Solanum villosum* at 300 mg/kgbwt and *S. nigrum* var. *sarrachoides* at 150 mg/kgbwt and 300 mg/kgbdwt produced a significant reduction in body weight compared to the untreated diabetic mice. The results can be attributed to improving glycaemic control imparted by the antidiabetic elements in the plant. Many plants extracts have been shown to ameliorate body weight loss (Nasser *et al.,* 2014)

Solanum villosum extract at 150 mg/kgbwt produced weight loss that was not significantly different when compared to the untreated diabetic mice. This finding can be attributed to the reduced potency of the extract for antihyperglycemic control and points to a dose-dependent potency of *Solanum villosum*. Other researchers have recorded a dose-dependent activity in *Solanum nigrum* (Lettre& Linn, 2010; Tiwari& Jain, 2012). It is worthwhile to note that all diabetic- treated mice were unable to gain their initial weight at the end of the experiment. Glibenclamide treated mice had a significant loss of 15% of their initial weight. This phenomenon can be attributed to the severe extent of damage of β-cells of the Islets of Langerhans in the pancreases with only minimal residual effect functional capacity being regained.

5.6 Conclusions

As per the results from this study, the following conclusions are made;

1. Both plants possess phytochemicals that impart medicinal properties. *Solanum nigrum* var.*sarrachoides* showed the presence of all phytochemical assessed while *Solanum villosum* lacked phytosterols and coumarins.
2. Both *Solanum nigrum* var.*sarrachoides and Solanum villosum* did not show any toxicity. It is therefore concluded that both plants have a relatively wide margin of safety.
3. Both plants showed significant anti-diabetic effects on mice at 300mg per Kg bodyweight hence validating their use in folklore medicine.
4. Both plants showed a dose-dependent amelioration of bodyweight loss that matched the antihyperglycemic activity.
5. Comparatively, *Solanum nigrum* var. *sarrachoides s*howed to be more effective in ameliorating diabetic effects.

5.7 Recommendations

Given the findings of this study, the following recommendations are made;

1. More research to be done to quantify the phytochemicals present in *S. villosum* and *S. nigrum* var. *sarrachoides.*
2. Further validation of the antidiabetic properties in other animal models to corroborate the findings in this study.
3. Additional toxicity studies to be done to ascertain further the safety properties of *villosum* and *S. nigrum* var.*sarrachoides.*
4. It is recommended that more robust studies be carried out on the species in the *Solanum nigrum* complex.
5. Additional studies to determine the optimal dosages for effective use of *S. villosum* and *S. nigrum* var. *sarrachoides* for management of diabetes.

REFERENCES

Aba, P. E., & Asuzu, I. U. (2018). Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants : A review, ***9***(June), 85–96.

Abiola, D., Sathyapalan, T., & Hepburn, D. (2016). Management of type 1 and type 2 diabetes requiring insulin. *Prescriber*, ***27***(9), 50–57.

A**bu, M., Nyeem, B., Mamun, A., Rashid, U., Nowrose, M., Hossain, M. A., & Abu, C. M. (2017)**. Solanum nigrum (Maku): A review of pharmacological activities and clinical effects, ***3***(1), 12–17.

Ahir, K. B., Patel, B. G., Patel, S. B., & Mehta, F. A. (2013). Effect of Solanum nigrum L. on blood glucose concentration and lipid profile in normal and STZ-induced diabetic rats. *Pharmacognosy Communications*, ***3***(23)

Al-Noaemi, M. C., & Shalayel, M. H. F. (2011). Pathophysiology of gestational diabetes mellitus: the past, the present and the future. *Gestational Diabetes*, 91–114.

Alberti, K. G. M. M., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Medicine : A Journal of the British Diabetic Association*, ***15***(7), 539–553

Alves, C., Casqueiro, J., & Casqueiro, J. (2012). Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian Journal of Endocrinology and Metabolism*, ***16***(7), 27.

Antu, K. A., Riya, M. P., Mishra, A., Anilkumar, K. S., Chandrakanth, C. K., Tamrakar, A. K., … Raghu, K. G. (2011). Antidiabetic Property of Symplocos cochinchinensis Is Mediated by Inhibition of Alpha Glucosidase and Enhanced Insulin Sensitivity, ***9*(9), 1–13.**

American Diabetes Association. (2004). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, ***27***(Supplement 1), S5–S10.

Ammari, F. (2004). Long-term complications of type 1 diabetes mellitus in the western area of Saudi Arabia. *Diabetologia Croatica*, ***33** (2)*, 59–63.

Anandan, R., Sindhu, R., Logeshwari, M. N., Nadu, T., Kolambakkam, C., Nadu, T., … Nadu, T. (2015). Screening of Solanum Nigrum for Its Phytochemical and Antimicrobial Activity Against Respiratory Tract Pathogens,***3***(3), 210–215.

Atanu, F., Ebiloma, U., & Ajayi, E. (2011). A Review of the Pharmacological Aspects of Solanum nigrum Linn. *Biotechnology and Molecular Biology Reviews*, ***6***(1), 001–007.

Becker, T., & Hux, J. (2011). Risk of acute complications of diabetes among people with schizophrenia in Ontario, Canada. *Diabetes Care*, ***34***(2), 398–402. h

Bellah, S. F., Alam, F., & Faisal, S. S. (2016). Phytochemical screening of Solanum nigrum L , S . myriacanthus Dunal , S . myriacanthus Dunal , Solanum melongena and Averrhoa bilimbi in Bangladesh, *J.medicinal plants **5**(7)456-553*

Booth, G. L., Luo, J., Park, A. L., Feig, D. S., Moineddin, R., & Ray, J. G. (2017). Influence of environmental temperature on risk of gestational diabetes. *Canadian Medical Association Journal*, ***189***(19), E682–E689.

Brennan, E., McEvoy, C., Sadlier, D., Godson, C., & Martin, F. (2013). The Genetics of Diabetic Nephropathy. *Genes*, ***4***(4), 596–619.

Brugnara, L., Murillo, S., Novials, A., Rojo-Martínez, G., Soriguer, F., Goday, A., … Ortega, E. (2016). Low physical activity and its association with diabetes and other cardiovascular risk factors: A nationwide, population-based study. *PLoS ONE*, ***11***(8), 1–12.

Cahn, A., & Cefalu, W. T. (2016). Clinical Considerations for Use of Initial Combination Therapy in Type 2 Diabetes, ***39***(August), 137–145.

Chamberlain, J. J., Rhinehart, A. S., Jr, C. F. S., & Neuman, A. (2016). *Annals of Internal Medicine Diagnosis and Management of Diabetes*: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes.

Chaudhury, A., Duvoor, C., Reddy Dendi, V. S., Kraleti, S., Chada, A., Ravilla, R., … Mirza, W. (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Frontiers in Endocrinology*, *8*(JAN).

Chauhan, A., Ruby, K. M., Shori, A., & Dwivedi, J. (2012). Solanum nigrum with dynamic therapeutic role: A review. *International Journal of Pharmaceutical Sciences Review and Research*, ***15***(1), 65–71.

Coustan, D. R. (2013). Gestational diabetes mellitus. *Clinical Chemistry*, ***59***(9), 1310–1321.

Chen, J., Jing, X., Liu, X., Volkmann, A., Chen, Y., Liu, Y., Id, J. M. (2019). Assessment of factors affecting diabetes management in the City Changing Diabetes (CCD) study in Tianjin *Plose One)*, **1–13.**

Davies, G., Lobanova, L., Dawicki, W., Groot, G., Gordon, J. R., Bowen, M., … Arnason, T. (2017). Metformin inhibits the development , and promotes the resensitization , of treatment- resistant breast cancer, 1–22.*PLoS ONE***12**(12): e0187191

Deepthi, B., Sowjanya, K., Lidiya, B., Rs, B., & Ps, B. (2017). A Modern Review of Diabetes Mellitus : An Annihilatory Metabolic Disorder.*iMedPub Journals* : Approach considerations Pathophysiology, 1–5.

Dryden, M., Baguneid, M., Eckmann, C., Corman, S., Stephens, J., Solem, C., … Haider, S. (2015). Pathophysiology and burden of infection in patients with diabetes mellitus and peripheral vascular disease: Focus on skin and soft-tissue infections. *Clinical Microbiology and Infection*, ***21*,** S27–S32.

Edmonds, J. M., & Chweya, J. A. (1997). Black Nightshades.efficacy and phytochemical screening of selected plants used in.

El-busaidy, H., Dawood, M., Kasay, A., Mwamlole, C., Koraya, N., & Parpia, H. (2014). How Serious is the Impact of Type II Diabetes in Rural Kenya? *The Open Diabetes Journal*,***7***(1), 1–4.

Fishbein, H., & Palumbo, P. (1995). Acute metabolic complications in diabetes mellitus. *"Diabetes In America,"****131***(5), 283–92.

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of Diabetic Complications. *Physiological Reviews*, ***93***(1), 137–188.

Gan, Y.-H. (2013). Host Susceptibility Factors to Bacterial Infections in Type 2 Diabetes. *PLoS Pathogens*, ***9***(12),

Gardiner, T. A., Archer, D. B., Curtis, T. M., & Stitt, A. W. (2007). Arteriolar involvement in the microvascular lesions of diabetic retinopathy: Implications for pathogenesis. *Microcirculation*, ***14***(1), 25–38.

Gogoi, P. (2012). Phytochemical Screening of Solanum nigrum L and S.myriacanthus Dunal from Districts of Upper Assam, India. *IOSR Journal of Pharmacy (IOSRPHR)*, ***2***(3), 455–459.

Golen, L. W. Van, Veltman, D. J., Ijzerman, R. G., Deijen, J. B., Heijboer, A. C., Barkhof, F., … Diamant, M. (2014). Effects of Insulin Detemir and NPH Insulin on Body Weight and Appetite-Regulating Brain Regions in Human Type 1 Diabetes : A Randomized Controlled Trial, ***9***(4), 1–7.

Goud, B. J., Dwarakanath, V., & Chikka swamy, B. K. (2015). Streptozotocin - A Diabetogenic Agent in Animal Models. *Human Journals*, ***3***(1), 253–269.

Haththotuwa, R. (2016). Cardiovascular complications of diabetes. *InnovAiT: Education and Inspiration for General Practice*, ***9***(11), 694–701.

Heyne, G. W., Melberg, C. G., Doroodchi, P., Parins, K. F., Kietzman, H. W., Everson, J. L., … Lipinski, R. J. (2015). Definition of critical periods for hedgehog pathway antagonist-induced holoprosencephaly, cleft lip, and cleft palatee0120517. *PLoS ONE*, ***10***(3), 1–14.

Home, P., Riddle, M., Cefalu, W. T., Bailey, C. J., Bretzel, R. G., Prato, S. Del, … Raz, I. (2014). Insulin therapy in people with type 2 diabetes: Opportunities and challenges. *Diabetes Care*, ***37***(6), 1499–1508.

International Diabetes Federation Diabetes Atlas (2019).*Idf diabetes atlas*.

International Diabetes Federation (2015): Diabetes Atlas 7th Edition. Diabetes by country.http://www.diabetesatlas.org/,Accessed 30th Nov 2016

Invest, D., & Kir, W. (2010). Sulfonylurea action re-revisited, ***1***(1), 37–39.

I**rons, B. (2013).** New Pharmacotherapies for Type 2 Diabetes. *PSAP - Cardiology and Endocrinology*, *35*, 21.

I**slam, S., Koya, D., & Portha, B. (2013).** Animal Models of Diabetes and Its Associated Complications. *Journal of Diabetes Research*, *2013*, 1.

Ja**ckiewicz, J. (2016).** Recent Trends in the Development of Gurson's Model. *Recent Trends in Fracture and Damage Mechanics*, ***3***(1), 417–442.

Jagatheeswari, D., Bharathi, T., & Ali, H. S. J. (2013). Black Night Shade (Solanum nigrum L .) - An Updated Overview, ***4***(2), 288–295.

Joshi, S. R., Gupta, S., Gupte, S., Divakar, H., Misra, S., Thanawala, U., … Magon, N. (2013). Diagnosis and Management of Gestational Diabetes Mellitus : Indian. *The Association of Physician of India*, 202–204.

J**un, H. S., & Yoon, J. W. (2001).** The role of viruses in Type I diabetes: Two distinct cellular and molecular pathogenic mechanisms of virus-induced diabetes in animals. *Diabetologia*, ***44***(3), 271–285.

Kamau, L. N., Mbaabu, M. P., Mbaria, J. M., Karuri, G. P., & Kiama, S. G. (2016). Knowledge and demand for medicinal plants used in the treatment and management of diabetes in Nyeri County, Kenya. *Journal of Ethnopharmacology*, ***189,*** 218–229.

K**amau, L. N., Mbaabu, P. M., Karuri, P. G., Mbaria, J. M., & Kiama, S. G. (2017).** Medicinal plants used in the management of diabetes by traditional healers of Narok County, Kenya. *Tang [Humanitas Medicine]*, ***7***(2), 10.1-10.10.

Kanungo, A., Jhingan, A., Sahay, R. K., Muruganathan, A., Das, A. K., Hospital, A. G., & Colony, K. P. N. (2014). Consensus Evidence-based Guidelines for Insulin Therapy in Patients with Type 1 Diabetes Mellitus as per Indian Clinical Practice, ***62***, 26–33.

Karunakar, t. (2017). Studies on phytochemical analysis of ethanolic extract of leaves of solanum nigrum l ., ***4***(4), 378–383.

K**asali, F., Kadima, J., & Mpiana, P. (2013).** Assessment of antidiabetic activity and acute toxicity of leaf extracts from Physalis peruviana L. in guinea-pig. *Asian Pacific Journal of*, ***4***(6), 14–19.

Katsuda, Y., Ohta, T., Shinohara, M., Bin, T., & Yamada, T. (2013). Diabetic mouse models. *Open Journal of Animal Sciences*,***3***(4), 334–342.

King, A. J. F. (2012). The use of animal models in diabetes research. *British Journal of Pharmacology*, ***166***(3), 877–894.

Kokwaro, J. O. (1993): Medicinal plants of East Africa, (2nd Edition). Nairobi: East African Literature bureau.

Konstantinos Papatheodorou, Nikolaos Papanas, Maciej Banach, Dimitrios Papazoglou, andMichael E. (2016). Complications of Diabetes. *Complications of Diabetes 2016*, **4**(7), 3–5.

Lam, S. C. H., Chung, J. W. Y., Fan, K. L., & Wong, T. K. S. (2010). Non-invasive blood glucose measurement by near infrared spectroscopy: Machine drift, time drift and physiological effect. *Spectroscopy*, ***24***(6), 629–639.

Leiter, E. H., & Schile, A. (2013). Genetic and Pharmacologic Models for Type 1 Diabetes. *Curr Protoc Mouse Biol*, ***3***(1), 9–19.

Lettre, D. P., & Linn, S. (2010). Anti-Hyperglycemic potential of aqueous extract of leaves of, ***2***(3), 168–179.

Lim, A. K. H. (2014). Diabetic nephropathy ? Complications and treatment. *International Journal of Nephrology and Renovascular Disease*,***7***, 361–381.

Liu, Z., Fu, C., Wang, W., & Xu, B. (2010). Prevalence of chronic complications of type 2 diabetes mellitus in outpatients - a cross-sectional hospital based survey in urban China. *Health and Quality of Life Outcomes*, ***8***(1), 62.

Mamun-or-Rashid, A., Shamim Hossain, M., Hassan, N., Kumar Dash, B., Ashrafuzzaman Sapon, M., & Kumer Sen, M. (2014). A review on medicinal plants with antidiabetic activity. *Journal of Pharmacognosy and Phytochemistry JPP*, ***149***(34), 149–159.

Mane, P. B., Antre, R. V, & Oswal, R. J. (2012). Antidiabetic Drugs : An Overview. *International Journal of Pharmaceutical and Chemical Sciences*, ***1***(1), 301–306.

Matasyoh, L. gomezgani, & Bosire, A. N. (2016). The Solanum nigrum Complex (Black Night Shade) Grown in the Rift Valley, Western and Nyanza Provinces of Kenya. *Journal of Life Sciences*, ***10***(5), 228–232.

MC Deeds, JM Anderson, AS Armstrong, DA Gastineau, HJ Hiddinga, A Jahangir, N., & Eberhardt, and Y. K. (2011). Single Dose Streptozotocin Induced Diabetes: Considerations for Study Design in Islet Transplantation Models. *Laboratory Animals*, ***45***(3), 131–140.

Meek, C. L., Lewis, H. B., Patient, C., Murphy, H. R., & Simmons, D. (2015). Diagnosis of gestational diabetes mellitus: falling through the net. *Diabetologia*, ***58***(9), 2003–2012.

Mujesh, R., & Namita, P. (2013). Medicinal Plants with Antidiabetic Potential - A Review. *American-Eurasian Journal of Agriculture and Environmental Science*, ***13***(1), 81–94.

Muller, L. M. A. J., Gorter, K. J., Hak, E., Goudzwaard, W. L., Schellevis, F. G., Hoepelman, A. I. M., & Rutten, G. E. H. M. (2005). Increased Risk of Common Infections in Patients with Type 1 and Type 2 Diabetes Mellitus, **5**(6), 281–288.

Nasser Singab, A., & Youssef, F. S. (2014). Medicinal Plants with Potential Antidiabetic Activity and their Assessment. *Medicinal & Aromatic Plants*, ***3***(1), 1–12.

OECD . (2001). Acute Oral Toxicity – Acute Toxic Class Method, (Guidelines), 1–14.

O**'brien, P. D., Sakowski, S. A., & Feldman, E. L. (2014).** Mouse models of diabetic neuropathy. *ILAR Journal*, ***54***(3), 259–272.

O**monkhua, A. A., Adebayo, E. A., Saliu, J. A., Ogunwa, T. H., & Adeyelu, T. T. (2014).** Liver function of Streptozotocin- Induced Diabetic Rats Orally Administered Aqueous **R**oot-Bark Extracts of Tetrapleura tetraptera (Taub)., ***22,*** 99–106.

Ontita, E., Onyango, C., Onwonga, R., & Nyamongo, D. (2016). Indigenous Knowledge on the Uses of African Nightshades (Solanum nigram L.) Species among Three Kenyan Communities. *Asian Journal of Agricultural Extension, Economics & Sociology*, ***14***(3), 1–8.

Onyango, C. M., Ontita, E. G., Ndemo, R., Desterio, N., & Gapusi, J. R. (2016). Status and Production Practices of Vegetable African Nightshade (S olanum nigrum L .) in Selected Communities of Kenya, ***13(***3), 1–12.

Pasquel, F. J., & Umpierrez, G. E. (2014). Hyperosmolar hyperglycemic state: A historic review of the clinical presentation, diagnosis, and treatment. *Diabetes Care*, ***37***(11), 45-51

Patel, a, Biswas, S., Shoja, M. H., Ramalingayya, G. V, & Nandakumar, K. (2014). Protective effects of aqueous extract of Solanum nigrum Linn. leaves in rat models of oral mucositis. *The Scientific World Journal*, *2014*, 345939.

Petrikkos, G., Skiada, A., Lortholary, O., Roilides, E., Walsh, T. J., & Kontoyiannis, D. P. (2012). Epidemiology and clinical manifestations of mucormycosis. *Clinical*

Infectious Diseases, ***54(***SUPPL. 1), 23–34

Peyrot, M., Rubin, R. R., Lauritzen, T., Skovlund, S. E., Snoek, F. J., Matthews, D. R., … Kleinebreil, L. (2005). Resistance to insulin therapy among patients and providers: Results of the cross-national Diabetes Attitudes, Wishes, and Needs (DAWN) study. *Diabetes Care*, ***28***(11), 2673–2679.

Pop-Busui, R., Boulton, A. J. M., Feldman, E. L., Bril, V., Freeman, R., Malik, R. A., … Ziegler, D. (2017). Diabetic neuropathy: A position statement by the American diabetes association. *Diabetes Care*, ***40***(1), 136–154.

Rees, D. A., & Alcolado, J. C. (2005). Animal models of diabetes mellitus. *Diabetic Medicine : A Journal of the British Diabetic Association*, ***22***(4), 359–70.

Sartorius, T., Peter, A., Schulz, N., Drescher, A., Bergheim, I., & Weigert, C. (2014). Cinnamon Extract Improves Insulin Sensitivity in the Brain and Lowers Liver Fat in Mouse Models of Obesity,***9***(3), 1–12.

Schmid, H. (2007). New options in insulin therapy. *Jornal de Pediatria*,***83***(8), 146–154.

Sengottaiyan, A., Aravinthan, A., Sudhakar, C., Selvam, K., Srinivasan, P., Govarthanan, M., … Selvankumar, T. (2016). Synthesis and characterization of Solanum nigrum-mediated silver nanoparticles and its protective effect on alloxan-induced diabetic rats. *Journal of Nanostructure in Chemistry*,***6***(1), 41–48.

Shejja, V. ., Reddy, M. . A., Joseph, J., & Reddy.N, D. (2010). Insulin therapy in diabetes management. *International Journal of Pharmaceutical Sciences Review and Research*, ***2***(2), 98–105.

Siddiqui, A. A., Siddiqui, S. A., Ahmad, S., Siddiqui, S., Ahsan, I., & Sahu, K. (2013). Diabetes: Mechanism, pathophysiology and management-A review. *International*

Journal of Drug Development and Research, ***5***(2), 1–23.

Silva, E. F. F., Ferreira, C. M. M., & Pinho, L. de. (2017). Risk factors and complications in type 2 diabetes outpatients. *Revista Da Associacao Medica Brasileira (1992)*,***63***(7),

Silvia, P., Claudia, M.-C., Ana, M., Ailce, B., Olinda, M., Marta, A., & Laura, R. (2016). Cardiovascular safety of type 2 diabetes medications: Review of existing literature and lcinical implications. *International Journal of Endocrinology and Metabolism*, ***15(***2), 170–198.

Stanifer, J. W., Cleland, C. R., Makuka, G. J., Egger, J. R., Maro, V., Maro, H., … Philippin, H. (2016). Prevalence, risk factors, and complications of diabetes in the Kilimanjaro region: A population-based study from Tanzania. *PLoS ONE*, ***11***(10), 1–13.

Stein, S. A., Lamos, E. M., Davis, S. N., & Facp, M. (2013). A review of the efficacy and safety of oral antidiabetic drugs. *Expert Opin Drug Saf.*, ***12***(2), 153–175.

Sw, K., & Bokelmann, W. (2017). Agrotechnology African Indigenous Vegetables and their Production Practices : Evidence from the HORTINLEA Survey in Kenya, *6*(3).

Swinnen, S. G., Hoekstra, J. B., & DeVries, J. H. (2009). Insulin therapy for type 2 diabetes. *Diabetes Care*, *32 Suppl 2*.

Taab, A., & Andersson, L. (2009). Seed dormancy dynamics and germination characteristics of Solanum nigrum, 490–498.

Tests, D., & Diabetes, F. O. R. (2015). 2. Classification and diagnosis of diabetes. *Diabetes Care*, ***38***(3), S8–S16.

Tiwari, V. K., & Jain, S. K. (2012). Hypoglycemic Activity of Ethanolic Extract of Solanum nigrum Linn . Leaves on Alloxan Induced Diabetes Mellitus in Rats. *International Journal of Pharmaceutical and Phytopharmacological Research*, ***1,*** 26–28.

Tolonen, N. (2015). ***Lipid profile and micro- and macrovascular complications in type 1 diabetes****. Helsinki : [N. Tolonen], 2015. 115 p*

Trease, G. and Evans, C. (2000): Pharmacognosy: New Delhi: Harcourt Brace and Company, Asia Pvt. Ltd.

Umamageswari, M. (2017). Antidiabetic Activity of Aqueous Extract of Solanum nigrum Linn Berries in Alloxan Induced Diabetic Wistar Albino Rats. *Journal of Clinical and Diagnostic Research*, ***11*****(**7), 19–22.

Victoria, H., Reimar, W., Ole, H., & Nicoli, L. (2011). Diabetes in Sub Saharan Africa 1999-2011: Epidemiology and public health implications. a systematic review. *BMC Public Health.*

von Herrath, M., & Nepom, G. T. (2009). Animal models of human type 1 diabetes. *Nat. Immunol.*, ***10***(2), 129–132.

Wagio, R. S. (2016). Assessing Diversity of Solanum nigrum L. Grown in Kenya, 77.

Wais, M., Nazish, I., Samad, A., Beg, S., Abusufyan, S., Ajaj, S. A., & Aqil, M. (2012). Herbal drugs for diabetic treatment: an updated review of patents. *Recent Pat Antiinfect Drug Discov*, ***7***(1), 53–59.

Wang, J. (2001). Glucose Biosensors : 40 Years of Advances and Challenges. *Eletroanalysis*, *1312*, 983–988.

Wang, J. (2008). Electrochemical Glucose Biosensors. *Chemical Reviews*, ***108***(2), 814–825.

Wang, J., Huang, M., Yang, J., Ma, X., Zheng, S., Deng, S., … Yang, X. (2017). Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. *Food & Nutrition Research*, ***61***(1) 156-161

W**ang, Y., Xiang, L., Wang, C., Tang, C., & He, X. (2013).** Antidiabetic and Antioxidant Effects and Phytochemicals of Mulberry Fruit (Morus alba L.) Polyphenol Enhanced Extract. *PLoS ONE*, ***8***(7).

WHO. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Medicine*, ***15***(7), 539–553.

World Health organization (2014): Global status report on non-communicable diseases. Geneva.

World Health organization (2016): Global report on diabetes. Geneva: WHO

World Health Organization. (2019). Global Report on Diabetes. *Isbn*, *978*, 88.

Yoo, E. H., & Lee, S. Y. (2010). Glucose biosensors: An overview of use in clinical practice. *Sensors*, *10*(5), 4558–4576.

Zebish, A., Virginia, P., Pallavi, S., & Manisha, P. (2016). Assessment of Nutritional , Anti Nutritional and Antioxidant Properties of Underutilised Leaves of, ***5***(8), 1285–1292.

Zhang, L., Chen, Q., Li, L., Kwong, J. S. W., Jia, P., Zhao, P., … Sun, X. (2016). Alpha-glucosidase inhibitors and hepatotoxicity in type 2 diabetes: a systematic review and meta-analysis. *Scientific Reports*, ***6***(1), 32649.

Zyl, D G Van, Bösenberg, L. (2008). The mechanism of action of oral antidiabetic drugs : A review of recent literature Insulin secretagogues. *Jemdsa*, ***13***(3), 80–88.