EVALUATION OF ANTI-DIABETIC EFFECTS OF PHYTOCHEMICALS FROM *Urtica dioica* (Stinging nettle), *Salvia officinalis* (Sage), *Psidium guajava* (Guava) and *Citrus limon* (Lemon) USING AN EXPERIMENTAL MICE MODEL

KANANA FAITH MBITI

A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Biochemistry of Egerton University

EGERTON UNIVERSITY

AUGUST, 2020

DECLARATION AND RECOMMENDATION

Declaration

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

Signature: …… Date: 21/8/2020

Kanana Faith Mbiti

SM14/14429/15

Recommendation

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

Signature: …… Date: 25/8/2020

Prof. J. Matasyoh, PhD

Department of Chemistry

Egerton University

Signature: …… Date: 21/08/2020

Dr. C. Mwendia, PhD

Department of Biochemistry and Molecular Biology

Egerton University

DEDICATION

To my parents, siblings and friends for their moral, emotional, and financial support throughout my study period.

ACKNOWLEDGMENTS

I wish to acknowledge Egerton University for the opportunity granted to undertake my master's degree. My sincere appreciation goes to my supervisors Prof. Josphat Matasyoh and Dr. Charles Mwendia for their keen supervision, support, advice and guidance throughout this research project. Special thanks to Chemistry Department of Egerton University for allowing me to use their facilities to achieve objectives of this study. I also wish to acknowledge Kenya Medical Research Institute (KEMRI) for providing Swiss Albino mice used in this study. My gratitude goes to Chemistry and Biochemistry laboratory technicians of Egerton University for their assistance during the entire period of my project. I also wish to thank my colleagues and friends; Ms. Sum Winnie, Ms. Lucy Aketch, Ms. Divina Nyamboki and Ms. Velma Wekesa for their unconditional support during the lab work as well as in the development of this thesis. Special gratitude goes to Dr. Nyaga and Mr. Areba of the Department of Veterinary Parasitology, Microbiology and Pathology of Egerton University for their guidance and assistance during the animal model experiments. Much appreciation to Mr. Kibet of Chemistry department, Mr. Mutumba and Ms. Lucy from KEMRI for their support throughout my lab work. Am highly indebted to Prof. Makanya of Chiromo Campus- Nairobi University for helping in the preparation of histological slides and Dr. Mutahi of the Department of Veterinary Parasitology, Microbiology and Pathology of Egerton University for his guidance during interpretation of histopathology results. Special thanks to Prof. S. T. Kariuki for helping in the identification of the study materials. My deepest appreciation goes to my loving parents and siblings for their love, prayers, encouragement, and financial support. Above all, I thank the Almighty God for His love, mercy, providence, blessings, and good health.

ABSTRACT

Diabetes mellitus affects millions of people worldwide. In developing countries, diabetes mellitus causes significant morbidity and mortality to both children and adults. Although extensive research has been conducted on diabetes mellitus, effective treatment has not yet been reported. Oral anti-diabetic drugs and insulin used in clinical management are expensive, unavailable or have side effects. Therefore, there is need for safer and effective bio-active drugs from medicinal plants. The aim of this study was thus to investigate the anti-diabetic efficacy of phytochemicals from *Urtica dioica, Salvia officinalis, Psidium guajava* and *Citrus limon* leaf extracts. Three months old fresh leaves of the plants were collected from Egerton University's Botanical Garden for extraction of phytochemicals. Methanol was used as the primary solvent for extraction. The resultant plants extracts were partitioned with water, ethyl acetate and hexane and concentrated using a rotary vacuum evaporator to obtain ethyl acetate, hexane, and aqueous crude extracts. Diabetes was induced in the experimental mice using a single intraperitoneal injection of alloxan monohydrate at a dose of 200 mg/kg body weight. The crude extracts were subjected to preliminary *in vivo* bio-assays for 7 days where they were administered orally to experimental mice. Ethyl acetate extract of *S. officinalis* and methanol extract of *C. limon* did not reveal any anti-diabetic effect while the aqueous extract of *S. officinalis* showed significant hypoglycemic effect, $p< 0.05$ compared to other extracts. It was, therefore, subjected to further hypoglycemic studies where fasting blood sugar and live weights of various groups were monitored at intervals of 72 hours for 15 days. There was a significant drop in blood sugar levels of the groups treated with *S. officinalis* aqueous extract at 400 mg/kg and 600 mg/kg dosage levels from 452.00 ± 11.13 Mg/dl and 431.00 ± 10.65 Mg/dl to 256.33 ± 5.12 Mg/dl and 256.67 ± 8.74 Mg/dl and weight gain improvement from 28.05 ± 0.39 g and 27.38 ± 0.52 g to 29.32 ± 0.42 g and 28.55 ± 0.38g respectively compared to controls, $p< 0.05$. Histopathological analysis of liver and kidney tissues obtained from euthanized mice did not reveal any significant changes compared to the controls. This indicated that the extracts did not confer adverse effects on these tissues. Phytochemical tests of the extracts revealed presence of flavonoids, sterols, saponins, tannins, alkaloids, and triterpenes. Flavonoids and triterpenes particularly have been documented to possess anti-diabetic effects on alloxan induced mice. Results from this study indicate that *U. dioica, S. officinalis, C. limon* and *P. guajava* extracts are potential anti-hyperglycemic and can be used in modulating blood glucose with exception of ethyl acetate extract of *S. officinalis* and methanol extract of *C. limon.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
FBS	Fasting Blood Sugar
GLUT 2	Glucose Transporter 2
GLUT 4	Glucose Transporter 4
HLA	Human Leucocyte Antigen
HPL	Human Placental Lactogen
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IPR	Institute of Primate Research
KEMRI	Kenya Medical Research Institute
MHC	Major Histocompatibility Complex
NCD	Non-Communicable Disease
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NO	Nitric Oxide
PIT	Pancreatic Islet Transplant
SEM	Standard Error of the Mean
SPSS	Statistical Package for Social Sciences
STZ	Streptozotocin
TLC	Thin Layer Chromatography
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Diabetes mellitus (DM) is an endocrine system condition with high global prevalence. It is estimated that over 100 million people globally develop diabetes mellitus annually. In 2015, International Diabetes Federation (IDF) estimated the number of diabetic patients globally to be 415 million (IDF, 2015). In 2019 the number had risen to 463 and it's expected to reach 642 million by 2040. In Kenya, diabetic cases have been on rise increasing from 478, 000 in 2015 to 552, 400 in 2019. At the time, diabetes mellitus is the fastest growing non-communicable disease with increased healthy problems that impacts hugely on the economy (Sen *et al*., 2016). The most affected people are found in low-income and middle-income countries. The high incidence of diabetes is associated with high prevalence in obesity cases, sedentary lifestyle, decreased physical activity, and altered diet. Diabetes mellitus is essentially a multifactorial metabolic disorder characterized by hyperglycemia and leads to premature death and acute metabolic side effects. The disease ranks fourth as a cause of death in most countries. Over three million deaths are attributed to diabetes-related complications annually (Sen *et al*., 2016).

Diabetes mellitus is due to abnormality in carbohydrate metabolism resulting from impaired production or utilization of insulin hormone. It can also occur as a result of insensitivity of the target tissues to insulin (Maiti *et al*., 2004) leading into type I and type II diabetes mellitus respectively. Progression of diabetes mellitus slows down production and secretion of insulin hormone thereby causing progressive hyperglycemia. Consequently, hyperglycemia aggravates insulin resistance and also declines secretion of insulin leading to a condition known as glucotoxicity. Although the mechanism leading to progressive failure of pancreatic beta cell is not known fully, several factors are involved such as glucotoxicity chronic inflammation, genetic determinants, and lipotoxicity which occurs as a result of high levels of free fatty acids acting on the beta cells. The human body maintains blood sugar levels at a very narrow range and this is enabled by two endocrine hormones, insulin and glucagon. Insulin lowers blood glucose levels while glucagon raises blood sugar levels by causing liver to release glucose into the blood (Rahimi, 2015). In diabetic state, insulin production is impaired causing elevation of blood sugar in the blood. This predisposes diabetic patients into various life-threatening complications. For instance, in unmanaged diabetes mellitus incidences, hyperglycemia causes macrovascular and microvascular complications. These include; nephropathy, neuropathy, arteriosclerosis, and retinopathy.

Insulin injections and oral hypoglycemic agents are the current drugs of choice in treatment and management of diabetes mellitus. However, these compounds have several adverse and side effects in addition to long-term diabetes complications. Despite the extensive use of insulin and other hypoglycemic agents, diabetes, and its associated secondary complications remains a core medical problem in the society. It is thus important to find essential and effective compounds with fewer side effects as an alternative to the existing medications. More attention has been directed towards medicinal plants as complementary or alternative treatment for diabetes and other diseases (Eddouks *et al*., 2014; Jamila & Mostafa, 2014). Plants are an exemplary source of drugs and many of the drugs in the market have been derived directly or indirectly from the medicinal plants. Although, herbal medicine usage has significantly increased in recent years, it is an age-long practice in several parts of the world. Use of plants in medicine is mainly exploited for preventive and curative purposes. Nowadays, developed countries stress that greater proportion of medicines supplied to their communities should have herbal origin (Behradmanesh *et al*., 2012). The plants are a major source of food supplements effective in controlling blood glucose and preventing the long-term complications associated with type I and type II diabetes (Gallagher *et al*., 2003). Though, therapeutic properties of herbs have been acclaimed in traditional systems, few herbal drugs possessing antidiabetic properties have not been documented and formulated (Wadkar *et al*., 2008). Consequently, there has been debate regarding some aspects of the herbal remedies such as; scientific evidence for the efficacy, lack of quality control, and potential adverse effects such as reproductive toxicity, cardiotoxicity, nephrotoxicity, and hepatotoxicity.

Nevertheless, due to the rapidly increasing incidence of diabetes mellitus, considerable attention has been directed in identifying plants with antidiabetic potential. The reliance on herbal medicine is attributed to their availability, affordability, and reduced toxicity compared to conventional medicine. Traditional medicine plays a key role in identifying bioactive molecules which show high hypoglycemic effects. This is because over 80% of chemical drug compounds have been isolated from natural materials (Njeru *et al*., 2015). In developing countries like Kenya, herbal medicine is the first option used mainly by patients as a form of treatment. People have also embraced traditional medicine in Kenya after the resolution made by WHO in 2003 where it recommended traditional healers to be included in the management of health care (Njeru *et al*., 2015). As a result, herbal remedies are promising in the therapy of diabetes mellitus. Nonetheless, there is a part that the scientists ought to play in ensuring the herbal remedies acclaimed in traditional system are

scientifically justified. Thus, this study investigates alternate remedies for diabetes from the plant kingdom focusing on four plants; *Urtica dioica* (stinging nettle), *Salvia officinalis* (sage), *Psidium guajava* (guava) and *Citrus limon* (lemon).

1.2 Statement of the Problem

Diabetes mellitus is a disease characterized by chronic hyperglycemia affecting many people globally. The International Diabetes Federation estimated the number of people with diabetes worldwide as 463 million in 2019. The number was also predicted to rise to about 642 million by the year 2040. In Africa, 19.4 million people are suffering from diabetes while in Kenya, 552, 400 cases of diabetes were reported in 2019. These statistics, however, may not be the exact figures since most of the diabetic cases go unreported especially those living in rural areas. As a consequence, the number of diabetic cases keeps on rising day by day making the disease a major threat to the economy. Despite the fact that conventional drugs used in the management of diabetes mellitus are in the market, they have adverse side effects, are expensive for common usage and unavailable to rural poor. Also, diabetes mellitus is a lifestyle disease whereby obesity is a contributing factor. Noting that it is not easy to stop people taking food rich in fat and calorie, diabetes will remain a devastating disease. As a result of the lifestyle behavior, the incidences and prevalence of diabetes mellitus are on steady rise globally. It is therefore, necessary to turn to herbal medicine as they are comparatively less toxic and cheaper.

1.3 Objectives

1.3.1 General Objective

To evaluate the anti-diabetic effects of phytochemicals from *U. dioica, S. officinalis, P. guajava,* and *C. limon* in diabetic induced mice.

1.3.2 Specific Objectives

i) To evaluate the anti-diabetic efficacy of the plants extracts in diabetic mice.
ii) To determine the anti-diabetic phytochemicals present in *U. dioica, S. officinalis, P. guajava,* and *C. limon* plant extracts.
iii) To assess the cytotoxic effects of the plant extract with anti-diabetic potential on experimental mice.

1.4 Hypotheses

i) The plants extracts have no significant hypoglycemic effect on blood sugar levels in diabetic mice.

ii) The *U. dioica, S. officinalis, P. guajava,* and *C. limon* extracted phytochemicals do not have anti-diabetic effect on diabetes induced mice.

iii) The plant extract has no significant cytotoxic effects on kidney and liver cells of the experimental mice.

1.5 Justification

There are many therapeutic options in the management of diabetes mellitus such as lifestyle management, weight control, exercise, medical nutrition therapy, oral-glucose lowering drugs, insulin injections and medical nutrition therapy. However, most of these management options have failed. The conventional drugs, for instance, are expensive and accompanied with many adverse physiological and metabolic effects. Due to these shortcomings, an alternative must be sought from medicinal plants. Several researchers have opted to try natural remedies to replace the synthetic drugs. A study released by WHO (2015) shows that 80% of the world population rely on medicinal plants to cater for their primary health care needs. This offers a lead to exploring medicinal plants for the purposes of identifying anti-diabetic drugs. Many plant preparations with anti-diabetic effects have been confirmed which are relatively non-toxic, affordable, efficient and readily available. Nevertheless, plants with anti-hyperglycemic activities are increasingly being sought by health care professionals and diabetic patients. Since the efficacy of medicinal extracts differs depending on the environment and climatic conditions, there is need to study and confirm the reported anti-diabetic potential of the extracts from *U. dioica*, *S. officinalis, P. guajava,* and *C. limon*.

CHAPTER TWO
LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus (DM) is a global non-communicable disease (NCD) whose prevalence is rapidly increasing in the whole world. Diabetes mellitus is a major healthcare problem that is experienced by many people due to a defect in the endocrine system. It is a complex metabolic disorder that leads to syndromes such as stroke, heart attack and peripheral vascular disease (Patel *et al*., 2011). High glucose levels in the blood (hyperglycemia) which is an indicator of diabetes, is as a result of reduced/lack of insulin secretion by the β-cells of the islets of Langerhans in the pancreas, decreased sensitivity of the target tissues to insulin, or due to a combination of these two factors (WHO, 2014; IDF, 2013). This leads to Type I and Type II diabetes mellitus respectively.

The symptoms of diabetes mellitus are similar in both types (Type I and Type II), but they differ in intensity. In Type I, symptoms develop more rapidly while in Type II they are insidious in onset. A diabetic person shows polyuria and glycosuria leading to dehydration, unrelenting thirst and ultimately to polydipsia and polyphagia. Diabetes can lead to numerous complications which are classified into either acute, sub-acute or chronic. Complications associated with the acute form of diabetes mellitus are; hyperosmolar, hyperglycemia, diabetic ketoacidosis and non-ketotic syndrome (Kitabchi *et al*., 2009). Sub-acute complications include polyuria, thirst, visual blurriness, weight loss and lack of energy (Unwin *et al*., 2009). Chronic complications, on the other hand, are nephropathy, neuropathy, hypertension, hepatopathy, cardiomyopathy, diabetic foot ulcers, retinopathy and reproductive damage (Kuchake & Upasani, 2013). Chronic hyperglycemia leads to increase in production of oxygen free radicals due to autoxidation of glucose and glycation of the body proteins. This, therefore, generates oxidative stress which results in secondary complications affecting various body organs such as the kidneys, eyes, arteries and nerves (Henriksen *et al*., 2011). Uptake and storage mechanisms of glucose are also impaired as well as the activity of key enzymes in carbohydrate metabolism such as glucokinase, phosphofructokinase, and pyruvate kinase (Saravanan & Ponmurugan, 2012). The chronic form of diabetes is the most severe and leads to death if not well managed. The management of chronic complications, however, is expensive and cost countries hundreds of millions annually. The magnitude of diabetes mellitus differ in terms of the symptoms while complications differ depending on the geographical location and race which leads to differences in morbidity and mortality rate between nations.

2.1.1 Types of Diabetes Mellitus

The two common types of diabetes mellitus are; Type I which is also known as insulin dependent diabetes mellitus (IDDM) and Type II similarly termed as non-insulin dependent diabetes mellitus (NIDDM) (Ayeleso *et al*., 2012). Other forms of diabetes have been identified which are steroid diabetes (Type III) and gestational diabetes (Type IV). Insulin dependent diabetes mellitus is due to the insufficiency, or complete lack of insulin secretion by the pancreas and results due to autoimmune destruction of beta cells, the action of chemical toxins or invasion by a virus (Wang *et al*., 2014). Type II results due to insulin resistance, and the primary reason is obesity and lack of exercise, thereby limiting responsiveness to the target tissue to both exogenous and endogenous insulin. Even though the development of the disease is associated with genetic factors, environmental factors also play a vital role and therefore, the risk increases proportionally with the body mass index (Lehtovirta *et al*., 2010).

Chronic glucocorticoids therapy cause steroid diabetes. Drugs such as prednisolone and dexamethasone act by opposing insulin action and stimulating gluconeogenesis especially in the liver. This results in increased hepatic glucose output, insulin resistance, hyperlipidemia and hyperglycemia (Heather *et al*., 2012). On the other hand, gestational diabetes occurs in pregnant women whereby their blood sugar levels rises, without a previous history of diabetes. It's caused by Human placental lactogen (HPL) that leads to glucose intolerance hence stimulating insulin resistance (Tripathi & Verma, 2014). It usually occurs in about 4% of all pregnancies worldwide. It has also been reported that mothers with gestational diabetes have a 30-50% chance of developing type II diabetes mellitus later in life (Bastaki, 2005). Risk factors of gestational diabetes include ethnicity or race, obesity and family history of diabetes.

Type II is the most common form of diabetes, affecting approximately 85-90% of the diabetic patients and is prevalent in adults of over 40 years. However, some data have shown that adolescents and children are also at a risk of Type II diabetes mellitus (Shi & Hu, 2014). Type I, affects about 10% of the individuals and it is majorly associated with children and young adults while gestation diabetes takes about 5%. Type I diabetes mellitus is usually due to genetic and/or environmental factors. Inherited susceptibility to Type I diabetes mellitus depends on various genes at different chromosome loci. One of the strongest linkage is found with human leukocyte antigen (HLA)-D genes which are located on chromosome six within the Major Histocompatibility Complex (MHC) region. Other 20 chromosomal regions are

known to predispose to Type I diabetes development. Environmental factors are also known to trigger auto immunity. These causes a type of diabetes which is T-cell mediated autoimmune disease. It is characterized by total destruction of insulin-secreting β-cells located in islets of Langerhans in the pancreas (Shi & Hu, 2014). Autoimmune diabetes could be due to; endogenous retroviral genome especially in diabetic islets, destruction of beta cell by certain cytokines and islet inflammation (insulitis). Therefore, Type I diabetes is divided into Type IA and Type IB. Type IA is due to autoimmune or immune-mediated diabetes while type 1B is idiopathic meaning its cause is unknown. Type IA is the most common affecting about 90% of type I diabetic patients while Type IB represents 10% of Type I diabetic cases (Bastaki, 2005). Type IA is characterized by the presence of anti-glutamic acid decarboxylate (anti-GAD), islet cell antibody (ICA), and insulin antibodies that result during beta cell destruction by the autoimmune process. Some autoimmune diseases such as Addison's disease, Hashimoto's thyroiditis, and Grave's disease are associated with Type I diabetes mellitus. For Type 1B, the etiological basis is unknown with some patients demonstrating permanent insulinopenia (insulin deficiency) hence prone to ketoacidosis (Bastaki, 2005). The Africans and people of Asian origin are the most prevalent for Type IB.

Type II diabetes mellitus results from environment and genetic risk factors. However, the greatest risk factor is due to genetic predisposition such as insulin resistance, receptor defects, post- receptor defects and decreased insulin secretion. They occur due to a single gene disorder that usually affect secretion of insulin by the pancreatic beta cell or the inability of the adipose tissue, liver and muscle cells to respond to insulin action. Therefore, insulin resistance and progressive loss of beta cell function remains to be the main physio-pathologic defect responsible for hyperglycemia development and hence development of Type II diabetes. Insulin resistance has been associated with obesity which is a predisposing factor and causes down regulation of insulin receptors (Bastaki, 2005). Change in lifestyle from traditional pattern to a more modernized or "Westernized" culture is noted as the major contributor to Type II diabetes mellitus. Other risk factors include; poor diet and lack of exercise, infections such as cystic fibrosis, Cushing's syndrome, pancreatic cancer as well as acute and chronic pancreatitis. Vast majority of individuals diagnosed with Type II diabetes have one of the following metabolic defects. Either their beta cells are defective in secreting insulin when there is elevated blood glucose or the target cells does not respond to increased insulin secretion (Ayeleso *et al*., 2012). The hyperglycemic condition generated causes the pancreatic organ to produce more insulin causing hyperinsulinemia, so as to compensate for the lack of cellular response to the hyperglycemic condition. Over a period of time, increased

insulin resistance leads to high demand for insulin production which causes excessive stress on beta cells hence complete beta cell failure.

2.1.2 Prevalence of Diabetes Mellitus

Although diabetes was a rare disease in the past, it has become a major medical and social problem in recent years. In 2013, diabetes caused 5.1 million deaths globally, an increase of 0.5 million compared to 2011. It's anticipated that over 592 million people with diabetes by the year 2035, may die (IDF, 2015). In the year 2000, about 177 million people had diabetes globally and by 2015 the number had risen to 415 million which is predicted to increase to about 642 million by the year 2040 (IDF, 2015). Currently, in the year 2020 diabetes mellitus cases stands at 463 million globally. In Africa, 14.2 million people were diabetes mellitus positive in 2015 and in 2020 the number had risen to 19.4 million. Consequently, this number is estimated to rise up to 34.2 million by 2040. In Kenya, 478, 000 cases of diabetes were reported in 2015 and in 2020, the number had risen to 552, 400. However, the data may not be accurate since most of the diabetic patients; around two-thirds are undiagnosed (IDF, 2015). The affected class of people are those in low-middle income in developing countries.

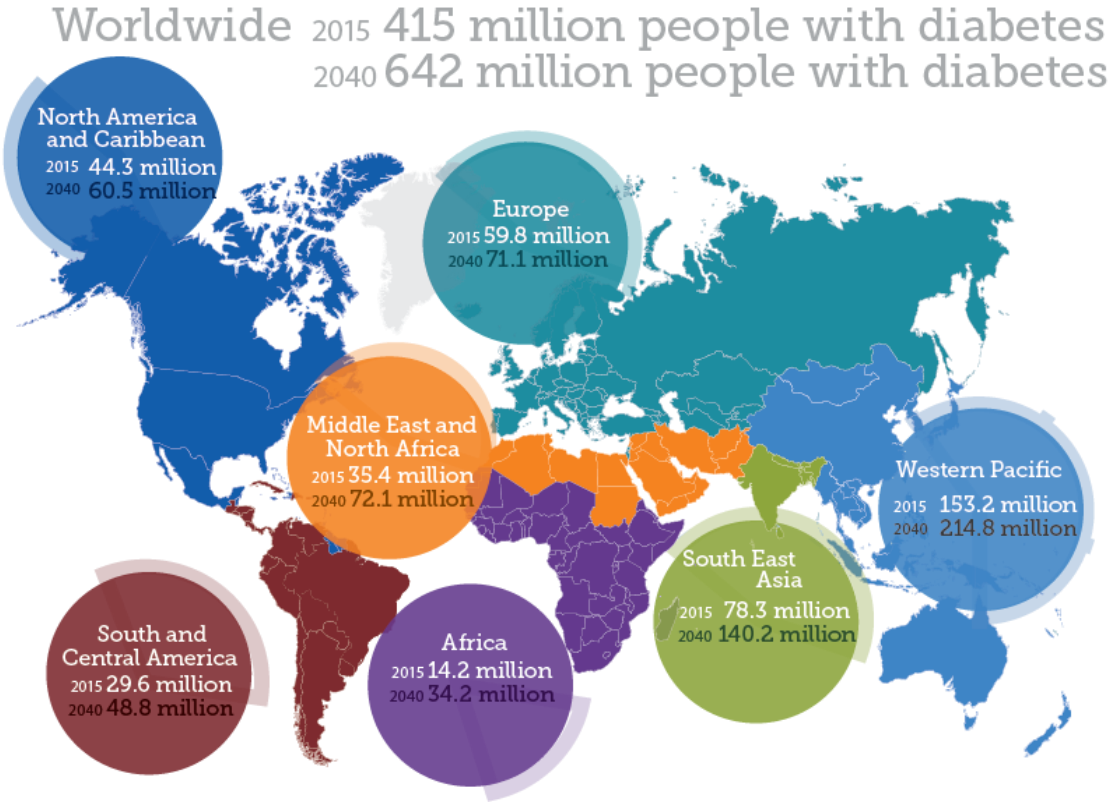


Figure 1: Global distribution of diabetes (IDF, 2015)

2.2 Control of Plasma Sugar Levels

During carbohydrate digestion, starch, lactose and sucrose are completely hydrolyzed to glucose, galactose and fructose. These monosaccharides are then absorbed into hepatic portal blood and carried to the liver. Galactose and fructose are converted rapidly to glucose by various enzymes found in the liver. Therefore, the sugar that leaves the liver in the hepatic vein is usually in the form of glucose (Rahimi, 2015). Carbohydrates are also transported in the form of glucose in plasma and erythrocytes. Blood glucose concentration is regulated at a relatively constant level of 80 Mg/dl. This is regulated mainly by two hormones (insulin and glucagon) produced by the islet of Langerhans in the pancreas. Insulin hormone is released by the beta cells while glucagon by alpha cells. Insulin is normally released when blood glucose level is elevated. Together with other gastrointestinal tract hormones, they act to lower blood sugar levels (Rahimi, 2015). On the other hand, glucagon is produced and released when blood sugar level is below normal and hence works to bring the blood glucose level back to normal concentration. It does this mainly by stimulating glycogenolysis in the liver hence causing release of glucose into the blood.

Insulin hormone lowers blood sugar by increasing the transport of glucose to the muscle and fat cells. It stimulates glucose transporter GLUT4 translocation from various intracellular sites to the plasma membrane, thereby increasing the rate of GLUT4 vesicle exocytosis (Heather *et al*., 2012). GLUT 4 is normally found in various vesicles that cycle continuously from intracellular stores to plasma membrane. High insulin concentration in blood promotes triacylglycerides synthesis and uptake of branched chain amino acids (valine, leucine, and isoleucine) by the muscle. This in turn facilitates protein synthesis, inhibits intracellular protein degradation, promotes glycogenesis and suppresses the process of gluconeogenesis in the liver (Solomon *et al*., 2008). The final effect of insulin is reduction of blood glucose levels. In any event that insulin production and/or release is absent, or there is inhibition of the regulatory mechanism, the result is prolonged elevation of blood glucose level.

2.2.1 Opportunistic Infections Associated with Diabetes Mellitus

Diabetes mellitus predisposes patients to opportunistic infections and pathogens. It is the opportunistic infections that increase the morbidity and mortality rate of people suffering from diabetes mellitus disease. The hyperglycemic environment that is created by high levels of blood sugar in the body, creates a conducive environment that favors immune dysfunction and hence leading to high occurrence of infections. Immune dysfunction that is affected

include; depression of the antioxidant system, damage to the neutrophil function, and depression of the humoral immunity (Henriksen *et al*., 2011). Once the immune system is weakened, the patient is predisposed to various infections. For instance, diabetic patients are at a high risk of yeast infections and urinary tract infections due to decreased antibacterial activity of urine and dysmotility of the urinary and gastrointestinal tract. The *Candida albicans* is the common yeast that affects diabetic individuals. It colonizes the mouth, nose, and vagina and in the process it impairs the ability of white blood cells in fighting infections. Also, due to reduced blood flow to the extremities and nerve damage, diabetic patients are prone to foot infections and surgical site infections.

2.2.2 Conventional and Non-Pharmacological Treatment of Diabetes Mellitus

Diabetes mellitus management and treatment has either been conventional (use of synthetic drugs) or traditional (non-pharmacological). The aim of managing and treating diabetes mellitus is to alleviate the symptoms and complications thereby increase longevity and save life. Traditional method of managing diabetes mellitus relies mainly on non-pharmacological approach especially exercise and diet. Lifestyle management is the most suitable traditional option for diabetes mellitus intervention. Exercising and taking the right diet have been shown to improve the state of type II diabetic patients. An increase in the daily energy expenditure and reduction in weight, decreases insulin resistance and increases glucose tolerance. In fact, exercise and weight reduction have been shown to improve insulin resistance (Solomon *et al*., 2008). Therefore, overweight diabetic patients should consume food with low fat content especially the saturated fat, restrict calorie intake, and increase intake of unrefined carbohydrate content. As such, diabetic patients are advised to watch their weight and diet as a preventive measure towards the diabetes mellitus disease.

Conventional treatment involves the use of pharmacological interventions such as herbal treatment and conventional treatment options. There is diverse literature review that has examined several anti-diabetic herbal agents. Over 1200 plant species globally has been reported to have hypoglycemic properties (Baharvand-Ahmadi *et al*., 2016). The extracts from these plants are used in making fresh juices or concoction that it's taken orally in management of diabetes mellitus traditionally. They have been shown to be effective in treating diabetes mellitus symptoms such as polyphagia, polydipsia, and polyuria. Several animal studies on alloxan or streptozotocin induced diabetic mice or rats have demonstrated the effectiveness of plants extracts in lowering blood sugar levels (Bastaki, 2005).

Apart from herbal medicine, insulin treatment has been effective in the management of type I diabetes mellitus. Insulin injections are known to maintain long-term normal glycaemia without the risk of low hypoglycemia. Insulin also has been effective in slowing or preventing the progression of complications associated with diabetes mellitus especially the chronic microvascular complications. However, continual and increased use of insulin has been shown to have adverse side effect such as weight gain and poor glycemic control on the patients (Bastaki, 2005). In addition to insulin injections, several other insulin analogues are currently in the market. They include; short- and rapid-acting insulin analogues such as insulin lispro (Humalog), insulin glulisine (Apidra), and insulin aspart (Novolog) and long-acting insulin analogues such as insulin detemir and insulin glargine (Lantus) which is mainly injected at bedtime (Bastaki, 2005).

Other option for conventional treatment is use of oral glucose-lowering agents such as thiazolidinediones, sulfonylureas, meglitinides, biguanides and alpha-glucosidase inhibitors. The sulfonylureas include the first generation drugs such as the chlorpropamide, tolazamide, acetahexamide, and tolbutamide. The second generation include the glibenclamide, glimepiride, gliclazide, and glipizide (Bastaki, 2005). The sulfonylureas lower blood sugar levels by stimulating release of insulin hormone from the pancreatic beta cells by binding to the sulfonylurea receptors located on the beta cell plasma membrane. This in turn causes cell membrane depolarization hence entry of insulin through opened voltage-gated calcium ions channels (Bastaki, 2005). However, they also have adverse side effects on long-term use such as hyopoglycemis, weight gain, headache, gastrointestinal disturbances, and bone fracture (Monami *et al*., 2014). The biguanides includes the metformin (commonly used), buphormin and phenformin, although the use of the latter two were rejected due to increased fatal lactic acidosis and risk of cardiovascular that increased mortality rates. Metformin lowers blood sugar levels by improving insulin resistance associated with type II especially in the adipose tissue, skeletal muscle, and liver. Metformin also reduces hepatic glucose output while at the same time increasing the peripheral uptake of glucose. It has been reported to have side effects such as abdominal discomfort, diarrhea, dizziness, tiredness, and nausea. Metformin is commonly used in combination with sulfonylureas in treatment of diabetes mellitus (Bastaki, 2005).

Thiazolidinediones which are agonists for nuclear peroxisome proliferator-activated receptor-gamma, lower insulin resistance in peripheral tissue. Commonly used thiazolidinediones are pioglitazone (Actos) and Rosiglitazone (Avandia). The troglitazone was withdrawn since it used to cause severe hepatic toxicity after use. They also have adverse

side effects such as gastro-intestinal and visual disturbances, anemia, and fluid retention that may cause heart failure. The other class of conventional drugs are the meglitinide analogues such as repaglinide and nateglinide. They improve insulin secretion during the early-phase (Monami *et al*., 2014). They are derived from sulfonylurea moiety hence act by binding to the beta cell receptors hence inducing insulin sectretion. Repaglinide has been shown to be five times more effective than glibenclamide in stimulating secretion of insulin from the beta cells. Unfortunately, they are also known to cause hypoglycemia, hypersensitivity, and gastrointestinal disturbances. Finally, the alpha-glucosidase inhibitors delays digestion of the complex carbohydrates thereby decreasing the upsurge of postprandial plasma glucose levels in the blood (Bastaki, 2005). They include the acarbose, voglibose, and miglitol that inhibits brush border enzymes in small intestine from hydrolyzing polysaccharides, and oligosaccharides to monosaccharides. Their prolonged use may lead to diarrhea, abdominal bloating, and flatulence. The main advantage of alpha-glucosidase inhibitors is that they do not lead to hypoglycemia. All the aforementioned glucose-lowering agents have been shown to be effective in treatment and management of diabetes mellitus so far. However, due to their shortcomings such as adverse sides, there remains a need to find alternative medicines that are more potent and less or none adverse side effects. Additionally, they are costly and inaccessible to many people especially the rural folks (Piero *et al*., 2012).

2.2.3 Diabetes Mellitus Therapy Management Progress

Since the discovery of insulin in 1921 by Banting and Best, tremendous developments in pathogenesis and treatment of diabetes mellitus has since advanced tremendously. Insulin which is manufactured from highly purified recombinant human cells, remains the best option available for diabetes mellitus (Tripathi & Verma, 2014). Type I diabetic patients require lifelong insulin injections. Administration of insulin via infusion pumps is available though not affordable to many. Lifestyle interventions are the cornerstone of type II diabetes management irrespective of concomitant pharmacological anti-diabetic therapy. Weight loss and muscle gain delay the onset of type II diabetes (Bastaki, 2005). Oral pharmacological agents for type II diabetes mellitus such as insulin secretogogues, stimulate glucose metabolism and the glucose sensor-glucokinase- GLUT2 thereby lowering blood sugar levels. For instance, sulfonylureas, repaglinide and meglitinides are short-acting insulin releaser which acts directly on the beta cells of the islet of Langerhans thereby closing ATP-sensitive K+ channels (Bastaki, 2005). Closure of potassium channels causes the beta cell membrane to be depolarized and opens the voltage-gated calcium channels. Consequently,

this leads to influx of calcium ions and exocytosis of insulin hormone into the blood. As a result, glucose levels in the blood is reduced.

Metformin is a basal hepatic glucose production suppressor, known to counter insulin resistance. It lowers blood sugar through reduction of hepatic glucose output as a consequence of inhibiting gluconeogenesis and glycogenolysis processes. It also increases insulin-stimulated glucose uptake and glycogenesis in skeletal muscle (Bastaki, 2005). Other drugs such as alpha glucosidase inhibitors cause delay in intraluminal production of glucose. Acarbose for example, competitively inhibits alpha glucosidases which are associated with brush border membrane of the small intestines and hence inhibiting digestion of sucrose and complex polysaccharides. The effectiveness of these oral anti-diabetic drugs, however, is temporary and after a period of 4-5 years of therapy, beta cell failure can no longer be compensated (Bastaki, 2005).

The first option, therefore, for complicated long-term type I and type II diabetic patients is cell transplant therapy or stem cell therapy. The aim of the cell-based therapies is to produce functional insulin-secreting beta cells that will normalize production of insulin hence curing diabetes. The destroyed beta cells of a diabetic patient are replaced with healthy ones from a diabetic-free person (Rahim *et al*., 2018). Usually, the islet of Langerhans which is a cluster of insulin producing cells are obtained from a donor and transplanted to pancreas of the diabetic patient. This method is particularly helpful for patients suffering from type I diabetes mellitus since in most cases there is total destruction of beta cells. Some successful cases for pancreatic islet transplants (PIT) have been witnessed (Tripathi & Verma, 2014). Stem cell therapy offers promising results in treatment of diabetes mellitus and in elimination of insulin injections which may lead to hypoglycemia after pro-longed use. However, the shortcoming of this therapy is that it is associated with the need for chronic immune suppression. There is increased vulnerability of pancreatic islets to instant blood inflammatory reaction after transplant, poor vascularization and diabetogenic immunosuppressive drug exposure (Rahim *et al*., 2018). After transplant the patient becomes vulnerable to bacterial and viral infections due to the weak immune system and low white blood cell count. Although researchers are exploiting the use of immunosuppressive agents to prevent the autoimmune attack, the therapy still poses some critical risks to patients. Additionally, shortage of human pancreatic organs for transplantation also remains a big challenge. Due to the ability of stem cells to self-renew and differentiate into various cells, there is a possibility to form cancer cells. Due to these risks, the medical specialists should be

cautious on the therapy. Further research also is necessary to ascertain the effectiveness of stem cell therapy (Tripathi & Verma, 2014).

Gene therapy is another alternative method that is receiving tremendous effort from various researchers. Gene therapy involves transduction method using either viral vector or non-viral vector to treat type I diabetes mellitus (Wong *et al*., 2010). The therapy involves replacement of the defective insulin gene or suppression of the autoreactive T cells in order to prevent destruction of islets cells of Langerhans. Careful consideration is critical when selecting the vector. It should be one that result in high transduction efficiency, not eliciting a strong immune response, ability to transduce both non-dividing and dividing cells, and ensure long-term expression of the transgene (Chellappan *et al*., 2018). For instance, the adenoviral vectors are able to transduce both dividing and non-diving cells, while the retroviral only transduces the dividing cells. So, once the effective islet gene is incorporated into the vector, it is directed to the patients' islets cells. The vector passes through the membrane of the islet and transduces the sac of islets cells within. After delivery, the normal gene is expressed and new proteins for normal islets are synthesized. This may also result in deletion of the defective gene causing diabetes mellitus (Wong *et al*., 2010). Gene therapy has been effective in treating diabetes mellitus, but it is a costly procedure. Nevertheless, by continual exploration of new strategies, there is hope of a breakthrough in prevention or better still cure of this disease. Since no effective cure has been reported yet, and the increased shortcomings of options already in use, there is need for alternative approach such as the herbal medicine.

2.3 Medicinal Plants

Medicinal plants refer to different types of plants used in herbal medicine. These plants are rich sources of ingredients that can be used in the drug development and synthesis (Rasool, 2012). WHO (2015) reported that about 80% of the world population depend on herbal medicine for primary health care. Plants extracts are used in the synthesis of several agents which include fungicides, insecticides and antibiotics. Medicinal plants have been exploited in the development of natural products due to their ability to produce numerous bioactive secondary metabolites. These secondary metabolites such as flavonoids, alkaloids, terpenoids, and saponins have been shown to possess anti-diabetic potential (Eddouks *et al*., 2014). Thus, due to their effectiveness, medicinal plants extracts have been recommended as an alternative or a complimentary treatment for diabetes as well as other diseases such as cardiovascular, cancer and neurological disorders (Jamila & Mostafa, 2014). Herbal medicine is preferred over the synthetic drugs due to low side effects, low cost, readily available, and

effectiveness (Nasri & Shirzad, 2013). Additionally, crude extracts of various plants are known to exert different actions on several diseases. Their synergistic and antagonistic effects of herbs' secondary metabolites also offer an interesting area for exploitation (Rahimi, 2015). Over 1200 traditional plant with anti- diabetic activity has been reported to date worldwide (Baharvand-Ahmadi *et al*., 2016) for diabetes mellitus management. However, according to Baharvand-Ahmadi *et al*. (2016) only a small fraction of these plants have been scientifically evaluated. Plant derivatives with purported hypoglycemic properties have been used for long in traditional healing systems and in folk medicine around the world such as in Jewish, Native American Indian, Chinese, Mexican and East Indian. Most of these plants are still in local use by various societies throughout the world (Rahimi, 2015). They have therefore, generated extraordinary interest as a potential sources of diabetic control.

A number of active compounds isolated from plants have been documented to lower blood sugar levels in diabetic-induced animal models and in people suffering from Type II diabetes. Their mode of action has been shown to either stimulate insulin synthesis or release from pancreatic beta cells or by causing their regeneration (Rao *et al*., 2010). In several parts of Kenya, various antidiabetic plants are used by the traditional healers in the management of diabetes mellitus. Regardless of the high percentage of Kenyans relying on herbal medicine, traditional medicines remain the least understood among other medical systems. It's only a small portion of Kenyan indigenous plants that have been investigated and active compounds responsible for blood sugar lowering effects determined (Piero *et al*., 2012). This study, therefore, sought to study and confirm the anti-diabetic effects of *U. dioica*, *S. officinalis*, *C. limon* and *P. guajava* plants. These plants are already used in the management of diabetes mellitus in several regions of Kenya especially Eastern and Western Kenya. The plants were selected on the basis of ethnobotanical information through collaboration with local herbalists around Egerton community.

2.3.1 *Urtica dioica* (Stinging Nettle)

Urtica dioica is an herbaceous perennial flowering plant. It belongs to the kingdom Plantae, order Rosales, family Urticaceae, genus *Urtica* and *U. dioica* species. It is known as common nettle, stinging nettle, devils leaf, or net plant in English. It is also known by different local dialects such as *thafai* in Kikuyu. It is native to Asia, Europe, Western North America and parts of Africa, although cultivated in other parts of the world. It grows 0.6 m in height with soft, green leaves and bright yellow stolons and rhizomes. The leaves are oppositely arranged on the stem and they range from 7-15 cm long. The stem and leaves bear

stinging hairs (trichomes) containing acetylcholine, histamine and formic acid, which when touched can sting causing irritation and skin blisters (Kataki *et al*., 2012).

The herb is used traditionally in the treatment of various diseases globally. Currently it is being used in the traditional systems in the management of diabetes mellitus (Safari *et al*., 2016). The fresh juice, for instance, has been reported to relieve pain, counter symptoms of allergies, treat fever, diabetes mellitus, and increase thyroid function (Said *et al*., 2015). The leaves are either placed in water and boiled for few minutes or placed in a pre-boiled water for extraction of the active compounds. The obtained concoction is drunk with the aim of fighting various diseases. Human clinical trials have been performed on the roots extracts of the stinging nettle for the treatment of symptoms that accompany benign prostate hyperplasia (Wilt *et al*., 2000). Roots, leaves and stem have been shown to possess antioxidant, antidiabetic, antifungal, anti-inflammatory, antipyretic, antimicrobial and analgesic properties (Safari *et al*., 2016; Kataki *et al*., 2012).

Figure 2: *Urtica dioica* (Jan & Singh, 2017)

2.3.2 *Salvia officinalis* (Sage)

Salvia officinalis is an evergreen, perennial subshrub with grayish leaves, woody stems and blue to purplish flowers. It belongs to kingdom Plantae, Order Lamiales, Family Lamiaceae, Genus *Salvia*, and *S. officinalis* species. It has numerous common names such as sage, garden sage, common sage, true sage, kitchen sage, golden sage and culinary sage. It has It is native to the Mediterranean region but has been naturalized in many parts of the world.

Figure 3: *Salvia officinalis* (Lemle, 2018).

Sage has been used in herbal medicine for long periods of time. Sage tea which is boiled dried sage leaves water infusion has been studied in the treatment of Alzheimer's disease, acute pharyngitis, sore throats, and hot flushes during menopause (Bommer *et al*., 2009). Sage has been reported to have anti-inflammatory, antibacterial, and anti-fungal properties (Salah *et al*., 2016). It is also known for lipid profiling and antioxidant properties (Schapowal *et al*., 2009).

2.3.3 *Psidium guajava* (Common Guava)

Psidium guajava is a small evergreen tree known as Guava in English or Amrood in Hindi. It belongs to kingdom Plantae, order Myrtales, family Myrtaceae, genus *Psidium*, and *P. guajava* species. It's native to Caribbean, South America and Central America though now cultivated extensively in tropical and subtropical regions. Its use and medicinal properties are ascribed in traditional medicine in Kenya. The fruits have high concentrations of water soluble vitamins and free sugars. The leaves and peels of ripe and unripe fruit have demonstrated antidiabetic effect in alloxan and STZ induced rodents (Bagri *et al*., 2016). The activity of the leaves is associated to various chemical compounds such as; pentacyclic triterpenoids, tannins, quercetin, β-sitosterol, flavanone-2 2'-ene, and volatile oil rich in eugenol, cineole and crypto nine (Bagri *et al*., 2016).

Figure 4: *Psidium guajava* (Joseph & Priya, 2011)

2.3.4 Citrus limon (Lemon)

Citrus limon is a perrenial evergreen plant that grows up to 10 to 20 feet in height. It belongs to kingdom Plantae, order Sapindales, family Rutaceae, genus *Citrus,* and *C. limon* species. It is widely grown in Africa and Asia. It is also distributed across the globe due to its edible fruit which contains high concentration of citric acid.

Figure 5: *Citrus limon* (Britanica, 2016)

Citrus limon is rich in flavonoids, polyphenols, tannins, terpenes, ascorbic acid, essential oil, and pectin (Miyake *et al*., 2006). These chemical compounds contribute to its effectiveness in managing various diseases. Different parts of this plant such as the leaves, root, and fruit peel are used in treatment of diabetes mellitus, as an anticancer, diuretic,

anthelmintic, and sedative (Naim *et al*., 2012). Also, its used in treatemnt of circulatory system diseases and respiratory tract diseases (Miyake *et al*., 2006). Studies involving animal models have shown that *C. limon* leaves, seeds, ripe and unripe fruit peel possess anti-diabetic potential (Naim *et al*., 2012).

2.4 Plant Phytochemicals

Plants have diverse phytochemicals that protect the plants against pathogens and predators. They are also involved in pollination by attracting the pollinating insects. Common phytochemicals present in various plant species include alkaloids, flavonoids, saponins, anthraquinones, steroids, tannins, glycosides, terpenoids, carotenoids and essential oil. These phytochemicals have medicinal properties and can act as templates to development of new drugs (Colegate & Molyneux, 2007).

2.4.1 Saponins

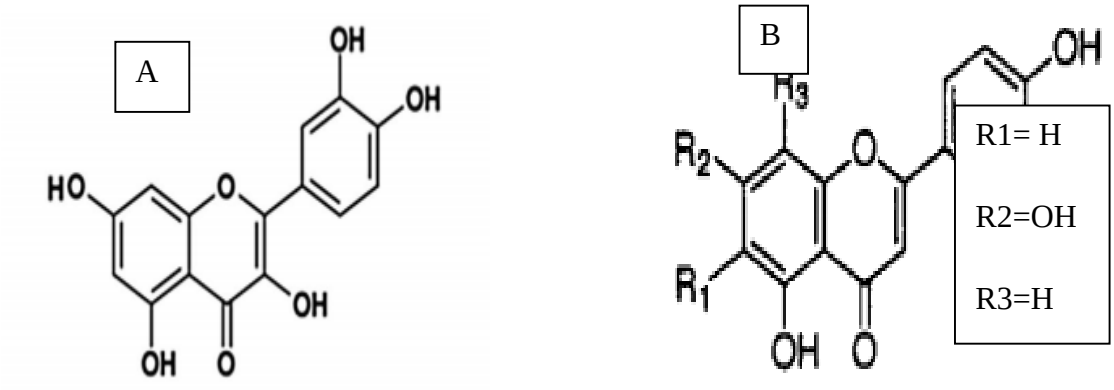
Saponins are surface-active glycosides which are naturally occurring and produced mostly by plants, some bacteria and lower marine animals. Due to their distinctive foaming characteristic, these class of saponins got their name from the soapwort plant Saponaria (Sen *et al.,* 1998). The structure of saponins usually consist of a sugar moiety (galactose, glucose, xylose and glucuronic acid) with a glycoside linkage and attached to a sapogenin (hydrophobic aglycon) which can either be a steroid or triterpenoid. The combination of water-soluble and non-polar sapogenin side chains, gives the saponins an ability to foam (Francis *et al.,* 2002).

2.4.2 Cardiac Glycosides

Cardiac glycosides are so named due to their ability to poison the heart (Majak & Benn, 2001). Their distinguishing characteristic is a steroidal aglycone that can either be bufadienolides or cardenolides. Na^+-K^+-adenosinetriphosphatase found in the cardiac muscle of the heart, is the main pharmacological receptor of cardiac glycosides. Inhibition of this receptor, therefore, by either cardenolides or bufadienolides affects intracellular electrolyte concentrations resulting in forceful myocardial contraction (Schoner & Scheiner-Bobis, 2007). Cardiac glycosides have been used in congestive heart failure treatment in humans. Nevertheless, they have been known to be toxic when consumed by domestic herbivores at their natural concentrations in plants (Majak & Benn, 2001).

2.4.3 Flavanoids

Flavonoids are widely distributed in various foods and beverages and they are polyphenols of plant origin. The free form occurs as glycosides or aglycones. The most common classes include; flavanones, flavones, isoflavones, flavonols, anthocyanidins and catechins (Nijveldt *et al.,* 2001). A basic backbone C6-C3-C6 phenyl-benzopyran, is shared by all flavonoids (Harnafi & Amrani, 2007). Due to the presence of hydroxyl groups in flavonoids, they are involved in scavenging oxygen derived free radicals. Due to this property as well as their anti-oxidant nature, they have been reported in the treatment of diabetes mellitus (Nijveldt *et al.,* 2001).



(A) Flavonoid isolated from stinging nettle (B) Flavonoid isolated from sage

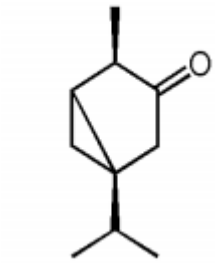
2.4.4 Tannins

Tannins are common in various plants and are present virtually in every plant part. Tannins are divided into two classes; hydrolysable and condensed. Condensed tannins also known as proanthocyanidins are derived from flavonoid monomers while hydrolysable tannins contain gallic acid as multiple esters with D-glucose (Serafini *et al.,* 1994). Monocots and gymnosperms produce only condensed tannins whereas dicots can produce either hydrolysable tannins, condensed tannins, or a mixture of both types (Serafini *et al.,* 1994).

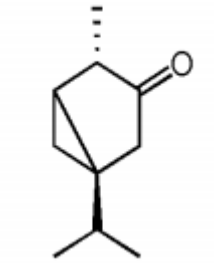
2.4.5 Essential Oils

Essential oils are volatile compounds produced by aromatic plants. They are characterized by a very strong odor and it represents about 10% of the plant kingdom (Sangwan *et al.,* 2001). They exist in several plant parts such as leaves, flowers, stem, seeds, fruits and roots. They are stored in distinct brittle secretory structures, such as secretory hairs, glands, and trichomes (Sangwan *et al.,* 2001). The total essential oil composition is found in minute quantities in plants and it rarely exceeds 1%. The chemical composition of essential oils within plants of same species vary depending on climatic conditions, genetic

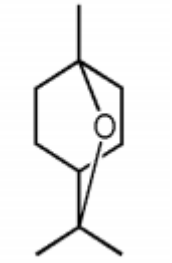
composition of the plant, and the period the plant material was collected (Sangwan *et al.,* 2001). The commonly used method in extraction of essential oils is hydro-distillation where a Clevenger type apparatus is used (Abad *et al.,* 2012). Due to the presence of several active ingredients present in essential oils, they have a broad spectrum of bioactivity such as antibacterial, antiviral, antifungal, anti-diabetic, insecticidal and anti-parasitic (Abad *et al.,* 2012). Major compounds isolated from *S. officinalis* essential oil are Camphor, Viridiflorol, Manool, 1,8-cineole, β-thujone, and α-thujone



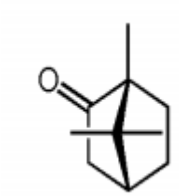
α-thujone



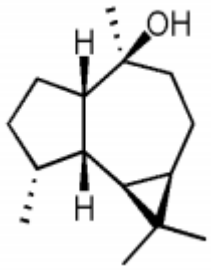
β-thujone



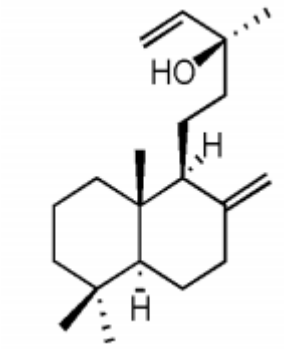
1,8-cineole



Camphor



Viridiflorol



Manool

2.5 Criteria for Selection of Medicinal Plants for Drug Discovery

Fabricant and Farnsworth (2001) described standard approach for selection of plants with potential bioactivity. First, there is random selection of plant(s) followed by chemical screening (phytochemical approach). Classes of phytochemicals such as tannins, glycosides, saponins and so on are sought that could have potential bioactivity. Secondly, there is random selection followed by bioassay on the test organism(s). In this method, all plant parts that are available are collected, irrespective of either experience or prior knowledge. However, this approach is laborious and costly (Fabricant & Farnsworth, 2001). Thirdly, follow-up of the bioactivity reports of the plant(s). The available published reports on the bioactivity of various plants are explored (Cos *et al.,* 2006). Finally, follow-up of various uses of ethno medicinal plants against the infectious agents (ethno medicinal approach). It's

based on written or oral information obtained from folklore, herbalism, or traditional medical systems. Ethno medicinal knowledge can as well be obtained from various sources, such as review articles, books, and computer databases (Cos *et al.*, 2006).

2.6 Animal Models in Study of Diabetes Mellitus

Animal models are used to ascertain the effectiveness of the plant's extracts, to determine their toxicity levels, and elucidate their mode of action. In later stages, clinical trials on human experiments can be performed when their safety is guaranteed. The commonly used animal models are the mouse, rats, rabbits, guinea pigs and hamster (Tripathi & Verma, 2014). Rats and mice remains to be an excellent model in medical research due to similarity of genes with humans. Almost all human genes that are known to be associated with diseases, are also encoded in the rat genome and they appear to be highly conserved throughout the mammalian evolution (Etuk, 2010). The animal models for the study of Type I and Type II diabetes can be obtained by two ways; induced by chemicals or surgical manipulations/dietary and/or by combination of the two. New genetically modified animal models such as transgenic, tissue-specific knockout and generalized knock-out mice have been engineered for study of diabetes and other diseases. The frequently used chemically-induced models include streptozotocin and alloxan induced mice (King, 2012).

2.6.1 Diabetogenic Compounds and their Mode of Action

Chemicals such as alloxan, streptozotocin (STZ), monosodium glutamate and dithizone are used in induction of diabetes in experimental animals. Viruses and genetically diabetic animals have also been used in various studies. Chemicals are preferred over the rest as the body changes that occur during the induction of diabetes and after can clearly be observed (King, 2012). Alloxan and STZ are mostly used since they are similar in structure with glucose, hence compete with glucose. However, Lee *et al*. (2010) indicated an adverse toxicity of these chemicals on other non-target organs.

a) Alloxan as a Diabetogenic Agent

Alloxan, similarly known as 2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil is a diabetogenic compound that is used to induce Type I diabetes in animal models. It’s a urea derivative that leads to selective necrosis of the beta cells of the Islets of Langerhans (Etuk, 2010). Alloxan is known to have two distinct pathological effects through which it leads to selective necrosis of beta cells. It specifically inhibits the glucokinase enzyme which acts as the glucose sensor of the beta cells hence causing selective inhibition of glucose-induced

insulin secretion. It also causes a state of insulin-dependent diabetes by its ability to induce Reactive Oxygen Species (ROS). Formation of free radicals leads to fragmentation of beta cell DNA (Szkudelski, 2001). By varying the dosage of alloxan, different grades of disease severity can be induced in the experimental animals (Iranloye *et al*., 2011). However, the route of administration and the animal species used determines the dosage level of alloxan (Federiuk *et al*., 2004).

b) Streptozotocin (STZ) as a Diabetogenic Agent

STZ [2-deoxy-2-(3-(methyl-3-nitrosoureido)-d-glucopyranose] is a monofunctional nitrosourea derivative and a naturally occurring chemical compound that is used in induction of Type I diabetes and Type II when used in multiple low doses. Moreover, STZ has been used in the treatment of metastatic cancer of islets of Langerhans and gastrointestinal cancers in combination with other chemotherapeutic drugs, although severe toxicity has been observed in many of the patients (Brentjens & Saltz, 2001). Once STZ has been injected into the experimental animal, it reaches the cells of the pancreas via the Glucose transporter-GLUT2 and hence causes DNA alkylation (Szkudelski, 2001). It leads further to stimulation of poly adenosine diphosphate ribosylation and release of Nitric Oxide (NO) and thereby causing the destruction of beta cells and eventually inducing insulin dependent diabetes (Patel *et al*., 2006; Mythilli *et al*., 2004).

2.6.2 Phases of Diabetes Mellitus Induction in Model Study Animals

Injection of diabetogenic compounds into the experimental animals, induces tetraphasic blood glucose response. After alloxan administration, transient hypoglycemic phase occurs which lasts for about 30 minutes. This could be as a result of inhibition of glucosidase enzyme thereby leading to increased insulin secretion (Lenzen, 2008). The second phase which is characterized by increased blood glucose concentration, starts 1 hour after alloxan administration. It lasts for about 2-4 hours. Once the toxin reaches the beta cells, it destroys beta cells hence inhibition of insulin secretion. After 4-8 hours' hypoglycemic phase starts again lasting for many hours. Changes that occur in this phase are irreversible. Organelles such as inner and the outer mitochondria membranes loose structural integrity and cisternae of Golgi complex and rough endoplasmic reticulum rupture (Mythili *et al*., 2004). The last phase, permanent diabetic hyperglycemia phase, occurs within 24-48 hours after administration of the alloxan. However, the non-beta cells and extra pancreatic parenchyma cells remain intact demonstrating selective toxicity of the Alloxan (Lenzen, 2008; Mythili *et al*., 2004).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

The plants samples were collected from the Botanical Garden of Egerton University. The Botanical garden is a 300-acre piece of land in Egerton University at an altitude of 2127 meters and 1° 37″ south of Equator.

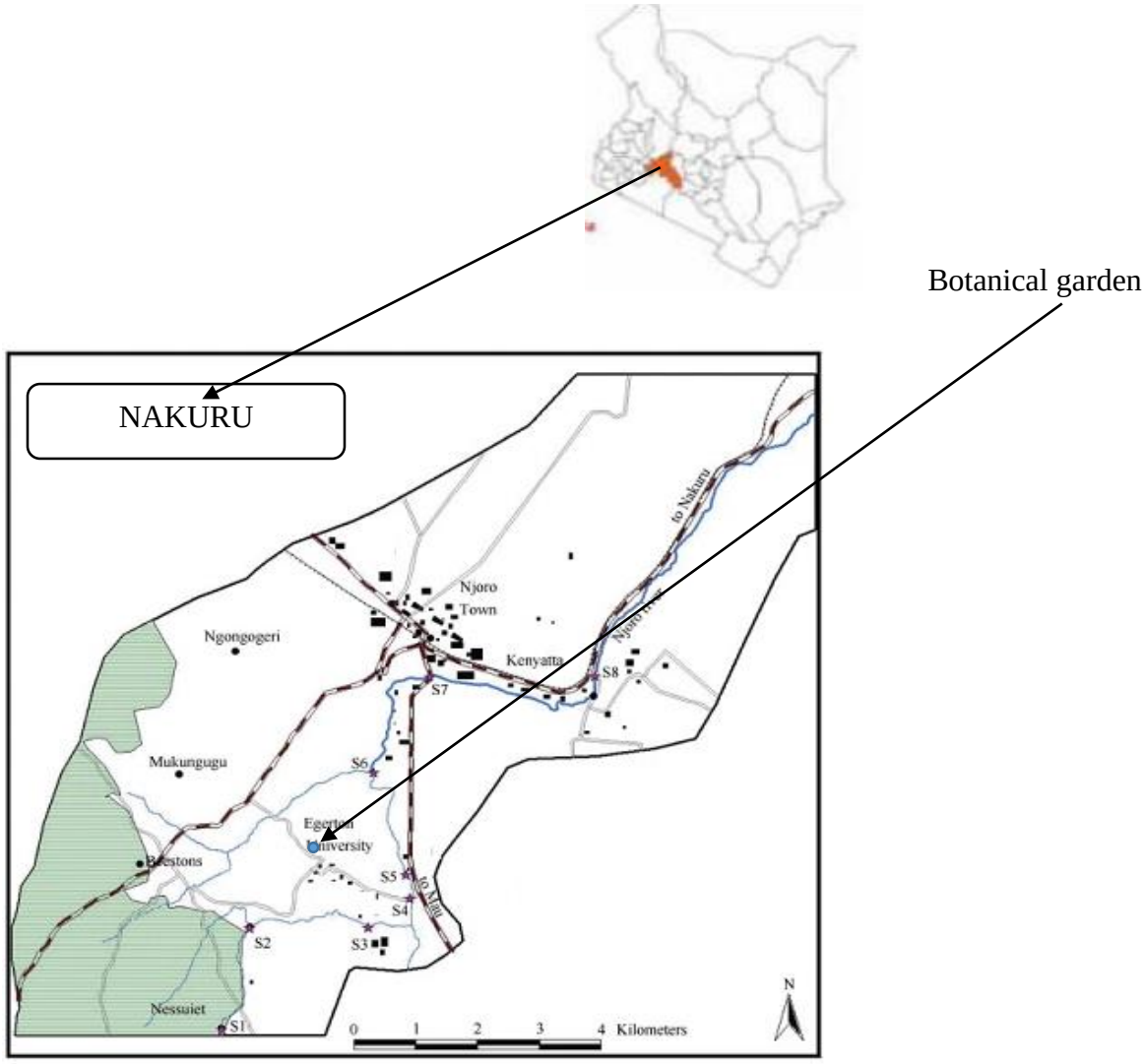


Figure 6: A map of Njoro sub-county showing Botanical garden in Egerton university

Egerton University is located in Njoro-sub county, Nakuru county, Kenya. The Botanical garden was established in the year 2002 as a center for research, teaching, recreational facility and environmental conservation. The botanical garden hence harbors threatened and rare plant species collected all over the country. It also has a wide range of medicinal plants that are common in various parts of the country.

(http://nakurupostnews.com). Thus, the Botanical garden was an excellent site for collection of plant samples used in this study.

3.2 Plants Materials

Identification and selection of the plants used in the current study was based on ethnobotanical information. This was through literature search and collaboration with local herbalists around Egerton University and a botanist for identification. With the help of a taxonomist, *U. dioica, S. officinalis, C. limon* and *P. guajava* plants were selected for this study. Young fresh leaves of the selected plants (two to three months old) were collected and taken to Biotechnology laboratory of Egerton University for drying. Young leaves were preferred to old leaves since phytochemicals are produced maximally at this stage. Also, old leaves are known to produce biomass that dilute phytochemicals (Nobossé *et al*., 2018). As a result, minute quantities of phytochemicals are produced. Leaves were also preferred over other plants parts such as stem or roots, since they regenerate in a short period hence avoiding destroying the whole plant. Additionally, they are known to produce variety of phytochemical compared to other plant parts. The leaves were dried under shade to a constant weight for a period of three weeks. A portion of wet *S. officinalis* leaves were set aside for extraction of essential oil. The dried leaves were turned periodically for even drying and to avoid rotting. Drying under shade ensured the active ingredients were retained by avoiding photo-decomposition and destruction of the phytochemicals.

3.3 Extraction of Non-Volatile Compounds

Extraction of the crude extracts (non-volatile compounds) was by methanol, hexane and ethyl acetate solvents. They were procured from Froma Commercial Supplier. Since the solvents were General Purpose Reagents (GPR), they were distilled using simple distillation before use. This was done to remove any impurities present and increase purity of the solvents. Once the solvents were distilled, the solvents were stored in glass bottles awaiting their use. After the leaves had dried to a constant weight, they were ground into a fine powder using a blending machine (Thomas-Wiley Laboratory Mill Model 4). From the powdered material, 500 g was soaked in 1.5 L of distilled methanol at room temperature (22˚C -25˚C) for 72 hours with intermittent shaking to allow maximum extraction of phytochemicals. Extraction was carried out exhaustively and filtration done using Whatman no. 1 filter paper. The obtained filtrate was concentrated to dryness using a rotary evaporator machine (BUCHI-R 205) under reduced pressure to remove the solvent. Methanol extract was obtained which was left to dry completely in a fume hood at room temperature.

3.3.1 Solvent-solvent Partitioning of Methanol Crude Extract

The concentrated methanol crude extract was divided into two parts; one portion approximately ¼ of the total yield was stored in a vial at 4ºC awaiting anti-diabetic bio-assays. The remaining portion was placed in 500 ml separating funnel and suspended in distilled water. Hexane solvent was added and mixed properly to ensure the two layers (aqueous and hexane) mix thoroughly. This was achieved through gentle swirling by tipping the separating funnel upside down for about two minutes. The pressure that built inside the separating funnel while swirling was relieved occasionally by opening the stopcock. Thereafter, the funnel was allowed to stand in a retort stand to resolve completely the mixture into two layers. Since hexane is less dense than water, the hexane portion formed the top layer. The two layers were then collected into separate conical flasks. Fresh hexane solvent was added to the aqueous portion and repeatedly extracted until the top hexane layer became colorless. The hexane portion was then concentrated to dryness under vacuum using rotary evaporator machine. The obtained hexane extract was placed in a vial and stored at 4˚C awaiting anti-diabetic assays. Thereafter, ethyl acetate solvent was added into the remaining aqueous phase in the separating funnel and exhaustively separated as in the case of hexane. After collection of ethyl acetate portion in a conical flask, it was concentrated to obtain ethyl acetate crude extract which was also stored at 4˚C. The remaining aqueous portion was dried in a freeze dryer for 48 hours to obtain the aqueous extract and stored in a freezer at -20 ºC.

3.3.2 Thin Layer Chromatography (TLC)

Ethyl acetate crude extracts of *U. dioica* and *S. officinalis* were further subjected to Thin layer chromatography analysis. The TLC was carried out on silica GF 254nm, (Merck, Germany), 0.25 mm thickness. The dry ethyl acetate extract was reconstituted in ethyl acetate solvent and mixed thoroughly in an ultrasonic cleaner in order to attain homogeneity. Several combinations of different solvent mixtures were tested and the best solvent system picked for use as the mobile phase. The ethyl acetate sample was spotted on 2×5 aluminum backed TLC plates using a capillary tube. The sample was spotted half a centimeter from the base of the TLC plate that was made by a ruler and pencil. The plate was then placed in 100 ml beaker containing 10 ml of the appropriate solvent system and covered with an aluminum foil. It was left to develop up to 4 cm up the plate. It was them removed and solvent front marked with a pencil. Thereafter, the developed chromatogram was visualized under the UV lamp (Uvitec-LF-204.LS) at 365nm and 254nm. After carrying out various solvent mixtures, 7:3 (ethyl acetate: hexane mixture) was found to give a better profiling for *S. officinalis* and 9:1 (ethyl

acetate: hexane mixture) for *U. dioica*. The extracts were then subjected to column chromatography. The hexane crude extract could not be subjected to TLC since it carries fats and sugars.

3.3.3 Column Chromatography

A portion ($^3/_4$) of *S. officinalis* and *U. dioica* ethyl acetate crude extract was subjected to column chromatography. The sample was first mixed with silica gel and ethyl acetate solvent in a round bottomed flask since the sample was sticky and could not dissolve in the solvent. The mixture was then evaporated to dryness in a rotor vapor to allow proper adsorption of the sample into the silica gel. Silica gel 60 0.06-0.2 mm (70-230 mesh ASTM) and the solvent system were mixed and packed in a chromatographic column (50 cm length and 20 mm diameter) supported by a chromatography column holder. The adsorbed sample was then placed above the layer of the silica gel and a thin layer of silica gel added above the sample to ensure the sample layer is not disturbed during further addition of the solvent system. The column was eluted gradually and the flow rate maintained at approximately 5 ml/min. At the end of the process, the column was eluted using pure methanol. Fractions of equal volumes were collected on test tubes and TLC performed on each fraction. Fractions with similar TLC patterns were pooled together and concentrated using a rotary vapor. Four fractions were obtained from *S. officinalis* ethyl acetate crude extract which were labeled as F1, F2, F3, and F4. The *U. doica* ethyl acetate extract yielded five fractions which were also labeled as F1, F2, F3, F4, and F5.

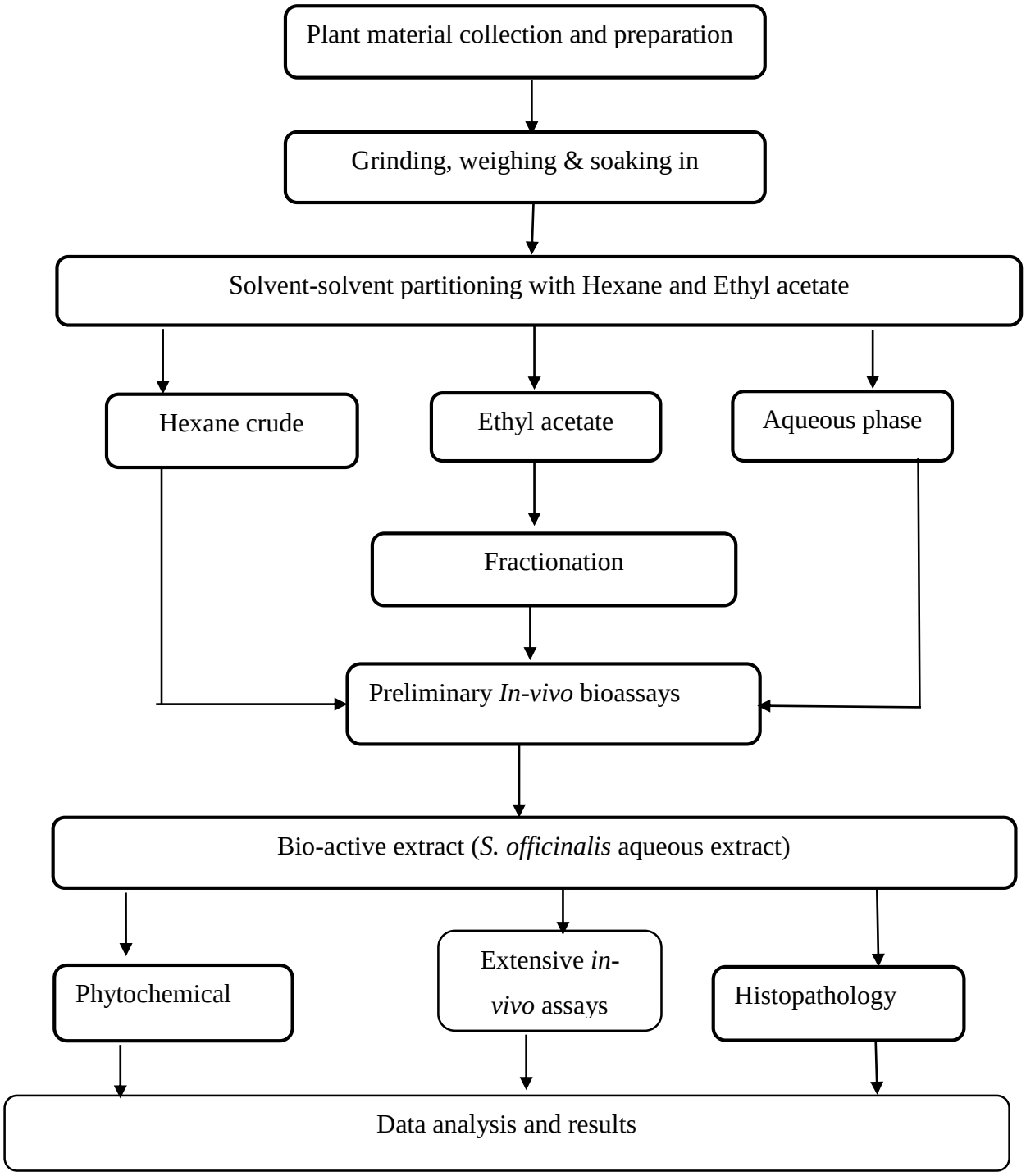


Figure 7: Summary of extraction of non-volatile compounds and the bio-assays

3.4 Extraction of Volatile Compounds

The fresh leaves of *S. officinalis* were weighed, washed under running tap water to remove dirt and cut into small pieces. They were then hydro-distilled in a modified Clevenger apparatus. The hydro-distillation process was carried out for 4 hours until the oil distillation process ceased. The essential oil obtained was dried over anhydrous sodium sulfate, put into a

glass vial, sealed and refrigerated at 4^{o}C awaiting anti-diabetic bio-assays. The *S. officinalis* essential oil was tested on experimental mice at 0.2 ml/kg and 0.4 ml/kg.

3.5 Ethical Considerations

The animal experimental protocol was approved by the Institute of Primate Research (IPR) Animal Care and Ethics Committee in collaboration with Egerton University. The ethical approval form is attached in appendix 15.

3.6 Experimental Mice

Healthy Swiss White albino male mice were used in *in vivo* bioassays in this study. Swiss albino mice are outbred strains that originated from Lausanne, Switzerland. They are best suited for biomedical research since they show genetic variability between the animals. In addition, they have some genetic similarities with human beings such as similar number of protein coding genes. Therefore, eight weeks old Swiss White albino male mice (weighing 25-30 g) were procured from Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. The animals were housed in standard polypropylene cages, six mice in each with wood shaving as beddings. Before and during the experiments mice were fed with standard mice cubes obtained from Unga Ltd, Nairobi (crude protein 18.1%, crude fibre 7%, calcium 0.8%, phosphorous 0.8%, and fat 8%). The feed and water were available *ad libitum* except during the day of blood sampling where animals were fasted overnight but with *ad libitum* water. The mice were acclimatized to standard laboratory environmental conditions [temperature (23 ± 2˚ C) dark and light cycles (12:12 h) for a period of 14 days.

3.6.1 Induction of Diabetes Mellitus in Mice

Diabetes was induced in the experimental mice through administration of a single intraperitoneal dose of alloxan (200 mg/kg). This was done 5 days before the start of the experiment to allow stable hyperglycemia to develop (King, 2012). The animals were fasted overnight before injection of the alloxan monohydrate chemical (Sigma-Aldrich, St. Louse, MO, USA). Each mouse averaging 30 g received 6 mg of the alloxan chemical. The alloxan solution was prepared by dissolving alloxan monohydrate in distilled water to a final concentration of 20 mg/ml. The solution was prepared immediately before injection since the chemical is relatively unstable (King, 2012) and kept in cold ice throughout the injection period. Each mouse received 0.3 ml of alloxan solution.

After 5 days (with access to food and water), the experimental animals were screened for hyperglycemia by determining their fasting blood glucose using the glucose oxidase kit

(The SoftStyle Glucometer and SoftStyle Blood Glucose Test Strips from Chem-labs Limited, Nairobi Kenya). Experimental mice with fasting blood sugar greater than 200 Mg/dl were selected for the study. For ease of identification, mice were marked with wet picric acid.

3.6.2 Experimental Design for the Preliminary Study

A total of 36 mice were used per plant. For each plant, mice were divided into six groups each with six mice. The first group was administered with methanol extract, the second group with ethyl acetate extract, the third group with hexane extract, and the fourth group with aqueous extract. The firth group was diabetic control and the sixth group was negative control (normal mice) which were given distilled water only. The extracts were fed to the experimental mice for a period of 7 days. This was meant to give insight into the extract with better hypoglycemic effects to pave way for the actual hypoglycemic study. Similarly, the same procedure was repeated for *U. dioica* and *S. officinalis* ethyl acetate fractions whereby six mice were used per fraction. The fractions were administered to the experimental mice for a 10-day period. For *U. dioica* fractions, five groups of six diabetic mice each were administered with extracts of F1, F2, F3, F4, and F5 respectively. The diabetic group and normal (negative) group acted as control groups and were given distilled water only. In *S. officinalis* fractions; F1, F2, F3, and F4 extracts were administered to group I, II, III, and IV of diabetic mice respectively. Group V and VI diabetic mice were administered with *S. officinalis* essential oil at 0.2 ml/kg and 0.4 ml/kg dosage levels. Diabetic and negative control groups were given distilled water.

3.6.3 Preparation of the Plants Extracts

The methanol, ethyl acetate, hexane, and aqueous crude extracts solutions of *U. dioica* (300 mg/kg), *S. officinalis* (400 mg/kg), *P. guajava* (500 mg/kg), and *C. limon* (400 mg/kg) were prepared for use in *in-vivo* assays. Equal dosage concentrations were used for all the extracts of the same plant. The aqueous extracts of the four plants were dissolved directly in distilled water. Methanol, ethyl acetate and hexane extracts were dissolved first in tween 80 to enhance their solubility and then distilled water used to top-up to the final concentration. In preparation of stock solution for animal administration, extracts should be constituted in an appropriate volume not exceeding 10 ml/kg according to Andrews & McErlane, (2015). Therefore, in every experiment, each mouse received 0.3 ml of the extract. This was the appropriate volume because large volumes cause unnecessary stress to the mice by overloading their stomachs. In 300 mg/kg of *U. dioica* each mouse averaging 30 g received 9

mg of the extract. The stock solution was prepared to the final concentration of 30 mg/ml. In 400 mg/kg of *S. officinalis* and *C. limon* each mouse received 12 mg of the extract. The extracts were constituted to a final concentration of 40 mg/ml. Lastly, each mouse received 15 mg of the *P. guajava* extract and was constituted to a final concentration of 50 mg/ml.

The dosage concentration of the fractions was half the total dose of the crude extracts. The ethyl acetate fractions of *U. dioica* were administered at 150 mg/kg whereby each mouse received 4.5 mg of the fraction. Similarly, *S. officinalis* ethyl acetate fractions were administered at 200 mg/kg, each mouse receiving 6 mg of the extract. The extracts were prepared to a final concentration of 15 mg/ml and 20 mg/ml respectively. The prepared samples were then stored at 4˚c awaiting assays. Stock preparation of the extracts and calculations are shown in appendix 14.

3.6.4 Administration of the extracts to the experimental mice

Extracts were administered orally by use of an intra-gastric gavage fitted with 2 ml syringe. The syringe was first pre-filled with the extract. Afterwards, the mouse was removed from the cage and restrained in an upright position by holding the mouse's skin on the shoulders. The mouse was held in such a manner that the head and neck were immobilized while the front legs were extended out to the side. However, the mouse was breathing freely. The gavage needle was then inserted into the mouse' mouth on the left side, in front of the first molar and behind the front teeth. The needle was directed along the hard palate and gently pushed towards the back of the throat. Then the extract dosage was injected slowly and steadily while ensuring the mouse was breathing normally. After the last bit of the extract was injected from the syringe, the gavage was removed steadily from the mouse' mouth. The mouse was then returned to its cage.

3.6.5 Transition from Preliminary Study to the Actual Hypoglycaemic Test

After the preliminary screening of the extracts for 7 days, results were analyzed to determine the most potent extract. The preliminary study was to offer insight into the hypoglycemic effect of the extracts. The resulted revealed that aqueous extract of *S. officinalis* was the most effective as it lowered blood sugar levels of the mice significantly compared to other extracts. Therefore, it was subsequently selected for extensive study at the dosage levels of 400 mg/kg and 600 mg/kg. For the 400 mg/kg and 600 mg/kg of the extract, each mouse averaging 30g received 12 mg and 18 mg of the *S. officinalis* aqueous extract

respectively. The extracts were reconstituted in 1% tween 80 and then distilled water was used to top up to the final concentration of 40 mg/ml and 60 mg/ml respectively.

3.6.6 Hypoglycaemic Activity Test

Mice were randomized into five groups of 6 mice each before initiation of the experiment. Group I (negative control-healthy mice not induced with diabetes, not fed with the extract), Group II (diabetic control-mice induced with diabetes, not fed with the extract), Group III (diabetic mice given 400 mg/kg of the extract), Group IV (diabetic mice given 600 mg/kg of the extract) and Group V (positive control- diabetic mice given a standard drug-glibenclamide at 2 mg/kg). Each mouse averaging 30 g received 0.06 mg of the glibenclamide drug. 2 mg of the drug was constituted in 10 ml of the distilled water to a final concentration of 0.2 mg/ml. Plant extracts and glibenclamide drug were administered orally to the respective groups of mice every day for a period of 15 days while Group I and Group II control groups were given distilled water. Baseline blood glucose levels of all the animals were taken before extract administration and fasting blood sugar (FBS) determined at intervals of 72 hours (3 days) for 360 hours (15) days. Blood was withdrawn by tail snipping from each animal for blood sugar analysis. The mouse was gently and securely restrained and the tail sterilized with 70% alcohol. The tail tip was cut with sanitized scissors and a single drop of blood was collected in the glucose test strip already fixed in the glucometer. The blood sugar level values were then recorded. Afterwards, the tail tip was wiped with wet cotton soaked in 70% alcohol to prevent further blood loss.

Table 1: Experimental design of *in vivo* bio-assay

	Design of treatment	**Dose (mg/kg bw)**
Group I	Negative control (Normal non-diabetic) + distilled water only	0.3 ml
Group II	Diabetic control + distilled water only	0.3 ml
Group III	Diabetic mice + *S. officinalis* aqueous extract	400
Group IV	Diabetic mice + *S. officinalis* aqueous extract	600
Group V	Positive control (Diabetic mice + Glibenclamide)	2

3.6 7 Determination of Body Weights of the Experimental mice

Baseline body weights of all the experimental mice in the hypoglycaemic study, were measured and recorded before and after induction of diabetes mellitus. Consequently, the weights were measured during the entire study period at intervals of 72 hours. An electronic beam balance, model type: BL-220H (Shimadzu®, Japan) was used.

3.7 Microscopic Examination of Liver and Kidney Histopathology

At the end of the experiment, the animals were sacrificed by ether euthanasia. The mice were placed in a closed container and diethyl ether introduced. Since diethyl ether is an inhalant anesthetic, the mice inhaled the vapor causing respiration to cease. They were then dissected to obtain kidney and liver organs for histopathology tests. Animal carcasses were disposed through incineration. The liver and kidney organs were fixed and preserved in 10% (v/v) formalin and then processed for paraffin embedding procedures according to Feldman & Wolfe, (2014). Sections 7μm thick were cut, mounted onto slides and stained with hematoxylin and eosin dye (H & E). They were then viewed under light microscopy (HumaScope Advanced^{LED} Binocular digital microscope) for evaluation. Observation was made on variations in the tissue characteristics in terms of structure, morphology and changes in appearance, size, and color of the tissues. Comparison of the various tissues was made between the different treatment groups against the controls.

3.8 Phytochemical Analysis of the Extracts

Phytochemicals present in various extracts was determined by carrying out various texts for alkaloids, flavonone alglycones, atheracene aglycones, sterols and triterpenes, steroid glycosides, tannins, saponins, and flavanone glycoside. All the methanol, ethyl acetate, hexane, and aqueous extracts of *U. dioica*, *S. officinalis*, *P. guajava*, and *C. limon* were analyzed. Similarly, the fractions of *U. dioica* and *S. officinalis* were tested for the presence or absence of the various classes of phytochemicals. All the texts were done qualitatively by following standard procedures according to Harborne, (1998).

3.8.1 Determination of alkaloids (Basic)

3 mg of the dried extract was placed in 10 ml test tube and dissolved in 3 ml of 2% hydrochloric acid. The mixture was stirred to form a soluble solution. From the obtained acidic aqueous solution, 0.5 ml was transferred to another test tube and 3 drops of Mayer's reagent were added. The Mayer's reagent was made beforehand by mixing 1.36 g of mercuric

chloride and 5 g of potassium iodide in 100 ml of distilled water. The solution was shaken formation of an opalescence, a cream colored precipate confirmed presence of alkaloids.

3.8.2 Determination of flavonone aglycones and atheracene aglycones

Approximately, 3 mg of the extract was placed in a test tube and dissolved in 2 ml of 50% methanol. The test tube was kept in a water bath maintained at 4˚C for 5 minutes. Formation of a red or orange color indicated presence of flavonone aglycones. Similarly, in a test tube containing 1 ml of 25% ammonium solution, 3 mg of the extract was added and the mixture shaken for 5 minutes. Formation of a red color showed presence of atheracene aglycones.

3.8.3 Determination of sterols

3 mg of the extract was dissolved in appropriate solvent and evaporated to dryness. The obtained residue was dissolved in 0.5 ml of acetic acid followed by addition of 0.5 ml of chloroform. The resulting solution was transferred to a dry test tube and 2 ml of concentrated sulfuric acid added slowly along the sides of the test tube. Formation of a brownish-red ring at the contact zone of the two liquids and a green supernatant confirmed presence of sterols.

3.8.4 Determination of flavanone glycoside

5 mg of the extract was placed in a test tube and dissolved in 2 ml of 50% methanol. The mixture was allowed to mix properly by heating in a water bath. A small piece of metallic magnesium was added to the solution and reaction allowed to take place for five minutes. Afterwards, 6 drops of concentrated hydrochloric acid were added along the sides of the test tube. Change in color of the solution to red indicated presence of flavonols while color change to orange confirmed presence of flavanones.

3.8.5 Determination of saponins

5 mg of the extract was first dissolved in 10 ml of the appropriate solvent. That is, the methanol, ethyl acetate, hexane, and aqueous extracts were dissolved in methanol solvent, ethyl acetate solvent, hexane solvent, and distilled water respectively. From the resultant solution, 2 ml was measured and transferred into a clean test tube. It was mixed with equal volume of methanol solvent in the ratio of 1:1. Consequently, 2 ml of the solution was placed in a test tube and shaken for 15 minutes. Formation of a froth indicated presence of saponins.

3.8.6 Determination of tannins

A mixture approximately 1 ml of methanol and aqueous extract was prepared with a solution of 0.1% ferric chloride. The solution was then boiled in a water bath to reflux the hydrochloric formaldehyde solution. In case catechol tannins were present, they condensed as a red precipitate which were filtered off. The resultant solution was then neutralized with sodium acetate and followed by addition of 3 drops of ferric chloride. Formation of a deep blue color indicated presence of gallic tannins while condensation of a red precipitate confirmed presence of catechol tannins.

3.8.7 Determination of steroid Glycosides and Triterpenes

5 mg of the extract was dissolved in 0.5 ml of acetic anhydride followed by 0.5 ml of chloroform. The mixture was shaken for about 5 minutes for proper mixing. The solution was transferred into a dry test tube and 2 ml of concentrated sulfuric acid added at the bottom. Formation of a violet coloration or ring at the interface confirmed presence of steroid glycosides or triterpenes.

3.9 Data Analyses

The fasting blood sugar levels data was entered in an excel worksheet and expressed as the Mean ± Standard Error of the Mean (Mean ± S.E.M). The data was analyzed using Statistical Package for Social Sciences (SPSS) software (IBM SPSSS Statistics 23). One-way analysis of variance (ANOVA) followed by Turkey post hoc test was used to analyze mean differences between various treatment groups. p values <0.05 were considered to be statistically significant. The histopathological data was analyzed qualitatively by examining tissue structure, regularities of cell shapes, distribution of cells in tissue, and general morphological characteristics. Comparison was made between the tissues of the treatment against the controls. The phytochemical data was qualitatively analyzed by observing color change in every test. The data was entered in a table form whereby presence of a phytochemical was indicated by a positive (+) sign while absence of a phytochemical by a negative (-) sign.

CHAPTER FOUR

RESULTS

4.1 Preliminary Screening of the Crude Extracts

Preliminary screening results of *U. dioica* (stinging nettle), *S. officinalis* (sage), *P. guajava* (guava) and *C. limon* (limon) extracts for hypoglycemic effect is as shown in Table 2. Before induction of diabetes (-ve 5 days), the mean fasting blood sugar levels of all the groups of mice were in the same range (80.33 ± 0.76 mg/dl – 91.33 ± 1.48 mg/dl). On day 0, they had reached diabetic levels of ≥ 200 mg/dl.

Table 2: Effects of crude extracts of *U. doica*, *S. officinalis*, *P. guajava* and *C. limon* on fasting blood sugar levels of alloxan-induced diabetic mice.

Blood sugar levels (Mg/dl)				
Plant	**Extracts**	**FBG level at day -5**	**FBG level at day 0**	**FBG level at day 7**
U. doica extracts at 300 mg/kg	Methanol	88.00 ± 1.20^{a}	486.00 ± 1.60^{bc}	441.33 ± 2.01^{b}*
	Ethyl acetate	88.33 ± 1.52^{a}	482.00 ± 0.97^{b}	466.33 ± 9.81^{c}*
	Hexane	89.00 ± 0.73^{a}	487.67 ± 1.28^{c}*	457.00 ± 2.30^{c}*
	Aqueous	86.00 ± 1.20^{a}	487.67 ± 0.84^{c}*	435.67 ± 1.28^{b}*
	Negative Control	90.33 ± 0.76^{a}	88.67 ± 0.21^{a}*	88.33 ± 1.28^{a}*
	Diabetic Control	87.67 ± 0.56^{a}	482.67± 0.76^{b}	549.00 ± 0.73^{d}
S. officinalis extracts at 400 mg/kg	Methanol	90.00 ± 1.59^{a}	497.00 ± 2.78^{c}	488.00 ± 2.82^{c}*
	Ethyl acetate	84.33 ± 2.76^{a}	482.33 ± 0.42^{b}	519.00 ± 10.30^{d}*
	Hexane	89.67 ± 0.56^{a}	490.67 ± 3.01^{bc}	473.00 ± 2.73^{c}*
	Aqueous	88.33 ± 1.52^{a}	487.67 ± 2.58^{bc}	426.33 ± 2.95^{b}*
	Negative Control	86.33 ± 1.69^{a}	85.00 ± 0.73^{a}*	83.00 ± 0.73^{a}*
	Diabetic Control	82.67 ± 3.37^{a}	488.00 ± 2.82^{bc}	558.00 ± 1.73^{e}
P. guajava extracts at 500 mg/kg	Methanol	87.33 ± 0.76^{a}	483.00 ± 0.73^{b}	462.33 ± 4.93^{b}*
	Ethyl acetate	86.67 ± 2.74^{ab}	499.50 ± 3.01^{c}	481.33 ± 2.60^{c}*
	Hexane	84.33 ± 1.52^{ab}	482.67 ± 0.56^{b}	475.67 ± 1.52^{bc}*
	Aqueous	80.33 ± 0.76^{a}	483.33 ± 0.92^{b}	457.00 ± 5.38^{b}*
	Negative Control	88.33 ± 1.52^{b}	89.33 ± 0.56^{a}*	88.00 ± 1.10^{a}*
	Diabetic Control	85.67 ± 2.23^{ab}	493.33 ± 7.34^{bc}	551.17 ± 7.0^{d}

C. limon extract	Methanol	87.00 ± 0.73^{a}	485.00 ± 1.93^{b}	493.33 ± 3.19^{d}*
at 400 mg/kg	Ethyl acetate	84.00 ± 1.83^{a}	487.33 ± 1.17^{b}	440.00 ± 0.97^{b}*
	Hexane	91.33 ± 1.48^{a}	483.33 ± 1.28^{b}	446.33 ± 1.12^{b}*
	Aqueous	86.00 ± 3.48^{a}	488.67 ± 2.93^{b}	481.33 ± 4.37^{c}*
	Negative Control	87.67 ± 1.12^{a}	87.33 ± 1.87^{a}*	87.00 ± 0.73^{a}*
	Diabetic control	88.00 ± 0.89^{a}	489.33 ± 2.23^{b}	559.67 ± 1.84^{e}

The means were expressed as Mean ± S.E.M (n=6). Statistical analysis of data was done by One-way ANOVA followed by Turkey Post-hoc test. Values in the same column with different superscript are statistically different: (*p<0.05, Turkey's test).

The diabetic control groups showed a gradual increase in the mean FBS levels throughout the 7-day period. The mean FBS of the negative control groups remained constant throughout the study as shown in Table 2. The aqueous extract of *S. officinalis* and *U. doica* fed on diabetic mice, showed a higher decrease in fasting blood sugar levels compared to other extracts. The mice fed with the two extracts recorded a drop in their mean fasting blood sugar levels from 487.67 ± 2.58 mg/dl and 487.67 ± 0.84 mg/dl – 426.33 ± 2.95 mg/dl and 435.67 ± 1.28 mg/dl respectively. The results revealed significant drop in blood sugar levels of the diabetic mice after treatment with extracts of stinging nettle, sage, guava, and lemon plants.

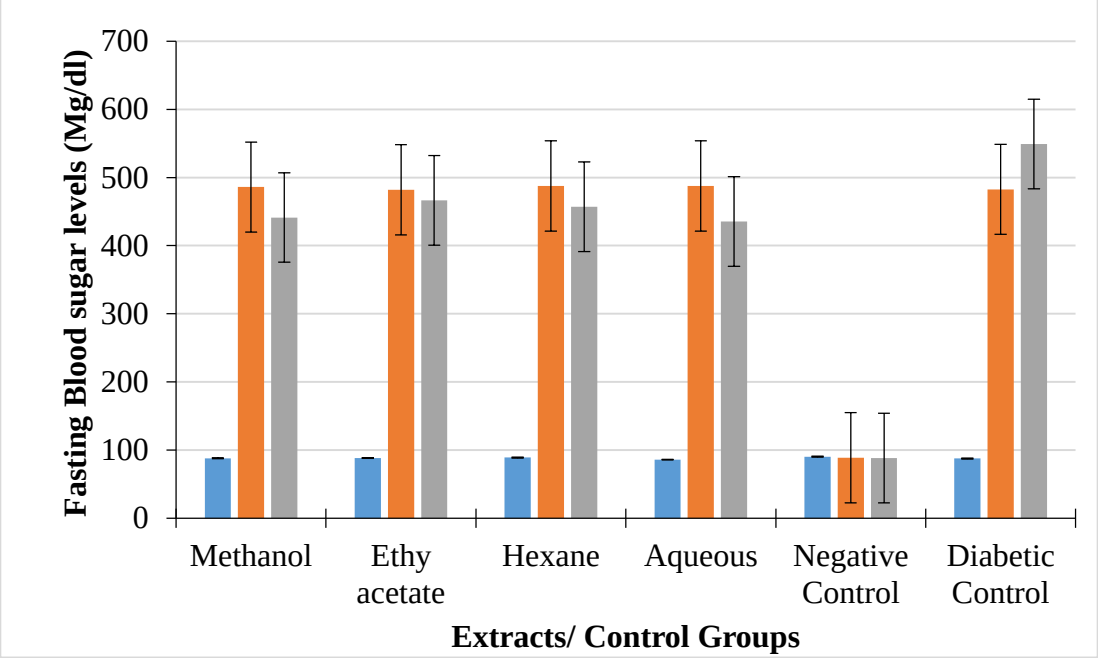


Figure 8: Mean fasting blood sugar levels extracts of alloxan-induced diabetic mice after administration of *U. dioica* crude

The methanol, ethyl acetate, hexane, and aqueous extracts of *U. dioica* (stinging nettle) recorded a significant decrease ($p<0.05$) in fasting blood sugar levels of alloxan-induced diabetic mice. The decrease was evident 7 days after administration of the extracts to diabetic mice. The aqueous extract recorded the highest decrease in fasting blood sugar levels of diabetic mice, followed by methanol extract, hexane extract, and finally the ethyl acetate had the least significant effect (figure 8).

Similarly, in *S. officinalis* (sage) plant, the aqueous extract lowered the fasting blood sugar levels of diabetic mice significantly compared to other extracts. It was followed by hexane and methanol extract. Nevertheless, the ethyl acetate did not lower blood sugar levels of diabetic mice, but rather recorded a hyperglycemic effect by increasing fasting sugar levels from 482.33 ± 0.42 mg/dl to 519.00 ± 10.30 mg/dl.

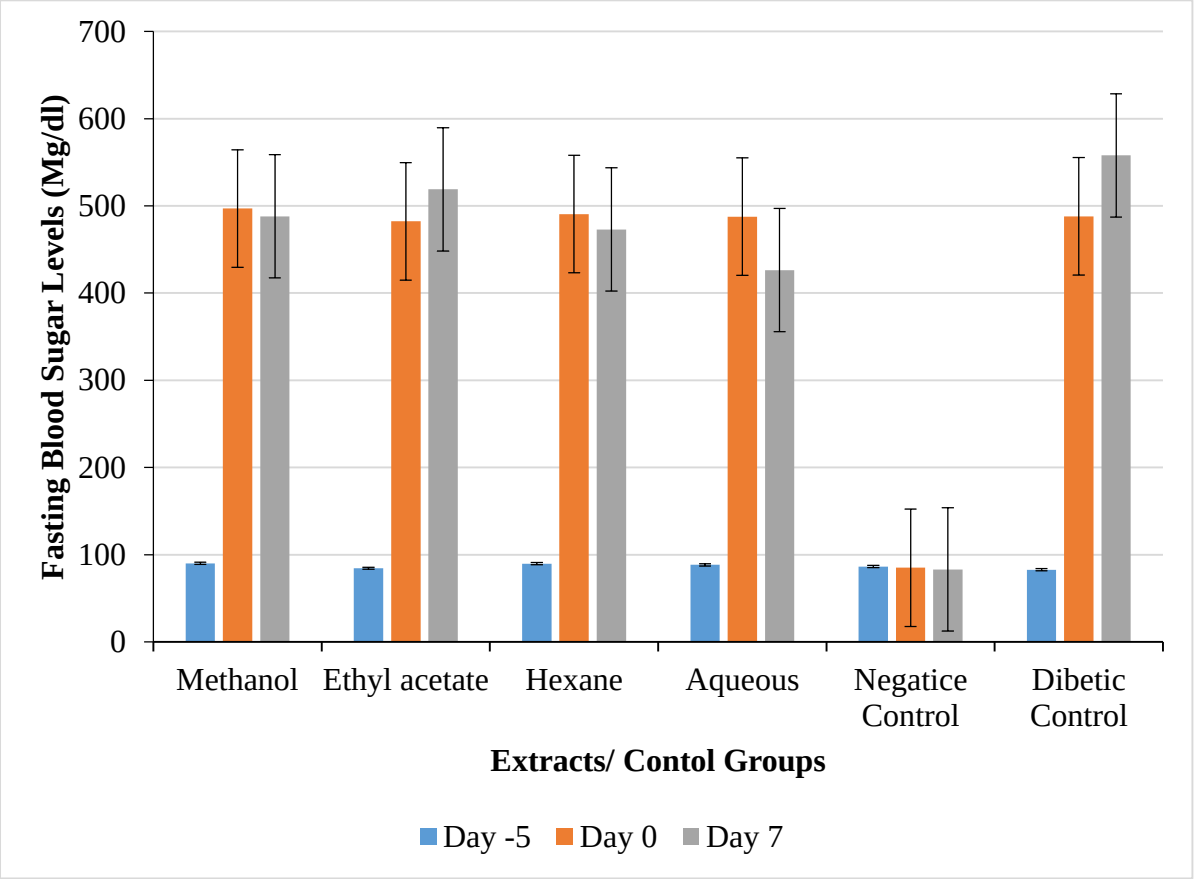


Figure 9: Mean fasting blood sugar levels of alloxan-induced diabetic mice after administration of *S. officinalis* crude extracts

Results of *P. guajava* plant revealed that methanol, ethyl acetate, hexane, and aqueous extracts had a significant effect in lowering fasting blood sugar levels of alloxan-induced diabetic mice. The aqueous extract recorded the highest drop in blood sugar levels of diabetic mice from 483.33 ± 0.92 mg/dl to 457.00 ± 5.38 mg/dl. It was followed by methanol extract, ethyl acetate extract, and hexane extract respectively.

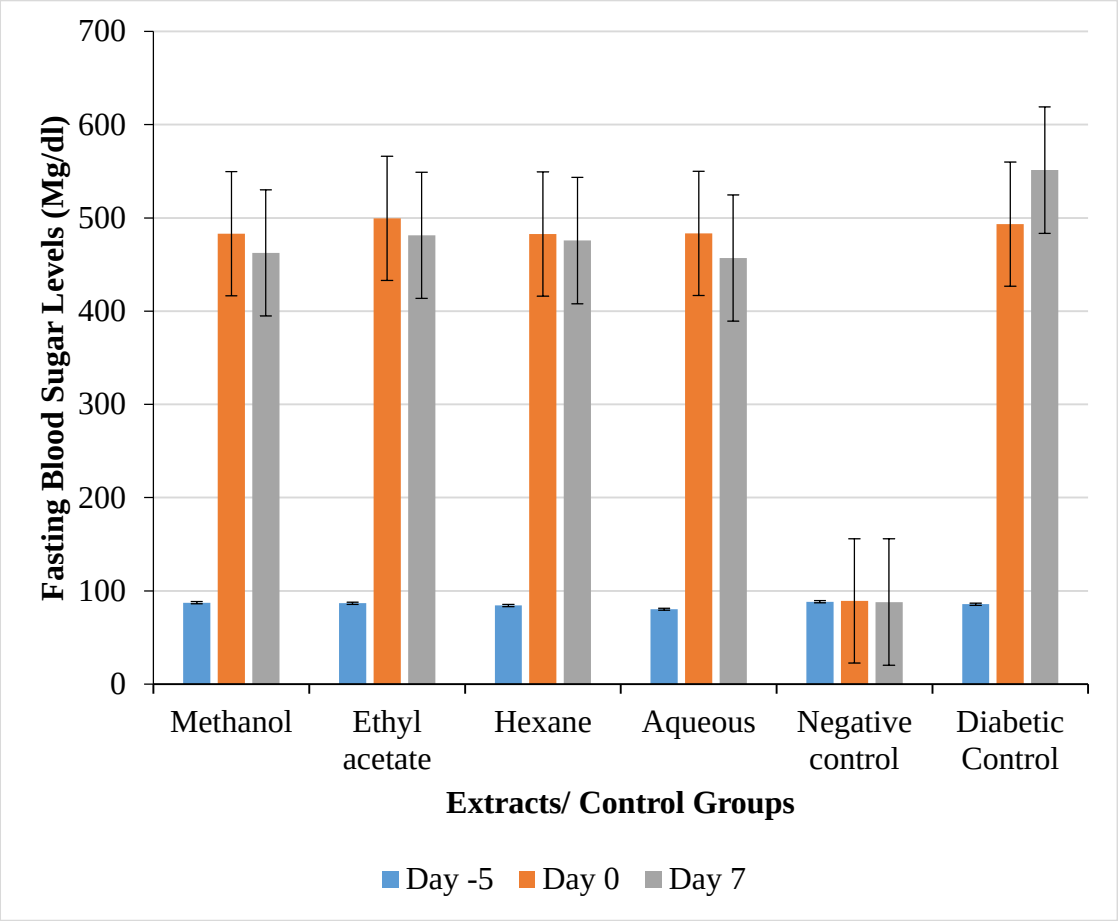


Figure 10: Mean fasting blood sugar levels of alloxan-induced diabetic mice after administration of *P. guajava* crude extracts

Analyses of data for *C. limon* plant showed that ethyl acetate lowered fasting blood sugar levels of alloxan-induced mice significantly compared to others. It was followed by hexane and aqueous extract respectively. Nevertheless, the methanol did not lower the fasting blood sugar levels after administration to diabetic mice for 7 days. Rather, the blood sugar levels had risen from 485.00 ± 1.93 mg/dl –and 493.33 ± 3.19 mg/dl respectively.

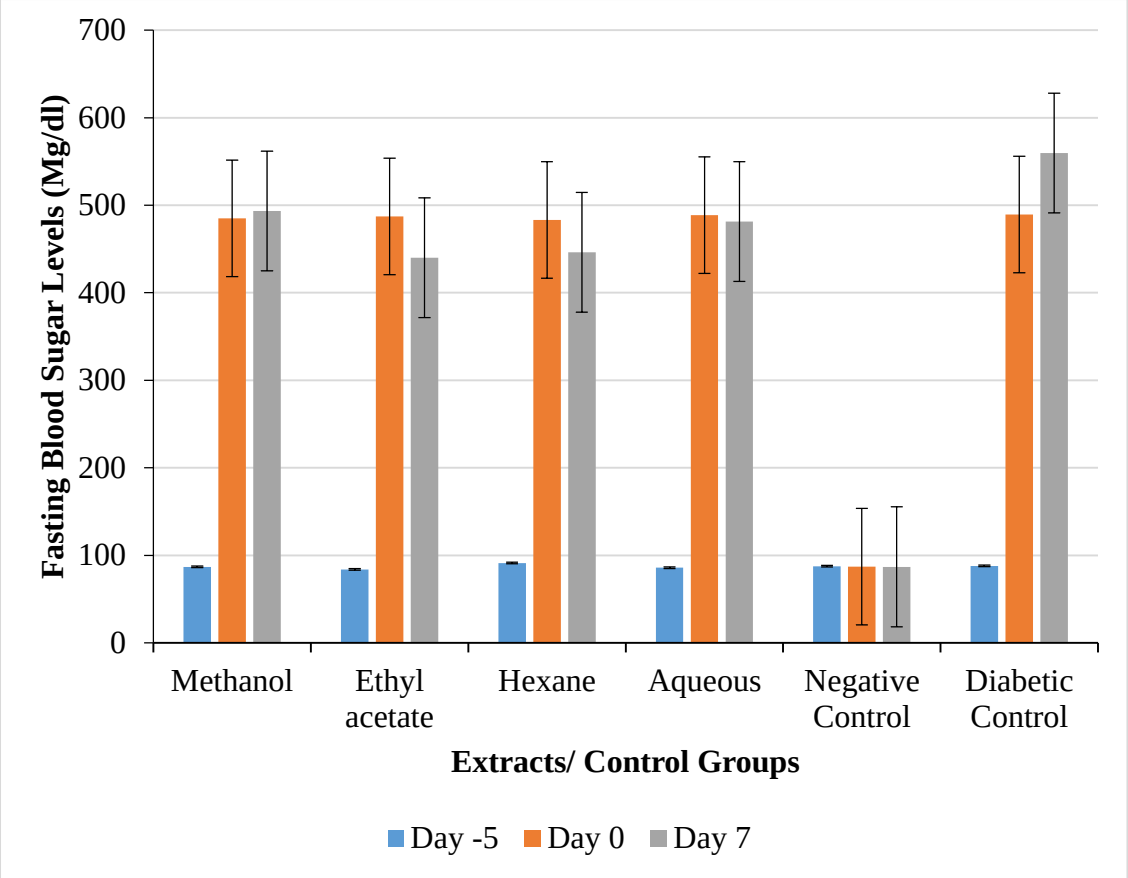


Figure 11: Mean fasting blood sugar levels of alloxan-induced diabetic mice after administration of *C. limon* crude extracts

4.2 Hypoglycemic Effects of *U. dioica* and *S. officinalis* Ethyl Acetate Fractions

The hypoglycemic results of *U. dioica* and *S. officinalis* ethyl acetate fractions and the essential oil of *S. officinalis* are indicated in Table 3. The data was analyzed 10 days after administration of the fractions to alloxan-induced diabetic mice. Additionally, the essential oil obtained from *S. officinalis* was tested together with the fractions. There was a significant decrease in fasting blood sugar levels of alloxan-induced diabetic mice after treatment with *U. dioica* and *S. officinalis* fractions as well as essential oil of *S. officinalis* for 10 days. Before induction of diabetes, all the groups of mice had mean fasting blood sugar levels ranging from 79.33 ± 1.87 mg/dl – 89.33 ± 0.56 mg/dl. Diabetes induction elevated FBS to levels ranging from 490.67 ± 0.56 mg/dl – 507.33 ± 0.56 mg/dl. After 10 days, the extracts lowered the blood sugar levels significantly to 426.33 ± 1.84 mg/dl - 482.67 ± 5.83 mg/dl (*$p<0.05$).

Table 3: Effects of *S. officinalis* fractions, *U. dioica* fractions and *S. officinalis* essential oil on fasting blood sugar levels of alloxan- induced diabetic mice

Blood sugar levels (mg/dl)				
Plant	**Fractions**	**FBG levels at -5 days**	**FBG levels at 0 days**	**FBG levels at 10 days**
S. officinalis (200 mg/kg)	F1	88.00 ± 0.37*^{b}	492.33 ± 0.56^{b}	470.33 ± 1.38^{b}*
	F2	84.33 ± 1.74^{ab}	497.33 ± 0.76^{cd}	472.00 ± 2.90^{b}*
	F3	85.67 ± 2.56^{ab}	491.33 ± 0.21^{b}	461.67 ± 2.69^{b}*
	F4	87.67 ± 0.76*^{b}	494.00 ± 1.00^{bc}	461.17 ± 7.40^{b}*
	0.2 ml/kg essential oil	84.00 ± 1.83^{ab}	500.00 ± 2.56*^{d}	465.67 ± 4.97^{b}*
	0.4 ml/kg essential oil	85.67 ± 0.42^{ab}	507.33 ± 0.56^{e}*	482.67 ± 5.83^{b}*
	Negative Control	89.33 ± 0.56*^{b}	86.67 ± 0.76^{a}*	90.33 ± 1.12^{a}*
	Diabetic Control	79.33 ± 1.87^{a}	494.67 ± 0.56^{bc}	562.17 ± 8.67^{c}
U. dioica (150 mg/kg)	F1	83.33 ± 1.23^{ab}	496.67 ± 0.56^{b}	475.67 ± 4.96^{d}*
	F2	87.67 ± 3.31^{ab}	490.67 ± 0.56^{b}	426.33 ± 1.84^{b}*
	F3	85.33 ± 2.43^{ab}	500.50 ± 5.27^{b}	454.17 ± 8.22^{c}*
	F4	84.00 ± 1.61^{ab}	500.33 ± 7.92^{b}	467.00 ± 5.41^{cd}*
	F5	79.67 ± 1.48*^{a}	494.00 ± 3.47^{b}	481.00 ± 3.86^{d}*
	Negative Control	87.00 ± 0.37^{ab}	88.67 ± 0.42^{a}*	89.33 ± 0.56^{a}*
	Diabetic Control	89.00 ± 0.45^{b}	496.67 ± 0.56^{b}	564.67 ± 1.28^{e}

The means were expressed as Mean ± S.E.M (n=6). Statistical analysis of data was done by one-way ANOVA followed by Turkey Post-hoc multiple comparison tests. Values in the same column with different superscript are statistically different: (*$p<0.05$, Turkey's test). (F1- Fraction 1; F2- Fraction 2, F3- Fraction 3, F4- Fraction 4, F-5 Fraction 5 (The fractions were obtained after fractionation of ethyl acetate crude extract of *U. dioica* and *S. officinalis* plants).

In *U. dioica*, the F2 fraction demonstrated better results compared to other fractions by reducing the mean blood sugar levels of diabetic mice from 490.67 ± 0.56 mg/dl – 426.33 ± 1.84 mg/dl. This was followed by fraction 3 (F3), fraction 4 (fraction 4), fraction 1 (F1), and finally fraction 5 (F5).

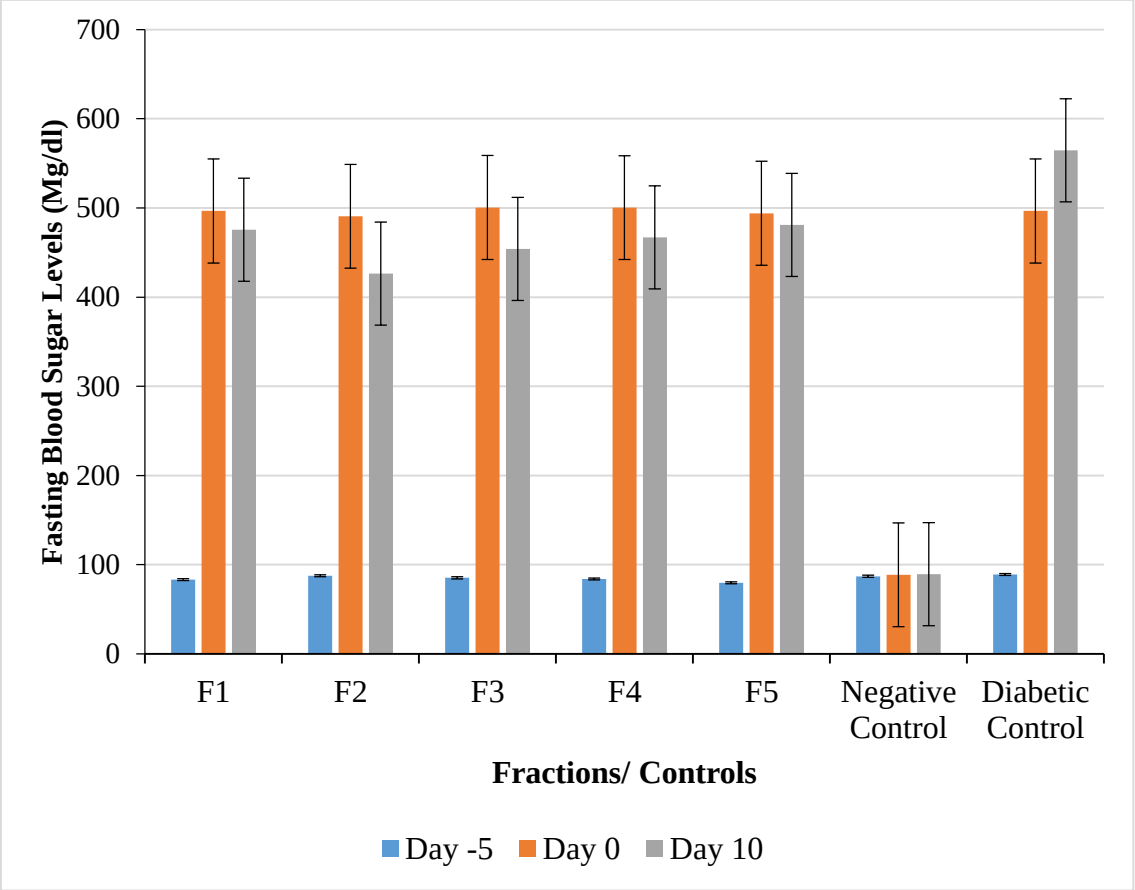


Figure 12: Mean fasting blood sugar levels of alloxan-induced diabetic mice after administration of *U. dioica* fractions

On the other hand, in *S. officinalis* fractions, F4 fraction gave better results as compared to other fractions by lowering the mean fasting blood sugar levels from 494.00 ± 1.00 mg/dl – 461.17 ± 7.40 mg/dl. This was followed by fraction 3 (F3), fraction 2 (F2), and fraction 1 (F1) respectively. Similarly, the essential oil of *S. officinalis* at 0.2 ml/kg and 0.4 ml/kg lowered FBS levels from 500.00 ± 2.56 mg/dl and 507.33 ± 0.56 mg/dl to 465.67 ± 4.97 mg/dl and 482.67 ± 5.83 mg/dl respectively. The drop in fasting blood sugar levels of alloxan-induced mice was significant with a p value of 0.000 (Appendix 1) compared to the diabetic control.

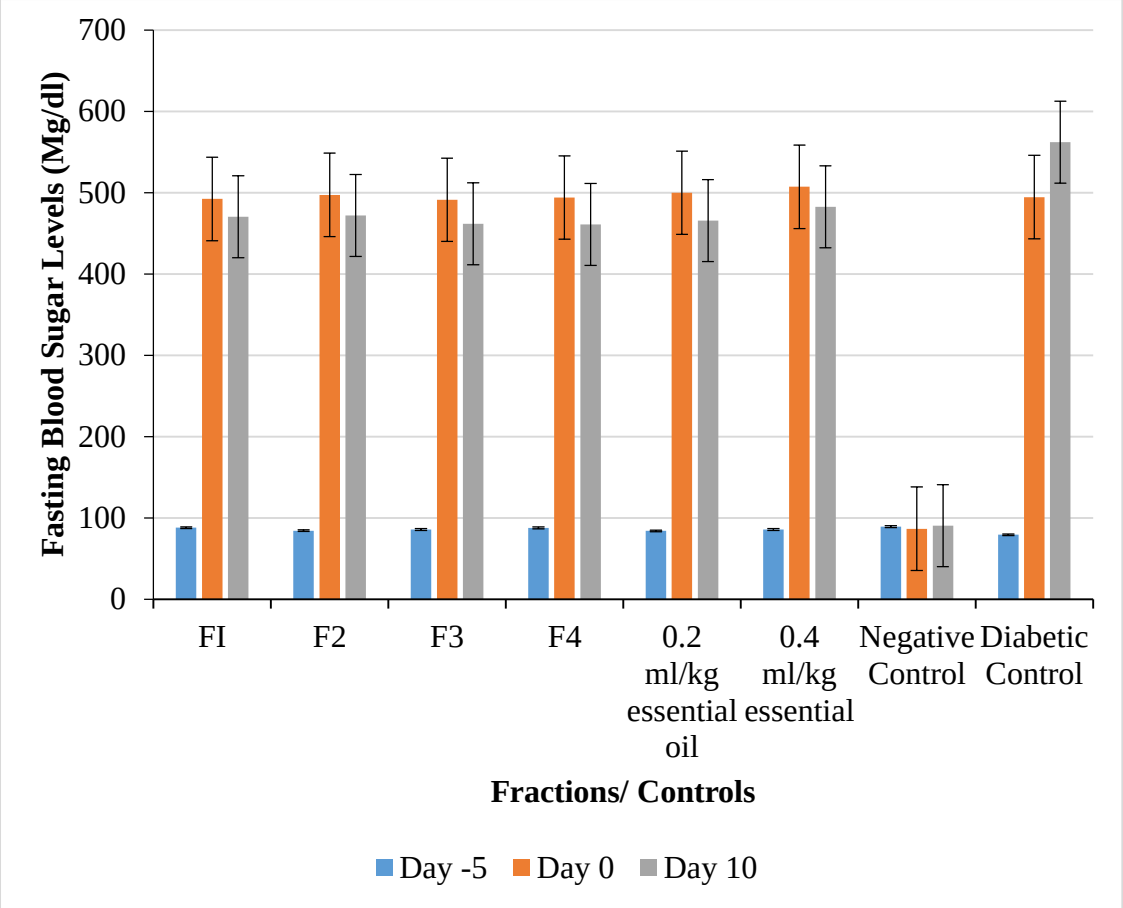


Figure 13: Mean fasting blood sugar levels of alloxan-induced diabetic mice after administration of *S. officinalis* fractions and essential oil

4.3. Hypoglycemic Activity Test

Before induction of diabetes (-5 days) the mean fasting blood sugar levels of all the groups ranged from 81.83 ± 1.38 mg/dl – 86.33 ± 1.91 mg/dl. Five days after induction of diabetes mellitus to experimental mice, the fasting blood sugar levels rose to diabetic levels, ≥ 200 mg/dl (Group II having a mean (± S.E.M) of 444.67 ± 14.07 mg/dl, Group III; 452.00 ± 11.13 mg/dl, Group IV; 431.00 ± 10.65 mg/dl, and Group V; 451.83 ± 5.88 mg/dl). The mean fasting blood sugar levels of the diabetic control group (Group II) was consistently high throughout the study period. By the 15th day, it had shot to 480.67 ± 4.65 mg/dl. Group III (diabetic mice fed with 400 mg/kg) recorded a gradual decrease in the mean fasting blood sugar levels and by the end of the study the mean had dropped to 256.33 ± 5.12 mg/dl. The same trend was observed in Group IV (diabetic mice fed with 600 mg/kg), where the mean fasting blood sugar levels dropped to 256.67 ± 8.74 mg/dl. Similarly, the mean fasting blood sugar levels of Group V (fed with glibenclamide drug) had dropped from to 300.00 ± 7.17 mg/dl.

Table 4: Effect of *S. officinalis* aqueous extract on fasting blood sugar levels of various treatment groups after administration to alloxan-induced diabetic mice.

Fasting Blood Sugar (FBS) Levels (mg/dl)					
Time/	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
-5 Days	85.17 ± 1.66[a]	85.67 ± 2.42[a]	81.83 ± 1.38[a]	85.50 ± 2.68[a]	86.33 ± 1.91[a]
0 Days	84.33 ± 1.45[a]*	444.67 ± 14.07[b]	452.00 ± 11.13[b]	431.00 ± 10.65[b]	451.83 ± 5.88[b]
Day 3	83.83 ± 1.83[a]*	454.67 ± 12.62[c]	412.83 ± 9.39[bc]	377.33 ± 17.22[b]*	452.67 ± 8.07[c]
Day 6	86.67 ± 1.67[a]*	446.67 ± 10.47[c]	374.83 ± 4.11[b]*	371.67 ± 14.24[b]*	395.83 ± 9.70[b]*
Day 9	82.67 ± 1.09[a]*	457.33 ± 4.29[c]	340.67 ± 8.30[b]*	327.67 ± 12.02[b]*	361.33 ± 15.11[b]*
Day 12	85.00 ± 1.93[a]*	473.00 ± 5.74[d]	287.17 ± 6.64[b]*	290.33 ± 9.58[b]*	337.00 ± 13.74[c]*
Day 15	86.50 ± 2.83[a]*	480.67 ± 4.65[d]	256.33 ± 5.12[b]*	256.67 ± 8.74[b]*	300.00 ± 7.17[c]*

The means were expressed as Mean ± S.E.M (n=6). Statistical analysis of data was done by one-way ANOVA followed by Turkey Post-hoc multiple comparison tests. Values in the same row with different superscript are statistically different: (*p<0.05, Turkey's test).

Group I (Negative control- normal mice not fed with S. officinalis aqueous extract), Group II (Diabetic control- diabetic mice not fed with S. officinalis aqueous extract), Group III (Diabetic mice fed with 400 mg/kg aqueous extract of S. officinalis for 15 days), Group IV (Diabetic mice fed with 600 mg/kg aqueous extract of S. officinalis for 15 days), Group V (Positive control- diabetic mice fed with 2 mg/kg glibenclamide drug for 15 days).

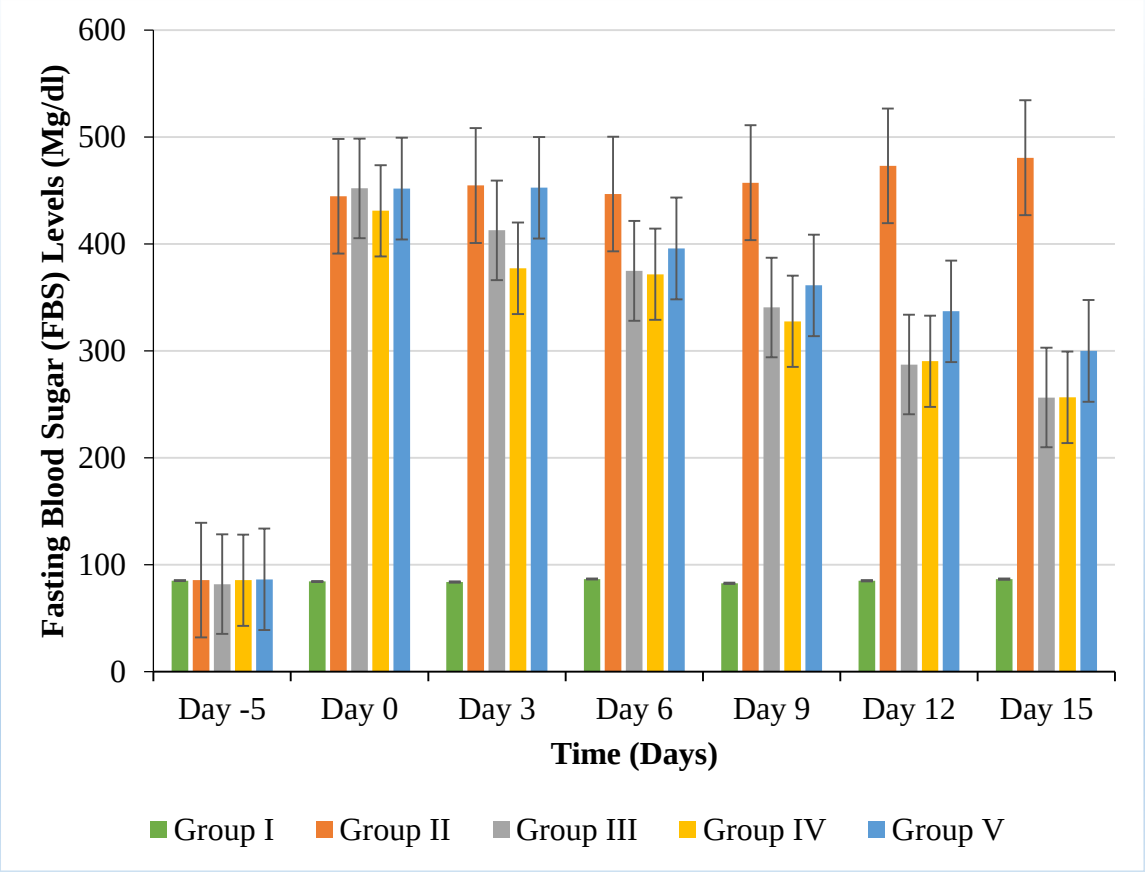


Figure 14: Mean fasting blood sugar levels of alloxan-induced diabetic mice after administration of *S. officinalis* aqueous extract for 15 days

4.3.1 Body Weights of the Experimental Mice

The mean body weight measurements of the experimental mice before induction of diabetes mellitus (-5 days), ranged from 26.77 ± 0.19 g to 28.25 ± 0.36 g. 5 days after induction of diabetes (0 days), the mean weight of Group II (diabetic control group) was 27.60 ± 0.58 g, Group III (diabetic mice fed with 400 mg/kg) was 28.05 ± 0.39 g, Group IV (diabetic mice fed with 600 mg/kg) was 27.38 ± 0.52 g and Group V (diabetic mice fed with glibenclamide drug) was 26.45 ± 0.22 g. However, Group I (normal non-diabetic mice not fed with the extract) had mean weight of 27.85 ± 0.22 g. Induction of diabetes caused a gradual decrease in the body weight of the experimental mice groups. Group I, exhibited consistent weight (27.77 ± 0.21 g to 27.87 ± 0.24 g) throughout the study. On the contrary, Group II exhibited a gradual weight decrease to the end of the study (27.92 ± 0.55 g to 25.72 ± 0.86 g).

Table 5: Effects of *S. officinalis* aqueous extract on live body weights (g) of various experimental groups 15 days after administration to alloxan-induced diabetic mice

Live body weights (g)					
Time/ Treatment	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
-5 days	27.77 ± 0.21[a]	27.92 ±0.55[a]	28.25 ±0.36[a]	27.57 ± 0.50[a]	26.77 ± 0.19[a]
0 days	27.85 ± 0.22[a]	27.60 ± 0.58[a]	28.05 ± 0.39[a]	27.38 ± 0.52[a]	26.45 ± 0.22[a]
Day 3	27.92 ± 0.21[b]	27.23 ± 0.55[ab]	27.73 ± 0.41[b]	27.07 ± 0.61[ab]	25.87 ± 0.19[a]
Day 6	27.80 ± 0.22[ab]	26.78 ± 0.78[ab]	28.62 ± 0.41[b]	27.60 ± 0.49[ab]	26.55 ± 0.32[a]
Day 9	27.93 ± 0.22[ab]	26.48 ± 0.82[a]	29.1 ± 0.38[b]*	28.17 ± 0.48[ab]	27.08 ± 0.38[ab]
Day 12	27.90 ± 0.20[ab]	25.97 ± 0.86[a]	28.82 ± 0.42[b]*	28.22 ± 0.41[b]*	27.27 ± 0.36[ab]
Day 15	27.87 ± 0.24[b]*	25.72 ± 0.86[a]	29.32 ± 0.42[b]*	28.55 ± 0.38[b]*	27.55 ± 0.36[ab]

Values are expressed as Mean ± S.E.M (n=6). Statistical analysis of data was done by one-way ANOVA followed by Turkey Post-hoc multiple comparison tests. Values in the same row with different superscript are statistically different: *$p<0.05$.

Group I (Normal mice (Negative control) not fed with S. officinalis aqueous extract), Group II (Diabetic control not fed with S. officinalis aqueous extract), Group III (Diabetic mice fed with 400 mg/kg aqueous extract of S. officinalis for 15 days), Group IV (Diabetic mice fed with 600 mg/kg aqueous extract of S. officinalis for 15 days), Group V (Diabetic mice (Positive control) fed with 2 mg/kg glibenclamide drug for 15 days).

Group II (diabetic control) demonstrated signs of polyphagia as they demonstrated excessive appetite by eating more feed compared to other groups. They also showed signs of polydipsia (unrelenting thirst)- they consumed large volumes of water and polyuria- they released large volume of urine compared to other groups. Group III, IV, and V, exhibited an improvement in the weight gain from day 6 to day 15. However, the weight improvement in Group V was not statistically significant compared to the diabetic control mice groups. On day 3, 6, and 9 the increase in weight gain of Group III, IV, and V was not statistically

significant, p value- 0.21, 0.40, 0.10, and 0.006 respectively. However, at day 12 and 15, the weight gain was significant, p value- 0.006 and 0.001 respectively.

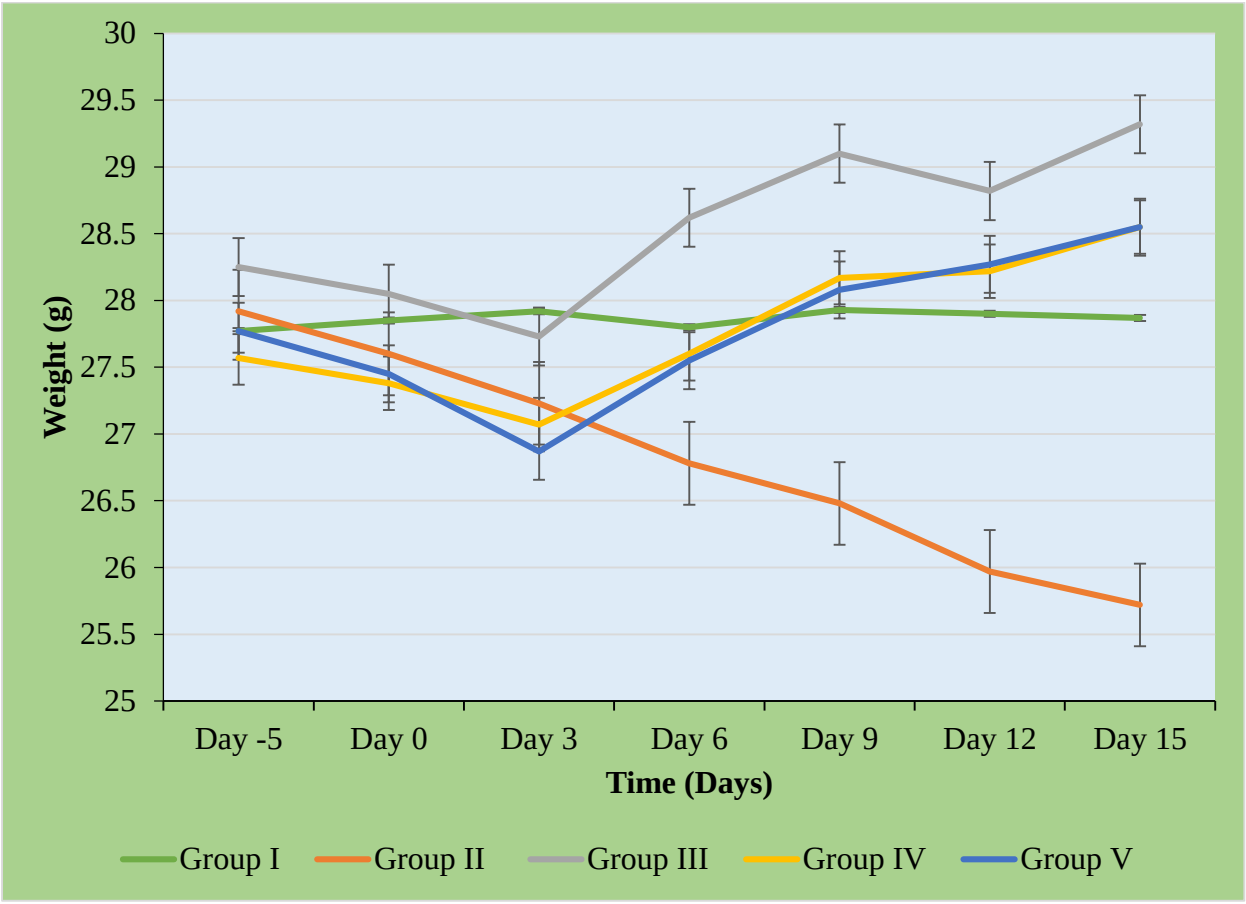


Figure 15: Mean body weight of alloxan-induced diabetic mice after administration of *S. officinalis* aqueous extract for 15 days

4.4 Histopathological Tests

Results for liver and kidney histopathology are presented below. Results were compared between the control groups (normal control mice and diabetic control mice) versus the mice groups administered with *S. officinalis* aqueous extract and glibenclamide drug.

4.4.1 Liver Histopathology

Liver sections of Group I mice group (Negative control group) showed normal cellular architecture characterized with normal hepatic cells with distinct nuclei and general liver hepatic cords being evident (Plate 1A). The nuclei were at the division stage either undergoing mitosis or meiosis. Severe cellular degenerative changes, however, were observed in liver sections of Group II- diabetic control group (Plate 1B). The general

architectural structure of the liver cell was lost with severe necrosis and fibrosis being noted. Dense nuclei (bluish in color) of different sizes were visible with abnormal eosinophilic background (Plate 1B).

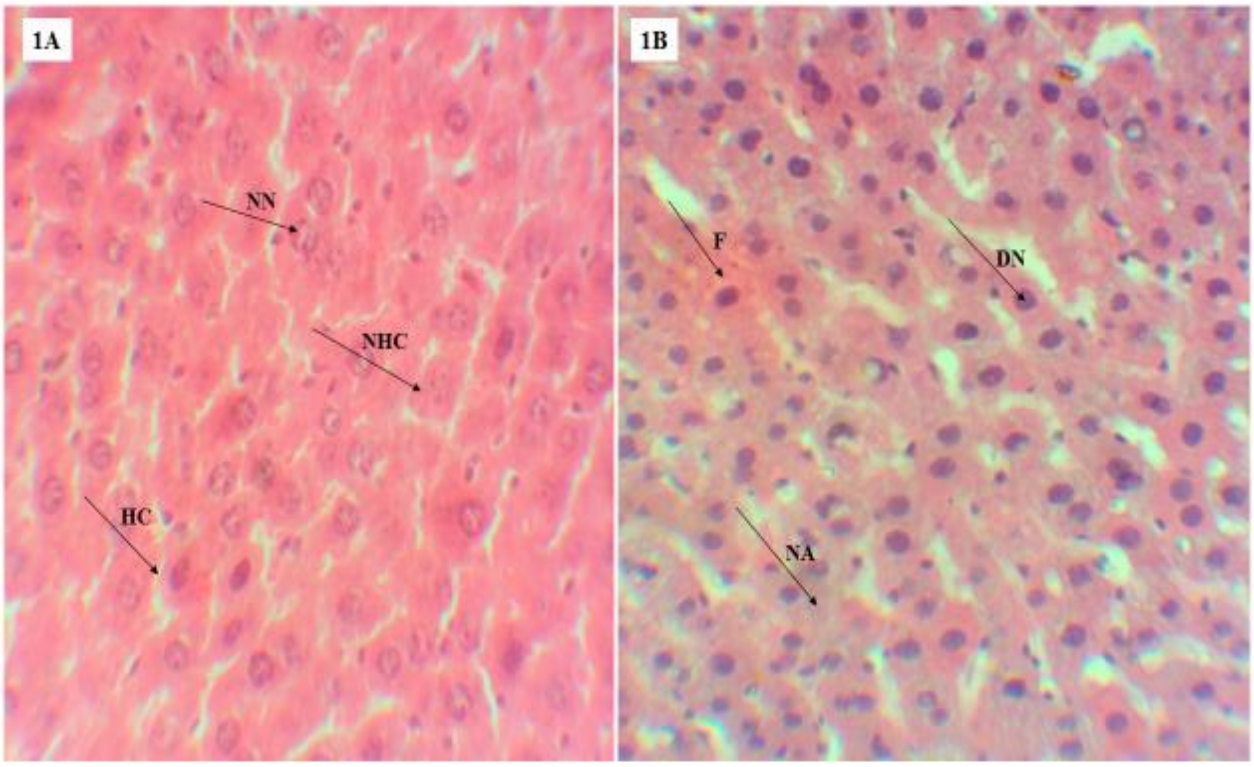


Plate 1: Histological plates of liver sections of Group I and Group II mice control groups (Magnification: H & E, ×40): Group I (1A) showed normal hepatic cells (NHC), hepatic cords (HC), and normal nucleus (NN). Group II (1B) exhibited fibrosis (F), dense nucleus (DN), and necrotic areas (NA).
Group I- Negative control- normal mice not fed with *S. officinalis* aqueous extract. Group II- Diabetic control- diabetic mice not fed with *S. officinalis* aqueous extract.

Liver sections of Group III and Group IV diabetic induced mice, treated with 400 mg/kg and 600 mg/kg *S. officinalis* aqueous extract respectively, also demonstrated severe liver cellular degeneration. The general architectural structure of the cells was lost and severe necrosis noted (Plate 2A & Plate 2B). The tissue mass of Group III and IV mice was uniform with no evidence of architectural structure. The nuclei were of different sizes and were deeply stained contrary to normal mice nucleus. The normal architectural structure of the nucleus was also destroyed.

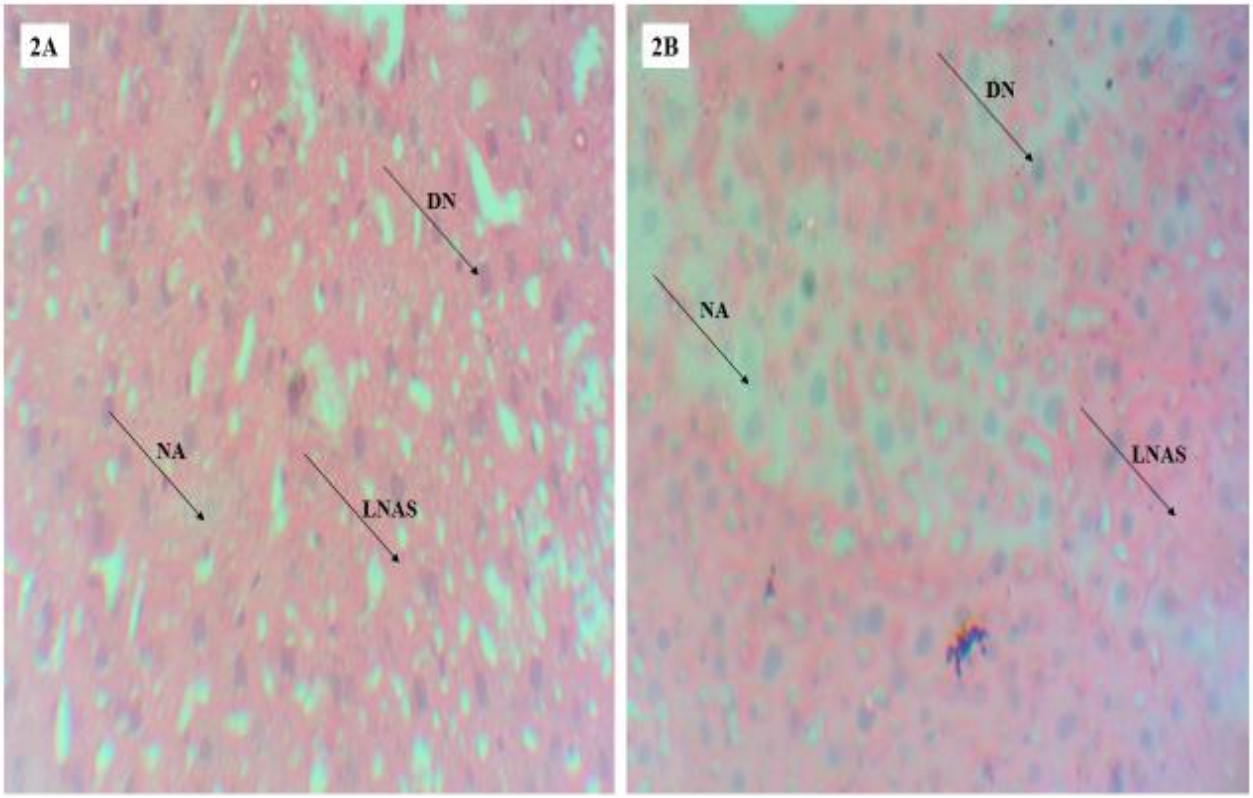


Plate 2: Histological plates of liver sections of Group III and Group IV experimental mice groups

(Magnification: H & E, ×40): Group III (2A) and Group IV (2B) exhibited dense nucleus (DN), necrotic areas (NA), and lack of normal architectural structure (LAS).

Group III- Diabetic mice fed with 400 mg/kg aqueous extract of *S. officinalis* for 15 days.

Group IV- Diabetic mice fed with 600 mg/kg aqueous extract of *S. officinalis* for 15 days.

Similar degenerative changes were seen in Group V diabetic induced mice (Positive control group) treated with 2 mg/kg glibenclamide drug. The nucleus of most cells was destroyed having spindle shape or spherical shape. Necrosis areas were also noted throughout the tissue. The tissue also demonstrated pink eosinophilic areas around the central vein and grey areas further from the central vein. Nuclei with hallow in the pink areas and no hallow in grey areas were observed (Plate 3). There was also renal hemorrhage in the central vein.

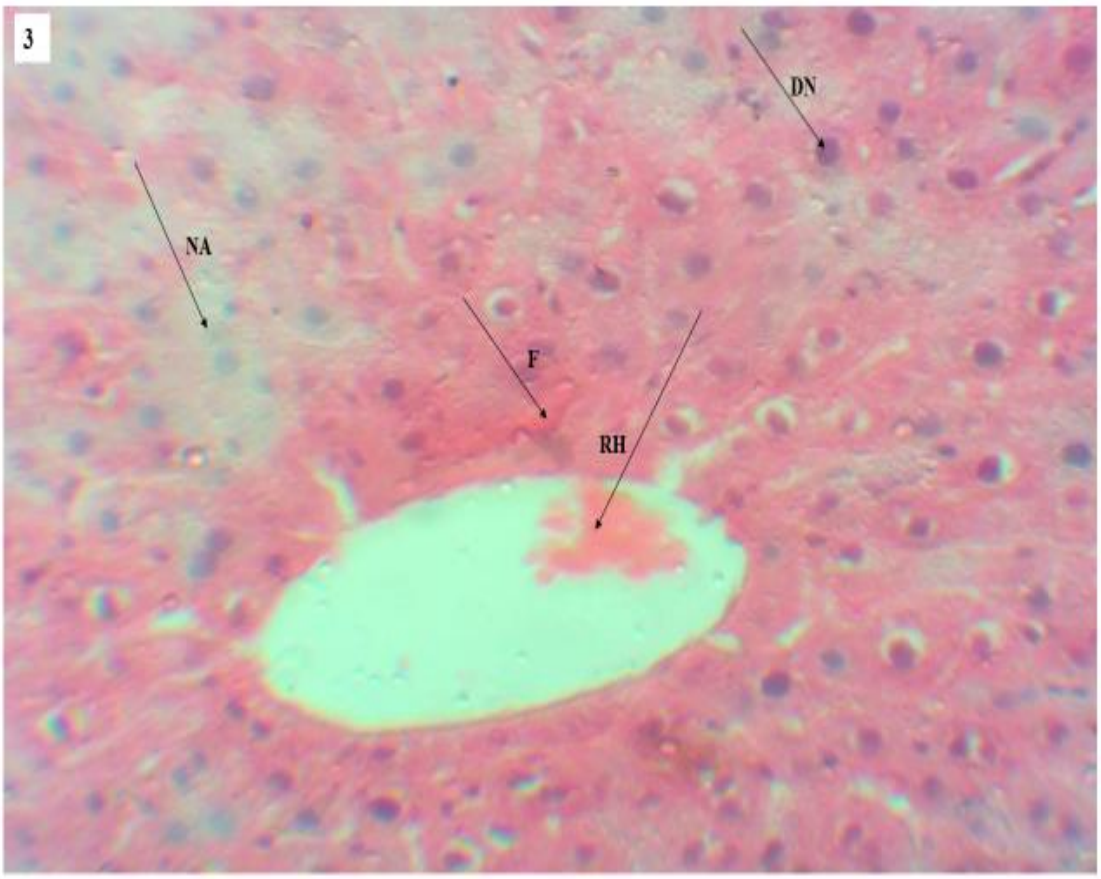


Plate 3: Histological plate of liver section of Group V experimental mice group (Magnification: H & E, ×40): Group V (3) showed necrotic areas (NA), dense nucleus (DN), fibrosis (F), and renal hemorrhage (RH).

Group V- Positive control- diabetic mice fed with 2 mg/kg glibenclamide drug for 15 days.

4.4.2 Kidney Histopathology

The kidney sections of the Group I-Negative control (normal mice) showed normal renal architecture with distinct glomeruli, bowman's capsule and renal tubules (Plate 4A). The kidney sections of Group II (diabetic control mice) indicated severe cellular degenerative changes with poorly formed glomerulus, fibrosis and several necrotic areas. Renal tubular fibrosis and renal hemorrhage was evident. The normal renal architecture was completely lost in alloxan-induced diabetic mice (Plate 4B).

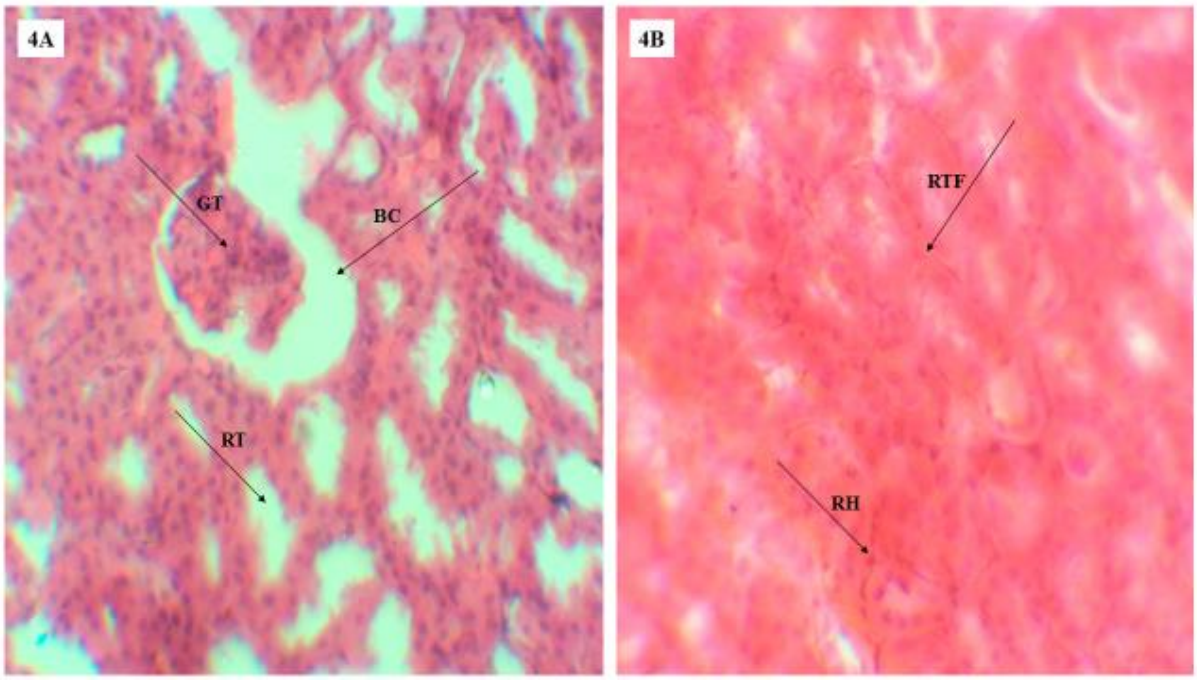


Plate 4: Histological plates of kidney sections of Group I and Group II mice control groups (Magnification: H & E, ×40): Group I (4A) showed normal Bowman's capsule (BC), glomerulus tuft (GT), and renal tubules (RT). Group II (4B) showed renal tubular fibrosis (RTF) and renal hemorrhage (RH).
Group I- Negative control- Normal mice not fed with *S. officinalis* aqueous extract. Group II- Diabetic control mice not fed with *S. officinalis* aqueous extract.

Kidney sections of Group III treated with 400 mg/kg aqueous extract of *S. officinalis* showed mild histopathological changes as compared to Group IV mice (treated with 600 mg/kg *S. officinalis* aqueous extract) and Group V mice (treated with 2 mg/kg of glibenclamide drug). However, the normal morphological structure of a kidney tissue was lost in most of the tissues. In Group III, tubular cells necrosis with intratubular debri and tubular disruption of the normal kidney structure was observed. There was severe cellular injury causing complete break of continuity of the tubules. Renal hemorrhage and congestion of blood vessels was also visible (Plate 2C). In Group IV, there was tissue structure disruption with only faint appearance. The tubules were affected causing them to coalesce together. Erythrocytic infiltration and distortion of the normal kidney structure was visible. Elongated pinkish material filled the intertubular tissue (intertubular fibrosis) (Plate 2D).

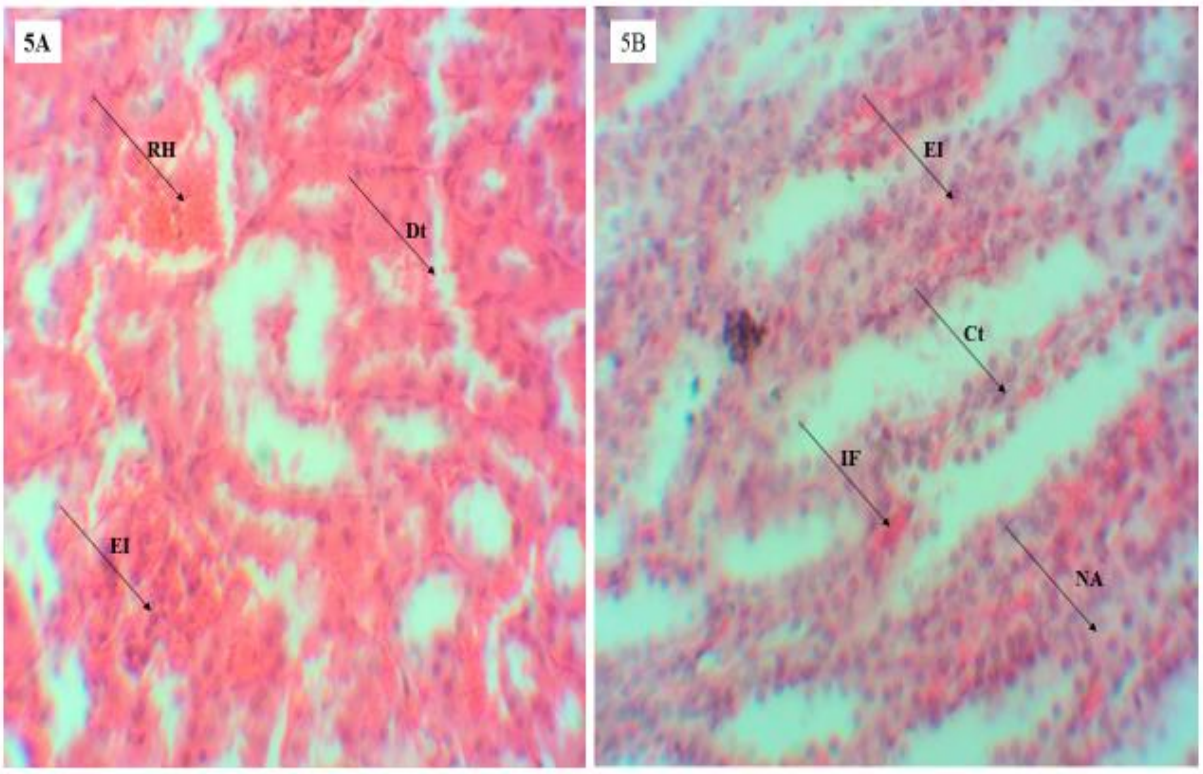


Plate 5: Histological plates of kidney sections of Group III and Group IV experimental mice groups

(Magnification: H & E, ×40): Group III (5A) showed renal hemorrhage (RH), erythrocytic infiltration (EI), and discontinuity of renal tubules (Dt). Group IV (5B) showed erythrocytic infiltration (EI), intratubular fibrosis (IF), coalescing of renal tubules (Ct), and necrotic areas (NA).

Group III- Diabetic mice fed with 400 mg/kg aqueous extract of *S. officinalis* for 15 days.
Group IV- Diabetic mice fed with 600 mg/kg aqueous extract of *S. officinalis* for 15 days.

Glibenclamide fed mice (Group V) demonstrated variable degrees of tubular atrophy, fibrosis, and atrophy of the glomerulus. There were enlarged interstitial tissues and the structure of the tubules and the glomerulus disrupted (Plate 6). Severe necrosis was also visible. The kidney sections of Group V showed severe necrosis and degenerative changes compared to Group III and IV treated with 400 mg/kg and 600 mg/kg of aqueous extract of *S. officinalis* respectively.

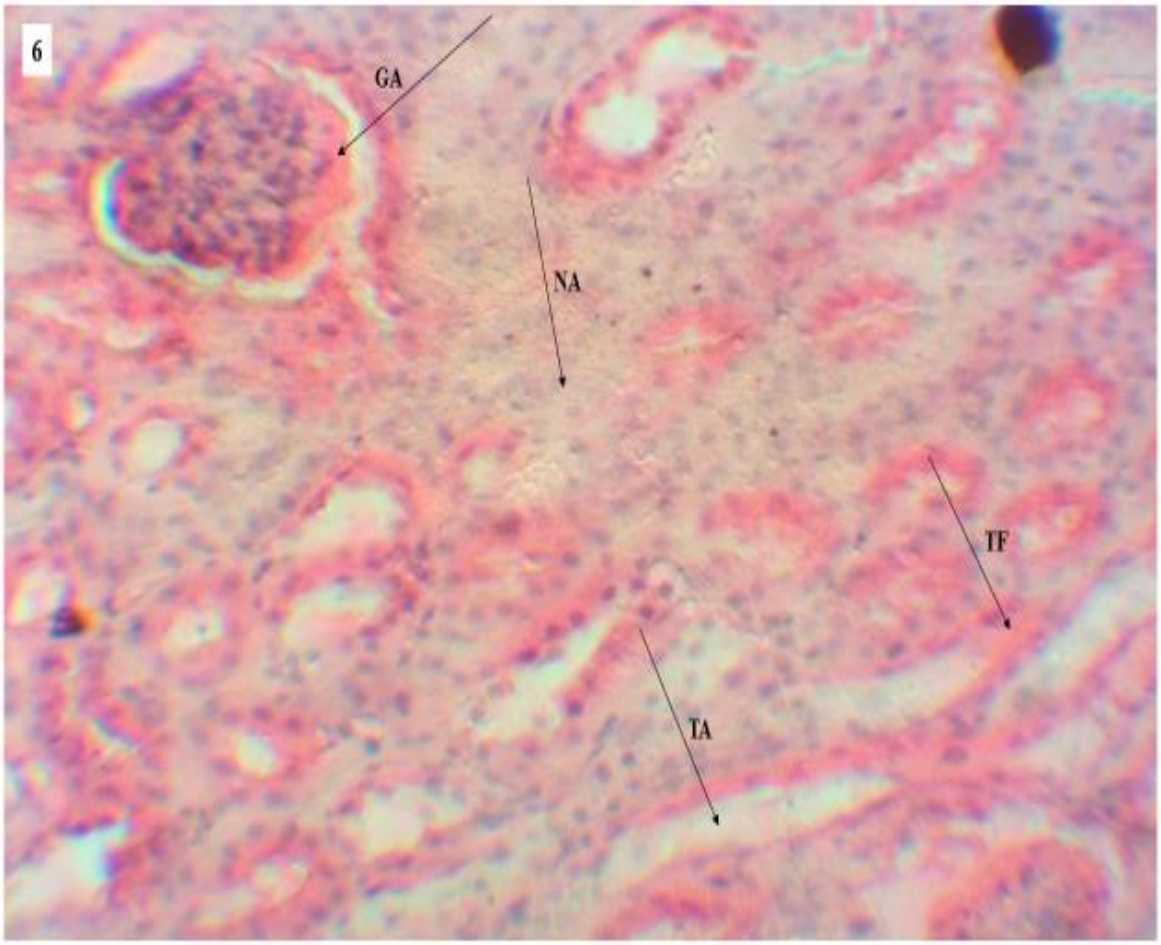


Plate 6: Histological plate of kidney section of Group V experimental mice group (Magnification: H & E, ×40): Group V (6) showed glomerulus atrophy (GA), tubular fibrosis (TF), tubular atrophy (TA), and necrotic areas (NA).

Group IV- Positive control- diabetic mice fed with 2 mg/kg glibeclamide drug for 15 days.

4.5 Phytochemical Screening for Chemical Composition of the Plant's Extracts

Different classes of phytochemicals were present in different plants and extracts. However, all the extracts *U. dioica, S. officinalis, P. guajava* and *C. limon* revealed presence of sterols, steroid glycosides, and triterpenes. In addition, all *P. guajava* extracts revealed presence of saponins, and tannins catechol while *C. limon* extracts showed presence of tannins catechol.

Table 6: Results of the phytochemical screening of the crude extracts

Plant	Extract	Results for phytochemical analysis										
		Flavonone aglycones	**Flavonols**	**Flavanone**	**Saponins**	**Tannins Catechol**	**Tannins Gallic**	**Atheracene aglycones**	**Sterols**	**Steroid glycosides**	**Triterpenes**	**Alkaloids**
U. dioica (Stinging nettle)	Methanol	+	+	-	+	+	+	+	+	+	+	-
	Ethyl acetate	+	+	+	-	-	-	+	+	+	+	+
	Hexane	-	-	-	-	+	-	+	+	+	+	-
	Aqueous	+	-	+	+	+	+	-	+	+	+	+
S. officinalis (Sage)	Methanol	+	+	+	-	+	-	-	+	+	+	+
	Ethyl acetate	+	+	-	-	-	+	+	+	+	+	-
	Hexane	-	-	-	-	+	+	-	+	+	+	-
	Aqueous	+	-	+	+	+	-	+	+	+	+	+
P. guajava (Guava)	Methanol	+	+	+	+	+	+	+	+	+	+	+
	Ethyl acetate	+	+	+	+	+	+	+	+	+	+	-
	Hexane	-	-	-	+	+	-	-	+	+	+	-
	Aqueous	+	+	+	+	+	-	+	+	+	+	+
C. limon (Lemon)	Methanol	+	-	+	-	+	-	+	+	+	+	-
	Ethyl acetate	+	+	+	-	+	-	-	+	+	+	+
	Hexane	-	-	-	+	+	-	-	+	+	+	-
	Aqueous	+	-	+	+	+	-	+	+	+	+	-

(The sign (+) indicates presence of phytochemical and (-) absence of phytochemical)

Table 7: Results of the phytochemical screening of *S. officinalis* and *U. dioica* fractions

Fractions	Results for phytochemical analysis										
	Flavonone aglycones	**Flavonols**	**Flavanone**	**Saponins**	**Tannins Catechol**	**Tannins Gallic**	**Atheracene aglycones**	**Sterols**	**Steroid glycosides**	**Triterpenes**	**Alkaloids**
F1 fraction of *U. dioica*	-	-	-	-	-	-	-	-	-	-	+
F2 fraction of *U. dioica*	+	-	+	-	-	-	-	-	-	-	-
F3 fraction of *U. dioica*	+	-	+	-	-	-	-	-	-	-	-
F4 fraction of *U. dioica*	+	+	-	-	-	-	-	+	+	+	+
F5 fraction of *U. dioica*	+	+	-	-	-	-	-	+	+	+	+
F1 fraction of *S. officinalis*	+	-	+	-	-	-	-	-	-	-	-
F2 fraction of *S. officinalis*	+	-	+	-	-	-	+	+	-	-	-
F3 fraction of *S. officinalis*	+	+	-	-	-	-	+	+	+	+	-
F4 fraction of *S. officinalis*	+	-	+	-	-	-	-	+	-	+	-

(The sign (+) indicates presence of phytochemical and (-) absence of phytochemical)

The F1 fraction of *U. dioica* contained only alkaloids while F2 and F3 fractions contained flavonones aglycones and flavanones. The F1 fraction of *S. officinalis* had the same chemical composition as F2 and F3 fractions of *U. dioica*. All the fractions except the F1 fraction of *U. dioica*, showed presence of flavonone aglycones and either flavonone or flavonols.

CHAPTER FIVE

DISCUSSION

5.1 Preliminary Screening of the Crude Extracts

Extracts of *U. dioica*, *S. officinalis*, *P. guajava,* and *C. limon* plants were found to lower fasting blood sugar levels significantly in alloxan induced diabetic mice. All the *U. dioica* extracts at a dose of 300 mg/kg lowered FBS levels significantly ($p<0.05$) compared to the diabetic control mice group. The *U. dioica* aqueous extract recorded a significant drop compared to other extracts by lowering the mean fasting blood sugar levels from 487.67 ± 0.84 mg/dl to 435.67 ± 1.28 mg/dl. Ethyl acetate extract, on the other hand, recorded the lowest drop in fasting blood sugar levels of alloxan-induced mice from 482.00 ± 0.97 mg/dl to 466.33 ± 9.81 mg/dl. These results were consistent with those reported by Dar *et al*. (2013) where the aqueous extract of *U. dioica* at 300 mg/kg produced significant drop in blood sugar levels of Wistar rats compared to methanol, hexane, ethyl acetate, aqueous, and chloroform extracts in glucose tolerance test. Similar to our results, the ethyl acetate extract was the least effective in lowering blood sugar levels compared to other extracts and the control group (Dar *et al*., 2013).

Aqueous extract of *S. officinalis* exhibited the highest drop in fasting blood sugar levels of alloxan-induced mice from 487.67 ± 2.58 mg/dl to 426.33 ± 2.95 mg/dl compared to other extracts of *S. officinalis* (methanol, hexane, ethyl acetate extracts). Similar results were reported by Salah *et al*. (2016) who documented the hypoglycemic character of *S. officinalis* aqueous extract. The Ethyl acetate extract of *S. officinalis*, however, increased the blood sugar levels from 482.33 ± 0.42 mg/dl to 519.00 ± 10.30 mg/dl indicating lack of hypoglycemic effect.

Aqueous extract of *P. guajava* as well lowered fasting blood sugar levels of alloxan-induced compared to other studied *P. guajava* extracts (methanol, hexane, and ethyl acetate extracts) from 483.33 ± 0.92 mg/dl to 457.00 ± 5.38 mg/dl. However, according to Basha & Kumari (2012), methanol extract of *P. guajava* revealed high antidiabetic potential compared to aqueous and hexane extracts when tested for antidiabetic activity. This could be attributed to geographical differences since environmental changes determine the presence or absence of secondary metabolites. Basha & Kumari (2012) attributed the blood lowering effect of *P. guajava* extracts to presence of tannins, flavonoids, and triterpenoids. Similar to their results, in this study we identified similar phytochemicals in our extracts with absence of tannins in hexane and aqueous extracts.

In *C. limon* extracts, ethyl acetate extract administered in alloxan-induced mice recorded a significant drop in fasting blood sugar of diabetic mice from 487.33 ± 1.17 mg/dl to 440.00 ± 0.97 mg/dl compared to other *C. limon* extracts (methanol, hexane, and aqueous) fed on diabetic mice. Contrary to these observations, the methanol extract administered on diabetic mice recorded an increase in fasting blood sugar levels from 485.00 ± 1.93 mg/dl to 493.33 ± 3.19 mg/dl. Nevertheless, Vasu *et al.* (2017) demonstrated the hypoglycemic effect of methanolic extract of *C. limon* peel. Environmental conditions could be the reason for the variability in these results (Hajzadeh *et al*., 2011). Generally, in all the plants, aqueous extracts lowered fasting blood sugar levels of alloxan-induced mice significantly compared to other extracts.

The efficacy of these methanol, ethyl acetate, and aqueous extracts of *U. dioica*, *S. officinalis*, *P. guajava*, and *C. limon* could be attributed to the presence of various phytochemicals. Flavonoids and terpenes have been documented to possess anti-diabetic effect (Balasubashini *et al*., 2004). They are known to possess insulin like-effect, thereby bringing down the blood sugar levels. Ferulic acid and quercertin are flavonoids documented to have an effect on pancreatic beta cells of alloxan-induced diabetic rats. They cause proliferation of beta cells of islets of Langerhans thereby causing them to secrete more insulin (Mahesh and Menon, 2004). Flavonols and flavanones are classes of flavonoids which were identified in most of the crude extracts in this study. They possess anti-oxidant activity which scavenges free radicals generated during the progression of diabetes mellitus (Balasubashini *et al*., 2004). This offers protection against possible damage to various tissues especially the liver, kidney, and eyes organs. It has been suggested that the extract's antioxidant property modulate the oxidative stress created in a diabetic state which arises when there is an imbalance between the radical generating and radical scavenging systems (Mahesh and Menon, 2004). In this study, all the aqueous extracts of all the plants used showed presence of flavanones. This could be the reason why they demonstrated better hypoglycemic effect compared to other extracts. A study conducted by Mukundi *et al.* (2017) reported that aqueous extracts of *U. dioica* possesses flavonoids, alkaloids, phenols, saponins, and tannins. Another study conducted by Salah *et al.* (2016) indicated that aqueous extract of *S. officinalis* leaves contained flavonoids, glycosides, terpenes, alkaloids, saponins, and steroids. These results were consistent with the data obtained in this study.

Nevertheless, other classes of phytochemicals identified could also be responsible for hypoglycemic effect exhibited in by alloxan-induced diabetic mice in this study. For instance, the hexane crude extracts of all the plants did not reveal any presence of flavonoids but

significantly lowered blood sugar levels of diabetic mice. The phytochemicals that were identified in the hexane extracts in this study were; tannins, sterols, tritepenes, and saponins. Tannins are polyphenols known to possess antioxidant activities, hence, responsible for free radical scavenging effect important in diabetic state (de Almeida Melo *at al*., 2005). Studies by Pan *et al.* (2003) and Rao & Gurfinkel, (2000) have also demonstrated the hypoglycemic activity exhibited by alkaloids and saponins. The blood sugar lowering effect of hexane extracts in alloxan-induced diabetic mice recorded in this study could therefore be as a result of tannins, sterols, triterpenes, and saponins phytochemicals.

The hyperglycemic effect of *S. officinalis* ethyl acetate extract and methanol extract of *C. limon* could be attributed largely to the composition of the secondary metabolites and the phytochemicals present in this extracts. The type of secondary metabolites present in an extract, influences greatly the bio-activity of the extract. The two extracts (ethyl acetate extract of *S. officinalis* and methanol extract of *C. limon*) showed presence of flavonols, tannins, atheracene glycosides, sterols, triterpenes, flavanone. It can be suggested that these metabolites could be working in an antagonistic manner thereby inhibiting the activity of each other as reported by Njeru *et al*. (2005). Nevertheless, it's important for further studies to be carried out to ascertain lack of hypoglycemic effect of these extracts. Also, they could be investigated whether they possess stimulants effects by causing increased blood sugar levels of diabetic mice. The Global Diabetes Community states that certain stimulant drugs increase blood sugar levels when consumed on a regular basis. In the long-run, the resulting high blood sugar levels may lead to insulin resistance hence development of Type II diabetes (Bowyer *et al*., 2017).

Alloxan monohydrate is known to induce hyperglycemia in experimental animals through selective destruction of the insulin-producing beta cells in the islet of langerhans. As a result, insulin production is affected leading to reduced insulin hormone levels in the blood. Consequently, blood glucose levels increase leading to diabetes mellitus (Rohilla & Ali, 2012). Thus, the aim of anti-diabetic drugs is to lower the elevated blood sugar levels in the blood of a diabetic patient. The plants extracts used in this study, lowered blood sugar levels of alloxan-induced mice due to the activity of the phytochemicals present. The extracts could be working through; causing regeneration of the destroyed pancreatic β-cells, protecting the intact functional beta cells from further damage, increasing plasma membrane permeability, or stimulating insulin secretion, thereby lowering blood glucose levels of diabetic mice (Eidi & Eidi, 2009). The exact mode of action through which these extracts lower blood sugar levels has not yet been validated. It is thought they act through various mechanisms, which

include; plants extracts possessing insulin-like activity, increasing peripheral utilization of glucose, decreasing the rate of glycogenolysis, increasing synthesis of hepatic glycogen, or inhibition of intestinal glucose absorption (Bnouham *et al*., 2006).

5.2 Hypoglycemic Effects of *U. dioica* and *S. officinalis* Fractions

All the ethyl acetate fractions of *U. dioica* (F1, F2, F3, F4, and F5) and *S. officinalis* (F1, F2, F3, and F4) had a significant hypoglycemic effects compared to diabetic control at $p<0.05$ after administration to alloxan-induced diabetic mice. The ethyl acetate crude extract of *S. officinalis* did not possess hypoglycemic effect after administration to diabetic mice. However, after fractionation, the fractions significantly lowered blood sugar levels of the diabetic mice with a p value of 0.000. This could be attributed to the fact that the hypoglycemic compounds in the crude extract may be in such low concentrations to have an effect. They could also be acting in an antagonistic manner (Njeru *et al*., 2005). Once fractionated, any antagonism is removed and their concentration increased, thereby exhibiting a hypoglycemic effect. Similarly, ethyl acetate extract fractions of *U. dioica* demonstrated better hypoglycemic results as compared to the crude extract after administration to diabetic mice, except for F5 fraction (Table 3). Compared to crude extracts that had numerous classes of phytochemicals, the fractions had specific class of phytochemicals. For instance, the F1 fraction of *U. dioica* had only alkaloids while F2 and F3 fractions of *U. dioica* and F1 fraction of *S. officinalis* had flavanone only. Thus, fractionation of crude extracts is important as it helps in determining the specific class of secondary metabolites present and in identification of chemical compounds.

Similarly, the essential oil of *S. officinalis* at 0.2 ml/kg and 0.4 ml/kg reduced fasting blood sugar levels of diabetic mice significantly from 500.00 ± 2.56 mg/dl and 507.33 ± 0.56 mg/dl to 465.67 ± 4.97 mg/dl and 482.67 ± 5.83 mg/dl respectively. This demonstrated the potency of *S. officinalis* essential oil in lowering of blood sugar levels. Nevertheless, a study by Eidi *et al*. (2005), did not demonstrate any hypoglycemic effect of *S. officinalis* essential oil at concentrations of 0.042 ml/kg, 0.125 ml/kg, 0.2ml/kg, and 0.4 ml/kg. This could be attributed to differences in the active components of essential oil in these two experiments as a result of environmental variability (Ben Farhat *et al*., 2009).

5.3 Hypoglycemic Activity Test

Intraperitoneal injection of alloxan (200 mg/kg) into the experimental mice, led to development of a stable hyperglycemia after 5 days. Administration of 400 mg/kg and 600 mg/kg *S. officinalis* aqueous extract to diabetic mice for 15 days, led to a significant decrease

in blood sugar levels compared to the diabetic control group with a p value of 0.000. The mice group fed with glibenclamide drug also showed a significant drop in sugar levels although the drop was lower compared to groups of mice fed with *S. officinalis* aqueous extract. The mean fasting blood sugar levels for the diabetic mice control group was consistently high throughout the study. Thus, the obtained results demonstrate the hypoglycemic effect of the *S. officinalis* aqueous extract which could be attributed to the presence of flavanone, sterols, saponins, triterpenes, and alkaloids. The results were in agreement with those reported by Eidi & Eidi, (2009) and Salah *et al*. (2016) where they demonstrated the hypoglycemic effect of *S. officinalis* aqueous extract. Thus, it can be inferred that aqueous extract of *S. officinalis* is a potent hypoglycemic agent and its traditional use in the management of diabetes mellitus is justified.

It has also been noted that the route of administration of the extract generally determines extract absorption, bioavailability and hence its activity. For instance, a study by Hajzadeh *et al*. (2011) found that aqueous extract of *S. officinalis* administered intraperitoneal at a dose of 430 mg/kg to STZ-diabetic mice did not possess any hypoglycemic activity. Contrary to this results, *S. officinalis* aqueous extract was administered orally in this study and significantly lowered the fasting blood sugar levels of diabetic mice. Thus, it is important to critically evaluate the best route to use in administering plants extracts to experimental animals. Nevertheless, oral administration is recommended since it mimics the commonly used route in administration of traditional herbal medicine (Mukundi *et al*., 2017).

5.3.1 Body Weights of the Experimental Mice

The mean weight of diabetic mice control group recorded a gradual decrease throughout the study (27.92 ± 0.55 g to 25.72 ± 0.86 g). The mean weight of normal control group, on the other hand, was relatively constant from the start to the end of the experiment (27.77 ± 0.21 g to 27.87± 0.24 g). The results obtained were in agreement with those recorded by Eidi & Eidi, (2009). The loss of weight observed in the diabetic mice control group, could be as a result of muscle wasting. Normally, in a diabetic state, carbohydrate utilization is poor, therefore, there is stimulation of protein breakdown to provide amino acids necessary for gluconeogenesis to take place. This results in muscle wasting and consequently weight loss (Eidi & Eidi, 2009). The diabetic mice groups fed with the *S. officinalis* aqueous extract (400 mg/kg and 600 mg/kg) and glibenclamide drug demonstrated a gradual increase in weight gain. The groups administered with the *S. officinalis* aqueous extract, however, recorded a higher weight gain compared to the group fed with glibenclamide. In fact, weight

improvement for glibenclamide fed group was not statistically different from the diabetic control (Table 5). This, therefore, shows that the *S. officinalis* aqueous extract is more potent compared to the standard glibenclamide drug in enhancing weight gain in a diabetic state. The results from this study were consistent with those recorded by Eidi & Eidi, (2009) who showed the weight improvement effect of *S. officinalis* aqueous extract. Thus, this validates the traditional claim of *S. officinalis* aqueous extract in use as a hypoglycemic agent and in improving weight of diabetic patients.

5.4 Histopathology of Liver and Kidney Tissues

Histological results of liver tissues of the diabetic control mice group demonstrated severe degenerative changes characterized by severe necrosis and loss of the normal architectural structure of the liver cell. Uncontrolled diabetes mellitus leads to increase in the production of oxygen free radicals due to autoxidation of glucose and glycation of the body proteins. This generates oxidative stress which results in secondary complications that affects various body organs such as the liver and the kidneys (Henriksen *et al*., 2011). The elevation of oxidative stress in a diabetic state causes inflammation and cellular necrosis of the liver as observed in tissue sections in this study (Plate 1B). Administration of *S. officinalis* aqueous extract on the diabetic mice at the dosage levels of 400 mg/kg and 600 mg/kg did not indicate any significant histopathological changes compared to the diabetic control. The cells were characterized by severe necrosis and fibrosis. Studies by Lima *et al*. (2007) revealed that *S. officinalis* aqueous extract increased liver injury of mice induced with carbon tetrachloride (CCl_4). Contrary to these results, Amin & Hamza (2005), demonstrated hepatoprotective effects of aqueous extract of *S. officinalis* on azathioprine-induced toxicity in rats. The differences in these reports could be attributed to differences in phytochemicals present due to environmental variability (Ben Farhat *et al*., 2009).

Kidney sections of the diabetic control group showed severe cellular damage characterized with tubular cellular necrosis, fibrosis, poor structure of the tubules and the general kidney structure lost (Plate 4B). Garcia-Compean *et al*. (2009) demonstrated that mitochondria oxidative stress generated due to diabetes leads to generation of free radicals that induces inflammation, necrosis, and fibrosis to the kidney cells. This could have resulted in degenerative changes observed in the diabetic mice control group. Group III fed with 400 mg/kg *S. officinalis* aqueous extract showed mild degenerative changes. Most of the tubules were observed with discontinuity in some areas. Group IV fed with 600 mg/kg *S. officinalis* aqueous extracts indicated tissue structure disruption with the tubules coalescing together.

The general morphology of the glomerulus was also disrupted and erythrocytic infiltration observed (Plate 5B). Nevertheless, studies by Ashour *et al*. (2017), reported the nephroprotective effect of *S. officinalis* aqueous extract on chemically induced renal toxicity to albino rats. Variable degrees of tubular atrophy, fibrosis, enlarged interstitial tissue, and glomerulus atrophy were noted in Group V (Plate 6). The kidney sections of Group V fed with glibenclamide drug showed severe necrosis and degenerative changes compared to the diabetic mice groups treated with *S. officinalis* aqueous extract. This indicates potency of *S. officinalis* aqueous extract in offering protection against further damage of the tissues from the alloxan effect compared to the standard glibenclamide drug.

The histopathological changes observed in the experimental groups, were not significantly different from the controls. Moreover, the hepatoprotective and nephroprotective effect of the aqueous extract of *S. officinalis* could not be fully established. This could be attributed to the fact that the cells were already destroyed beyond repair or the treatment period was short to give an effect. Nevertheless, the extract was not cytotoxic to the liver and kidney cells as no further damage was observed and no deaths of the animals was recorded during the study period. The extract could have, therefore played a huge role in causing attenuation of the oxidative stress and also enhancing the antioxidant defense system as highlighted by the study of Ashour *et al*. (2017).

5.5 Scope and Limitation of the Study

This study went into depths of determining how plants extracts lower blood sugar levels of alloxan-induced mice. The study examined the hypoglycemic effect of methanol, ethyl acetate, hexane, and aqueous extract of *Urtica dioica* (stinging nettle), *Salvia officinalis* (sage), *Psidium guajava* (guava), and *Citrus limon* (lemon). It also analyzed the effects of the extracts on liver and kidney tissues of diabetic mice. This was intended to shed light on the protective effects of the plants extracts on the damaged tissues of the mice due to the effect of alloxan.

The main limitation experienced in this study was the variability of fasting blood sugar levels of diabetic mice. Some mice had drastic increase and decrease of blood sugar levels making it hard to make inference concerning the effect of the plant's extracts. Also, some mice were resistant to alloxan induction. This brought significant challenge especially to the number of mice that were meant to be used and also led to unforeseen delays. Additionally, injection of alloxan more than once to the resistant mice led to unnecessary pain to the animal.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the results obtained in this study, the crude extracts of *U. dioica*, *S. officinalis*, *P. guajava* and *C. limon* plants demonstrated hypoglycemic effect by significantly lowering blood sugar levels of alloxan-induced diabetic mice. As a result, these extracts are justified to be used in the management of diabetes mellitus as traditionally claimed. However, there was exception of ethyl acetate extract of *S. officinalis* and methanol extract of *C. limon* which indicated a hyperglycemic effect by further increasing the blood sugar levels of diabetic mice.

From the phytochemical analysis of the plants extracts, it is evident that the plants extracts possess diverse classes of chemical compounds. Fractionation of the crude extracts revealed specific chemical compounds present in each fraction. The antidiabetic effect of the extracts could, therefore, be attributed to the presence of phytochemicals such as flavonoids, alkaloids, phenols, saponins, tannins, sterols, and triterpenes due to their ability to scavenge free radicals generated during the progression of diabetes mellitus.

Histopathology analysis of liver and kidney tissues revealed severe degenerative changes. The aqueous extract of *S. officinalis* did not reveal significant hepatoprotective and nephroprotective effect. Nevertheless, the extract did not have any cytotoxic effect since no death or further tissue damage occurred compared to tissues of diabetic mice control group during the study.

6.2 Recommendations

i) Further studies should be carried out to explore the efficacy of the extracts of *U. dioica*, *S. officinalis*, *P. guajava*, and *C. limon* from other regions of Kenya since the bio-activity of medicinal plants vary from place to place due to environmental conditions.

ii) It is suggested that the exact phytochemical (s) responsible for hypoglycemic effect of the extract be determined. This can be achieved through structure elucidation of the bio-active compound(s).

iii) More studies should be carried out to determine the mode of action of the aqueous extract of *S. officinalis* and its protective effect on liver and kidney cells of the diabetic mice.

REFERENCES

Abad, M. J., Bedoya, L. M., Apaza, L., & Bermejo, P. (2012). The Artemisia L. genus: a review of bioactive essential oils. *Molecules*, **17**: 2542-2566.

Andrews, K., & McErlane, S. (2015). Oral dosing (gavage) in adult mice and rats' standard operating procedures (SOP). *UBC Animal Care Guidelines*, **1**: 1-7.

Amin, A., & Hamza, A. A. (2005). Hepatoprotective effects of *Hibiscus*, *Rosmarinus* and *Salvia* on azathioprine-induced toxicity in rats. *Life sciences*, **77**: 266-278.

Ashour, M. B., Ahmed, O. M., Abd El Mawgoud, A. A., & Ali, M. A. (2017). Assessment of the Preventive Effects of *Salvia officinalis* and *Ruta graveolens* Ethanolic Leaf Extracts on Chlorpyrifos-and Methomyl-induced Renal Toxicity and Oxidative Stress in Albino Rats. *International Journal of Prevention and Treatment*, **6**: 34-44.

Ayeleso, A. O., Oguntibeju, O. O., & Brooks, N. (2012). Flavonoids and their antidiabetic potentials. *Bioactive Phytochemicals: Perspectives for Modern Medicine*, **1**:79-106.

Bagri, P., Ali, M., Aeri, V., & Bhowmik, M. (2016). Isolation and antidiabetic activity of new lanostenoids from the leaves of *Psidium guajava*. *International Journal of Pharmacy and Pharmaceutical Sciences*, **8**: 14-18.

Baharvand-Ahmadi, B., Bahmani, M., Tajeddini, P., Naghdi, N., & Rafieian-Kopaei, M. (2016). An ethno-medicinal study of medicinal plants used for the treatment of diabetes. *Journal of Nephropathology*, **5**: 44-68.

Balasubashini, M. S., Rukkumani, R., Viswanathan, P., & Menon, V. P. (2004). Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **18**: 310-314.

Basha, S. K., & Kumari, V. S. (2012). *In vitro* antidiabetic activity of *Psidium guajava* leaves extracts. *Asian Pacific Journal of Tropical Disease*, **2**: 98-100.

Bastaki, A. (2005). Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism*, **13**: 111-132.

Behradmanesh, M., Ahmadi, M., & Rafieian, K. M. (2012). Effect of glycogol on blood glucose level of patients with type II diabetes. *Iranian Journal of Endocrinology and Metabolism*, **14**: 163-168.

Ben Farhat, M., Jordán, M. J., Chaouech-Hamada, R., Landoulsi, A., & Sotomayor, J. A. (2009). Variations in essential oil, phenolic compounds, and antioxidant activity of Tunisian cultivated *Salvia officinalis* L. *Journal of Agricultural and Food Chemistry*, **57:** 10349-10356.

Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A., & Legssyer, A. (2006). Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). *International Journal of Diabetes and Metabolism*, **14**: 1-17.

Bommer, S., Klein, P., & Suter, A. (2009). A multicenter open clinical trial to assess the tolerability and efficacy of sage tablets in menopausal patients with hot flushes. *Planta Medica*, **75**: 159-181.

Bowyer, J. F., Tranter, K. M., Sarkar, S., George, N. I., Hanig, J. P., Kelly, K. A., ... & O'callaghan, J. P. (2017). Corticosterone and exogenous glucose alter blood glucose levels, neurotoxicity, and vascular toxicity produced by methamphetamine. *Journal of Neurochemistry*, **143**: 198-213.

Brentjens, R., & Saltz, L. (2001). Islet cell tumors of the pancreas: the medical oncologist's perspective. *Surgical Clinics of North America*, **81**: 527-542.

Britannica, E. (2016). Rutaceae plant family. *Encyclopedia Britannica*, **1**: 1-2.

Chellappan, D. K., Sivam, N. S., Teoh, K. X., Leong, W. P., Fui, T. Z., Chooi, K., ... & Dahiya, R. (2018). Gene therapy and type 1 diabetes mellitus. *Biomedicine & Pharmacotherapy*, **108**: 1188-1200.

Colegate, S. M., & Molyneux, R. J. (2007). An introduction and overview. In *Bioactive Natural Products*, **5**: 10-18.

Cos, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. *Journal of Ethnopharmacology*, **106**: 290-302.

Dar, S. A., Ganai, F. A., Yousuf, A. R., Balkhi, M. U. H., Bhat, T. M., & Sharma, P. (2013). Pharmacological and toxicological evaluation of *Urtica dioica*. *Pharmaceutical Biology*, **51**: 170-180.

de Almeida Melo, E., Mancini Filho, J., & Guerra, N. B. (2005). Characterization of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum*). *LWT-Food Science and Technology*, **38**: 15-19.

Eddouks, M., Chattopadhyay, D., De Feo, V., & Cho, W. C. S. (2014). Medicinal plants in the prevention and treatment of chronic diseases 2013. *Evidence-based Complementary and Alternative Medicine*, **7**: 20-35.

Eidi, A., & Eidi, M. (2009). Antidiabetic effects of sage (*Salvia officinalis*) leaves in normal and streptozotocin-induced diabetic rats. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, **3**: 40-44.

Eidi, M., Eidi, A., & Zamanizadeh, H. (2005). Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, **100**: 310-313.

Etuk, E. U. (2010). Animal models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*, **1**: 130-134.

Fabricant, D. S & Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Journal of Environmental Health Perspective*, **109**: 69–75.

Federiuk, I. F., Casey, H. M., Quinn, M. J., Wood, M. D., & Ward, K. W. (2004). Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. *Comparative Medicine*, **54**: 252-257.

Feldman, A. T., & Wolfe, D. (2014). Tissue processing and hematoxylin and eosin staining. *In Histopathology*, **1180**: 31-43

Francis, G., Kerem, Z., Makkar, H. P., & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, **88**: 587-605.

Gallagher, A. M., Flatt, P. R., Duffy, G., & Abdel-Wahab, Y. H. A. (2003). The effects of traditional antidiabetic plants on *in vitro* glucose diffusion. *Nutrition Research*, **23**: 413-424.

Garcia-Compean, D., Jaquez-Quintana, J. O., Gonzalez-Gonzalez, J. A., & Maldonado-Garza, H. (2009). Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. *World Journal of Gastroenterology*, **15**: 280-295.

Hajizadeh, M., Rajaei, Z., Ghamami, G., & Tamiz, A. (2011). The effect of *Salvia Officinalis* Leaf Extract on Blood Glucose in sterptozotocin-diabetic Rats. *Pharmacologyonline*, **1**: 213-220.

Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. *In Phytochemical Methods*, **15**: 1-300.

Harnafi, H., & Amrani, S. (2007). Flavonoids as potent phytochemicals in cardiovascular diseases prevention. *Pharmacognosy Reviews*, **1**: 193-211.

Heather, A., Ferris, C., & Kahn, C. R. (2012). New mechanisms of glucocorticoid-induced insulin resistance: Make no bones about it. *The Journal of Clinical Investigation*, **122**: 3854-3857.

Henriksen, E. J., Diamond-Stanic, M. K., & Marchionne, E. M. (2011). Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radical Biology and Medicine*, **51**: 993-999.

International Diabetes Federation. (2013). IDF Diabetes Atlas, 6th edition. *International Diabetes Federation*.

International Diabetes Federation. (2015). IDF Diabetes Atlas, 7th edition, *International Diabetes Federation*.

Iranloye, B. O., Arikawe, A. P., Rotimi, G., & Sogbade, A. O. (2011). Anti-diabetic and anti-oxidant effects of *Zingiber Officinale* on alloxan-induced and insulin-resistant diabetic male rats. *Nigerian Journal of Physiological Sciences*, **26**: 1-17.

Jamila, F., & Mostafa, E. (2014). Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. *Journal of Ethnopharmacology*, **154**: 76-87.

Jan, K. N., & Singh, S. (2017). Stinging nettle (*Urtica dioica*): a reservoir of nutrition and bioactive components with great functional potential. *Journal of Food Measurement and Characterization*, **11**: 423-433.

Joseph, B., & Priya, M. (2011). Review on nutritional, medicinal and pharmacological properties of guava (*Psidium guajava*). *International Journal of Pharmacy and Biological Sciences*, **2**: 53-69.

Kataki, M. S., Murugamani, V., Rajkumari, A., Mehra, P. S., Awasthi, D., & Yadav, R. S. (2012). Antioxidant, hepatoprotective, and anthelmintic activities of methanol extract of *Urtica dioica* Leaves. *Pharm Crops*, **3**: 38-46.

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

Kitabchi, A. E., Umpierrez, G. E., Miles, J. M., & Fisher, J. N. (2009). Hyperglycemic crises in adult patients with diabetes. *Diabetes Car*, **32**: 1335–1343.

Kuchake, V. G., & Upasani, C. D. (2013). Effect of Vitamin E and C Plus Reduced Glutathione in Treatment of Diabetic Nephropathy. *International Journal of Pharmacy Research & Review*, **2**: 1-5.

Lee, J. H., Yang, S. H., Oh, J. M., & Lee, M. G. (2010). Pharmacokinetics of drugs in rats with diabetes mellitus induced by alloxan or streptozocin: comparison with those in patients with type I diabetes mellitus. *Journal of Pharmacy and Pharmacology*, **62**: 1-23.

Lehtovirta, M., Pietiläinen, K. H., Levälahti, E., Heikkilä, K., Groop, L., Silventoinen, K., & Kaprio, J. (2010). Evidence that BMI and type 2 diabetes share only a minor fraction of genetic variance: a follow-up study of 23,585 monozygotic and dizygotic twins from the Finnish Twin Cohort Study. *Diabetologia*, **53**: 1314-1321.

Lenzen, S. (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, **51**: 216-226.

Lemle, K. L. (2018). *Salvia officinalis* used in pharmaceutics. *Materials Science and Engineering*, **294**: 012037.

Lima, C. F., Fernandes-Ferreira, M., & Pereira-Wilson, C. (2007). Drinking of *Salvia officinalis* tea increases CCl4-induced hepatotoxicity in mice. *Food and Chemical Toxicology*, **45**: 456-464.

Mahesh, T., & Menon, P.V. (2004). Quercetin alleviates oxidative stress in streptozoticin induced diabetic rats. *The Journal of Nutrition*, **135**: 2555-2565.

Maiti, R., Jana, D., Das, U. K., & Ghosh, D. (2004). Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, **92**: 85-91.

Majak, W., & Benn M. H. (2001). Glycosides. *Plant Toxicants*, **3**: 299–349.

Miyake, Y., Suzuki, E., Ohya, S., Fukumoto, S., Hiramitsu, M., Sakaida, K., ... & Furuichi, Y. (2006). Lipid-Lowering Effect of Eriocitrin, the Main Flavonoid in Lemon Fruit, in Rats on a High-Fat and High-Cholesterol Diet. *Journal of Food Science*, **71**: 633-637.

Monami, M., Dicembrini, I., Kundisova, L., Zannoni, S., Nreu, B., & Mannucci, E. (2014). A meta-analysis of the hypoglycaemic risk in randomized controlled trials with sulphonylureas in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, **16**: 833-840.

Mukundi, M. J., Mwaniki, N. E., Piero, N. M., Murugi, N. J., Kelvin, J. K., Yusuf, A. A., ... & Alice, M. N. (2017). Potential Anti-Diabetic Effects and Safety of Aqueous Extracts of *Urtica dioica* Collected from Narok County, Kenya. *Pharmaceutica Analytica Acta*, **8**: 2153-2165.

Mythili, M., Vyas, R., Akila, G., & Gunasekaran, S. (2004). Effect of streptozotocin on the ultrastructure of rat pancreatic islets. *Microscopy Research and Technique*, **63**: 274-281.

Naim, M., Amjad, F. M., Sultana, S., Islam, S. N., Hossain, M. A., Begum, R., ... & Amran, M. S. (2012). Comparative study of antidiabetic activity of hexane-extract of lemon peel (*Limon citrus*) and glimepiride in alloxan-induced diabetic rats. *Bangladesh Pharmaceutical Journal*, **15**: 131-134.

Nasri, H., & Shirzad, H. (2013). Toxicity and safety of medicinal plants. *Journal of Herbal Medicine and Pharmacology*, **2**: 21-22.

Nijveldt R. J., Van Nood, E. L. S., Van Hoorn, D. E., Boelens, P. G., Van Norren, K., & Van Leeuwen, P. A. (2001). Flavanoids: a review of probable mechanism of action and potential application. *American Journal of Clinical Nutrition,* **74**: 418-425.

Njeru, S. N., Obonyo, M. A., Nyambati, S. O., & Ngari, S. M. (2015). Antimicrobial and cytotoxicity properties of the crude extracts and fractions of *Premna resinosa* (Hochst) Schauer (Compositae): Kenyan traditional medicinal plant. *BMC Complementary and Alternative Medicine*, **15**: 295-311.

Nobossé, P., Fombang, E. N., & Mbofung, C. M. (2018). Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Science & Nutrition*, **6**: 2188-2198.

Pan, G. Y., Huang, Z. J., Wang, G. J., Fawcett, J. P., Liu, X. D., Zhao, X. C., ... & Xie, Y. Y. (2003). The antihyperglycaemic activity of berberine arises from a decrease of glucose absorption. *Planta Medica*, **69**: 632-636.

Patel, D. K., Kumar, R., Prasad, S. K., Sairam, K., & Hemalatha, S. (2011). Antidiabetic and *in vitro* antioxidant potential of *Hybanthus enneaspermus* in streptozotocin–induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine,* **1**: 316-322.

Patel, R., Shervington, A., Pariente, J. A., Martinez-Burgos, M. A., Salido, G. M., Adeghate, E., & Singh, J. (2006). Mechanism of Exocrine Pancreatic Insufficiency in Streptozotocin-Induced Type 1 Diabetes Mellitus. *Annals of the New York Academy of Sciences*, **1084**: 71-88.

Piero, N. M., Joan, M. N., Cromwell, K. M., Joseph, N. J., Wilson, N. M., Daniel, M., & Eliud, N. N. (2012). Hypoglycemic activity of some Kenyan plants traditionally used to manage diabetes mellitus in Eastern Province. *Journal of Diabetes & Metabolism*, **2:** 2155-2169.

Rahim, F., Arjmand, B., Shirbandi, K., Payab, M., & Larijani, B. (2018). Stem cell therapy for patients with diabetes: a systematic review and meta-analysis of metabolomics-based risks and benefits. *Stem Cell Investigation*, **5**: 40-47.

Rahimi, M. (2015). A Review: Anti Diabetic medicinal plants used for diabetes mellitus. *Environmental Pharmacology Life Sciences*, **4**: 163-180.

Rao, M. U., Sreenivasulu, M., Chengaiah, B., Reddy, K. J., & Chetty, C. M. (2010). Herbal medicines for diabetes mellitus: a review. *International Journal of Pharmarcy & Technology Research,* **2**: 1883-1892.

Rao, A. V., & Gurfinkel, D. M. (2000). The bioactivity of saponins: triterpenoid and steroidal glycosides. *Drug Metabolism and Drug Interactions,* **17**: 211-236.

Rasool, H. B. A. (2012) Medicinal Plants (Importance and Uses). *Pharmaceutica Analytica Acta*, **3**: 139-149.

Rohilla, A., & Ali, S. (2012). Alloxan induced diabetes: mechanisms and effects. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **3**: 819-823.

Safari, V. Z., Ngugi, M. P., Orinda, G., & Njagi, E. M. (2016). Anti-pyretic, Anti-inflammatory and Analgesic Activities of Aqueous Leaf Extract of *Urtica dioica* in Albino Mice. *Medicinal & Aromatic Plants*, **5**: 2167-0412.

Said, A. A. H., El Otmani, I. S., Derfoufi, S., & Benmoussa, A. (2015). Highlights on nutritional and therapeutic value of stinging nettle (*Urtica dioica*). *International Journal of Pharmacy and Pharmaceutical Sciences*, **7**: 8-14.

Salah, A., Al-Chalabi, H. M. M., Shukri, R. M., Mahmood, L. I., & Al-Anbar, B. K. (2016). Effect of *Salvia officinalis* (Sage) aqueous extract on liver and testicular function of diabetic albino male rats. *Journal of Babylon University/Pure and Applied Sciences*, **24**: 83-90.

Sangwan, N. S., Farooqi, A. H. A., Shabih, F., & Sangwan, R. S. (2001). Regulation of essential oil production in plants. *Plant Growth Regulation*, **34**: 3-21.

Saravanan, G., & Ponmurugan, P. (2012). Antidiabetic effect of S-allylcysteine: effect on thyroid hormone and circulatory antioxidant system in experimental diabetic rats. *Journal of Diabetes and its Complications*, **26**: 280-285.

Schapowal, A., Berger, D., Klein, P., & Suter, A. (2009). Echinacea/sage or chlorhexidine/lidocaine for treating acute sore throats: a randomized double-blind trial. *European Journal of Medical Research*, **14**: 406-417.

Schoner, W., & Scheiner-Bobis, G. (2007). Endogenous and exogenous cardiac glycosides and their mechanisms of action. *American Journal of Cardiovascular Drugs*, **7**: 173-189.

Sen, S., Chakraborty, R., & De, B. (2016). Prevalence of Diabetes and Its Economic Impact. *Diabetes Mellitus in 21st Century*, **2**: 27-34.

Sen, S., Makkar, H. P., & Becker, K. (1998). Alfalfa saponins and their implication in animal nutrition. *Journal of Agricultural and Food Chemistry*, **46**: 131-140.

Serafini M, Ghiselli A., & Ferro-Luzzi A. (1994). Red wine, tea and antioxidants. *Journal of Lancet*, **344**: 626-635.

Shi, Y., & Hu, F. B. (2014). The global implications of diabetes and cancer. *Journal of Lancet*, **383**: 1947-1963.

Solomon, T. P., Sistrun, S. N., Krishnan, R. K., Del Aguila, L. F., Marchetti, C. M., O'Carroll, S. M., & Kirwan, J. P. (2008). Exercise and diet enhance fat oxidation and reduce insulin resistance in older obese adults. *Journal of Applied Physiology*, **104**: 1313-1319.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, **50**: 537-546.

Tripathi, V., & Verma, J. (2014). Current updates of Indian antidiabetic medicinal plants. *International Journal of Research in Pharmacy and Chemistry*, **4**: 114-118.

Tripathi, V., & Verma, J. (2014). Different models used to induce diabetes: a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**: 29-32.

Unwin, N., Whiting, D., Gan, D., Jacqmain, O., & Ghyoot, G. (2009). *IDF Diabetes Atlas*.

Vasu, P., Khan, N. D., Khan, Z. H., & Mular, S. M. (2017). In vitro antidiabetic activity of methanolic extract of *Citrus limon*, *Punica granatum*, *Musa acuminata* peel. *International Journal of Advanced Research*, **3:** 804-806.

Wadkar, K. A., Magdum, C. S., Patil, S. S., & Naikwade, N. S. (2008). Antidiabetic potential and Indian medicinal plants. *Journal of Herbal Medicine and Toxicology*, **2**: 45-50.

Wang, L., Waltenberger, B., Pferschy-Wenzig, E. M., Blunder, M., Liu, X., Malainer, C., & Schuster, D. (2014). Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ). *Biochemical Pharmacology*, **92**: 73-89.

Wilt, T. J., Ishani, A., Rutks, I., & MacDonald, R. (2000). Phytotherapy for benign prostatic hyperplasia. *Public Health Nutrition*, **3**: 459-472.

Wong, M. S., Hawthorne, W. J., & Manolios, N. (2010). Gene therapy in diabetes. *Self/Non-self*, **1**: 165-175.

World Health Organization. (2014). About diabetes. World Health Organization

World Health Organization. (2015). WHO traditional medicine strategy 2002–2005. Geneva: World Health Organization.

APPENDICES

Appendix A: Analysis of variance (ANOVA) of Fasting Blood Sugar (FBS) levels of the crude extracts and Ethyl acetate fractions

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
U. dioica						
Fasting blood sugar levels (Mg/dl) at -5 days	Between Groups	62.222	5	12.444	2.029	.103
	Within Groups	184.000	30	6.133		
	Total	246.222	35			
Fasting Blood Sugar levels (Mg/dl) at 0 days	Between Groups	786370.222	5	157274.044	24404.59	.000
	Within Groups	193.333	30	6.444		
	Total	786563.556	35			
Fasting Blood Sugar levels (Mg/dl) at day 7	Between Groups	778381.889	5	155676.378	4818.045	.000
	Within Groups	969.333	30	32.311		
	Total	779351.22	35			
S. officinalis						
Fasting Blood Sugar levels (Mg/dl) at -5 days	Between Groups	264.889	5	52.978	2.010	.106
	Within Groups	790.667	30	26.356		
	Total	1055.556	35			
Fasting Blood Sugar levels (Mg/dl) at 0	Between Groups	817302.222	5	163460.444	5097.519	.000

days	Within Groups	962.000	30	32.067		
	Total	818264.222	35			
Fasting Blood Sugar levels (Mg/dl) at 7 days	Between Groups	906707.222	5	181341.444	1299.835	.000
	Within Groups	4185.333	30	139.511		
	Total	910892.556	35			
P. guajava						
Fasting Blood Sugar levels (Mg/dl) at -5 days	Between Groups	244.889	5	48.978	2.681	.041
	Within Groups	548.000	30	18.267		
	Total	792.889	35			
Fasting Blood Sugar levels (Mg/dl) at 0 days	Between Groups	797549.472	5	159509.894	2457.990	.000
	Within Groups	1946.833	30	2457.990		
	Total	799496.306	35			
Fasting Blood Sugar levels (Mg/dl) at 7 days	Between Groups	824681.917	5	164936.383	1454.109	.000
	Within Groups	3402.833	30	113.109		
	Total	828084.750	35			
C. limon						
Fasting Blood Sugar levels (Mg/dl) at -5 days	Between Groups	177.333	5	35.467	1.814	.140

	Within Groups	586.667	30	19.556		
	Total	764.000	35			
Fasting Blood Sugar levels (Mg/dl) at 0 days	Between Groups	797754.333	5	159550.867	6697.564	.000
	Within Groups	714.667	30	23.822		
	Total	798469.000	35			
Fasting Blood Sugar levels (Mg/dl) at 7 days	Between Groups	843620.556	5	168724.111	4787.254	.000
	Within Groups	1057.333	30	35.244		
	Total	844677.889	35			
***U. dioica* fractions**						
Fasting Blood Sugar (FBS) at day -5	Between Groups	355.810	6	59.302	2.770	.026
	Within Groups	749.333	35	21.410		
	Total	1105.143	41			
Fasting Blood Sugar (FBS) at day 0	Between Groups	855710.905	6	142618.484	1603.914	.000
	Within Groups	3112.167	35	88.919		
	Total	846998.119	41			
Fasting Blood Sugar (FBS) at day 10	Between Groups	842747.952	6	140457.992	1156.668	.000
	Within Groups	4250.167	35	121.433		
	Total	846998.119	41			

***S. officinalis* fractions** Fasting blood sugar (FBS) levels on day -5	Between Groups	404.000	7	57.714	4.356	.001
	Within Groups	530.000	40	13.250		
	Total	934.000	47			
Fasting Blood sugar (FBS) levels on day 0	Between Groups	883831.917	7	126261.702	17415.407	.000
	Within Groups	290.000	40	7.250		
	Total	884121.917	47			
Fasting Blood Sugar (FBS) levels on day 10	Between Groups	853006.667	7	121858.095	783.108	.000
	Within Groups	6224.333	40	155.608		
	Total	859231.000	47			

Appendix B: Analysis of variance (ANOVA) of Fasting Blood Sugar (FBS) levels and weight measurement of various groups administered with *S. officinalis* aqueous extract

ANOVA

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
FBS group results for *S. offficinalis* aqueous extract						
Fasting Blood Sugar (FBS) levels on day -5	Between Groups	74.867	4	18.717	.731	.579
	Within Groups	639.833	25	25.593		
	Total	714.700	29			
Fasting Blood Sugar (FBS) levels on day 0	Between Groups	625703.867	4	156425.967	276.225	.000
	Within Groups	14157.500	25	566.300		
	Total	639861.367	29			
Fasting Blood Sugar (FBS) levels on day 3	Between Groups	581034.200	4	145258.550	197.753	.000
	Within Groups	18363.667	25	734.547		
	Total	599397.867	29			
Fasting Blood Sugar (FBS) levels on day 6	Between Groups	484623.800	4	121155.950	236.935	.000
	Within Groups	12783.667	25	511.347		
	Total	497407.467	29			

Fasting Blood Sugar (FBS) levels on day 9	Between Groups	463187.200	4	115796.800	209.191	.000
	Within Groups	13838.667	25	553.547		
	Total	477025.867	29			
Fasting Blood Sugar (FBS) levels on day 12	Between Groups	465779.333	4	116444.833	268.698	.000
	Within Groups	10834.167	25	433.367		
	Total	476613.500	29			
Fasting Blood Sugar (FBS) levels on day 15	Between Groups	474811.467	4	118702.867	538.824	.000
	Within Groups	5507.500	25	220.300		
	Total	480318.967	29			

Weight group results for *S. offficinalis* aqueous extract

Weight (g) on day -5	Between Groups	7.391	4	1.848	2.008	.124
	Within Groups	23.003	25	.920		
	Total	30.395	29			
Weight (g) on day 0	Between Groups	9.273	4	2.318	2.258	.091
	Within Groups	25.673	25	1.027		
	Total	34.947	29			
Weight (g) on day 3	Between Groups	15.528	4	3.882	3.516	.021
	Within Groups	27.602	25	1.104		

	Total	43.130	29			
Weight (g) on day 6	Between Groups	16.551	4	4.138	2.951	.040
	Within Groups	35.052	25	1.402		
	Total	51.603	29			
Weight (g) on day 9	Between Groups	24.471	4	6.118	4.140	.010
	Within Groups	36.943	25	1.478		
	Total	61.415	29			
Weight (g) on day 12	Between Groups	28.343	4	7.086	4.711	.006
	Within Groups	37.603	25	1.504		
	Total	65.947	29			
Weight (g) on day 15	Between Groups	43.620	4	10.905	7.247	.001
	Within Groups	37.620	25	1.505		
	Total	81.240	29			

Appendix C: Homogenous subsets of Fasting blood sugar (FBS) levels of plants extracts at -5 days in the preliminary studies of *U. doica, S. officinalis, P. guajava* and *C. limon*

Fasting blood sugar levels (Mg/dl) at -5 days

Tukey HSD[a]

		Subset for alpha = 0.05	
Plants extract	**N**	**a**	**b**
U. dioica			
Aqueous	6	86.0000	
Diabetic control	6	87.6667	
Methanol	6	88.0000	
Ethyl acetate	6	88.3333	
Hexane	6	89.0000	
Negative control	6	90.3333	
Sig.		.051	
S. officinalis			
Diabetic control	6	82.6667	
Ethyl acetate	6	84.3333	
Negative control	6	86.3333	
Aqueous	6	88.3333	
Hexane	6	89.6667	
Methanol	6	90.0000	
Sig.		.164	
P. guajava			
Aqueous	6	80.3333	
Hexane	6	84.3333	84.3333
Diabetic control	6	85.6667	85.3333
Ethyl acetate	6	86.6667	86.6667
Methanol	6	87.3333	87.3333
Negative control	6		88.3333
Sig.		.079	0.592

C. limon

Ethyl acetate	6	84.0000
Aqueous	6	86.0000
Methanol	6	87.0000
Negative control	6	87.6667
Diabetic control	6	88.0000
Hexane	6	91.3333
Sig.		.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix D: Homogenous subsets of Fasting blood sugar (FBS) levels of plants extracts at 0 days

Fasting Blood Sugar levels (mg/dl) at 0 days				
Tukey HSD[a]				
		Subset for alpha = 0.05		
Plants extract	**N**	**a**	**b**	**c**
U. dioica				
Negative control	6	89.3333		
Ethyl acetate	6			499.5000
Diabetic control	6		493.3333	493.3333
Methanol	6		483.0000	
Hexane	6		482.6667	
Aqueous	6			
Sig.		1.000	.228	.769
S. officinalis Negative control	6	85.0000		
Ethyl acetate	6		482.3333	
Aqueous	6		487.6667	487.6667
Diabetic control	6		488.0000	488.0000
Hexane	6		490.6667	490.6667
Methanol	6			497.0000
Sig.		1.000	.142	.076
P. guajava Negative control	6	89.3333		
Hexane	6		482.6667	
Methanol	6		483.0000	
Aqueous	6		483.3333	
Diabetic control	6		493.3333	493.3333
Ethyl acetate	6			499.5000
Sig.		1.000	.228	.769

C. limon			
Negative control	6	87.3333	
Hexane	6		483.3333
Methanol	6		485.0000
Ethyl acetate	6		487.3333
Aqueous	6		488.6667
Diabetic control	6		489.3333
Sig.		1.000	.300

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix E: Homogenous subsets of Fasting blood sugar (FBS) levels of plants extracts at 7 days

Fasting Blood Sugar levels (Mg/dl) at day 7

Tukey HSD[a]

		Subset for alpha = 0.05				
Plants extract	**N**	**a**	**b**	**c**	**d**	**e**
U. dioica						
Negative control	3	88.3333				
Aqueous	3		435.6667			
Methanol	3		441.3333			
Hexane	3			457.0000		
Ethyl acetate	3			466.3333		
Diabetic control	3				549.0000	
Sig.		1.000	.526	.077	1.000	
S. officinalis Negative control	6	83.0000				
Aqueous	6		426.3333			
Hexane	6			473.0000		
Methanol	6			488.0000		
Ethyl acetate	6				519.000	
Diabetic control	6					563.0000
Sig.		1.000	1.000	.268	1.000	1.000
P. guajava						
Negative control	6		88.0000			
Hexane	6			475.6667	475.6667	
Methanol	6		462.3333			
Aqueous	6		457.000			
Diabetic control	6					551.1667
Ethyl acetate	6			481.3333		
Sig.		1.000	.051	0.938		1.0000

C. limon

Negative control	6	87.0000				
Ethyl acetate	6		440.0000			
Hexane	6		446.3333			
Aqueous	6			481.3333		
Methanol	6				493.333	
Diabetic control	6					559.6667
Sig.		1.000	.452	1.0000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix F: Homogenous subsets of Fasting blood sugar (FBS) levels of *U. dioica* and *S. officinalis* E.A fractions at -5 days

Fasting Blood Sugar (FBS) at day -5

Tukey HSD[a]

		Subset for alpha = 0.05	
Fractions of E.A extract	**N**	**a**	**b**
U. dioica			
F5	6	79.6667	
F1	6	83.3333	83.3333
F4	6	84.0000	84.0000
F3	6	85.3333	85.3333
Negative control	6	87.0000	87.0000
F2	6	87.6667	87.6667
Diabetic control	6		89.3333
Sig.		.068	.363
S. officinalis			
Diabetic control	6	79.3333	
F2	6	84.0000	84.0000
0.2 ml/kg essential oil	6	84.3333	84.3333
F3	6	85.6667	85.6667
0.4 ml/kg essential oil	6	85.6667	85.6667
F4	6		87.6667
F1	6		88.0000
Negative control	6		89.3333
Sig.		.077	.210

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix G: Homogenous subsets of Fasting blood sugar (FBS) levels of *U. dioica* and *S. officinalis* E.A fractions at 0 days

Fasting Blood Sugar (FBS) at day 0

Tukey HSD[a]

Fractions of E.A extract	**N**	**Subset for alpha = 0.05**				
		a	**b**	**c**	**d**	**e**
U. doica						
Negative control	6	88.6667				
F2	6		490.6667			
F3	6		500.5000			
F4	6		500.3333			
F5	6		494.0000			
F1	6		496.6667			
Diabetic control	6		496.6667			
Sig.		1.000	.553			
S. officinalis						
Negative control	6	86.6667				
F3	6		491.3333			
F1	6		492.3333			
Diabetic control	6		494.6667	494.6667		
F4	6		494.0000	494.0000		
F2	6			497.3333	497.3333	
0.2 ml/kg essential oil	6				500.0000	
0.4 ml/kg essential oil	6					507.3333
Sig.		1.000	.406	.406	.678	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix H: Homogenous subsets of Fasting blood sugar (FBS) levels of *U. dioica* and *S. officinalis* E.A fractions at 10 days

Fasting Blood Sugar (FBS) at day 10

Tukey HSD[a]

Fractions of E.A extract	N	Subset for alpha = 0.05				
		a	b	c	d	e
U. dioica						
Negative control	3	89.3333				
F2	3		426.3333			
F3	3			454.1667		
F4	3			467.0000	467.0000	
F1	3				475.6667	
F5	3				481.0000	
Diabetic control	3					564.6667
Sig.		1.000	1.000	.423	.321	1.000
S. officinalis						
Negative control	6	90.3333				
F4	6		461.1667			
F3	6		461.6667			
0.2 ml/kg essential oil	6		465.6667			
F1	6		470.3333			
F2	6		472.0000			
0.4 ml/kg essential oil	6		482.6667			
Diabetic control	6			562.1667		
Sig.		1.000	.082	1.000		

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix I: Homogenous subsets of weight (g) of various groups treated with *S. officinalis* aqueous extract

Tukey HSD[a] (g)		Weight		
		Subset for alpha =0.05		
Groups of mice	N	1	2	
Weight (g) on day -5				
Positive control-glibenclamide drug (2 mg/kg)	6	26.7667		
S. officinalis aqueous extract (600 mg/kg)	6	27.5667		
Negative control	6	27.7667		
Diabetic control	6	27.9167		
S. officinalis aqueous extract (400 mg/kg)	6	28.2500		
Sig.		.086		
Weight (g) on day 0				
Positive control-glibenclamide drug (2 mg/kg)	6	26.4500		
S. officinalis aqueous extract (600 mg/kg)	6	27.3833		
Diabetic control	6	27.6000		
Negative control	6	27.8500		
S. officinalis aqueous extract (400 mg/kg)	6	28.0500		
Sig.		.077		
Weight (g) on day 3				
Positive control-glibenclamide drug (2 mg/kg)	6	25.8667		
S. officinalis aqueous extract (600 mg/kg)	6	27.0667	27.0667	
Diabetic control	6	27.2333	27.2333	

Negative control	6		27.9167
S. officinalis aqueous extract (400 mg/kg)	6		27.7333
Sig.		.194	.633
Weight (g) on day 6			
Positive control-glibenclamide drug (2 mg/kg)	6	26.5500	
Diabetic control	6	26.7833	26.7833
S. officinalis aqueous extract (600 mg/kg)	6	27.6000	27.6000
Negative control	6	27.8000	27.8000
S. officinalis aqueous extract (400 mg/kg)	6		28.6167
Sig.		.380	.086
Weight (g) on day 9			
Diabetic control	6	26.4833	
Positive control-glibenclamide drug (2 mg/kg)	6	27.0833	27.0833
Negative control	6	27.9333	27.9333
S. officinalis aqueous extract (600 mg/kg)	6	28.1667	28.1667
S. officinalis aqueous extract (400 mg/kg)	6		29.1000
Sig.		.149	.057
Weight (g) on day 12			
Diabetic control	6	25.9667	
Positive control-glibenclamide drug (2 mg/kg)	6	27.2667	27.2667
Negative control	6	27.9000	27.9000
S. officinalis aqueous extract (600 mg/kg)	6		28.2167
S. officinalis aqueous extract (400 mg/kg)	6		28.8167

Sig.		.077	.216
Weight on day 15			
Diabetic control	6	25.7167	
Positive control-glibenclamide drug (2 mg/kg)	6	27.5500	27.5500
Negative control	6		27.8667
S. officinalis aqueous extract (600 mg/kg)	6		28.5500
S. officinalis aqueous extract (400 mg/kg)	6		29.3167
Sig.		.103	.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix J: Homogenous subsets of FBS (Mg/dl) of various groups treated with *S. offficinalis* aqueous extract

Tukey HSD[a]		FBS (Mg/dl)			
		Subset for alpha = 0.05			
Groups of mice	N	1	2	3	4
FBS on day -5					
S. officinalis aqueous extract (400 mg/kg)	6	81.8333			
Negative Control	6	85.1667			
S. officinalis aqueous extract (600 mg/kg)	6	85.5000			
Diabetic control	6	85.6667			
Positive control-glibenclamide drug (2 mg/kg)	6	86.3333			
Sig.		.547			
Sig.		.634			
FBS on day 0					
Negative Control	6	84.3333			
S. officinalis aqueous extract (600 mg/kg)	6		431.0000		
Diabetic control	6		444.6667		
Positive control-glibenclamide drug (2 mg/kg)	6		451.8333		
S. officinalis aqueous extract (400 mg/kg)	6		452.0000		
Sig.		1.000	.555		
FBS on day 3					
Negative Control	6	83.8333			
S. officinalis aqueous extract (600mg/kg)	6		377.3333		

S. officinalis aqueous extract (400 mg/kg)	6		412.8333	412.8333
Positive control-glibenclamide drug (2 mg/kg)	6			452.6667
Diabetic control	6			454.6667
Sig.		1.000	.188	.087
FBS on day 6				
Negative Control	6	86.6667		
S. officinalis aqueous extract (600 mg/kg)	6		371.6667	
S. officinalis aqueous extract (400 mg/kg)	6		374.8333	
Positive control-glibenclamide drug (2 mg/kg)	6		395.8333	
Diabetic control	6			446.6667
Sig.		1.000	.368	1.000
FBS on day 9				
Negative Control	6	82.6667		
S. officinalis aqueous extract (600 mg/kg)	6		327.6667	
S. officinalis aqueous extract (400 mg/kg)	6		340.6667	
Positive control-glibenclamide drug (2 mg/kg)	6		361.3333	
Diabetic control	6			457.3333
Sig.		1.000	.128	1.000
FBS on day 12				
Negative Control	6	85.0000		

S. officinalis aqueous extract (400 mg/kg)	6		287.1667		
S. officinalis aqueous extract (600 mg/kg)	6		290.3333		
Positive control-glibenclamide drug (2 mg/kg)	6			337.0000	
Diabetic control	6				473.0000
Sig.		1.000	.999	1.000	1.000
FBS on day 15					
Negative Control	6	86.5000			
S. officinalis aqueous extract (400 mg/kg)	6		256.3333		
S. officinalis aqueous extract (600 mg/kg)	6		256.6667		
Positive control-glibenclamide drug (2 mg/kg)	6			300.0000	
Diabetic cntrol	6				480.6667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Appendix K: Multiple comparisons results of plants extracts

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Plants extract	(J) Plants extract	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
U. dioica							
Fasting blood sugar levels (Mg/dl) at -5 days	Diabetic control	Methanol	-3.33333	1.42984	1.000	-4.6823	4.0157
		Ethyl acetate	-.66667	1.42984	.997	-5.0157	3.6823
		Hexane	-1.33333	1.42984	.935	-5.6823	3.0157
		Aqueous	1.66667	1.42984	.849	-2.6823	6.0157
		Negative control	-2.66667	1.42984	.442	-7.0157	1.6823
Fasting Blood Sugar levels (Mg/dl) at 0 days	Diabetic control	Methanol	-3.33333	1.46566	.236	-7.7913	1.1246
		Ethyl acetate	.66667	1.46566	.997	-3.7913	5.1246
		Hexane	-5.00000*	1.46566	.021	-9.4579	-.5421
		Aqueous	-5.00000*	1.46566	.021	-9.4579	-.5421
		Negative control	394.00000*	1.46566	.000	389.5421	398.4579
Fasting Blood Sugar levels (Mg/dl) at day 7	Diabetic control	Methanol	107.66667*	3.28182	.000	97.6847	117.6486
		Ethyl acetate	82.66667*	3.28182	.000	72.6847	92.6486
		Hexane	92.00000*	3.28182	.000	82.0180	101.9820

		Aqueous	113.33333*	3.28182	.000	103.3514	123.3153
		Negative control	460.66667*	5.28182	.000	450.6847	470.6486
S. officinalis	Diabetic control	Methanol	-7.33333	2.96398	.164	-16.3486	1.6819
Fasting Blood Sugar		Ethyl acetate	-1.66667	2.96398	.993	-10.6819	7.3486
levels (Mg/dl) at -5 days		Hexane	-7.00000	2.96398	.202	-16.0152	2.0152
		Aqueous	-5.66667	2.96398	.983	-14.6819	3.3486
		Negative control	-3.66667	2.96398	.815	-12.6819	5.3486
Fasting Blood Sugar	Diabetic control	Methanol	-9.00000	3.26939	.094	-18.9441	-.9441
levels (Mg/dl) at 0 days		Ethyl acetate	5.66667	3.26939	0.522	-4.2775	15.6108
		Hexane	-2.66667	3.26939	.962	-12.6108	7.2775
		Aqueous	.33333	3.26939	1.000	-9.6108	10.2775
		Negative control	403.00000*	3.26939	.000	393.0559	412.9441
Fasting Blood Sugar	Diabetic control	Methanol	75.00000*	6.81936	.000	54.2583	95.7417
levels (Mg/dl) at 7 days		Ethyl acetate	44.0000*	6.81936	.000	23.2583	64.7417
		Hexane	90.00000*	6.81936	.000	69.2583	110.7417
		Aqueous	136.66667*	6.81936	.000	115.9249	157.4084
		Negative control	480.00000*	6.81936	.000	459.2583	500.7417
P. guajava	Diabetic control	Methanol	-1.66667	2.46757	.983	-9.1720	5.8387

Fasting Blood Sugar levels (Mg/dl) at -5 days		Ethyl acetate	-1.00000	2.46757	0.998	-8.5053	6.5053
		Hexane	1.33333	2.46757	.994	-6.1720	8.8387
		Aqueous	5.33333	2.46757	.285	-2.1720	12.8387
		Negative control	-2.66667	2.46757	.885	-10.1720	4.8387
Fasting Blood Sugar levels (Mg/dl) at 0 days	Diabetic control	Methanol	10.33333	4.65097	.258	-3.8130	24.4797
		Ethyl acetate	-6.166667	4.65097	.769	-20.3130	7.9797
		Hexane	10.66667	4.65097	.228	-3.4797	24.8130
		Aqueous	10.00000	4.65097	.290	-4.1464	24.1464
		Negative control	404.00000*	4.65097	.000	389.8536	418.1464
Fasting Blood Sugar levels (Mg/dl) at 7 days	Diabetic control	Methanol	88.83333*	6.14892	.000	70.1308	107.5359
		Ethyl acetate	69.83333*	6.14892	.000	51.1308	88.5359
		Hexane	75.50000*	6.14892	.000	56.7975	94.2025
		Aqueous	94.16667*	6.14892	.000	75.4641	112.8692
		Negative control	463.16667*	6.14892	.000	444.4641	481.8692
C. limon Fasting Blood Sugar levels (Mg/dl) at -5 days	Diabetic control	Methanol	1.00000	2.55314	.999	-6.7656	8.7656
		Ethyl acetate	4.00000	2.55314	.626	-3.7656	11.7656
		Hexane	-3.33333	2.55314	.780	-11.0989	4.4323
		Aqueous	2.00000	2.55314	.968	-5.7656	9.7656
		Negative control	.33333	2.55314	1.000	-7.4323	8.0989

Fasting Blood Sugar levels (Mg/dl) at 0 days	Diabetic control	Methanol	4.33333	2.81793	.644	-4.2377	12.9043
		Ethyl acetate	2.00000	2.81793	.979	-6.5710	10.5710
		Hexane	6.00000	2.81793	.300	-2.5710	14.5710
		Aqueous	.66667	2.81793	1.000	-7.9043	9.2377
		Negative control	402.00000*	2.81793	.000	393.4290	410.5710
Fasting Blood Sugar levels (Mg/dl) at 7 days	Diabetic control	Methanol	66.33333*	3.42756	.000	55.9081	76.7586
		Ethyl acetate	119.66667*	3.42756	.000	109.2414	130.0919
		Hexane	113.33333*	3.42756	.000	102.9081	123.7586
		Aqueous	78.33333*	3.42756	.000	67.9081	88.7586
		Negative control	472.66667*	3.42756	.000	462.2414	483.0919

*. The mean difference is significant at the 0.05 level.

Appendix L: Multiple comparison results of *U. dioica* E.A fractions, *S. officinalis* E.A fractions and the essential oil

Multiple Comparisons

Dependent Variable	(I) Fractions of E.A extract	(J) Fractions of E.A extract	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Tukey HSD							
***U. dioica* E.A fractions**							
Fasting Blood Sugar (FBS) at day -5	Diabetic control	F1	5.66667	2.67142	.363	-2.6840	14.0174
		F2	1.33333	2.67142	.999	-7.0174	9.6840
		F3	3.66667	2.67142	.812	-4.6840	12.0174
		F4	5.00000	2.67142	.511	-3.3507	13.3507
		F5	9.33333*	2.67142	.020	-.9826	17.6840
		Negative control	2.00000	2.67142	.988	-6.3507	10.3507
Fasting Blood Sugar (FBS) at day 0	Diabetic control	F1	.00000	5.44423	1.000	-17.0183	17.0183
		F2	6.00000	5.44423	.923	-11.0183	23.0183
		F3	-3.83333	5.44423	.991	-20.8517	13.1850
		F4	-3.66667	5.44423	.993	-20.6850	13.3517
		F5	2.66667	5.44423	.999	-14.3517	19.6850
		Negative control	408.00000*	5.44423	.000	390.9817	425.0183

Fasting Blood Sugar (FBS) at day 10	Diabetic control	F1	89.00000*	6.36221	.000	69.1121	108.8879
		F2	138.33333*	6.36221	.000	118.4455	158.2212
		F3	110.50000*	6.36221	.000	90.6121	130.3879
		F4	97.66667*	6.36221	.000	77.7788	117.5545
		F5	83.66667*	6.36221	.000	63.7788	103.5545
		Negative control	475.33333*	6.36221	.000	455.4455	495.2212
***S. officinalis* E.A fractions** Fasting blood sugar (FBS) levels on day -5	Diabetic control	F1	-8.66667*	2.20159	.004	-15.3844	-1.9489
		F2	-5.00000	2.10159	.279	-11.7177	1.7177
		F3	-6.33333	2.10159	.077	-13.0511	.3844
		F4	-8.33333*	2.10159	.007	-15.0511	-1.6156
		0.2 ml/kg essential oil	-4.66667	2.10159	.362	-11.3844	2.0511
		0.4 ml/kg essential oil	-6.33333	2.10159	.077	-13.0511	.3844
		Negative control	-10.00000*	2.10159	.001	-16.7177	-3.2823
Fasting Blood sugar(FBS) levels on day 0	Diabetic control	F1	2.33333	1.55456	.802	-2.6358	7.3025
		F2	-2.66667	1.55456	.678	-7.6358	2.3025
		F3	3.33333	1.55456	.406	-1.6358	8.3015
		F4	-.66667	1.55456	1.000	-4.3025	5.6358
		0.2 ml/kg essential oil	-5.33333*	1.55456	.028	-10.3025	-.3642
		0.4 ml/kg essential oil	-12.66667*	1.55456	.000	-17.6358	-7.6975
		Negative control	407.33333*	1.55456	.000	403.0308	412.9692

Fasting Blood Sugar(FBS) levels on day 10	Diabetic control	F1	91.83333*	7.20204	.000	68.8120	114.8547
		F2	90.16667*	7.20204	.000	67.1453	113.1880
		F3	100.50000*	7.20204	.000	77.9787	123.5213
		F4	101.00000*	7.20204	.000	77.9787	124.0213
		0.2 mg/kg essential oil	96.500000*	7.20204	.000	73.4787	119.5213
		0.4 mg/kg essential oil	79.50000*	7.20204	.000	56.4787	102.5213
		Negative control	471.83333*	7.20204	.000	448.8120	494.8547

*. The mean difference is significant at the 0.05 level.

Appendix M: Multiple comparison results (weight and FBS levels) of aqueous *S. officinalis* extract administered to various groups

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Groups of mice	(J) Groups of mice	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Weight (g) on day -5	Diabetic control	Negative control	.15000	.55382	.999	-1.4765	1.7765
		S. officinalis aqueous extract (400 mg/kg)	-.33333	.55382	.973	-1.9598	1.2932
		S. officinalis aqueous extract (600 mg/kg)	.35000	.55382	.968	-1.2765	1.9765
		Positive control-glibenclamide drug (2 mg/kg)	1.15000	.55382	.261	-.4765	2.7765
Weight (g) on day 0	Diabetic control	Negative control	-.25000	.58507	.993	-1.9683	1.4683
		S. officinalis aqueous extract (400 mg/kg)	-.45000	.58507	.937	-2.1683	1.2683
		S. officinalis aqueous extract (600 mg/kg)	.21667	.58507	.996	-1.5016	1.9350

	Positive control-glibenclamide drug (2 mg/kg)	1.15000	.58507	.311	-.5683	2.8683
Weight (g) on day 3	Diabetic control	Negative control	-.68333	.60665	.791	-2.4650	1.0983
		S. officinalis aqueous extract (400 mg/kg)	-.50000	.60665	.921	-2.2816	1.2816
		S. officinalis aqueous extract (600 mg/kg)	.16667	.60665	.999	-1.6150	1.9483
		Positive control-glibenclamide drug (2 mg/kg)	1.36667	.60665	.194	-.4150	3.1483
Weight (g) on day 6	Diabetic control	Negative control	-1.01667	.68363	.580	-3.0244	.9911
		S. officinalis aqueous extract (400 mg/kg)	-1.83333	.68363	.086	-3.8411	.1744
		S. officinalis aqueous extract (600 mg/kg)	-.81667	.68363	.754	-2.8244	1.1911
		Positive control-glibenclamide drug (2 mg/kg)	.23333	.68363	.997	-1.7744	2.2411
Weight (g) on day 9	Diabetic control	Negative control	-1.4500	.70184	.266	-3.5112	.6112
		S. officinalis aqueous extract (400 mg/kg)	-2.61667*	.70184	.008	-4.6779	-.5555

		S. officinalis aqueous extract (600 mg/kg)	-1.68333	.70184	.149	-3.7445	.3779
		Positive control-glibenclamide drug (2 mg/kg)	-.60000	.70184	.910	-2.6612	1.4612
Weight (g) on day 12	Diabetic control	Negative control	-1.93333	.70808	.077	-4.0129	.1462
		S. officinalis aqueous extract (400 mg/kg)	-2.85000*	.70808	.004	-4.9295	-.7705
		S. officinalis aqueous extract (600 mg/kg)	-2.25000*	.70808	.029	-4.3295	-.1705
		Positive control-glibeclamide drug (2 mg/kg)	-1.30000	.70808	.376	-3.3795	.7795
Weight (g) on day 15	Diabetic control	Negative control	-2.15000*	.70824	.040	-4.2300	-.0700
		S. officinalis aqueous extract (400 mg/kg)	-3.60000*	.70824	.000	-5.6800	-1.5200
		S. officinalis aqueous extract (600 mg/kg)	-2.83333*	.70824	.004	-4.9133	-.7533
		Positive control-glibenclamide drug (2 mg/kg)	-1.83333	.70824	.103	-3.9133	.2467
Fasting Blood Sugar	Diabetic control	Negative Control	.50000	2.92081	1.000	-8.0780	9.0780

(FBS)levels (Mg/dl) on day -5	*S. officinalis* aqueous extract (400 mg/kg)	3.83333	2.92081	.686	-4.7447	12.4114
	S. officinalis aqueous extract (600mg/kg)	.16667	2.92081	1.000	-8.4114	8.7447
	Positive control-glibenclamide drug (2 mg/kg)	-.66667	2.92081	.999	-9.2447	7.9114
Fasting Blood Sugar Diabetic control (FBS) levels (Mg/dl) on day 0	Negative Control	360.33333*	13.73924	.000	319.9830	400.6837
	S. officinalis aqueous extract (400 mg/kg)	-7.33333	13.73924	.983	-47.6837	33.0170
	S. officinalis aqueous extract (600 mg/kg)	13.66667	13.73924	.855	-26.6837	54.0170
	Positive control-glibenclamide drug (2 mg/kg)	-7.16667	13.73924	.984	-47.5170	33.1837
Fasting Blood Sugar Diabetic control (FBS) levels (Mg/dl) on day 3	Negative Control	370.83333*	15.64765	.000	324.8782	416.7885
	S. officinalis aqueous extract (400 mg/kg)	41.83333	15.64765	.087	-4.1218	87.7885
	S. officinalis aqueous extract (600 mg/kg)	77.33333*	15.64765	.000	31.3782	123.2885

	Positive control-glibenclamide drug (2 mg/kg)	2.00000	15.64765	1.000	-43.9551	47.9551
Fasting Blood Sugar (FBS) levels (Mg/dl) on day 6	Diabetic control Negative Control	360.00000*	13.05561	.000	321.6574	398.3426
	S. officinalis aqueous extract (400 mg/kg)	71.83333*	13.05561	.000	33.4907	110.1760
	S. officinalis aqueous extract (600 mg/kg)	75.00000*	13.05561	.000	36.6574	113.3426
	Positive control-glibenclamide drug (2 mg/kg)	50.83333*	13.05561	.005	12.4907	89.1760
Fasting Blood Sugar (FBS) levels (Mg/dl) on day 9	Diabetic control Negative Control	374.66667*	13.58365	.000	334.7732	414.5601
	S. officinalis aqueous extract (400 mg/kg)	116.66667*	13.58365	.000	76.7732	156.5601
	S. officinalis aqueous extract (600 mg/kg)	129.66667*	13.58365	.000	89.7732	169.5601
	Positive control-glibenclamide drug (2 mg/kg)	96.00000*	13.58365	.000	56.1066	135.8934
Fasting Blood Sugar (FBS) levels (Mg/dl) on day 12	Diabetic control Negative Control	388.00000*	12.01897	.000	352.7018	423.2982
	S. officinalis aqueous extract (400 mg/kg)	185.83333*	12.01897	.000	150.5352	221.1315

	S. officinalis aqueous extract (600 mg/kg)	182.66667*	12.01897	.000	147.3685	217.9648
	Positive control-glibenclamide drug (2 mg/kg)	136.00000*	12.01897	.000	100.7018	171.2982
Fasting Blood Sugar (FBS) levels (Mg/dl) on day 15	Diabetic control	394.16667*	8.56933	.000	368.9997	419.3337
	Negative Control	224.33333*	8.56933	.000	199.1663	249.5003
	S. officinalis aqueous extract (400 mg/kg)	224.00000*	8.56933	.000	198.8330	249.1670
	S. officinalis aqueous extract (600 mg/kg)	180.66667*	8.56933	.000	155.4997	205.8337
	Positive control-glibenclamide drug (2 mg/kg)					

*. The mean difference is significant at the 0.05 level.

Appendix N: Preparation of plants' extracts for administration to experimental mice

a) *U. dioica* (300 mg/kg)

If a mouse averaging 1000 g receives 300 mg of *U. dioica*, then a mouse averaging 30 g will receive 9 mg.

$\frac{30g}{1000g} \times 300 \text{ mg} = 9 \text{ mg}$

The extract was constituted in 10 ml/kg of Tween 80 and topped up with distilled water to the final concentration of 30 mg/ml.

$\frac{30g}{1000g} \times 10 \text{ ml} = 0.3 \text{ ml}$. Thus, each mouse received 0.3 ml of *U. dioica* extract.

b) *Salvia officinalis* and *Citrus limon* (400 mg/kg)

Each mouse received 12 mg of *Salvia officinalis* or *Citrus limon* extract

$\frac{30g}{1000g} \times 400 \text{ mg} = 12 \text{ mg}$

The extracts were dissolved in Tween 80 and made to a final concentration of 40 mg/ml.

Each mouse received 0.3 ml of the solubilized *Salvia officinalis* or *Citrus limon* extract.

c) *Psidium guava* (500 mg/kg)

Each mouse received 15 mg of the *Psidium guava* extract

$\frac{30g}{1000g} \times 500 \text{ mg} = 15 \text{ mg}$

$15mg \rightarrow 1 \text{ mouse} \rightarrow 0.3 \text{ ml}$ of the medium (Tween 80 and distilled water).

It was made to a final concentration of 50 mg/ml.

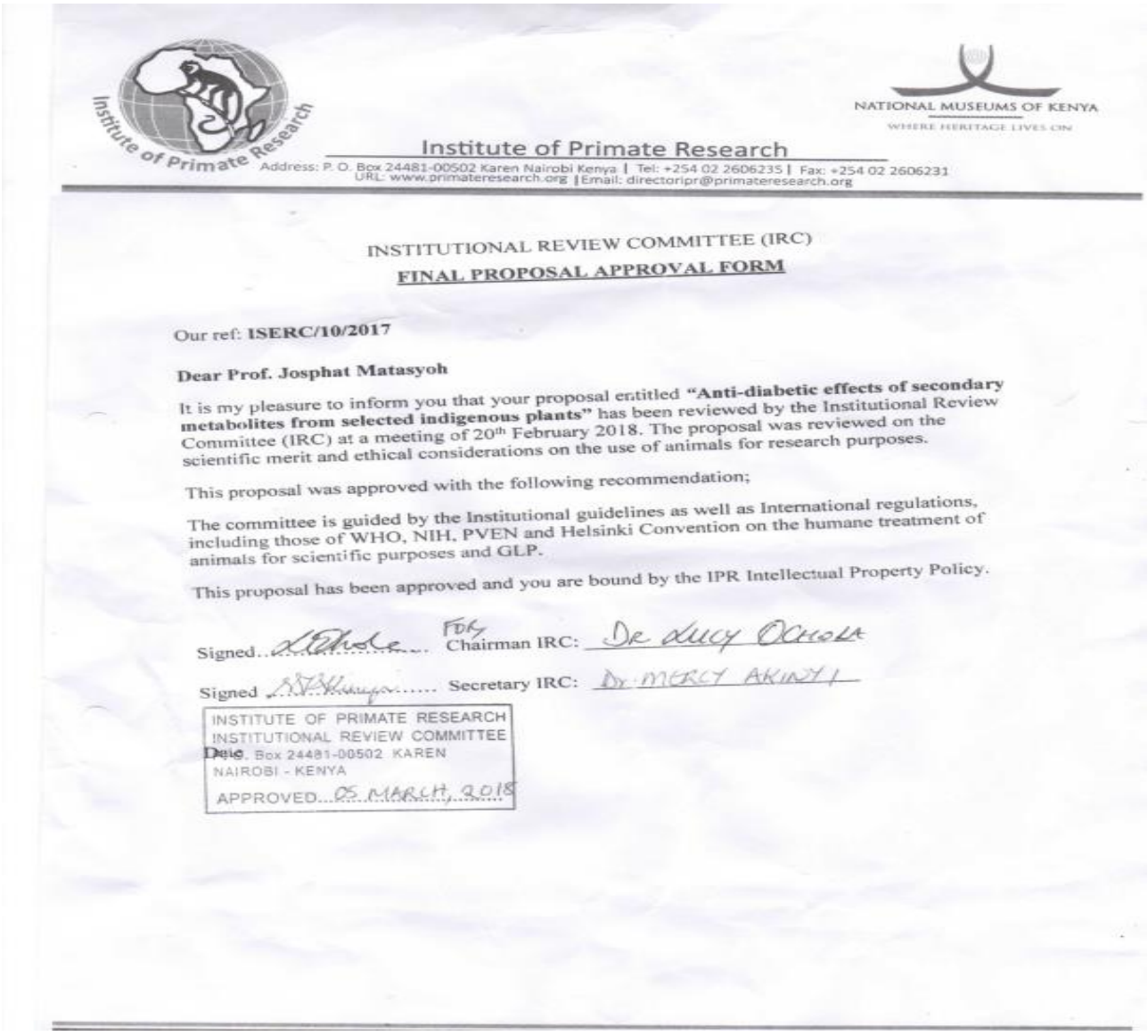
d) Alloxan (200 mg/kg)

Each mouse was administered 6 mg of alloxan

$\frac{30g}{1000g} \times 200 \text{ mg} = 6 \text{ mg}$

Alloxan was dissolved in distilled water and made to a concentration of 20 mg/ml. Each mouse received 0.3 ml of the alloxan solution.

Appendix O: Ethics Approval Form



Institute of Primate Research

Address: P. O. Box 24481-00502 Karen Nairobi Kenya | Tel: +254 02 2606235 | Fax: +254 02 2606231
URL: www.primateresearch.org | Email: directoripr@primateresearch.org

NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON

INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **ISERC/10/2017**

Dear Prof. Josphat Matasyoh

It is my pleasure to inform you that your proposal entitled **"Anti-diabetic effects of secondary metabolites from selected indigenous plants"** has been reviewed by the Institutional Review Committee (IRC) at a meeting of 20th February 2018. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes.

This proposal was approved with the following recommendation;

The committee is guided by the Institutional guidelines as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed..... FOR Chairman IRC: Dr Lucy Ochola

Signed ..... Secretary IRC: Dr. MERCY AKINYI

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P.O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED... 05 MARCH, 2018

Appendix P: NACOSTI Research Permit

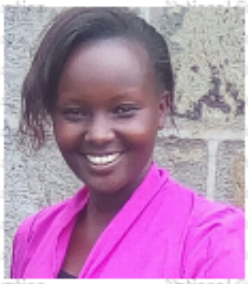
REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 128867

Date of Issue: 03/August/2020

RESEARCH LICENSE



This is to Certify that Miss.. Kanana Faith Mbiti of Egerton University, has been licensed to conduct research in Nakuru on the topic: ANTI-DIABETIC EFFECTS OF SECONDARY METABOLITES FROM SELECTED INDIGENOUS PLANTS for the period ending : 03/August/2021.

License No: **NACOSTI/P/20/4311**

128867

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix Q: Publication

Vol. XX(XX), pp. XXX-XXX, XXXXXX 2019
DOI: 10.5897/JMPRXXXXXXX
Article Number: XXXXXXXXXXX
ISSN: 1996-0875
Copyright ©2019
Author(s) retain the copyright of this article
http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

Hypoglycaemic effects of Salvia officinalis extracts on alloxan-induced diabetic Swiss albino mice

Kanana Faith Mbiti[1]*, Maina Charles Mwendia[1], Kibet Joseph Mutai[2] and Clement Josphat Matasyoh[3]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science, Egerton University, P. O. Box 536 Egerton, Kenya.
[2]Department of Veterinary Parasitology, Microbiology and, Pathology, Faculty of Veterinary Medicine and Surgery, Egerton University, P. O. Box 536 Egerton, Kenya.
[3]Department of Chemistry, Faculty of Science, Egerton University, P. O. Box 536 Egerton, Kenya.

Received 30 July, 2019; Accepted 26 September, 2019

Diabetes mellitus is the fourth killer disease globally. The available management strategies are quite expensive and sometimes unsafe. This necessitates the need for bio-active drugs from medicinal plants. *Salvia officinalis* (sage) has been in use in herbal medicine for a long time. However, the scientific validation for anti-diabetic effects of various extracts from this plant has been elusive. The present study aimed to determine and compare the anti-hyperglycaemic efficacy of methanolic, hexane, ethyl acetate, and aqueous extracts of *S. officinalis* in alloxan-induced diabetic mice. Dried and powdered leaves were extracted with methanol, hexane, ethyl acetate, and water solvents. Phytochemical screening of the extracts revealed presence of flavanone, sterols, saponins, tannins, alkaloids, and triterpenes. Diabetes was induced in the experimental mice using a single intraperitoneal injection of alloxan monohydrate at a dose of 200 mg/kg bwt. The extracts were subjected to preliminary *in vivo* bio-assays at dosage levels of 400 mg/kg for 7 days through oral administration. The aqueous extract demonstrated significant hypoglycaemic effect ($p<0.05$) hence subjected to further hypoglycaemic studies for 15 days. There was a significant decrease in blood sugar levels of groups treated with aqueous extract at 400 and 600 mg/kg doses from 452.00 ± 11.13 and 431.00 ± 10.65 mg/dL to 256.33 ± 5.12 and 256.67 ± 8.74 mg/dL. Weight gain improved significantly from 28.05 ± 0.39 and 27.38 ± 0.52 g to 29.32 ± 0.42 and 28.55 ± 0.38 g, respectively compared to controls ($p<0.05$). Histopathological studies revealed no significant changes in liver and kidney tissues. Besides, no significant cytotoxic effect was reported. Results from this study indicate that aqueous extract of *S. officinalis* is a potential anti-hyperglycaemic and can be used in modulating blood glucose levels.