AN INVESTIGATION OF THE EFFECT OF *KIGELIA AFRICANA* FRUIT FRACTIONS ON DIABETES BIO-MARKERS IN ALLOXAN MONOHYDRATE-INDUCED DIABETES WISTAR RAT MODELS

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9. *Tumelo Muyenga Akapelwa, Christian E Ezaela, Festus Mushabati, Samuel Dominion-Kayonde Bamitale, Dan Kibuule (2021). Commentary on the Antidiabetic Activity of Kigelia Africana. *Journal of Preventive and Rehabilitative Medicine, 3*(2), 21–23. https://journals.unza.zm/index.php/medicine/article/view/537*

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LIST OF ABBREVIATIONS

ALT:	Alanine transaminase
ANOVA:	Analysis of Variance
DMEM	Dulbecco's Modified Eagle Media
DPPH:	2,2-Diphenyl-1-Picrylhydrazyl
ELISA:	Enzyme linked immunoassay
FBS	Foetal Bovine Serum
HDL:	High Density Lipoproteins
GC-MS	Gas Chromatography – Mass Spectrophotometry
IDF:	International Diabetes Federation
KAFE:	*Kigelia africana* fruit extract
LDL:	Low Density Lipoproteins
PBS	Phosphate-buffered saline
TCAM:	Traditional Complementary and Alternative Medicine
TC:	Total Cholesterol
TAG:	Transient Axonal Glycoproteins
TFC:	Total Flavonoid Content
TPC:	Total Phenolic Content
T1DM:	Type one Diabetes Mellitus
T2DM:	Type two Diabetes Mellitus
VLDL:	Very Low-Density Lipoproteins

DECLARATION

I, Tumelo Muyenga, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof, has not been submitted for a degree at any other institution.

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Tumelo Muyenga		8/10/2025
Name of Student	**Signature**	**Date**

DEFINITION OF KEY TERMS

Antidiabetic drug: A drug that reduces blood glucose levels.

Phytochemistry*:* Chemicals or secondary metabolites synthesized by plants. In this case, phytochemistry referred to secondary metabolites that were present in the bioactive fraction of *Kigelia africana*.

Efficacy: the capacity to produce an effect. (In this case, the capacity to lower blood glucose levels) (Scott, 2010)

Drug safety: The frequency of adverse drug effects (that is; physical or laboratory toxicity that could possibly be related to the drug) that result from treatment; that is, they emerge during treatment and are not present before treatment, or they become worse during treatment compared with the pretreatment state (Lynch, 2022).

Traditional Medicine: The total sum of knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to different cultures used to maintain health and prevent, diagnose, and treat physical and mental illnesses (WHO, 2013).

Herbal medicines: herbs, herbal materials or preparations, and finished herbal products that contain active ingredients, plant parts or materials, or a combination of both (WHO,2013).

ABSTRACT

Diabetes affects 19 million people in Africa, with 60% of diabetic patients opting for traditional therapies due to cost-effectiveness, accessibility, and perceived safety. *Kigelia africana* (Bignoniaceae) is used as a traditional antidiabetic remedy, but data on the specific phytochemicals responsible for its benefits and safety are limited. This study investigated the antidiabetic properties of *Kigelia africana* fruit fractions and their effects on diabetes markers in alloxan-induced diabetic rats. The chemical composition of the bioactive fractions was determined, while cytotoxic and genotoxic effects of the crude extracts were also analysed.

The antidiabetic effects of the *Kigelia africana* fruit extract were compared with those of glibenclamide in 54 alloxan-induced diabetic rats. Fractions were obtained using liquid-liquid and column fractionation. The effects of *Kigelia* fractions on lipid profile, blood glucose, alanine aminotransferase, and creatinine levels were determined. Histopathological changes in the liver, kidneys, heart, brain, and pancreas were examined. In vitro bioactivity against alpha-amylase, alpha-glucosidase, glucose utility, and glucose uptake in Caco2 colorectal cells was determined for the bioactive ethyl acetate fraction. The phytochemical profile was obtained using Gas Chromatography-Mass Spectrometry (GC-MS). Total phenolic content, total flavonoid content, and free radical scavenging activity were determined. Mutagenicity and genotoxicity were assessed using quantitative fluorescence microscopy and compared with Ames results obtained in this study.

The ethyl acetate fraction at 1000mg/kg significantly reduced blood glucose levels to 8.16 ± 4.4 mmol/L from 28.42 ± 2.7 mmol/L after 28 days, comparable to glibenclamide

(p=0.15). In vitro studies showed its α-glucosidase inhibitory activity. Animals receiving *Kigelia* fruit fractions and extracts showed decreased weight ($p<0.05$). The ethyl acetate fraction produced a more favourable lipid profile than glibenclamide. Inter-group variations were observed in triglycerides (p=0.03), total cholesterol (p =0.001), and non-HDL cholesterol (p=0.0007) levels. Biochemical and histological examinations revealed improved cell viability in the liver, kidneys, and nervous system of treated rats.

No significant differences in DPPH inhibition, TPC, or TFC were observed amongst the fractions. GC-MS analysis showed that the most bioactive fraction contained 11"(2-cyclopenten-1-yl) undecanoic acid, (+)- and cyclopentane undecanoic acid, along with the indole alkaloids Akuammilan-17-ol-10-methoxy, N-nitroso-2-methyl-oxazolidine and epoxide Oxirane2.2″ -(1.4-butanediyl) bis-.

The aqueous extract had no effect on cell viability, whereas the ethyl acetate extract caused a concentration-dependent decrease in cell viability *(*IC50 = 414.8 ± 8.69 µg/mL). Fruit extracts showed similar effects on His+ revertants as the negative control, indicating no mutagenic activity. The highest concentration of ethyl acetate fruit extract increased the average nuclear area of Vero cells (IC50 EtOAc = 338.6± 1.058 µg/mL). After 48 hours, no significant changes were observed in the ratio of multi+dual to mononucleated Vero cells.

K. africana fruit fraction improved glucose and lipid profiles and the histoarchitecture of organs in diabetes-induced rats. It showed notable alpha-glucosidase inhibitory activity, whereas its alpha-amylase inhibitory activity was limited. At therapeutic doses, the extracts did not display genotoxic properties. This study identified the bioactive phytocomposition

of this fruit, highlighting its potential as a medicinal agent for the treatment of diabetes mellitus. It suggests a potential natural alpha-glucosidase inhibitor and phytocompounds that could serve as lead compounds for developing new antidiabetic medications.

Key words: *Kigelia africana*; Diabetes; Mutagenicity; Genotoxicity; GC-MS analysis; Phytochemistry

CHAPTER ONE: INTRODUCTION

1.1. Background

Diabetes mellitus, a metabolic syndrome characterised by hyperglycemia resulting from decreased insulin production and/or resistance, is projected to affect approximately 700 million people worldwide by 2030 (Saeedi *et al.*, 2019). Approximately 33 million people in Africa are expected to live with diabetes by 2030. Currently, the prevalence of diabetes in Zambia is 8.6% (IDF, 2022). Due to the nature of the disease, treatment is lifelong, which can sometimes prove to be a financial burden for both patients and various health ministries in Africa (Erzse *et al.*, 2019; Mwila *et al*., 2019). With the projected increase in the burden of diabetes, new antidiabetic drugs are needed to provide affordable and new methods for disease prevention and treatment.

Traditional medicine (TM) is the sum of knowledge, skills, and practices based on theories, beliefs, and experiences of different cultures. It is used to maintain health and prevent, diagnose, and treat physical and mental diseases (World Health Organization, 2013). Its application extends to all diseases, including diabetes. Sub-Saharan Africa reports a high prevalence of use of herbal medicines for several reasons, including difficulties in accessing hospitals, convenience of use, affordability of herbal medicines, and as an adjunct to traditional medicines (Ameade *et al.,* 2018; James *et al.*, 2018; Nyirongo *et al.*, 2021). In Zambia, approximately 92% of patients attending a diabetes clinic at the University Teaching Hospital in Lusaka use herbal medicines in addition to conventional medicines (Hikaambo *et al.*, 2022). Herbal medicines include herbs, herbal materials, preparations, and finished herbal products containing active ingredients, plant parts, or other herbal materials, or a combination of both (WHO, 2013).

Most people perceive herbal medicines as safe (Zhang *et al*., 2015). However, healthcare providers have expressed concerns regarding potential drug-herb interactions from both pharmacokinetic and pharmacodynamic perspectives (Ameade *et al.,* 2018). On the other hand, it is important to note that herbal medicines are a significant source of new molecules and drugs that can be used to treat diseases such as diabetes (Fagbohun *et al.,* 2020).

Kigelia africana Lam (Benth.) (*Mupolota* in silozi) is a medicinal plant that has recently attracted the attention of researchers because it is traditionally used to lower blood sugar levels (Muyenga *et al.*, 2021). Previous studies have shown that crude extracts from multiple parts of the plant (leaves, flowers, and fruits) lower blood sugar levels in diabetic mice (Muyenga *et al.*, 2015; Njogu *et al.,* 2018). However, very few studies have highlighted the compounds responsible for the observed antidiabetic activity, although these compounds may serve as pharmacophores for potential new antidiabetic drugs. Furthermore, understanding the phytochemical composition in relation to the ameliorative antidiabetic effects of this plant and determining the plant's safety profile can warrant the development of affordable local dosage forms for the management and control of diabetes and other metabolic conditions.

Therefore, this study used bioassay-guided fractionation to determine the ameliorative properties of fractionated extracts of *Kigelia africana* fruit (KAFE) and their phytochemical fingerprints. Additionally, the cytotoxicity and genotoxicity of the crude extracts were assessed to evaluate their long-term safety.

1.2. Statement of the problem

The burden of diabetes mellitus is substantial in low-income countries, with three out of four individuals with diabetes residing in low- and middle-income countries (Kibirige *et al*., 2019; IDF,

2022). In Zambia, it is estimated that 726,300 adults live with diabetes (IDF, 2022). A significant number of these patients also experience complications such as visual impairment, sexual dysfunction, and fatigue, as well as comorbidities such as obesity, stroke, and hypertension (Mwila *et al*., 2019; Nutakki *et al*., 2021). As a low-income country, Zambian patients face financial challenges that often lead to limited access to medication, poor compliance, and suboptimal glycaemic control (Mwila *et al*., 2019; Nyirongo *et al*., 2021). Consequently, many individuals resort to traditional medical practices, including the use of herbal medicines, which have limited scientific validity, safety, and efficacy (Rutubemberwa *et al*., 2013; Yousef *et al*., 2017). A study conducted at the University Teaching Hospital in Lusaka, Zambia, found a high prevalence of traditional medicine use among patients with diabetes (Hikaambo *et al*., 2022).

In Zambia, *Kigelia africana* has been traditionally used to treat diabetes mellitus and has received significant research attention in recent years (Bello *et al*., 2016; Muyenga *et al*., 2018). Although several studies have demonstrated that its crude extract reduces blood sugar levels (Muyenga *et al*., 2015; Njogu *et al*., 2018), there is a lack of information regarding its active antidiabetic phytoconstituents and their bioactivity in ameliorating diabetes. As the use of traditional remedies continues, there is a paucity of information on the safety profile of this plant, particularly concerning its long-term consumption for diabetes.

To address this knowledge gap, this study aimed to characterise the antidiabetic compounds of *Kigelia africana* and to assess their ameliorative properties. Additionally, this study aimed to evaluate the cell viability and mutagenic and genotoxic potential of the crude extract.

1.3.Study justification

A significant number of individuals with diabetes in Zambia and other Southern African countries have used *Kigelia africana* to manage their condition (Muyenga *et al.,* 2018; Dangana *et al.,* 2024). Although some studies have demonstrated the effectiveness of crude extracts in reducing blood glucose levels in rodents (Muyenga *et al.,* 2015; Njogu *et al.,* 2018), there is a paucity of research on the antidiabetic bioactive agents in this plant. While some studies have highlighted some antidiabetic compounds of the plant growing in India (Kumar *et al.,* 2012), it is important to note that differences in geographic regions may result in variations in phytochemistry (Pacheco-Hernández *et al*., 2021) and, thus, in the efficacy, potency, mechanism of action, and toxicity of herbal medicines. Therefore, extrapolating the results of these studies would be challenging. Identifying the bioactive compounds of the plant growing in Zambia and their probable mechanisms of action would contribute to pharmacophores that may be used to develop antidiabetic drugs and provide a new perspective on the mechanism of antidiabetic bioactivity of these compounds. Furthermore, determining its efficacy in ameliorating diabetes would provide the scientific evidence needed for its traditional use and serve as a stepping stone for clinical research.

Metformin and other antihyperglycemic drugs continue to play a crucial role in the management of Type II diabetes, even though they may have limitations related to adverse drug reactions, the need to increase dosages as tolerance develops, failure to prevent the escalation of the disease, weight gain, and, in some cases, the cost of the medicine among low-income countries (Feingold, 2000; Mutyambizi *et al*., 2018; Lu *et al*., 2024). Novel drugs that would both prevent the disease from escalating and be cost-effective, especially for low-income countries, could

change the existing landscape of diabetes management. Therefore, this study provides a locally available and affordable alternative therapy for patients with type II diabetes mellitus. Additionally, the bioactive compounds identified in this study may offer new avenues for the development of antidiabetic drugs.

Despite the widespread use of traditional medicines, there is a paucity of information regarding their safety (Tabuti *et al*., 2014; Matsabisa *et al*., 2022). This study provides a novel contribution to traditional medicine safety by illuminating the safety of crude *Kigelia* extracts in both cellular and genetic contexts, thus serving as a stepping stone towards the development of affordable yet safe herbal formulations and supplements for the management of type II diabetes and other metabolic conditions, such as obesity.

1.4.Scope of study

1.4.1. Geographical scope

Kigelia africana is a tree commonly found in riverine areas and floodplains throughout South, Central, and Western Africa (Olubunmi *et al.,* 2009; Joffe, 2023). This study focused on the antidiabetic properties of *Kigelia africana* fruit, which was sourced from the Kazungula district in the southern province of Zambia, specifically the riverine area of Singanga village in Kachola, Chief Sikute area. This region is well known for its abundance of *Kigelia* trees, also known as "Muzungula" in Tonga or "Mupolota" in Silozi. The fruit was collected during the rainy season, when it was plentiful and fresh in January 2022.

1.4.2 Content scope

Antidiabetic principles were identified and characterised using biofractionation. This study

determined how *Kigelia* improves blood glucose levels, lipid profiles, rat weight, and liver and kidney function in vivo. Histopathological changes in the brain, heart, liver, pancreas, and kidney were investigated. This study determined the in vitro antioxidant capacity, in vitro effects on carbohydrate metabolism enzymes, and glucose utilization in colorectal cells. Finally, the cellular toxicity and mutagenicity were determined using the Ames test, and the genotoxicity of the aqueous and ethyl acetate crude extracts was evaluated.

Specifically, a sample size of fifty-four (54) rats was used to conduct a bioactivity-guided assay of the aqueous and ethyl acetate crude extracts and the chloroform, ethyl acetate, and butanol fractions of the aqueous crude extract obtained using liquid–liquid fractionation (Emran *et al*., 2015). Plasma blood glucose levels were used to determine the bioactivity. At the end of the study, blood was drawn from the animals for biochemical tests, and their organs were subjected to histopathological analysis. Various in vitro analyses were conducted on the crude extracts and fractions, encompassing evaluations of mutagenicity and genotoxicity, DPPH inhibitory activity, total flavonoid and phenolic content, and in vitro antidiabetic properties. The latter included assessments of the effect on alpha-amylase and alpha-glucosidase enzymes, as well as glucose utilization and uptake in Caco2 colorectal cells. The ethyl acetate fraction, which exhibited the highest antidiabetic activity in rats, was subsequently fractionated using column fractionation into 13 fractions, which were then tested for their in vitro antidiabetic activity. The collected fractions and subfractions were subsequently analysed using GC-MS to identify the available chemical compounds.

1.5. Research questions

i. What is the effect of *Kigelia africana* fruit fraction on diabetes diagnostic markers

(blood glucose, body weight, lipid profile, alanine aminotransferase (ALT), and creatinine) in alloxan-induced diabetic rats?

ii. What is the phytochemical profile of the efficacious *Kigelia africana* fruit fractions that have shown efficacy?

iii. Does *Kigelia africana* have mutagenic or genotoxic effects on *Salmonella TA97*, *TA 98 TA100, Vero cells* and *CA3 hepatocytes*?

1.5.1. Research objective

The main objective of this study was to determine the safety of *Kigelia africana* fruit extracts and the phytochemistry of the efficacious *Kigelia africana* fruit fractions on diabetes markers in alloxan-induced diabetes rats.

1.5.1.1. Specific objectives

To answer the above Research Questions, the following specific objectives were raised:

i. To determine phytochemical profile of bioactive *Kigelia africana* fruit fractions

ii. To determine the bioactivity of *Kigelia africana* fruit fractions

iii. To determine the effect of the bioactive *Kigelia africana* fruit fractions on diabetes diagnostic markers (blood glucose, body weight, lipid profile, ALT and creatinine) in alloxan induced diabetic rats.

iv. To determine mutagenic/genotoxic properties of *Kigelia africana* fruit extract

1.6. Study limitations

This study only provided a phytochemical fingerprint of the bioactive fractions because of the lack of access to the NMR equipment required for chemical structural elucidation. Furthermore, the use of GCMS reduced the probability of identifying some of the compounds which may have

been identified if LCMS was available for the study.

The column fractions collected from the ethyl acetate liquid fraction were only determined on in vitro cell lines to limit the number of animals used in the experiment (13 fractions were collected). This was done in line with recommendations for humane animal research, which aims to improve the welfare of animals used in research (Hubrecht & Carter, 2019) since the sample size for in vivo tests would have been too large.

The study did not have a good positive control for the glucose uptake study, as we were unable to purchase them during the time of the study; thus, we only compared the results to cells that were not treated but still exposed to the same amount of glucose.

A significant part of the research was conducted during the COVID-19 pandemic, making data collection difficult. Due to insufficient funding, some studies that may have determined the antioxidant effects of fruit extracts in rats could not be conducted.

1.7. Study delimitations

This preclinical experimental research was conducted in a laboratory using an alloxan monohydrate diabetes induced Wistar rat model. This model was used because of the availability of rats and their ability to reflect the pathogenesis of diabetes and its complications in humans (Al-awar *et al*., 2016). The use of different fruit fractions instead of a crude extract enabled the study to collect compounds according to their different polarities, and thus identify the antidiabetic bioactive principles. These compounds may be used for drug discovery and development purposes. In this study, we provide a unique perspective on the safety of this plant extract by determining its cytotoxicity, genotoxicity, and mutagenicity, which adds to the body of knowledge regarding the safety profile of the plant. Fractionated antidiabetic bioactivity-guided assays for *Kigelia* africana

fruit extract growing in Zambia were conducted for the first time and revealed the presence of some unique fatty acids. It also provides a new perspective on the biological effects of some of the compounds identified via GC-MS analysis of the fractions and subfractions.

1.8. Research Paradigm

This study adhered to a positivist philosophical approach that assumed a realistic perspective. Consequently, observations were conducted, and quantitative statistical methods were employed to analyse the observations and test the formulated research objective. This study postulated that *Kigelia* could improve diabetes outcomes in alloxan monohydrate-induced diabetic rats and that it is neither mutagenic nor cytotoxic. Independent of the researcher, the outcomes were observed using a post-test randomised experimental design that utilised statistical methods to compare the effects of fruit fractions on diabetes-induced rats in contrast to those treated with normal saline (negative control) and glibenclamide (positive control). These methods were also employed to determine in vitro activity and toxicity. Although these results may not be entirely generalisable to human subjects, they provide insights into the safety of fruit extracts and their potential application in the management of diabetes in humans. Furthermore, this study lays the groundwork for future research on the effects of this plant extract in humans. Figure 1.1 outlines the methodology employed to test answer the research questions.

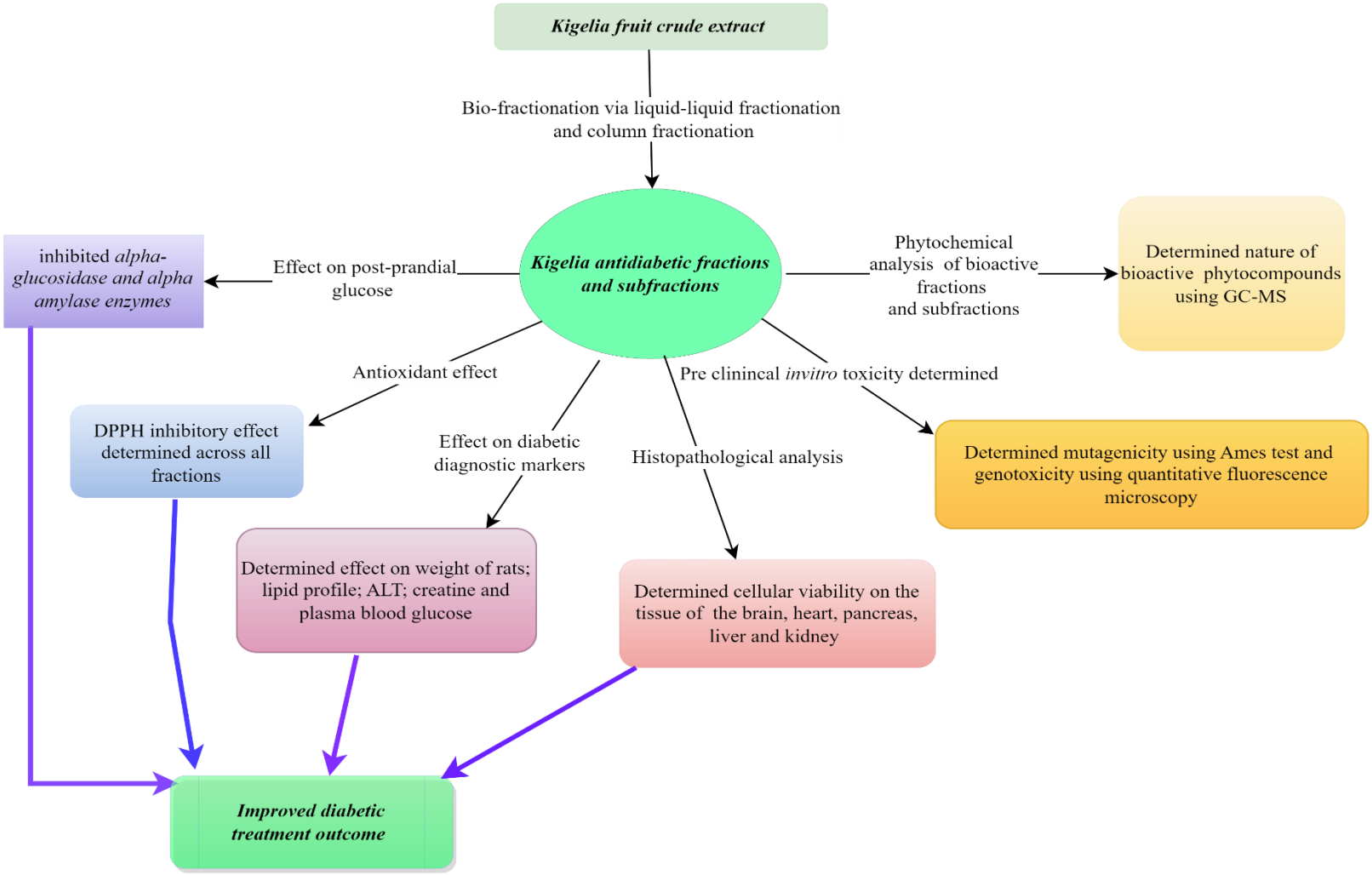


Figure 1.1: Research paradigm outline

CHAPTER TWO: LITERATURE REVIEW

This literature review focused on the burden of diabetes and existing limitations of conventional medicines in effectively managing this disease. It also examined the current gaps in the literature regarding the phytoconstituents of *Kigelia africana* and their potential application in diabetes management, as well as their impact on cellular and genetic materials. This review aimed to provide a comprehensive review of the knowledge on this subject and to identify areas where further research is required to fully understand the potential of *Kigelia africana* in the treatment of diabetes.

2.1. Diabetes disease burden in Africa, complications and pitfalls of existing antidiabetics

Approximately 19 million individuals in Africa currently have diabetes, with an additional 45 million exhibiting impaired glucose tolerance. Furthermore, approximately 60% of the population is unaware of their blood glucose levels (Asmelash & Asmelash, 2019; IDF, 2022). These statistics, reported by Asmelash and Asmelash (2019) and the International Diabetes Federation (IDF) (2022), highlight the need for increased awareness and access to proper medical care because, first, the prevalence of diabetes may be higher than projected. Second, many patients presenting to healthcare providers would already have diabetes complications due to late diagnosis and treatment. Finally, this may translate into an increase in mortality due to diabetes-related complications. However, this growing burden will increase pressure on available resources at the individual and national levels to access treatment. At the individual level, studies have reported that financial challenges are some of the major problems faced by patients living with diabetes (Mutyambizi *et al*., 2018; Moucheraud *et al*., 2019). The inconsistent availability of medicines in health

facilities, resulting in inconsistent access to treatment for patients, and the high cost of drugs used for managing diseases are significant challenges in managing the disease (Rutubemberwa *et al*., 2013; Ameade *et al*., 2018; Nyirongo *et al*., 2021). Consequently, patients seek alternative, cheaper, and more readily available treatment sources, such as medicinal plants. Currently, the prevalence of herbal medicine use among diabetics stands at a median range of 50% (Ekpor *et al.,* 2024). Given the above, there is a need to intensify research on local options for managing diabetes in Africa. A study such as this would justify the use of locally available treatments to reduce the financial burden associated with managing the disease in patients.

Diabetes mellitus (DM) is caused by insufficient insulin production in beta cells in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). In T2DM, there is a heterogeneously progressive loss of beta cell function, resulting in impaired insulin resistance (Lu *et al*., 2024). Several factors may cause the deterioration of beta cell function. These may include modifiable risk factors such as a sedentary lifestyle, smoking, and a BMI greater than or equal to 30 kg/m^2 (Hu *et al*., 2001). Other factors include genetic predisposition, inflammation, endoplasmic reticulum stress, oxidative and metabolic stress, and glucolipotoxicity (Voight *et al*., 2010; Galicia-Garcia *et al*., 2020; Alejandro *et al*., 2015). Elevated blood glucose levels initially trigger a compensatory response that increases insulin release to create a homeostatic environment because insulin release is mainly controlled by blood glucose levels, which regulate beta-cell function (Voight *et al*., 2010). This initial compensatory response leads to excess beta cell activity, which is often characterised by elevated plasma insulin levels and asymptomatic diabetes, especially when blood glucose levels are within the normal range. Therefore, a perpetual rise in blood

glucose coupled with elevated free fatty acids predisposes to beta cell damage and apoptosis, thus reducing beta cell mass and leading to beta cell failure, often leading to diabetes.

Another significant feature of T2DM is reduced insulin receptor sensitivity (insulin resistance), an impaired biological response to insulin stimulation in target tissues such as the muscle, adipose tissue, and liver (Freeman *et al*., 2025). Insulin release facilitates glucose transport from plasma via insulin-dependent glucose transporters, such as GLUT-4, located in the skeletal muscles (van Gerwen *et al*., 2023). Consequently, in cases of insulin resistance, the GLUT-4 transporter fails to mediate glucose transport from the plasma into the tissues effectively. Meanwhile, the GLUT-2 glucose transporter, which operates independently of insulin, is also affected by insulin resistance, leading to altered localisation and impaired functionality (Ait-Omar *et al*., 2011). In both instances, blood glucose regulation is compromised, resulting in hyperglycemia. Insulin resistance arises from various factors, including increased visceral adipose tissue, ageing, a sedentary lifestyle, the use of medications such as steroids, and genetic influences, which may include the presence of insulin receptor antibodies or abnormal insulin genes(Petersen & Shulman, 2006; Brown & Walker, 2016; Freeman *et al*., 2025). This condition is a component of metabolic dysfunction syndrome (Lu *et al*., 2024). Insulin resistance frequently precedes the onset of diabetes mellitus by approximately 10–15 years. Nevertheless, research has indicated that beta cell failure is a primary determinant of diabetes development in insulin-resistant individuals (Alejandro *et al*., 2015). Consequently, a detrimental cycle ensues, characterised by hyperglycemia, insulin resistance, impaired beta-cell function, and inadequate insulin secretion.

Research has demonstrated that the principal complications associated with diabetes mellitus are peripheral vascular disease and cardiovascular disease (Zelniker *et al*., 2019). These complications are linked to oxidative stress within the context of metabolic dysfunction syndrome (Bhatti *et al*., 2022). Literature further indicates that an elevated lipid profile contributes to insulin resistance and a long-term reduction in beta cell mass. Consequently, the management of diabetes mellitus necessitates the regulation of hyperglycemia and lipid levels, along with the enhancement of renal and cardiovascular functions, to effectively prevent its complications (Blaslov *et al*., 2018; Perreault *et al*., 2021; Watts, 2022).

The management of type 2 diabetes mellitus (T2DM) typically necessitates the administration of multiple pharmacological agents, either as monotherapy or in combination (Gupta *et al*., 2017; Ameada *et al*., 2018), in conjunction with lifestyle modification (Blaslov *et al*., 2018). These therapeutic strategies enhance insulin secretion, augment insulin sensitivity in tissues, or diminish hepatic glucose production, thereby reducing plasma glucose levels and lipotoxicity (Feingold, 2000; Thrasher, 2017). Given the progressive nature of diabetes, where monotherapy often fails to decelerate disease progression and control blood glucose levels adequately, combination therapy is imperative (Feingold, 2000; Perreault *et al*., 2021). While existing therapies have helped control diabetes mellitus over the years, we cannot overlook that these drugs have not been able to reverse the damage to the beta cell mass and improve its function over time (Cahn & Cefalu 2016). Furthermore, with oxidative stress playing a key role in the damage to beta cell mass and the development of complications of diabetes, there are very few existing conventional antidiabetic agents that provide antioxidant effects. This property

gives traditional medicines an advantage as they contain phytocompounds, such as polyphenols, which have antioxidant properties (Blahova *et al.,* 2021). Although metformin (a biguanide) is the backbone of diabetes treatment and is regarded as safe, it causes side effects such as lactic acidosis and gastrointestinal disturbances which often limit its dose escalation. It also causes vitamin B12 deficiency with long-term use. Sulfonylureas and Meglitinides, which are insulin secretagogues, have been associated with weight gain and hypoglycemia in patients. Sulfonylureas cannot be used in patients who do not tolerate sulfa drugs or have poor renal function (Feingold, 2000). Thiazolidinediones, which improve insulin sensitivity by acting as PPAR gamma receptor agonists, have limitations in their use because they may cause oedema, congestive heart failure, osteoporosis, and weight gain (Mudaliar *et al.,* 2003; Yki-Järvinen, 2004). Physicians may also prescribe medications that interfere with glucose metabolism by inhibiting the enzymes involved in carbohydrate metabolism and lipid levels to minimize the risk of cardiovascular and peripheral complications in patients with diabetes. Existing alpha-glucosidase inhibitors reduce postprandial glucose levels but predispose patients to gastrointestinal GIT adverse effects, which often cause patients to discontinue treatment. These drugs cannot be used in patients with poor renal function, are costly, and are unavailable to many patients in Africa. (Feingold, 2000; Bhattarai, 2017; Mwila *et al.,*2019). While recent drugs such as incretin mimetics have brought positive change to the management of diabetes, they do not prevent complications of diabetes and have been associated with serious adverse drug reactions like pancreatitis, significant weight loss and GIT disturbances (Jamie & Jacobs, 2013; Gou & Schwartz, 2023; Kamarullah et al., 2025). Ultimately, as the disease progresses, combination therapy is always required, thus

predisposing patients to more adverse drug reactions and possible drug-drug interactions. Identifying the drug responsible for the observed side effects in combination therapy patients can be challenging (Kibiti & Afolayan, 2015).

Finally, an important aspect in the management of diabetes is individualised therapy (Thrasher, 2017; Blaslov *et al.,* 2018; Perreault *et al.,* 2021). This involves setting glycemic targets with the patients and achieving them with preferred treatment options, guided by the cost of treatment, risks of hypoglycemia and other adverse drug reactions, lifestyle management, tolerability, route of administration, effect on weight, and the patient's preferred drugs (Thrasher, 2017). *Kigelia africana,* a traditional medicine used to lower blood glucose levels (Oyelami *et al.,* 2012; Muyenga *et al.,* 2018), is an option for patients. It effectively improves glucose metabolism, lowers lipid levels, and improves pancreatic well-being after damage by chemicals such as alloxan monohydrate in rodents (Fagbohun *et al.,* 2020). Hence, African patients should be provided with accessible and affordable medication options.

2.2. Kigelia africana, a traditional medicine with potential antidiabetic activity

Kigelia africana Lam (Benth.), also referred to as the "sausage tree", is a species belonging to the *Bignoniaceae* family. *Kigelia africana* is the sole species in the genus *Kigelia*. The tree is indigenous to the southern, eastern, and western African riverine regions, where it can reach a height of up to 24 m. It is also found in floodplains and open woodlands (Olubunmi *et al.,* 2009; Joffe, 2023). *Kigelia* produces pendulous, indehiscent woody fruits resembling sausages, which are suspended from long, fibrous stalks. The greyish-brown fruit can reach up to 1 m in length and 15 cm in diameter, with a mass of up to 10 kg. The

fruit contains numerous unwinged obovate seeds. Mature fruits can be found throughout the year; however, they are more abundant during the rainy season.

Traditionally, the plant has been utilised for the management of several health issues. Some of these include dermatological conditions, cancer, reproductive disorders, wound healing, pain, microbial infections, and diabetes (Grace *et al.,* 2002; Oyelami *et al.,* 2012; Nabatanzi *et al.,* 2020; Joffe, 2023). Among the *Kikuyu* people, the *Kigelia* fruit is an important component of the traditional brews used in various ceremonies (Mihegwa, 2024). However, the focus of this study was on the blood-glucose-lowering activity of the plant.

Figure 2.1: Kigelia africana in an open woodland area and its fruit on the tree

Traditional medicine is the total sum of knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to different cultures, used to maintain health and to prevent, diagnose, and treat physical and mental illness (World Health Organization, 2013).

Medicinal plants contain substances that are used for therapeutic purposes or as precursors

for drug synthesis (Sofowora *et al.,* 2013). Medicinal plants have been instrumental in the management of diseases throughout history as traditional medicine. Currently, these plants can be combined with modern medicines to achieve more effective results. (Habte *et al.,* 2017; Ameade *et al.,* 2018; James *et al.,* 2018). Managing diabetes with medicinal plants involves different mechanisms of action that produce their effects. For example, certain plants can stimulate insulin release from beta cells, whereas others can modify hepatic enzymes that are crucial for glucose metabolism. (Kibiti & Afolayan, 2015). Managing diabetes may be more effective with medicinal plants than conventional medications because of their various phytochemicals, which support multiple mechanisms of action within a single plant (Alam *et al.,* 2022). This leads to a synergistic effect that helps manage diabetes while reducing the need for multiple medications and minimizing the risk of side effects from different drug combinations.

It is essential to keep in mind that the diverse phytochemistry is determined by various factors such as geographical location, soil content, and plant disease exposure. (Salehi *et al.*, 2019). Thus, it is crucial to thoroughly examine medicinal plants from various geographical regions. It is important to consider the potential benefits of medicinal plants not only as a therapeutic option for diabetes management, but also as a source of functional foods for diabetes prevention and treatment. The affordability of traditional medicines such as *Kigelia africana* makes it a viable option for many African patients. However, like for any medicine efficacy and safety must be proven (Tabuti *et al.*, 2014).

The ethnopharmacological potential of *Kigelia* has garnered considerable scientific interest. The pharmacological properties elucidated in this review included antibacterial,

antioxidant, and antidiabetic activities. Hussain et al. (2016) demonstrated that the aqueous extract of *Kigelia* bark exhibited greater inhibition of *S. aureus, E. coli*, and *K. pneumoniae* growth than leaves and fruits (Hussain *et al.,* 2016). The efficacy of the aqueous extract of *Kigelia* was further corroborated by Binutu *et al.,* (1996), who demonstrated that both aqueous and methanolic extracts not only possess antibacterial activity but also exhibit broad-spectrum activity against various bacteria and fungi (Binutu *et al.,* 1996). The positive outcomes observed in these studies suggest that the bioactive phytoconstituents of Kigelia are predominantly polar in nature. These findings also provide a rationale for the traditional use of water as an extraction medium, which was employed in this study.

The antioxidant compounds in the management of chronic conditions, including diabetes, are significant because of their association with oxidative stress. Various parts of *Kigelia* have demonstrated radical scavenging activity and the presence of phenols and flavonoids, which are frequently associated with antioxidant activity (Hussain *et al.,* 2016; Nkadimeng, *et al.,* 2020; Ofosu *et al.,* 2020). Ogunlakin *et al.,* (2021) demonstrated the antioxidant effect of *Kigelia* growing in West Africa by elucidating Kigelia methanol extracts' free radical scavenging properties and their fractions against DPPH. Similarly, Raman *et al.,* (2022) illustrated that the fruit of *Kigelia pinata* obtained in Egypt possesses substantial ferric ion-reducing activity as well as DPPH scavenging activity. However, while most studies based their activity on the radical scavenging activity of *Kigelia*, few studies have substantiated its effect against markers of inflammation. Although plants growing in North and Western Africa may exhibit free radical-scavenging properties, these results may not be extrapolated to plants growing in Southern Africa because of

geographical and climatic variations. This study is the first to investigate the antioxidant properties of *Kigelia* growing in Zambia.

So far, studies have demonstrated that *Kigelia africana* is utilised in Zambia and across Africa to manage the symptoms of diabetes (Demoz *et al.,* 2015; Amuri *et al.,* 2018; Muyenga *et al.,* 2018). In Zambia, Muyenga *et al.,* (2015) elucidated how the crude aqueous fruit extract of *Kigelia* could reduce blood glucose levels in alloxan-induced diabetes mice models (Muyenga *et al.,* 2015). Similarly, Njogu *et al.,* (2018) in Kenya demonstrated the blood-glucose-lowering effect of the aqueous and ethyl acetate leaf and fruit extracts in alloxan-induced diabetes mice models. Although studies substantiating folklore information regarding its use to treat several ailments have been published, there remains a need to intensify investigations into the plant to facilitate clinical trials in human subjects and isolate molecules that researchers may use as pharmacophores for future drugs (Yuan *et al.,* 2017; Nabatanzi *et al.,* 2020). Further evidence of the efficacy of *Kigelia,* a local antidiabetic agent, could potentially result in an affordable treatment option for the local population.

2.4. Kigelia phytochemistry and its role in the management of diabetes

Medicinal plants are an important source of structurally unique molecules that provide safer treatment alternatives for the management of diabetes (Ameade *et al.*, 2018a). *Kigelia africana Lam (Benth)* fruit is a medicinal plant that is traditionally macerated or made into a decoction using water as a solvent and consumed as prescribed by the traditional healer (Bello *et al.,* 2016; Muyenga *et al.,* 2018), often twice daily after meals. As indicated previously, several scientists have in the recent past proved efficacy of the

plant extracts and shown the basic phytochemistry of the fruit extracts which is diverse and thus attributing to its diverse use (Gabriel and Olubunmi, 2009). However, researchers have suggested that the plant's phytochemistry may vary in different geographical regions due to soil diversity (Osman *et al.*, 2017). *Kigelia* has been reported to contain a wide variety of secondary metabolites, including saponins, terpenes, flavonoids, alkaloids, naphthoquinones, iridoids, glucosides, and anthraquinones (Bello *et al.*, 2016; Said *et al.,* 2019; Uhuo *et al.*, 2018). The fruit samples have been reported to be rich in iridoids and naphthoquinones (Bello *et al.,* 2016). Khan and colleagues., (2011) in India isolated different compounds among which included iridoid glycosides from the twigs of *Kigelia africana*. They reported that the plant extract stimulated GLUT-4 translocation to the cell surface in skeletal muscle cells. Shah *et al*. discussed the mechanism of action of plants containing naphthoquinones. This review reported that naphthoquinones are good candidates for antidiabetic activity because they have different effects on glucose metabolism. Naphthoquinones may reduce glucose uptake from the gastrointestinal tract, cause changes in carbohydrate-metabolising enzymes, affect glucose uptake in the muscles, increase insulin sensitivity in tissues, and improve insulin secretion (Shah *et al.,* 2017).

Kigelia has also been associated with the presence of flavonoids and other phenolic compounds which are related to their radical-scavenging activity and antioxidant capacity. Some of the flavonoids identified include quercetin and luteolin and their glycosides, while phenolic compounds such as kigelion have been identified and related to the observed antioxidant activity (Babbar *et al.*, 2014; Hussain *et al.,* 2016; Nabatanzi, Nkadimeng, *et al.,* 2020; Ofosu *et al.,* 2020). The antioxidant properties in the

management of chronic conditions, including diabetes, are significant because of their association with oxidative stress. In patients with diabetes, antioxidants may slow down the damage to the beta cells of the pancreas caused by oxidative stress and improve insulin secretion (Sarian *et al.*, 2017; Pastor *et al.,* 2021; Singh and Patil, 2022). Ogunlakin *et al.,* (2021) demonstrated the antioxidant effect of *Kigelia* growing in West Africa by elucidating *Kigelia* methanol extracts' free radical scavenging properties and their fractions against DPPH. Similarly, Raman *et al.,* (2022) illustrated that the fruit of *Kigelia pinata* obtained in Egypt possesses substantial ferric ion-reducing and DPPH scavenging activities. However, while most studies have based their activity on the radical scavenging activity of *Kigelia,* few studies have substantiated its effect against markers of inflammation. Although plants growing in North and Western Africa may exhibit free radical-scavenging properties, these results may not be extrapolated to plants growing in Southern Africa because of the geographical and climatic variations. This study is the first to investigate the antioxidant properties of *Kigelia* growing in Zambia.

Sarian *et al*. (2017) demonstrated that flavonoids are also associated with a reduction in alpha-glucosidase activity, which translates to reduced postprandial glucose levels in patients with diabetes. The reduction in α-glucosidase activity is key in the management of diabetes, as it reduces hyperglycemia and subsequent complications (Bischoff, 1995; Derosa and Maffioli, 2012; Cai *et al.*, 2013). However, anecdotal evidence from the price list in pharmacies indicates that the cost of treatment using this group of drugs is high, while the availability of this important group of antidiabetic drugs is scarce in Southern Africa. In a study by Swarnalatha and colleagues, the phytochemistry of the stem was positive for steroids, alkaloids, carbohydrates, phenols and flavonoids. Furthermore, they

demonstrated that the stem had α-amylase activity (Swarnalatha *et al.,* 2019). Fagbohun *et al.,* (2020) was able to demonstratre that *Kigelia* fruit extract improved the activity of the 3T3 L_1 adipocytes thus increasing insulin sensitivity. They further demonstrated that the fruit extract proliferated kidney cells (Fagbohun *et al.,* 2020). Therefore, it may be concluded that several flavonoids and phenols, through different mechanisms of action, have an ameliorative effect on diabetes mellitus (AL-Ishaq *et al.*, 2019).

Kigelia fruit is often pulverized along with its seeds before extraction thus, its fruit extracts do not just contain flavonoids, phenols and carbohydrates, but are also rich in fatty acids. *Kigelia* seeds have been documented to contain oleic and palmitic acids (Chivandi *et al.,* 2011). However, literature shows that most of the other fatty acids have not been highlighted and some African plants have some rare fatty acids (Avato *et al.,* 2022). There is a need to highlight them and determine their therapeutic or toxicological effects. Thus, through a bioassay-guided fractionation and GC-MS analysis, a phytochemical fingerprint of the fruit fractions would help ascertain the fatty acid content and its biological effects in diabetes and related metabolic conditions.

Thus, this study assessed the phytochemistry of *Kigelia africana* in relation to its ameliorative effect in diabetes-induced rats by determining the total phenolic and flavonoid contents and radical scavenging activities of fractions from the *Kigelia africana* fruit.

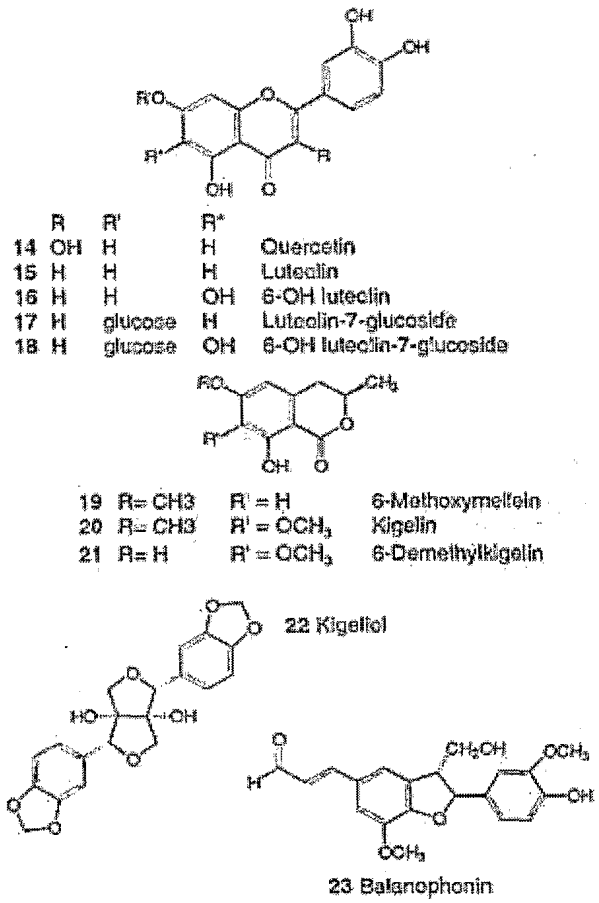


Figure 2.2: some of the flavonoids and phenols identified in Kigelia extracts

2.5. Mutagenic and Cytotoxicity Studies of *Kigelia*

There is a high prevalence of herbal medicine use among African patients. In Zambia, approximately 92% of patients with type 2 diabetes at the country's largest referral hospital use herbal remedies (Hikaambo *et al.*, 2022). Similarly, 68% of elderly patients in Uganda reportedly use traditional medicine as first-line treatment for any illness (Annie Logiel *et al.*, 2021). However, despite the reported high use of traditional medicines for managing and preventing diseases among African patients, little has been done to determine the quality of these medicines and little is known about their dosage and toxicity profiles

(Anywar *et al.*, 2021; Matsabisa *et al.*, 2022).

Although numerous studies have been conducted to demonstrate the efficacy of *Kigelia* extracts (Muyenga *et al.*, 2015; Fagbohun *et al.*, 2020; Byrdie, 2021), only a few have examined the acute and chronic toxicity of the plant (Farah *et al.*, 2018). Although Abdul-Hafeez *et al*. determined the mutagenic potential of *Kigelia* stem bark and leaf extracts (Abdul-Hafeez *et al.,* 2018), these results cannot be extrapolated to fruit extracts, because the phytochemistry of the fruit may differ from that of the bark and stem (Opeyemi Akintola *et al.*, 2020; Joshi, 2023). Moreover, differences in the geographical origin of the plant may result in variations in the phytochemical composition and, consequently, cytotoxic effects (Hadju *et al.*, 2021; Pacheco-Hernández *et al.*, 2021).

To provide high-quality traditional medicines, their efficacy and safety are vital. Drug safety can not only be limited to acute toxic exposure but may also include cyto- and genotoxicity. This is essential because mutagenic drugs often cause physiological, biochemical, and genetic changes in humans (Goayl *et al.* 2022). Therefore, studying the genotoxicity of drugs (including traditional medicines) used to treat chronic diseases is crucial. Studies on the genotoxic effects of antidiabetic drugs are needed in patients with diabetes, as these drugs can amplify mutations, some of which may have already been present in the patient (Habib and Rojna, 2013; Eremina *et al.*, 2021; Goayl *et al.*, 2022). However, there are very few cytotoxic studies of traditional medicines used to treat patients with long-term diseases, despite their continued high use (Anywar *et al.*, 2021). Therefore, in this study, the cytotoxic, genotoxic, and mutagenic potential of aqueous *Kigelia africana* crude extracts were determined.

2.6. Gaps identified in the literature review

Given the literature reviewed, it is essential to provide safe and effective traditional medicines that can serve as low-cost locally available treatment options for African patients. Although mutagenic properties of the stem bark of *Kigelia africana* have been reported, it is important to note that these findings may not apply to other plant parts because of potential chemical differences. Furthermore, there is a lack of literature discussing the genotoxicity and cytotoxicity of *Kigelia africana* extracts, despite their long-term use in certain conditions that may result in altered genetic information. Therefore, this study aimed to evaluate the safety of the fruit extracts in relation to their mutagenic, cytotoxic, and genotoxic properties, which are crucial for the development of drug dosage forms and for potential therapeutic applications in humans.

Although conventional medicines are effective in managing diabetes, they do not improve pancreatic function over time. In addition, drugs targeting carbohydrate-metabolising enzymes in T2DM as well as recent drugs like incretin mimetics are either too expensive or not readily available to patients who need them in most Southern African countries. Determination of the bioactivity of the plant extracts and fractions in this regard points to a readily available and inexpensive alternative, as well as to phytocompounds that may be used for drug development and discovery. Further, literature shows that oxidative stress predisposes patients experiencing glucolipotoxicity to macrovascular and microvascular complications. While some recent antidiabetic agents can slow down the progression of these complications, most conventional antidiabetic drugs do not prevent or slow down the development of complications and do not provide antioxidant properties which some

traditional medicines provide.

Despite the evidence that *Kigelia* can lower blood glucose levels, limited information is available on the phytocompounds responsible for its antidiabetic activity. Therefore, this study focused on bioactive fractions to explore their bioactivity in comparison with conventional treatments and to identify their active compounds.

CHAPTER THREE: MATERIALS AND METHODS

This section discusses the study's methodology based on the research objectives. This will include study design, instruments and reagents, population, sampling technique, data collection procedures, and analysis.

3.1. Study site

This study was conducted at the Mulungushi University School of Medicine and Health Sciences laboratories in Zambia in collaboration with the University of Namibia School of Pharmacy.

3.2.Materials, Equipment, and Laboratory Animals

3.2.1. Solvents and general reagents

Alloxan monohydrate A7413 was purchased from MERCK South Africa, Glibenclamide from BLISS GVS PHARMA LTD; Glucometer (On Call ®), and glucose sticks. silica gel 60-120 mesh was purchased from Chemsol Zambia. Solvents used were of analytical grade and included petroleum ether; ethyl acetate, n-butanol; anhydrous carbonate, chloroform; methanol, di-chloromethane; GC-MS standard pure helium gas

3.2.2 Equipment

Animal balance; cages; rat handling tunnels; Soxhlet; Rota vapor; electronic balance; Huma Star 600; Scion 436 GC-MS Single Quadruple; Biobase, BK-UV 1800 UV spectrophotometer; BioTek PowerWave XS spectrophotometer; Molecular Device ImageXpress Micro XLS Widefield Microscope

3.2.3 Materials for cell cultures

The chemicals were obtained commercially and were of analytical grade. They included:

The Ames test kit was procured from Sigma and consisted of commercially available strains of *Salmonella typhimurium* (*S. typhimurium*) TA97, TA98, and TA100. C3A hepatocytes were generated from human hepatoma cells acquired from the American Type Culture Collection. Vero cells were purchased from Cellonex (South Africa). Dulbecco's modified Eagle 's medium (DMEM) and Foetal Bovine Serum (FBS) were purchased from GE Healthcare Life Sciences (Logan, UT, USA). Phosphate-buffered saline (PBS) with and without Ca^{2+}, Mg^{2+}, and trypsin was purchased from Lonza (Wakersville, MD, USA). Hoechst 33342 nuclear dye and penicillin–streptomycin were purchased from Lonza (BioWhittaker, Verviers, Belgium). Hoechst 33342 nuclear dye, phospho- NF-κB p65 (Ser536) (93H1), rabbit monoclonal antibody, cyclooxygenase 2 (mAb, Cox2) (D5H5) XP Rabbit mAb (Alexa Fluor 488 conjugate), and inducible nitric oxide system (iNOS) (D6B6S) rabbit mAb (PE Conjugate) antibodies were purchased from Cell Signaling Technology, Inc. (Ser536) (93H1); Rabbit mAb, Cox2 (D5H5) XP; Horse serum and 1% non-essential amino acids obtained from Biowest. non-essential amino acids

3.2.4. Plant material

The fruit was harvested from the Kazungula district, in the riverine areas of Singanga village, Kachola, Chief Sikute area of Southern Province of Zambia, in January 2022, since fresh fruit is readily available during the rainy season. The specimen was authenticated at the University of Zambia School of Natural Sciences in the Department of Biological Sciences and mounted in the herbarium under specimen number 22420. The fresh fruit was pounded and allowed to air-dry in a shed. The dry, pounded fruit was ground and sieved to obtain a homogenous powder. The powder was stored at a temperature of 8-10 ºC. Figure

3.1 shows one of the *Kigelia* fruits that was used in the experiment before pulverisation and the powder of the *Kigelia* fruit after pulverisation.

Figure 3.1: Fresh whole fruit after harvest and dried powder after processing

3.2.5. Experimental animals

Healthy male Wistar albino (Sprague-Dawley) rats aged between 7-12 weeks (Jackson *et al*., 2017), with weights ranging between 150-250 grams were used in this study. They were obtained from the Department of Physiological Sciences at the University of Zambia, School of Medicine. Animals were considered healthy if they were alert, had intact fur, and no signs of inflammation of the feet or tail.

The animals were allowed to acclimatize to the laboratory environment for at least 14 days before the commencement of the study. During the study, they were allowed free access to animal feed and water, except for the day before the induction of diabetes, when they were fasted overnight. The animal feed was sourced from the National Milling Company Limited ® in Livingstone. However, during this period, they continued to have access to water. Animals had a normal light and day cycle since they were kept in a room with access to natural light and, thus, were kept at room temperature (approximately 25-28 degrees Celsius) with 35-40% humidity. All observations were performed in the morning to avoid the influence of circadian rhythms on the animals.

3.2.6. Cell cultures for cyto- and genotoxicity

Selected cell lines were maintained in polystyrene culture dishes treated with 10 cm in culture medium supplemented with 10% FBS and 1% (v/v) penicillin/streptomycin and incubated in a humidified incubator at 37 °C with 5% CO_2. Cultures were assessed daily for contamination using an Axiovert 40C inverted microscope (Carl Zeiss, Germany) and sub-cultured when they reached approximately 80% confluence. Furthermore, the culture medium was replaced every third–fourth day to stimulate and maintain optimal growth conditions.

3.3.Study design

This laboratory-based experimental study investigated the antidiabetic activity of *Kigelia africana* fruit extract and its fractions in alloxan-induced diabetic Wistar rats. Through bio-fractionation, the bioactivity of the *Kigelia africana* aqueous extract was determined using liquid-liquid fractionation and column fractionation of its ethyl acetate fraction. The collected fractions were examined for their effects on weight, glucose and lipid metabolism, kidney and liver function, as well as histopathological changes in the rats. Mechanistic in vitro bioactivity tests were conducted on the fruit extracts and their fractions, including effects on alpha-amylase, alpha-glucosidase, glucose absorption, and free radical scavenging activity. Phytochemical screening using GC-MS facilitated the collection of phytochemical fingerprints. The total phenolic and total flavonoid contents were also determined. Mutagenic properties, genotoxicity, and cytotoxicity of aqueous and ethyl acetate crude extracts were determined in vitro. A schematic representation of the experimental design is shown in Fig. 3.2.

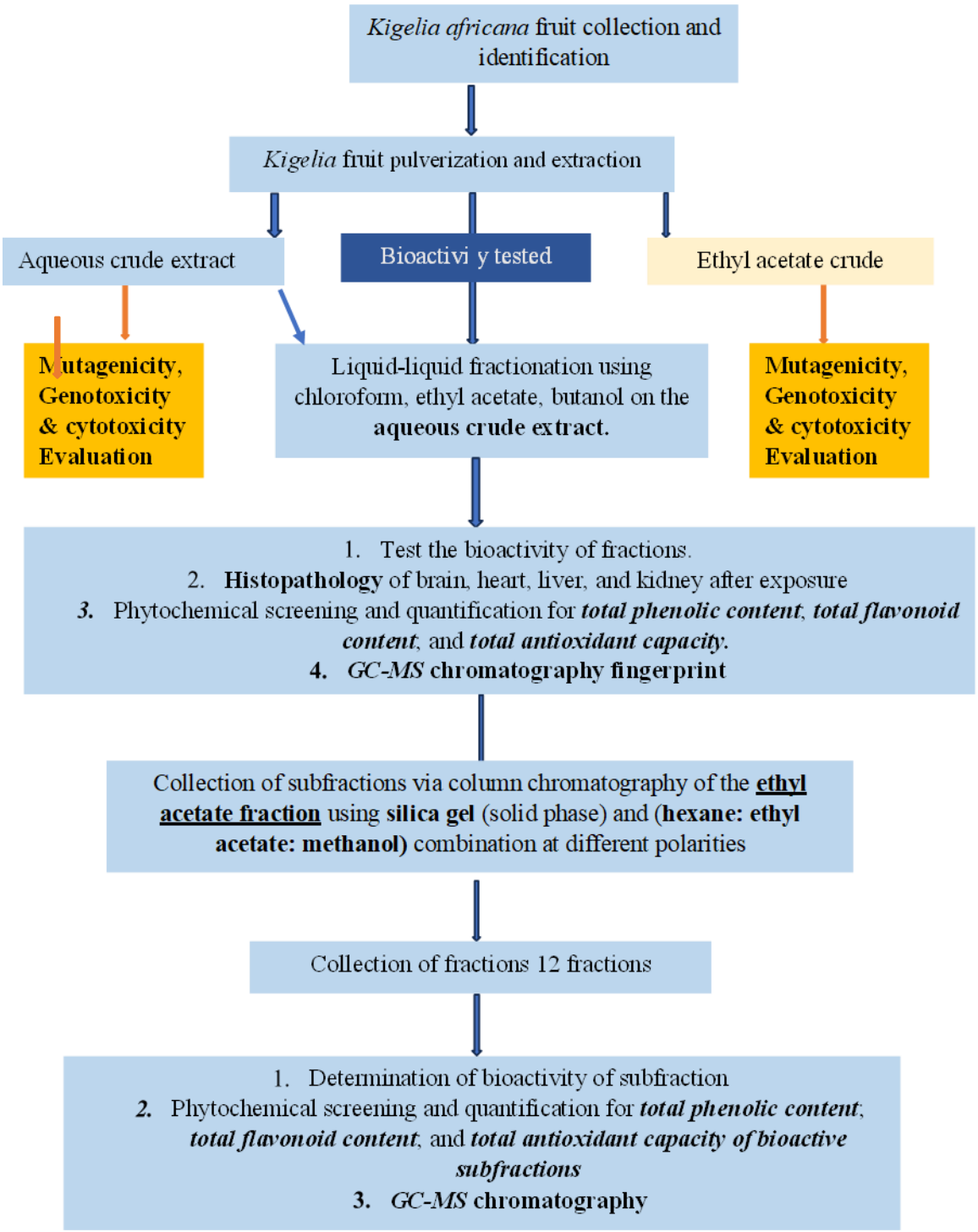


Figure 3.2: Study design layout

3.3.1. Experimental groups of rats

The bioactivity of the crude extract was compared by measuring the fasting blood glucose levels in the rats. Rats were divided into the following groups:

Group 1(Negative control)	• diabetic induced rats treated with **saline**
Group 2 (Positive control)	• diabetic rats treated with **Glibenclamide 0.3mg/kg** (Omoboyowa et al., 2017)
Group 3	• diabetic rats treated **ethyl acetate 1000mg/kg** crude extract
Group 4	• diabetic rats treated with **aqueous crude extract 1000mg/kg**

Figure 3.3: experimental groups for rats treated with the crude extract

To determine the bioactivity of the fractions, animals were grouped as follows:

Group 1 (Negative control)	• Diabetic-induced rats treated with **normal saline**
Group 2 (Positive control)	• Diabetic-induced rats treated with **Glibenclamide 0.3mg/kg**
Group 5	• Diabetic induced rats treated with **chloroform fraction 1000mg/kg**
Group 6	• Diabetic induced rats treated with **ethyl acetate fraction 1000 mg/kg**
Group 7	• Diabetic induced rats treated with **n-butanol fraction 1000 mg/kg**

Figure 3.4: experimental group of rats treated with Kigelia fractions

3.3.2. Inclusion and exclusion criteria of rats

3.3.2.1.Inclusion criteria

- rats aged between 7-12 weeks old.
- rats weighing between 150-250 grams.
- healthy animals

3.3.2.2.Exclusion criteria

- female rats
- rats older than 12 weeks.
- animals involved in other experiments.

3.3.3. Sample size calculation

To calculate sample size, the open epi statistical calculator (OpenEpi n.d.) was used with a minimum size, and the following assumptions were made with confidence intervals of 95% and 80% power of the study: the ratio of sample size for the groups = 1, two-tailed tests such as the unpaired t-test were used. Using the results presented by Muyenga *et al.,* (2015), and following the mean differences formula, the minimum sample size N for each group was to be 3. However, after a review of literature, we used the formular by *(Charan & Kantharia, 2013)*

$$Sample\ Size = 2SD^2 \left(Z^{\frac{\propto}{2}} + Z^{\beta}\right)^2 / d^2 \qquad \textit{(Equation 1)}$$

Where $Z^{\frac{\propto}{2}}$ = 1.96 and $Z^{\beta} = 0.842$

SD is standard deviation and is equal to 7.9

While d^2 is difference in mean values. The sample size was calculated to be 5.

Considering a 10% attrition rate, the corrected sample size was determined to be 5.5 using

equation 2 which is rounded off to six animals per group. Therefore, the study used 54 animals for the nine groups used in the study. The allocation of animals according to groups is presented in Figures 3.3 and 3.4.

$$corrected\ sample\ size = {sample\ size}\Big/{\left(1 - \left[\%\frac{attrition}{100}\right]\right)} \qquad (Equation\ 2)$$

3.3.4. Sampling technique

Random block sampling was used to assign animals to experimental groups based on their weights. This involved grouping the animals by weight and assigning them unique numbers, which were then randomly selected to determine the treatment group to which each animal belonged. To maintain internal validity, a research assistant was responsible for preparing the required dosages and treatments for the animals. Cages and animals were randomly selected to ensure that no cage was consistently the first or last.

3.4. Preparation of cell cultures

Selected cell lines were maintained in 10 cm treated polystyrene Petri dishes in the relevant culture medium supplemented with 10% FBS and 1% (v/v) penicillin/streptomycin and incubated in a humidified incubator at 37 °C with 5% CO2. Cultures were assessed daily for contamination using an Axiovert 40C inverted microscope (Carl Zeiss, Germany) and sub-cultured when they reached approximately 80% confluency. Furthermore, the culture medium was replaced every third to fourth day to stimulate and maintain optimal growth conditions.

3.5.Phytochemical investigations

3.5.1. Crude extraction

About 500g of *Kigelia africana* fruit powder was extracted using 2500 ml of water to collect the aqueous extract. The maceration was combined with shaking for a period of 72 hours. In comparison, another 500 g of fruit powder was extracted using 2500 ml of ethyl acetate in a Soxhlet extraction system at 60 ^{0}C for 24 hours. The chosen solvent systems allowed the extraction of polar and non-polar phytochemicals. The collected solutions were then dried using a Rota vapor at 40 ^{0}C. The collected, dried extract was weighed, and the extractive value was determined.

3.5.2 Screening tests for phytochemicals

The presence of phytochemical compounds in the samples was investigated using several different methods as described by (Jaradat *et al.,*2015; Gul *et al.*, 2017) The phytochemical compounds analyzed included alkaloids, saponins, steroid, terpenoids, glycosides, flavonoids, phenols, tannins, carbohydrates, amino acids and proteins and Fixed oils.

Test for Tannins/Phenols

Ferric Chloride Test

Three drops of 5% Ferric Chloride were added to 2 mL of the plant extract. A transient greenish to dark bluish color observed showed the presence of tannins.

Test for Saponins (Foam Test)

To 5 mL of each of the plant extracts, 5mL of distilled water was added and shaken vigorously. Formation of a stable and persistent foam indicated the presence of saponins.

Test for flavonoids

Alkaline Test

To 2 mL of the plant extract, 2 mL of 2% sodium hydroxide (NaOH) was added. Formation of a yellow color, which turned colorless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

Test for Alkaloids

Mayer's Test

To a few mL of the plant extract, 2 drops Mayer's reagent was added. A creamy or white precipitate showed a positive result for alkaloids.

Test for Glycosides

To 5mL of the plant extract, 2 mL of glacial acetic acid was added, and the contents were mixed. 2 drops of 5% ferric chloride were added to the mixture, followed by 2mL of concentrated sulphuric acid along the sides of the test tube. A greenish-bluish color formed in the lower layer indicated presence of glycosides.

Salkowski s Test for Terpenoids

Three mL of plant extract solution was mixed with 2 mL of chloroform, and 2 mL of concentrated H_2SO_4 was carefully added along the sides of the test tube to form a layer. Appearance of a reddish-brown color at the interface indicated the presence of terpenoids.

Test for Steroids

Three mL of chloroform was added to 2mL of plant extract. Then few drops concentrated Sulphuric acid were added along the sides of the test tube to form a lower layer. A reddish-brown ring was noted to show the presence of steroids.

Test for Carbohydrates

Benedict's Test

Two mL of the extract was mixed with 2 mL of Benedict's reagent. The mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicated the presence of reducing sugars.

Test for Proteins and amino acids

Biuret test

To 2mL of extract, 2 mL of 2% sodium hydroxide solution and 2 mL of copper (II) sulphate solution were added. A purple color indicated the presence of proteins.

Nitric acid Test

2mL of extract was mixed with 2mL of concentrated nitric acid. The appearance of yellow color indicated the presence of proteins.

Test for Fat

Stain test, the small quantity of crude extract was pressed between two filter papers; the stain on 1st filter paper indicated the presence of fixed oils.

The yield for each extract was recorded as a percentage of the original starting material of 10 g.

The Approximate quantities were used by scoring the color intensity as –ve for 0, +ve for 1, ++ve for 2 while +++ve for 3 according to (Sheikh et al., 2013) in comparison to the control.

3.5.3. Liquid-liquid fractionation

For the separation of fractions, the protocol of Malviya and Malviya (2017) was adopted with modifications. Two fractionation methods were employed in this study. First, the modified Kupchan method was used. This system involves liquid-liquid fractionation using solvents of different polarities, beginning with the solvent that is most non-polar to the

most polar solvent. (Kupchan, 1970; TriForC, 2016). The collected aqueous dried crude extract was dissolved in a methanol/water mixture at a ratio of 9:1. This solution was then suspended in chloroform and separated, leaving a methanol: water solution (see Figure 3.6). The collected chloroform fraction was used for the determination of antidiabetic bioactivity, and the methanol-water solution was further partitioned using ethyl acetate. The collected ethyl acetate fraction was used to determine the antidiabetic bioactivity, while the remaining aqueous phase was further separated with n-butanol. The collected butanol fraction was used to determine antidiabetic bioactivity, and the remainder was discarded. All collected fractions were then subjected to gas chromatography-mass spectrophotometry to accurately describe the active substances in the solvent fraction. The fraction that showed the best activity, in this case, the ethyl acetate fraction, was then subjected to column chromatography to collect sub-fractions.

3.5.4. Column chromatographic fractionation

About 50g of ethyl acetate fraction was mixed with 50 g of silica gel and left to dry on a filter paper in a fume hood. The dry silica gel and fraction were then smoothed to make a fine powder using a motor and pestle. For 400 g of silica, 1.330 L of hexane was used to form a slurry while ensuring that the column did not dry out.

Fractions of 250 ml were collected in a conical flask and concentrated using a Rotavapor. The collected concentrate was poured into clearly labelled vials and left to dry completely at room temperature in a fume hood.

Following collection in 320 vials, the fractions were grouped using TLC. Iodine was used to visualize TLC. Subsequently, the collected fractions were evaluated for *in vitro* bioactivity.

3.5.5. Determination of total phenolic content (TPC)

Total phenolic content (TPC) was determined as described by Makkar, H.P.S. *et al*. (2000). 1 ml of each partition extract prepared to a concentration of 10 mg/ml was added to the test tubes. 5 ml of 10% Folin-Ciocalteu Reagent (FCR) and 4 ml of 7% Na_2CO_3 were also added. The resulting, blue-colored mixture was incubated for 30 minutes at 40°C in a water bath before determining absorbance at 550 nm with a UV spectrophotometer. The samples were prepared in triplicate, and the results were determined using a standard gallic acid curve (20-100µg/ml). The phenolic content of each fraction and crude extract was determined using the standard gallic acid curve and expressed as gallic acid equivalent (GAE) per gram of dried plant extracts (GAE mg/g). Equation 3 was used to determine the total phenolic content of the extracts and their respective fractions.

$$GAE \times \frac{V}{m} \qquad \textit{(Equation 3)}$$

where GAE is the gallic acid concentration (mg/ml) determined from the calibration curve, V is the final volume of the partitioning extract (ml), and m is the weight of the plant extract (g).

3.5.6. Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) of the partition extracts was determined as described by Swarnalatha *et al*., (2019). 1 mL of each partition extract solution at a concentration of 2000 µg/mL was placed in three test tubes, followed by 0.5 mL of 5% NaNO3. The mixture was then allowed to stand for five minutes. Thereafter, 0.5 mL of 10% AlCl3 was also added, and the mixture was left to stand for a further six minutes before 2.0 mL of 1 M NaOH was added to the mixture. Finally, distilled water was added to reach a total volume

of 10 ml. The solution was left at room temperature for 20 minutes to allow the reaction to complete. Absorption was determined at 510 nm. The flavonoid content of each partition extract was determined by using a quercetin standard curve (20-100µg/ml). Values were expressed as mg/g quercetin equivalent (QE). The quantification of total flavonoid content was accomplished by applying Equation 4

$$X = C \times \frac{V}{m} \qquad \textit{(Equation 4)}$$

Where X is the flavonoid content, mg/g is the distribution extract in QE, C is the concentration of QE (mg/ml) determined from the calibration curve, V is the final volume of the distribution extract (ml) and m is the weight of the plant extract (g) (Masuku and Lebelo, 2019)

3.5.7. Gas chromatography-Mass Spectrophotometry (GC-MS) analysis

GC-MS analysis was performed on a Scion 436 GC-MS Single Quadruple equipped with a low-bleed, high-inertness SCION 5MS column (equivalent to 5% phenyl/95% dimethyl polysiloxane). Capillary column (30 × 250 × 0.25 m). Pure helium gas (99.99%) at a constant flow rate of 1 ml/min was used as the carrier gas. For spectral detection, an electron ionization energy method was used with a high ionization energy of 70 eV (electron volts) with a scan time of 0.2 s and fragments in the 40 to 600 m/z range. An injection quantity of 1 micro-L was used with a split ratio of 10:1 and an injection temperature of 250 °C (constant). The column oven temperature was set at 50°C for 3 min, increased at a rate of 10°C/min to 280°C, and the final temperature was increased to 300°C for 10 min. The phytochemicals present in the fractions were identified by comparing their retention times (min), peak areas, peak heights, and mass spectral patterns to the spectral

database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

3.6. Determination of bioactivity via *in vivo* and *in vitro* antidiabetic assays

3.6.1. Preparation of reagents for induction of diabetes.

After overnight fasting, a baseline glucose level was established. Rats were randomly divided into six groups. The animals were injected intraperitoneally with alloxan monohydrate calculated at a dose of 100 mg/kg body weight and reintroduced to the normal feeding cycle (Muyenga *et al.*, 2015). Alloxan monohydrate was used to induce diabetes. The alloxan monohydrate was freshly diluted with normal saline to obtain a 10% solution. The prepared alloxan solution was used immediately and was protected from sunlight. It takes approximately 72 hours for diabetes to be established in the animals following the administration of alloxan monohydrate. Therefore, fasting blood sugar was collected to determine the establishment of diabetes using the tail vein puncture 72 hours after administration of alloxan monohydrate. The animals remained alert, with no signs of inflammation on the tail or feet, and the fur coat was intact. A glucometer was used to assess blood glucose levels. Animals were considered diabetic if their fasting blood glucose levels were > 7 mmol/l.

3.6.2. Dosing and Toxicity Considerations

The animals were treated with an oral dose of 1000 mg/kg body weight of aqueous fruit fraction, for 28 days to test for activity. The doses were arrived at following a toxicity consideration observed from the review of literature that shows that *Kigelia africana* crude

extract and fractions have a large therapeutic index with no adverse effects observed at doses as high as 5000 mg/kg body weight (Farah *et al.,* 2017; Fagbohun *et al.*, 2020) and a previous study conducted by Muyenga *et al.,* (2015) where a dose dependent effect was determined with the maximum dose being 1000 mg/kg.

3.6.3. Collection of Blood for Biochemistry

At the end of the study, the rats were restrained and blood was drawn using a size 22G cannular from the heart of the rats. Blood was stored in an EDTA bottle and centrifuged. Blood samples were sent to the laboratory for biochemistry testing.

3.6.4. Liver, Kidney function and Lipid profile

Collected blood samples were tested using a Humar Star 600 machine to determine the concentrations of triglycerides, total cholesterol, HDL-cholesterol, and LDL. Blood samples were also assessed for serum alanine aminotransferase (ALT) and creatinine concentrations.

3.6.5. Histopathological assessment of vital organs

At the end of the study, the animals were euthanised by exposure to chloroform fumes. The animals were dissected, and the heart, liver, kidney, and brain were quickly harvested. The organs obtained were fixed in freshly prepared 10% v/v formaline and then processed for routine histological examination by staining with haematoxylin and eosin (H&E) to observe cellular changes. The samples were sliced using a microtome and placed on slides for observation.

3.7. *In vitro* tests for Postprandial glucose-lowering effect

3.7.1. Alpha-amylase inhibitory activity

Preparation of reagents for α-amylase inhibitory activity

To prepare the porcine pancreatin: 1 mg mL−1 in 1× phosphate buffer solution (PBS); prepared fresh and kept on ice

To prepare the starch solution: 2 mg mL−1 in distilled water; boiled with continuous stirring for 15 minutes until the solution turned clear; cooled to room temperature with continuous stirring and the volume of evaporated water was replaced

α-amylase inhibition was measured using the colourimetric assay method used to quantify starch-iodine complexes, as described by Xiao *et al*., (2006), with slight modifications. Briefly, in a 96-well microtitre plate, 15 µL of the test sample (aqueous crude extract, ethyl acetate fraction or acarbose as positive control) was incubated with 5 µL of porcine pancreatin for 10 min at 37 °C. The reaction was initiated by adding 20 µL starch solution and allowed to proceed for 30 min at 37 °C. The reaction was halted by adding 10 µL HCl (1 M in distilled water) and 75 µL iodine.

3.7.2. Alpha-glucosidase inhibitory activity

Preparation of reagents for alpha-glucosidase inhibitory activity

The reaction buffer: 67 mM potassium phosphate, pH 6.8 to which 3 mM reduced glutathione was added directly before use

To measure α-glucosidase inhibition, yeast α-glucosidase from *Saccharomyces cerevisiae* was utilised using the method described by Akinloye *et al.* (2012), with slight modifications. In a 96-well plate, 5 µL of the sample (aqueous crude extract, ethyl acetate

fraction or epigallocatechin as positive control) was incubated in the presence of 20 µL enzyme (50 µg mL−1) and 60 µL reaction buffer. The reaction was pre-incubated for 5 min at 37 °C, followed by the addition of 10 µL of substrate (10 mM p-nitrophenyl α-d-glucopyranoside). The reaction was allowed to proceed for 30 min at 37 °C, after which it was stopped by the addition of 25 µL of sodium carbonate (100 mM). Epigallocatechin gallate (ECGC) was included as a positive control for α-glucosidase inhibition, and the quantity of p-nitrophenol released was determined spectrophotometrically at 405 nm. The percentage of α-glucosidase inhibition was calculated using equation 5:

$$\%\alpha - glucosda\ inhb = \frac{(A405nm\ of\ control - A405nm\ of\ test\ sample)}{A\ 405nm\ of\ control} \times 100\% \quad \textit{(Equation 5)}$$

No enzymes and substrate controls were included for each sample to account for the absorbance of the extracts. The absorbance of the enzyme and substrate-free wells was subtracted from the absorbance readings of the wells containing enzyme and substrate.

3.7.3. Glucose uptake and utilization assay

Preparation of reagents for glucose uptake and utilization assay

To incubate the buffer: Roswell Park Memorial Institute medium (RPMI)-1640 diluted with PBS containing 0.1% bovine serum albumin (BSA) to a final glucose concentration of 8 mM

Glucose oxidase assay reagent: 3 mM phenol, 0.4 mM 4-aminoantipyrine, 0.25 mM EDTA and 2.5 U/mL horseradish peroxidase in 0.5 M PBS (pH 7.0) with 1 mU/mL glucose oxidase from *Aspergillus niger.*

Cells were seeded in 96 well plates (2 × 104 cells/well, 100 µL aliquots) and left overnight for attachment. Treatments were prepared in complete medium (31.25, 62.5 and 125 µg/mL for the aqueous extract and 16.125, 31.25 and 62.5 µg/mL for the ethyl acetate extract) and

added to cells and incubated for 24 hours. 10 µL spent culture/treatment medium was removed after 24 hours of incubation and transferred to new 96 well plates, which were sealed and stored at -20°C until required (A). The remaining medium was aspirated, and the cells were washed with 100 µL PBS. 25 µL incubation buffer was added to the cells. Cells were incubated for 4 hours. Culture medium (10 µL) was transferred to a new 96-well plate (B). Freshly prepared glucose oxidase reagent (200 µL) was added to the plates (A and B). The reaction mixture was then incubated for 15 min at room temperature. Absorbance was measured at 510 nm using a BioTek® Power Wave XS spectrophotometer (Winooski, VT, USA). Cell-free wells containing the incubation buffer and complete culture media were used as glucose standards. Glucose uptake and consumption were determined as a function of the concentration of glucose (mM) remaining and expressed as the difference between the mean of the standard and test samples. In order to prevent bias, the CaCO2 cells were subjected to cytotoxicity test using the Hoechst 33342 nuclear dye staining method. Hoechst 33342 is a cell-permeable stain that is excited by ultraviolet light and emits blue fluorescence at 469 to 490 nm.

3.7.4. Determination of DPPH radical scavenging activity (for *invitro* antioxidant effect)

Crude extracts and their fractions were prepared at concentrations of 0.1, 0.3 and 0.6 mg/ml and 1 ml of each concentration was placed in a test tube. DPPH (3 ml of DPPH was added to each test tube, and a 10 ml solution was prepared with 99% methanol. The test tubes were left in the shade at 25°C for 30 min to complete the reaction. After 30 minutes, the absorbance of the solutions was measured in triplicate using a spectrophotometer at 517 nm against DPPH in methanol, which served as a blank. The antioxidant capacity was

compared to that of ascorbic acid. It was computed using Equation 6:

$$AA\% = 1 - \left({}^{Ab\ sample}/_{Ab\ control}\right) \times 100\% \qquad \textit{(Equation 6)}$$

Where: "Ab sample" is the absorbance of the sample and "Ab control" is the absorbance of the negative control.

3.8. Mutagenic and genotoxicity assay

3.8.1. Ames' test for mutagenicity

The mutagenicity test of *Kigelia fruit* was performed as described by (Maron and Ames (1983) and Mushtaq *et al*. (2015). About 0.1 ml of 100 µg/mL *Kigelia africana* bioactive fraction and the crude extract were placed separately on a medium containing histidine and 0.1 ml *Salmonella typhimurium,* which is His -ve and allowed to incubate for 12 hours at a temperature of about 36.5 °C. The negative control of this test only contained His-ve *Salmonella typhimurium,* which was grown on the same histidine-containing medium. The number of colonies observed visually after incubation was compared between the two groups.

3.8.2. *C3A hepatocyte cytotoxicity assay*

The C3A hepatocytes generated from human hepatoma and acquired from the American Type Culture Collection were cultured in 10 cm culture dishes using a complete medium consisting of MEM supplemented with 1% non-essential amino acids, 10% foetal bovine serum (FBS), and 1% v/v penicillin/streptomycin. The cells were maintained at 37°C in a humidified environment containing 5% carbon dioxide (CO2). The cells were distributed into 96 well plates at a density of 5000 cells per well, with a total of 100µL aliquots.

Subsequently, the plates were incubated overnight at 37°C to allow cells to adhere. Dilutions of each extract were performed in complete medium at a concentration of 500 µg/mL. A 100 µL aliquot of each dilution was added to 100 µL of the cells adhered to the 96 well plate, resulting in final concentrations of 15.6, 31.25, 62.5, 125, and 250 µg/mL. A final concentration of 30 µM was used as the positive control for melphalan. The cells were then incubated for 48 hours at 37°C in an environment containing 5% carbon dioxide. The supernatant was removed from the cells by aspiration, and then 100 µL of a solution containing 5 µg/mL Hoechst 33342 in phosphate-buffered saline was added to each well. The plates were incubated for 30 minutes at 37°C prior to the addition of 10 µL of 100 µg/mL PI solution to each well. Images were promptly acquired using an ImageXpress Micro XLS widefield automated fluorescence microscope (Molecular Devices). This microscope was equipped with DAPI and Texas Red filters and a 10x Plan Fluor objective. Nine images were obtained per well, including approximately 70% of the entire well surface area. The Multiwavelength Cell Scoring module of the MetaXpress® software was utilised for image analysis. The number of viable cells (nuclei solely stained with Hoechst 33342) and non-viable cells (nuclei stained with both Hoechst 33342 and PI) were recorded and imported into Excel for subsequent analysis.

3.8.3. Genotoxic evaluation using Vero cells.

Vero cells purchased from Cellonex (South Africa) were used as the experimental model. The cells were distributed into 96 well plates at a density of 3000 cells per well, with each well containing 100 µL aliquots. Subsequently, the plates were incubated overnight to allow the cells to adhere. Subsequently, the cells were treated with 15.625, 31.25, 62.5,

125, 250, and 500 µg/mL of *Kigelia* aqueous and ethyl acetate crude extracts for 48 h under controlled conditions of 37°C and 5% CO2. Griseofulvin was employed as the positive control, with concentrations ranging from 0.94 µM to 30 µM. Following the incubation period, cells were fixed with a 4% formaldehyde solution for 15 min. A working solution of Hoechst 33342 was prepared in phosphate-buffered saline (PBS) supplemented with calcium (Ca) and magnesium (Mg) ions at a final concentration of 5 µg/mL. The fixative was removed by aspiration before introducing 100 µL of Hoechst 33342 working solution. The cells were stained for 15–30 minutes. Subsequently, the stained cells were examined using the DAPI filter on the ®ImageXpress Micro XLS Widefield Microscope, manufactured by Molecular Devices. The acquired pictures were analysed using the MetaXpress program and Micronuclei Application Module. The collected data were subsequently transferred to Microsoft Excel®, for analysis and processing.

3.9. Data management and analysis

Data were de-identified and entered into tables created using Microsoft Excel® on a password-protected computer accessible only to the researcher. The data were subsequently analysed using GraphPad Prism® software version 5. Data are presented as the mean ± SD.

The t-test; ANOVA, and Post hoc tests used: Dunn's Multiple Comparison Test for the one-way ANOVA; (Objective 1 and 2) Linear regression (Objective 2) and Non-linear regression to determine the IC50 (Objective 3 and Objective 1). The histopathological study was presented as descriptive data, whereas the phytochemical analysis data were scored according to the observed colour intensity.

3.10. Ethical considerations

Approval was sought from the School of Pharmacy prior to the commencement of the study. Ethical approval was obtained from the university's Research and Ethics Committee in Namibia and Zambia. Further approval for this study was obtained from the National Health Research Authority (Zambia). See the appendix I, II and III for research approval.

The animals were handled by trained personnel in accordance with the stipulated guidelines for animal handling to minimise discomfort and distress. The animals had access to food obtained from the National Milling Company ® in Zambia, except on the day when fasting was required to induce diabetes. The animals were maintained under normal circadian rhythms and ambient temperatures ranging between 25-28^{0}C. Adequate space was provided in the animals' housing facilities to prevent overcrowding.

Procedures such as the determination of blood glucose levels in the animals involved puncturing the tail vein with a small prick, a method adopted to minimise distress and pain in rats. At the conclusion of the study, the animals were euthanised and tissue samples were collected to determine histopathological changes in the organs. To minimise the number of rats used in the study, adjustments were made in accordance with the 3Rs principle (Hurbert & Carter, 2019), and cell cultures were used to study the subfractions of the aqueous extracts.

Verbal permission to collect *Kigelia* fruits was obtained from the village headman.

CHAPTER FOUR: RESULTS

4.1. Extractive values

4.1.1. Extractive value of Aqueous, Ethyl acetate crude extract and PE, Chloroform and Ethyl acetate aqueous fractions following liquid-liquid fractionation.

The extractive value for the aqueous extract (23%) was higher than that of the ethyl acetate extract (12.10%) as indicated in Table 4.1.1:

Table 4.1.1. ***Extractive values for aqueous and ethanolic crude extracts***

Description	**Water extract**	**Ethyl acetate extract**
Weight of plant before extraction	500g	41.76 g
Weight of plant extract	114.8g	60.5g
Extractive value	**23%**	**12.10%**

The chloroform fraction yielded the best extractive value (2.6%), whereas ethyl acetate yielded the lowest extractive value (1.5%). See Table 4.1.2

Table 4.1.2. ***Extractive value following liquid-liquid fractionation.***

Description	**Chloroform**	**Ethyl acetate**	**Butanol**
Weight of crude extract before fractionation	50g		
Weight of fraction collected	1.3g	0.75g	0.95g
Extractive value	**2.6%**	**1.5%**	**1.9%**

4.2. Phytochemical analysis

4.2.1. Phytochemical screening

The aqueous extract contained all phytochemicals tested at different intensities, whereas the butanol fraction had the least variety, although it was the only fraction with saponins.

The ethyl acetate fraction had the widest variety of phytochemicals among the fractions and was the only fraction that had alkaloids, as indicated in Table 4.2.1. The observed basic phytochemistry corresponds to the observed results of the total phenolic content, the total flavonoid content and the radical scavenging activities seen in sections 4.2.2, 4.2.3 and 4.4.4, respectively

Table 4.2.1. ***Phytochemical screening of crude extracts and fractions***

	Butanol Fraction	**Ethyl acetate fraction**	**Chloroform fraction**	**Ethyl acetate crude**	**Aqueous crude**
Proteins and amino acids	+	+++	++	-	+++
Fats	+	-	+	-	+
Carbohydrates	+++	+++	+++	+	+++
Tannins	-	+++	++	-	++
Saponins	+	-	-		+
Alkaloids	-	+	-	+	+
Flavonoids	-		--	+	++
Glycosides	-	+	+	+	+
Steroids and Terpenoids	-	+	-	+	+

4.2.2. Total flavonoid content

Quantification of the flavonoid content in the fractions and crude extracts was performed by determining the concentration of quercetin equivalents. The calibration curve is shown in Figure 4.1. There was a statistically significant difference in the total flavonoid content among the crude extracts and the fractions ($p= 0.014$). The aqueous crude extract had the highest total flavonoid content at 18.0 mg/g quercetin, while the ethyl acetate fraction

exhibited the highest flavonoid content, with a concentration of 3.1 mg/g quercetin. The butanol fraction exhibited the lowest recorded value of quercetin at 0.75 mg/g. No statistically significant difference was observed among the fractions, as indicated by the p-value of 0.06. Refer to table 4.2.2.

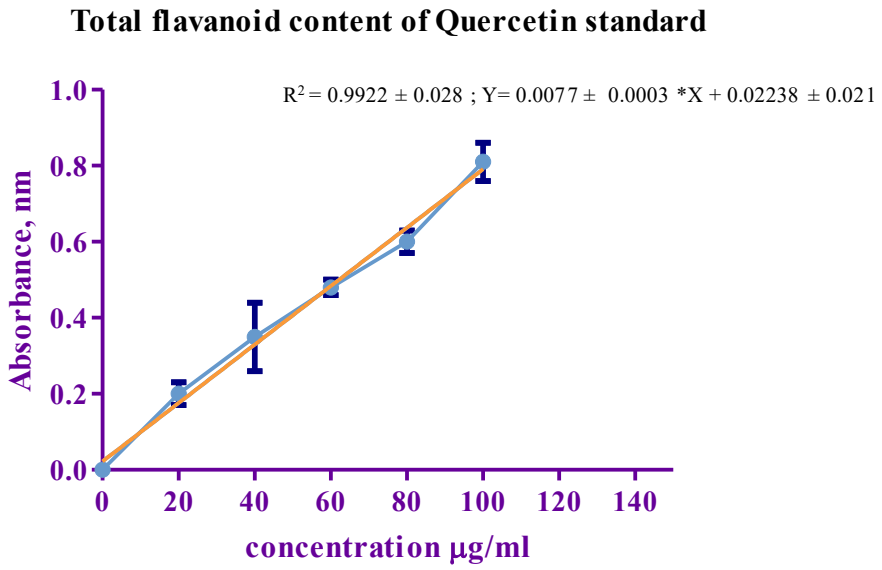


Figure 4.1: Quercetin standard curve

Table 4.2.2. ***Total Flavonoid content of crude extracts and fractions***

Description	Quercetin Concentration, µg/ml	Abs, WL = 510 nm	TFC, mg/g
Aqueous crude (2mg/ml)	**35.99 ±0.9**	**0.29 ± 0.01**	**18.0 ± 0.13**
Ethyl acetate Crude (2 mg/ml)	32.97 ± 0.87	0.27 ± 0.05	16.5 ± 0.12
Chloroform fraction (20 mg/ml)	52.39 ± 1.50	0.42 ± 0.20	2.6 ± 0.1
Ethyl acetate fraction (20 mg/ml)	**61.62 ± 1.11**	**0.05± 0.03**	**3.1 ± 0.05**
Butanol fraction (20mg/ml)	15.95 ± 0.9	0.02 ± 0.004	0.75 ± 0.02

TFC = Total flavonoid content; Abs = Absorbance; WL= wavelength. P= 0.014 across fractions and crude extracts. P=0.06 across all fractions.

4.2.3. Total phenolic content

The ethyl acetate fraction exhibited the highest measured total phenolic content, with a 2.95 mg/g gallic acid value. The chloroform extract had the lowest total phenolic concentration, at 2.45 mg/g gallic acid. There was no statistically significant variation, as indicated by the p-value of 0.329 (refer to Table 4.2.3). The calibration curve is given in figure 4.2.

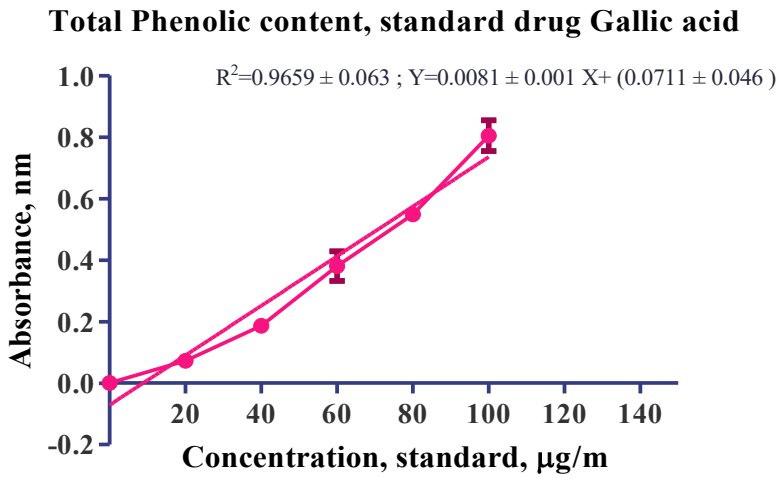


Figure 4.2: Graph of the standard drug Gallic acid

Table 4.2.3. ***Total Phenolic content (TPC) of crude extracts and fractions***

Description	Gallic acid conc, µg/ml	Abs, WL = 550 nm	TPC
Aqueous crude (10 mg/ml)	116.99 ± 1.3	0.87 ± 0.09	11.7 ± 0.98
Ethyl acetate crude (10 mg/ml)	118.37 ± 0.61	**0.88 ± 0.04**	**11.8 ± 0.13**
Chloroform fraction (20mg/ml)	49.09 ± 1.32	0.32 ± 0.02	2.45 ± 0.89
Ethyl acetate fraction (20mg/ml)	59.812 ± 1.12	**0.41 ± 0.01**	**2.95 ± 0.9**
Butanol fraction (20 mg/ml)	53.46 ± 0.89	0.36 ± 0.05	2.68 ± 0.5

P= 0.02 determined via one way ANOVA across crude extracts and fraction. P= 0.32 determined via one way ANOVA across all fractions

4.2.4 Gas Chromatography – Mass Spectrophotometry (GC-MS) of *Kigelia* fractions and bioactive subfractions

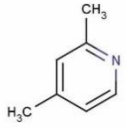
4.2.4. GC-MS of Kigelia fractions and subfractions

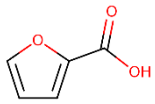
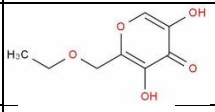
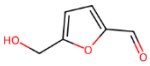
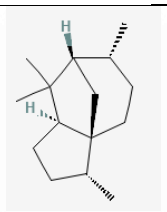
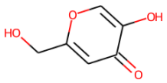
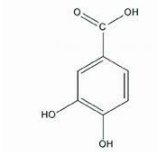
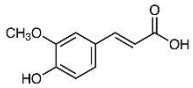
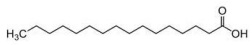
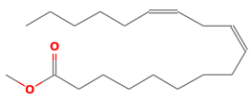
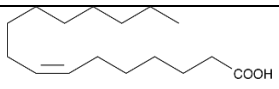
The chloroform fraction contained the highest number of compounds (49), followed by ethyl acetate (46) and butanol (25). Although the chloroform fraction had the highest number of compounds, the ethyl acetate fraction had the best glycaemic control and ameliorative properties. The corresponding chromatograms are shown in the appendix IX.

4.2.4.1. Phytochemicals in the ethyl acetate fraction

The ethyl acetate fraction showed 11 prominent peaks, as shown in Table 4.2.4. The peaks correspond to phenolic compounds like 2-furancarboxylic acid, which appeared at 6.761 minutes, 4H-Pyran-4-one 3,5-dihydroxy-2-methyl- at 8.754 minutes, 5-hydroxymethylfurfural at 9.25 minutes. There were also some alkaloids like pyridine, 2,4-dimethyl- a seen at 5.487 minutes- and fatty acids such as hexadecenoic acid and its derivative. The combination of these compounds is reflected in the high total phenolic and total flavonoid content of this fraction and in the good radical scavenging properties observed. This translated into good glycaemic outcomes for rats treated with the ethyl acetate fraction in comparison with the other fractions. The chromatogram is shown in Appendix 4-1.

Table 4.2.4. ***The phytochemicals for the Ethyl acetate fraction.***

Index	RT	Chemical structure	Name	MW	Formula
1	5.487		Pyridine, 2,4-dimethyl-	107	C_7H_9N

2	6.761		2-Furancarboxylic acid	112	$C_5H_4O_3$
3	8.754		4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	142	$C_6H_6O_4$
4	9.254		5-Hydroxymethylfurfural	126	$C_6H_6O_3$
5	11.362		1H-3a,7-Methanoazulene, octahydro-1,4,9,9 tetramethyl	206	$C_{15}H_{26}$
6	12.665		4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)	142	$C_6H_6O_4$
7	16.004		Benzoic acid, 3,4-dihydroxy-	154	$C_7H_6O_4$
8	17.169		trans-Ferulic acid	194	$C_{10}H_{10}O_4$
9	17.984		n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
10	18.381		2-Methyl-Z,Z-3,13-octadecadienol	280	$C_{19}H_{36}O$
11	19.679		cis-7-Hexadecenoic acid	254	$C_{16}H_{30}O_2$

RT- retention time; MW- molecular weight

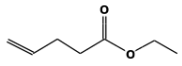
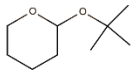
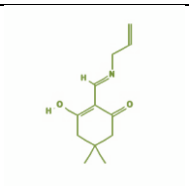
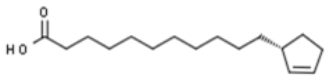
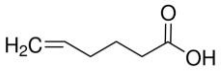
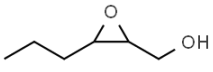
Among the original 13 fractions from the ethyl acetate fraction, G, J, H, and F exhibited very good α- glucosidase inhibitory activity. The GC-MS analysis of these fractions

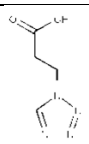
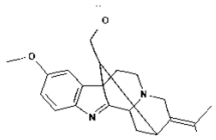
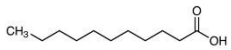
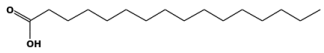
revealed multiple peaks, indicating the presence of fatty acids and phenolic and nitrogen-containing compounds. The main peaks in these subfractions were as follows:

4.2.4.2. Phytochemicals in Subfraction F of ethyl acetate fraction

Subfraction F exhibited the largest number of peaks. However, 11 peaks of interest, were observed as are indicated in Table 4.2.5. The highest peak corresponded to n-hexadecenoic acid, followed by undecanoic acid at 40.8 and 40.4 minutes, respectively. The corresponding chromatogram is under Appendix 4-4.

Table 4.2.5. ***The phytochemicals for the subfraction F***

Index	RT	Structure	Chemical name	MW	Formula
1	9.4		4-Pentenoic acid ethyl ester	128	$C_7H_{12}O_2$
2	10.27		2H-Pyran,2"(1,1-dimethyl ethoxy tetrahydro-)	158	$C_9H_{18}O_2$
3	16.92		Cyclohexane-1,3-dione,2"(2-hydroxyethyl amino methylene}-5,5-dimethyl-	211	$C_{11}H_{17}NO_3$
4	20.43		11"(2-Cyclopenten-1-yl) undecanoic acid, (+)-	146	$C_{16}H_{28}O_2$
5	24.66		5-Hexenoic acid	114	$C_7H_{14}O$
6	25.10		Oxirane, pentyl-	114	$C_6H_{10}O_2$
7	27.20		2,3-Epoxyhexanol	114	$C_7H_{14}O$

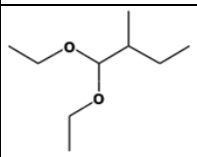
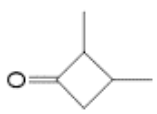
8	32.46		Propionic acid, 3-tetrazol-1-y1-	142	$C_4H_6N_4O_2$
9	38.43		Akuammilan-17-ol, 10-methoxy-	320	$C_{20}H_{24}N_2O_2$
10	40.49		Undecanoic acid	254	$C_{16}H_{30}O_2$
11	40.87		n-Hexadecenoic acid	186	$C_{11}H_{22}O_2$

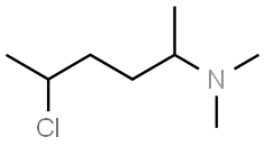
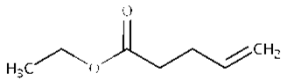
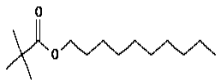
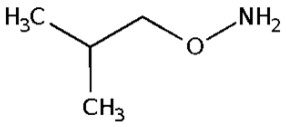
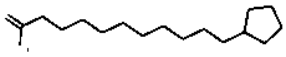
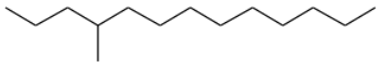
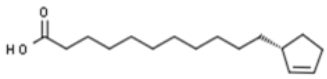
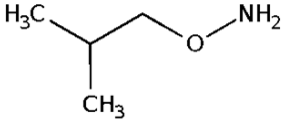
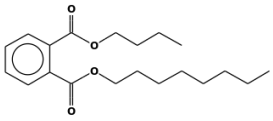
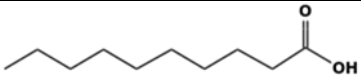
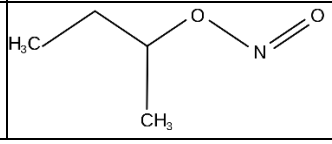
RT- retention time; MW- molecular weight

4.2.4.3. Phytochemicals in Subfraction G of ethyl acetate fraction

With subfraction G, we recorded 13 peaks, with the highest peak corresponding to n-decanoic acid at 39.95 minutes. At 30.06 minutes, the peak corresponds to 11"(2-cyclopenten-1-yl) undecanoic acid, (+)-. Other peaks of interest at 24.08 and 27.4 minutes corresponded to cyclopentane undecanoic acid and tridecane, 4-methyl- respectively. The highest peak corresponded to 1,2-benzene dicarboxylic acid and butyl octyl ester. Refer to table 4.2.6. The Chromatogram is shown in Appendix 4-5.

Table 4.2.6. ***The phytochemicals for subfraction G***

Index	***RT***	***Structure***	***Name***	***MW***	***Formula***
1	5.18		Butane. 1.1-diethoxy-	146	$C_{16}H_{30}O_2$
2	8.73		Cyclobutanone, 2,3-dimethyl-, cis-	98	$C_{16}H_{30}O_2$

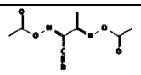
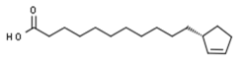
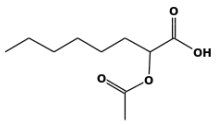
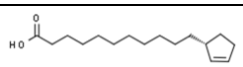
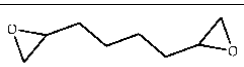
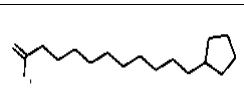
3	9.01		N-(4-Chloro-3,3-dimethyl-2-butylidene) met...	147	$C_7H_{14}ClN$
4	9.18		4-Pentenoic acid ethyl ester	128	$C_7H_{12}O_2$
5	10.09		2,2-Dimethylpropionic acid, decyl ester	242	$C_{15}H_{30}O_2$
6	21.29		Hydroxylamine, O-(2-methyl propyl)-	89	$C_4H_{11}NO$
7	24.03		Cyclopentane undecanoic acid	254	$C_{16}H_{30}O_2$
8	27.45		Tridecane, 4-methyl-	198	$C_{14}H_{30}$
9	30.04		11-(2-Cydopenten-1-yl) undecanoic acid, (...	252	$C_{16}H_{28}O_2$
10	35.65		Hydroxylamine, O-{2-methylpropyl}-	89	$C_4H_{11}NO$
11	39.74		1,2-Benzanedicarboxylic acid, butyl octyl ester		$C_{20}H_{30}O_4$
12	39.96		n-Decanoic acid	172	$C_{10}H_{20}O_2$
13	40.67		sec-Butyl nitrite	103	$C_4H_9O_2$

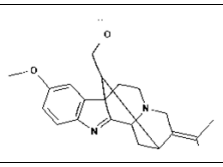
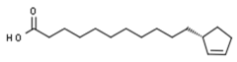
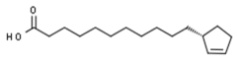
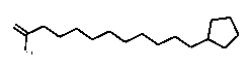
RT- retention time; MW- molecular weight

4.2.4.4. Phytochemicals in Subfraction H of ethyl acetate fraction

Subfraction H had 30 peaks, most of which corresponded to 11"(2-cyclopenten-1-yl) undecanoic acid (+)-. However, the highest peaks were observed around 38.11 minutes to 41.2 minutes. The phytocompounds observed here included akuammilan-17-ol- 10-methoxy 11-(2-cyciopenten-1-yl) undecanoic acid. (+)-, cyclopentane undecanoic acid methyl ester and cyclopentane undecanoic acid. Significant peaks were also observed at 26.3 and 26.5 minutes which corresponded to oxirane 2.2" -(1.4-butanediyl) bis- and cyclopentane undecanoic acid. At 23.05 minutes, (+)-2- hydroxy octanoic acid acetate was also observed. Another peak of interest was observed at 11.49 minutes and corresponded to N-nitroso-2-methyl-oxazolidine. Please refer to Table 4.2.7 and the corresponding chromatogram in Appendix 4-6.

Table 4.2.7. ***Phytochemicals for subfraction H***

In	*RT*	*Structure*	*Name*	*MW*	*Formula*
1	11.08		Butane nitrile, 2,3- dioxo-, dioxime, O, O' – diacetyl-	211	$C_8H_9N_3O_4$
In	*RT*	*Structure*	*Name*	*MW*	*Formula*
2	20.32		11-(2-cyciopenten-1-yl) undecanoic acid. (+)-	252	$C_{16}H_{28}O_2$
3	23.05		(+)-2- Hydroxy octanoic acid acetate	202	$C_{10}H_{18}O_4$
4	24.10		11-(2-cyciopenten-1-yl) undecanoic acid. (+)-	252	$C_{16}H_{28}O_2$
5	26.58		Oxirane 2.2" -(1.4-butanediyl) bis-	142	$C_8H_{14}O_2$
6	38.11		Cyclopentane undecanoic acid	254	$C_{16}H_{30}O_2$

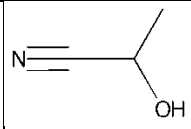
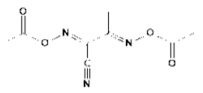
7	39.28		Akuammilan-17-ol- 10-methoxy-	324	$C_{20}H_{24}N_2O_2$
8	39.67		11-(2-cyciopenten-1-yl) undecanoic acid. (+)-	252	$C_{16}H_{28}O_2$
9	39.97		11-(2-cyciopenten-1-yl) undecanoic acid. (+)-	252	$C_{16}H_{28}O_2$
10	41.29		Cyclopentaneundecanoic acid methyl ester	268	$C_{17}H_{32}O_2$

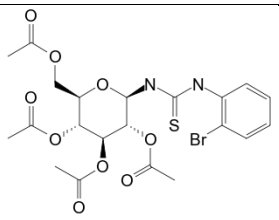
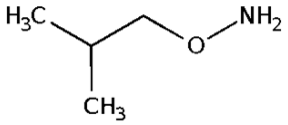
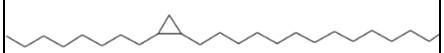
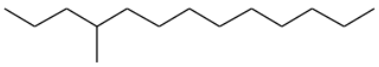
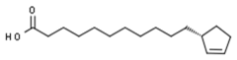
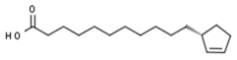
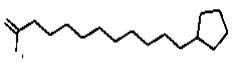
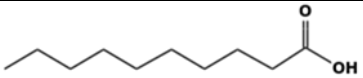
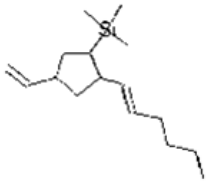
RT- retention time; MW- molecular weight

4.2.4.5. Phytochemicals in Subfraction J of ethyl acetate fraction

Subfraction J had 15 peaks. The peaks of interest were observed between 39.9 minutes and 41.3 minutes, corresponding to the following phytoconstituents: 1-ethenyl-3 (1-hexenyl)-4-trimethylsilyl cyclopentane, epoxy hexanol, and n-decanoic acid. In this subfraction, the continued presence of 11-(2-cyclopenten-1-yl) undecanoic acid was also noted at different times from 36 to 37 minutes. Significant peaks were detected at 11.22 minutes for butane nitrile, 2,3-dioxo-, dioxime, O, O' diacetyl-. At 8.9 minutes, propane nitrile, 2-hydroxy-, was observed. The data are shown in Table 4.2.8 and corresponds to the chromatogram in Appendix 4-7.

Table 4.2.8. ***Phytochemicals for subfraction J***

Index	RT	Chemical structure	Name	MW	Formula
1	8.997		Propane nitrile, 2-hydroxy-	71	C_3H_5NO
2	11.223		Butane nitrile, 2,3-dioxo-, dioxime, O,O" di...	211	$C_8H_9N_3O_4$

3	22 .998		1-(3-(4-Bromophenyl)-2-thioureido) ·1-deoxy-b-d-glucopyranose, 2,3,4,6 tetraacetate	560	$C_{21}H_{25}BrN_2O_9S$
5	27.454		Hydroxylamine, 0-(2-methyl propyl)	89	$C_4H_{11}NO$
6	35.430		Cyclopropane tetra decanoic acid, 2- octyl-	394	$C_{26}H_{50}O_2$
7	35.653		Tridecane, 4-methyl-	198	$C_{14}H_{30}$
9	37.009		11-(2-Cyclopenten-1-yl) undecanoic acid,	252	$C_{16}H_{28}O_2$
10	37.671		11-(2-Cyclopenten-1-yl) undecanoic acid,	252	$C_{16}H_{28}O_2$
11	37.735		Cyclopentane undecanoic acid	254	$C_{16}H_{30}O_2$
12	39.900		n-Decanoic acid	172	$C_{10}H_{20}O_2$
13	40.541		1-Ethenyl-3-(1-hexenyl}-4-trimethylsilylcy.. .	250	$C_{16}H_{30}Si$
14	40 .682		2,3-Epoxyhexanol	116	$C_{16}H_{12}O_2$

RT- retention time; MW- molecular weight

4.3. *In vivo* effects of plant extracts and fractions on biomarkers of diabetes in an animal model

4.3.1. Effect of crude extracts and fractions on weight of rats

A reduction in weight was observed during the 28 days of treatment across all groups.

There was a statistically significant difference in the weight observed among the treatment groups ($p<0.001$); however, the weight loss was not statistically different at the end of treatment from the first day of treatment. Rats treated with the ethyl acetate and chloroform fractions showed the largest decline in weight. Animals treated with normal saline died by the 21st day of treatment; thus, weight could not be measured at the end of treatment for comparison. Refer to tables 4.3.1 and 4.3.2 and figure 4.3.

Table 4.3.1. ***Percentage weight difference between the 1st day and the last day of treatment***

Treatment group	**Days of treatment ***		**% Weight difference**
	Day 1of treatment	**Day 28of treatment**	
Glibenclamide (3mg/kg)	199.9 ± 23.9 g	187.9 ± 38.3 g	- 6.01%
Aqueous crude (1g/kg)	201.4 ± 28.8 g	196.96 ± 27.7 g	-2.17%
EtOAc crude (1 g/kg)	234.4 ± 31.8 g	230.88 ± 28.8 g	-1.53%
EtOAc fraction (1g/kg)	207.32 ± 14.8 g	195.22 ± 23.3 g	-5.83%
Chloroform fraction (1g/kg)	212.16 ± 41.63 g	194.3 ± 35.28	-8.42%
Butanol fraction (1g/kg)	192.0 ± 27.7 g	189.2 ± 20.4 g	-1.45%

**p= 0.016, paired t-test used to compare weight between day 1 and day 28 of treatment.*

Table 4.3.2. ***Average weight (mean ± SD) (g) of rats during treatment***

	Neg-Control	**Aqueous crude (1g/kg)**	**EtOAc crude (1g/kg)**	**EtOAc fraction (1g/kg)**	**Chlr fraction (1g/kg)**	**But fraction (1g/kg)**	**Glibenclamide (3mg/kg)**
Day 1	198.1 ± 4.5 g	201.4 ± 28.8 g	234.4 ± 31.8 g	207.32 ± 14.8 g	212.16 ± 41.63 g	192.0 ± 27.7 g	199.9 ± 23.9
Day 7	194.3 ± 71.7 g	184.08 ± 20.8 g	208.17 ± 27.7 g	187.14 ± 9.2 g	207.1 ± 42.0	187.34 ± 26.8 g	205.7 ± 27.9
Day 14	191.6 ± 69.4 g	186.06 ± 26.4 g	217.72 ± 28.5 g	187.82 ± 10.1 g	204.48 ± 39.13	186.88 ± 21.3 g	184.1 ± 38.7

Day 21	182.1 ± 66.1 g	190.07 ± 25.9 g	223.22 ± 28.6*g	190.32 ± 14.1 g	200.46 ± 38.15**	185.68 ± 21.5 g	185.6 ± 33.3
Day 28	M	196.96 ± 27.7 g	230.88 ± 28.8 g	195.22 ± 23.3 g	194.3 ± 35.28	189.2 ± 20.4 g	187.9 ± 38.3

M- mortality; Chlr- Chloroform; EtOAc- Ethyl acetate; But- Butanol; Neg- Negative; Pos- Positive

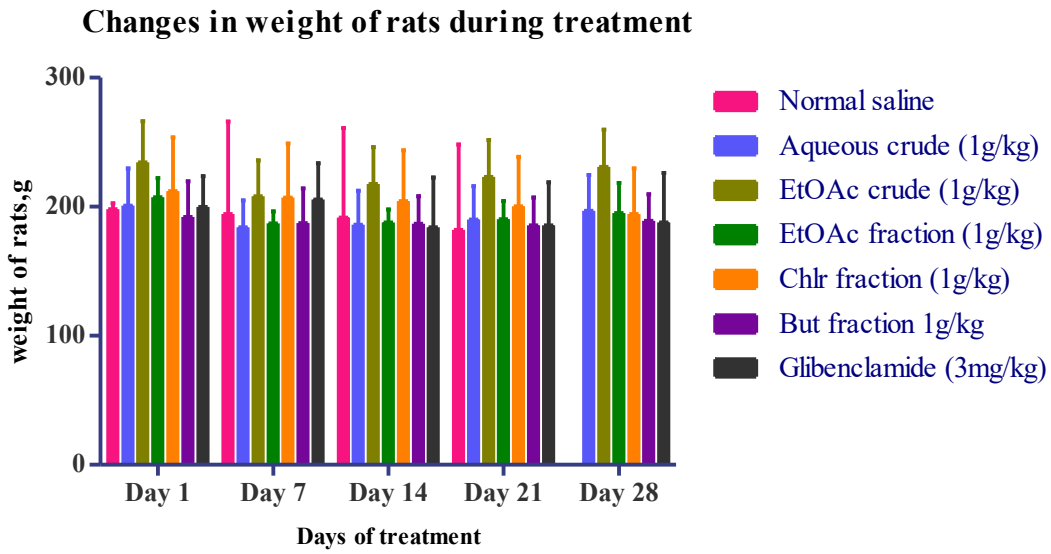


Figure 4.3: Changes in weight of rats during treatment

4.3.2 Effect of crude extracts and fractions on Blood glucose reading

After inducing diabetes using alloxan monohydrate, there was a significant change in blood glucose levels among the treated groups (p= 0.002). Blood glucose before induction ranged between 4.1-5.8 mmol/l. Post-induction blood glucose ranged from 11.3mmol/l to 31.4mmol/l (p=0.0006). After treatment, there was no statistically significant difference between the aqueous and ethyl acetate crude extracts (p=0.285). The aqueous crude extract and ethyl acetate extract had a better glucose-lowering effect than glibenclamide. There was a statistically significant difference in the glucose levels between the aqueous crude extract and the positive control (glibenclamide) (p=0.0014). The same was also observed

between the ethyl acetate crude extract and positive control (p=0.004). The ethyl acetate fraction showed the greatest reduction in blood glucose compared to the chloroform and butanol fractions (p= 0.002). Although the ethyl acetate fraction showed lower glucose levels on day 29, there was no significant difference compared with the positive control (p= 0.152). However, a statistically significant difference was observed between the ethyl acetate fraction and the aqueous crude extract (p= 0.024). No significant glucose-lowering activity was observed with the butanol and chloroform fraction, as there was no reduction in blood glucose readings throughout the days or treatment. By day 28 of treatment, all rats treated with normal saline (negative control) died of persistent hyperglycaemia, as shown in Table 4.3.3.

Table 4.3.3. ***Blood glucose changes across treatment groups from Pre-induction period to the 28th day of treatment***

Group ID/ day of treatment	Aqua extr (1g/kg)*	EtOAc extr (1g/kg)	-ve control*	+ve control (3mg/kg)*	Chlor fr (1g/kg)	EtOAc fr (1g/kg)	But fr (1g/kg)
Pre induction (mmol/l)	5.5 ±0.7	4.1 ±0.50	5.3 ±1.1	5.8 ±0.6	5.0 ±1.1	5.1 ±1.2	4.8 ±0.6
Post induction (mmol/l)	13.4 ±5.2	11.3 ±4.5	23.0 ±9.1	21.3 ±9.4	31.4 ±1.9	28.4 ±5.9	26.1 ±7.2
Treatment day 3 (mmol/l)	10.1 ±6.5	10.1 ±6.1	20.8 ±16.3	16.5 ±8.4	31.7 ±1.0	31.4 ±3.6	24.7 ±6.5
Treatment day 5 (mmol/l)	7.7 ±3.1	8.2 ±5.2	13.3 ±15.4	10.2 ±9.3	30.1 ±0.7	12.6 ±10.3	22.1 ±12.8
Treatment day 7 (mmol/l)	4.9 ±2.0	8.5 ±5.2	28.2 ±6.8	18.9 ±0.9	26.9 ±3.0	11.3 ±10.3	20.7 ±11.9
Treatment day 10 (mmol/l)	5.0 ±0.9	8.8 ±6.6	27.9 ±0.2	8.7 ±7.5	31.0 ±3.9	12.8 ±12.1	20.7 ±11.7
Treatment day 14 (mmol/l)	6.7 ±2.8	10.0 ±8.7	25.3 ±1.9	12.0 ±1.3	22.1 ±2.7	10.3 ±9.5	22.0 ±12.7
Treatment day 17 (mmol/l)	8.2 ±5.6	6.6 ±3.9	30.5 ±3.5	13.6 ±0.9	23.8 ±4.0	6.7 ±6.7	19.3 ±11.3
Treatment day 20 (mmol/l)	8.7 ±6.3	9.1 ±7.8	26.2 ±2.5	11.2 ±0.5	24.4 ±5.1	9.0 ±9.9	22.6 ±14.4
Treatment day 22 (mmol/l)	6.0 ±2.9	6.2 ±3.5	33.0 ±0.0	20.8 ±17.3	26.9 ±4.9	8.6 ±8.7	20.4 ±12.5
Treatment day 24 (mmol/l)	5.1 ±0.9	5.9 ±2.6	29.1 ±5.5	11.1 ±2.2	28.1 ±2.9	6.6 ±7.1	24.8 ±13.9
Treatment day 28 (mmol/l)	5.0 ±0.8	6.2 ±3.0	0.0[M]	9.9 ±1.3	29.5 ±4.6	8.2 ±9.8	25.3 ±14.3

*M-mortality for all members of the group by the 28th day of treatment. P= 0.002 One-way ANOVA conducted across all treatment groups; P=0.0006, t-test used to compare blood glucose between Post induction day and Day 28 of treatment. * P=0.0014; +ve control vs Aqueous & -ve control vs Aqueous extract*

4.3.3. Effect of crude extracts and fractions on Lipid profile

There was a statistically significant difference in the triglyceride levels after treatment (p=0.03). The animals receiving the positive control glibenclamide had the highest triglyceride levels (198.84 ± 43.21 mg/dl) while the rats treated with the aqueous crude extract had the lowest triglyceride levels (122.23 ± 44.68 mg/dl). Thus, the aqueous crude extract had the best triglyceride-lowering effect.

There was also a statistically significant difference in the total cholesterol levels (p= 0.001). Animals on butanol fraction had the highest total cholesterol levels at 3.01 ± 0.35 mmol/l while animals treated with the ethyl acetate fraction had the least total cholesterol levels 1.84 ±0.11 mmol/l, suggesting that the ethyl acetate extract had the best cholesterol lowering effect.

There was no statistically significant difference in the HDL cholesterol levels among the animal groups (p=0.08), although it was observed that animals treated on the butanol fraction had the lowest readings at 0.73 ± 0.49 mmol/l while animals treated with the chloroform fraction had the highest reading at 0.95 ± 0.02 mmol/l. There was a statistically significant difference among the non-HDL cholesterol levels among the animals (p= 0.0007). Animals treated on the butanol fraction had the highest non-HDL cholesterol levels at 2.24 ± 0.37mmol/l, while ethyl acetate crude extract had the lowest readings at 0.858 ± 0.17mmol/l. This was reflected in the glycemic controls observed in this group of rats. The poor glycemic control in rats treated with butanol may also have been associated with poor effect on HDL and LDL levels. See table 4.3.4.

Table 4.3.4. ***Lipid profile of rats after 28 days of treatment***

	Chloroform frac (1g/kg)	**Butanol frac (1g/kg)**	**EtOAc frac (1g/kg)**	**Aqueous crude (1g/kg)**	**Glibenclamide (3mg/kg)**	**EtOAc cr (1g/kg)**
Triglycerides mg/dl *	191.1 ±40.6	129.98 ± 87.71	143.27 ±49.5	122.23 ± 44.68	198.84 ± 43.21	141.4 ±4.3
Total cholesterol mmol/l **	2.75 ± 0.38	3.01 ± 0.35	1.84 ±0.11	1.89 ± 0.28	2.31 ± 0.20	1.62 ± 0.25
HDL-chol mmol/l	0.95 ± 0.02	0.73 ± 0.49	0.85 ± 0.05	0.83 ± 0.02	0.93 ±0.11	0.81 ± 0.03
Non-HDL chol mmol/l***	1.7 ± 0.38	2.24 ± 0.37	1.04 ± 0.07	1.08 ± 0.28	1.38 ± 0.31	0.858 ± 0.17
LDL-chol mmol/l	1.49 ± 0.26	1.85 ± 0.37	0.72 ± 0.06	1.07 ± 0.38	1.12 ± 0.51	0.84 ± 0.33
VLDL chol mmol/l	0.77 ± 0.24	0.84 ± 0.26	0.78 ± 0.09	0.57 ± 0.17	0.59 ± 0.45	0.60 ± 0.22
LDL/HDL	1.56 ± 0.24	2.42 ± 2.41	0.85 ± 0.07	1.28 ± 0.44	1.25 ± 0.7	0.97 ± 0.41
Cholesterol/HDL	2.91 ± 0.34	5.79 ± 3.5	2.16 ± 0.23	2.28 ± 0.35	2.54 ± 0.49	1.99 ± 0.17

(P<0.05),(P<0.001), ***(P<0.001); Triglyceride levels, Total Cholesterol levels and Non_ HDL cholesterol levels compared among groups of treatment using one way ANOVA. EtOAc- Ethyl acetate; frac-fraction; cr-crude extract; chol-Cholesterol*

4.3.4. Effect of crude extracts and fractions on Kidney and Liver function

Both the aqueous and ethyl acetate crude extracts as well as the butanol fraction appeared to have renoprotective effects in diabetes-induced rats. However, the chloroform fraction may have been toxic to both the kidney and liver since it had very high readings for both ALT and creatinine. The liver function test showed no statistically significant difference among the different animal groups. This could be associated with mild liver disease. The rats treated with the chloroform fraction had the highest alanine aminotransferase (ALT) activity at157.82 ± 64.1U/L while those treated with ethyl acetate crude extract had the lowest at 123.46 ± 22.1 U/L.

There was a statistically significant difference in the serum creatinine levels among the different groups of rats (p= 0.0019). The animals treated with the Ethyl acetate fraction had the highest serum creatinine at 92 ± 13.7umol/l, while those treated with the Ethyl acetate crude extract had the lowest serum creatinine at 47.82 ± 8.6 µmol/l. Table 4.3.5., presents the findings on ALT and serum creatinine.

Table 4.3.5. ***Average ALT levels and serum creatinine levels after treatment in different treatment groups of rats***

	Chlor fr (1g/kg)	**But fr (1g/kg)**	**EtOAc fr (1g/kg)**	**Aqueous crude (1g/kg)**	**Glib (3mg/kg)**	**EtOAc crude (1g/kg)**
ALT U/L	157.82 ± 64.1	128.48 ± 23.33	144.76 ± 54.5	127 ± 50.1	132.84 ±12.5	123.46 ± 22.1
Creatinine µmol/l **	85.7 ± 12.61	47.88 ± 26.32	92 ± 13.7	49.72 ± 8.89	49.15 ±8.74	47.82 ± 8.6
*** (p<0.001) determined using one-way ANOVA*						

4.3.5. Histopathological report of animals after treatment with crude extracts and fractions

The photomicrographs show different organs of the treated rats, including the frontal cortex (figure 4.8), hippocampus of the brain (figure 4.9), heart (figure 4.7), pancreas (figure 4.4), kidney (figure 4.5), and liver (figure 4.6) after 28 days of treatment. The organs were stained with hematoxylin and eosin (H&E) at 400 × magnification.

4.3.5.1. Histopathological changes in the pancreas following treatment with Kigelia africana extracts and fractions

The pancreatic cell mass of animals treated with extracts and fractions showed normal islets of Langerhans and minimal necrotic tissue due to apoptosis compared to the negative control, which had small islets with extensive necrotic tissue at the center and fibrous tissue. Animals treated with the aqueous extract and its ethyl acetate fraction displayed the lowest necrotic masses on the islets of Langerhans in diabetes-induced rats compared with the negative control. This could be a result of the possible cell proliferative effects of the extracts associated with the observed free radical scavenging activity of the extracts. However, the butanol fraction and the ethyl acetate crude extract had the most necrotic cells among the fractions. This is in tandem with the poor antioxidant activities of these two samples as observed in Section 4.4.4. Figure 4.4., shows these results.

Pancreas

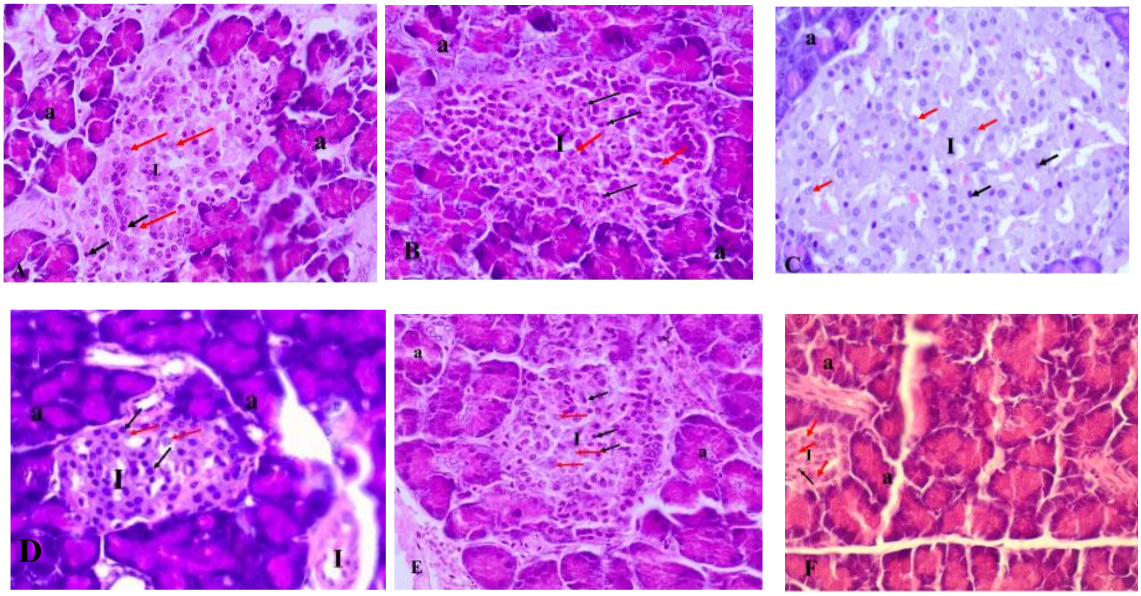


Figure 4.4: Histology of the pancreas after 28 days of treatment. H&E stain X400 magnification

I- Islet of Langerhans; a- acinar cells. Red arrows- cells with necrosis. Black arrows- healthy cells

A – Butanol fraction; B- Ethyl acetate fraction; C- Chloroform fraction; D- Glibenclamide (positive control); E- Aqueous crude extract; F- Normal saline (negative control)

4.3.5.2. Histopathological changes in the kidney and liver following treatment with Kigelia africana extracts and fractions

The cytoprotective effects of the extracts and fractions on the liver were evident (figure 4.6), with the tissues obtained from animals treated with the aqueous extract which exhibiting the lowest number of necrotic cells and the highest proportion of normal hepatocytes among all tested extracts. The presence of normal cells within liver tissue may indicate ongoing regenerative processes.

A typical cellular distribution was observed within the glomeruli of the kidneys (figure 4.5) of animals treated with the extracts and fractions. Moreover, subjects administered ethyl

acetate fraction, chloroform fraction, and glibenclamide displayed a normal urine space, in contrast to rats receiving normal saline treatment, which exhibited a markedly enlarged urine space. The good performance of chloroform fraction can be associated with the fraction's potent free radical scavenging activity observed in this study.

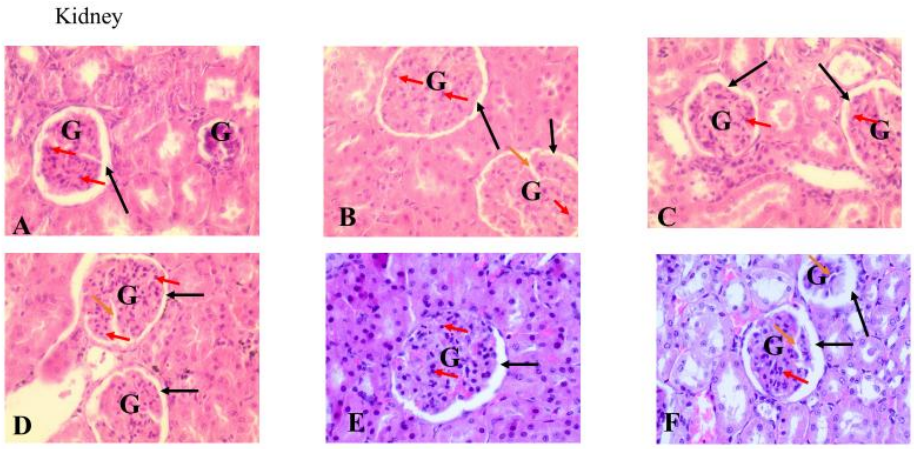


Figure 4.5: Histopathology of Kidneys after treatment. H&E stain X 400 magnification

G- Glomerulus; black arrow- urine space; orange arrow- capillary infiltration; red arrows- mesangial cells. A – Butanol fraction; B- Ethyl acetate fraction; C- Chloroform fraction; D- Glibenclamide (positive control); E- Aqueous crude extract; F- Normal saline (negative control)

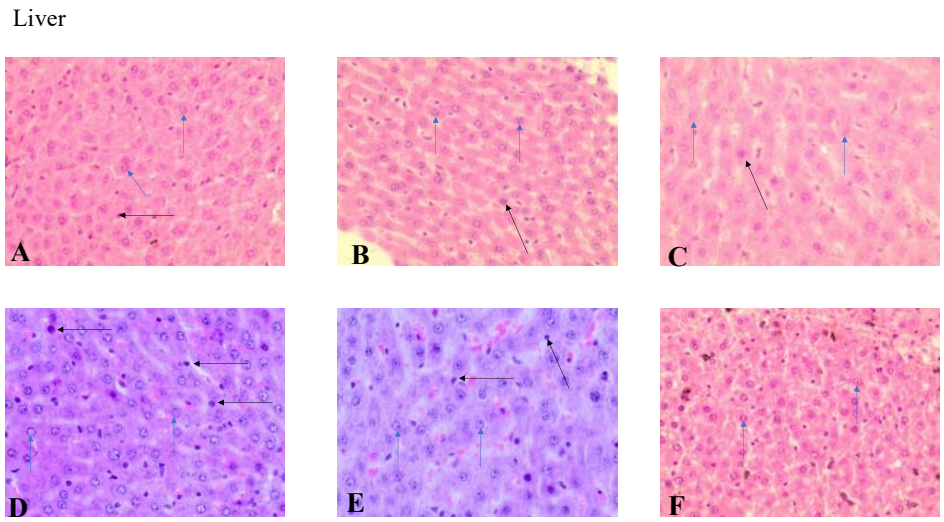


Figure 4.6: Histopathology of Liver cells after treatment H & E stain X 400

magnification

Blue arrows indicate degenerated hepatocytes, whereas black arrows indicate normal hepatocytes. A –Butanol fraction; B- Ethyl acetate fraction; C- Chloroform fraction; D- Glibenclamide (positive control); E- Aqueous crude extract; F- Normal saline (negative control)

4.3.5.3. Histopathological changes in the heart following treatment with Kigelia extracts and fractions

The histoarchitecture of the heart (figure 4.7) showed a normal distribution of cardiomyocytes. However, rats exposed to glibenclamide demonstrated a higher incidence of degenerated cells than those exposed to extracts and fractions. Consequently, the extracts exhibited superior cardioprotective properties compared with the positive control. Furthermore, rats treated with normal saline displayed disruption of cardiac architecture and a significant number of degenerating cardiomyocytes interspersed with normal cells.

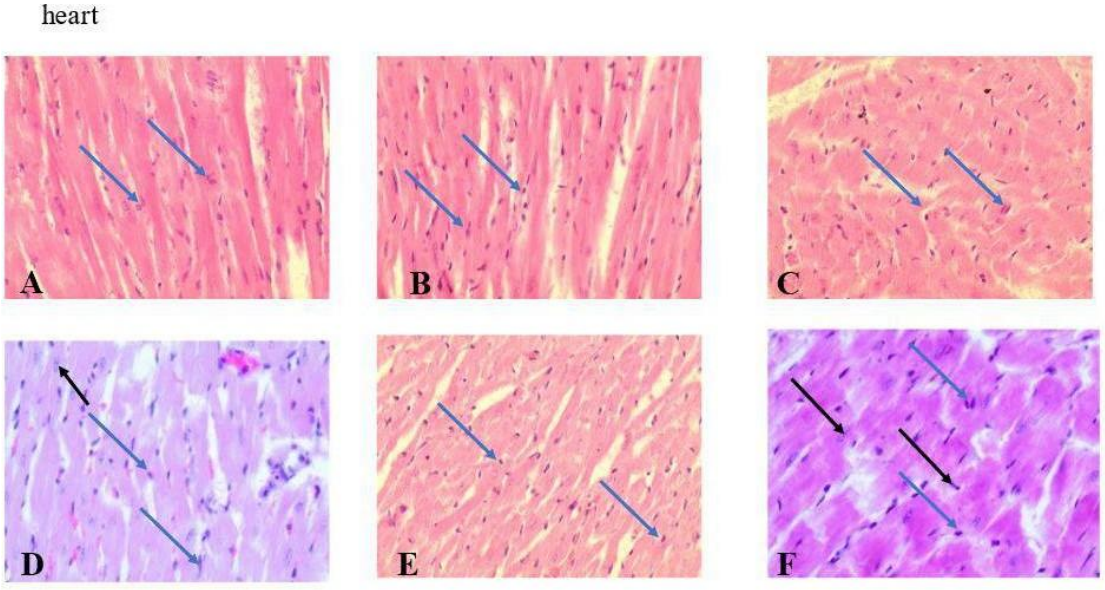


Figure 4.7: Histology of the heart following treatment. H&E stain X 400 magnification

A–Butanol fraction; B- Ethyl acetate fraction; C- Chloroform fraction; D- Glibenclamide

*(positive control); E- Aqueous crude extract; F- Normal saline (negative control); *Black arrows show necrotic cells; * Blue arrows show healthy cells.*

4.3.5.4. Histopathological changes in the brain following treatment with Kigelia extracts and fractions

In the brain, it was observed that the animals treated with the fractions exhibited the lowest incidence of necrotic tissue which appeared as pyknotic nuclei (shrunken and dark) in the frontal cortex; this is particularly seen with the chloroform fraction in the cortex (figure 4.8) as well as the hippocampus (figure 4.9). This finding suggested a potential cytoprotective effect on the cells in these organs related to the free radical scavenging properties of the extract. In the hippocampus of the brain, regenerating cells were present in animals treated with *Kigelia* extracts and fractions, with the aqueous crude extract yielding the highest number of regenerated cells.

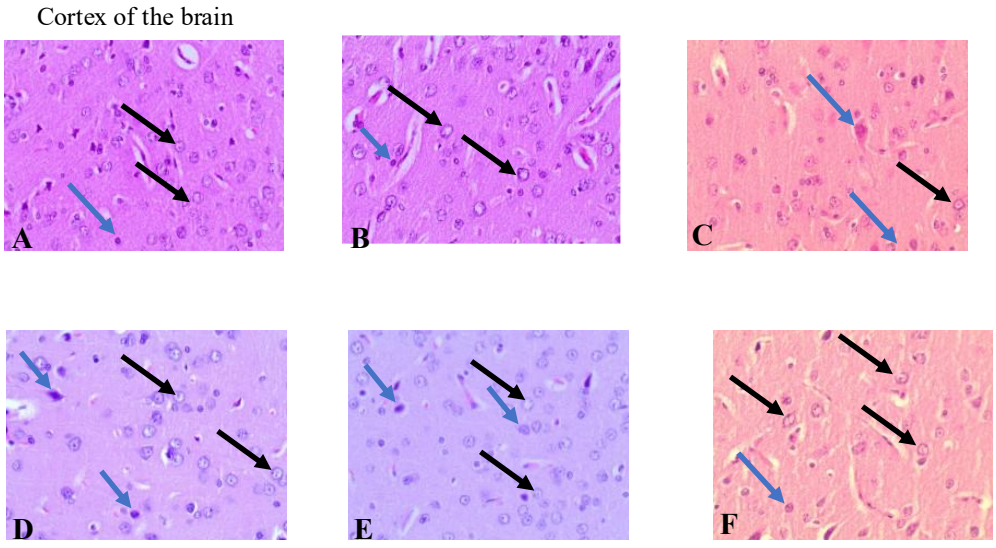


Figure 4.8: Histology of the cortex of the brain after 28 days of treatment. H&E stain X400 magnification

A: Butanol fraction; B: Ethyl acetate fraction; C: Chloroform fraction; D: Glibenclamide

*(positive control); E: Aqueous crude extract; F: Normal saline (negative control); *Black arrows show necrotic cells; * Blue arrows show healthy cells.*

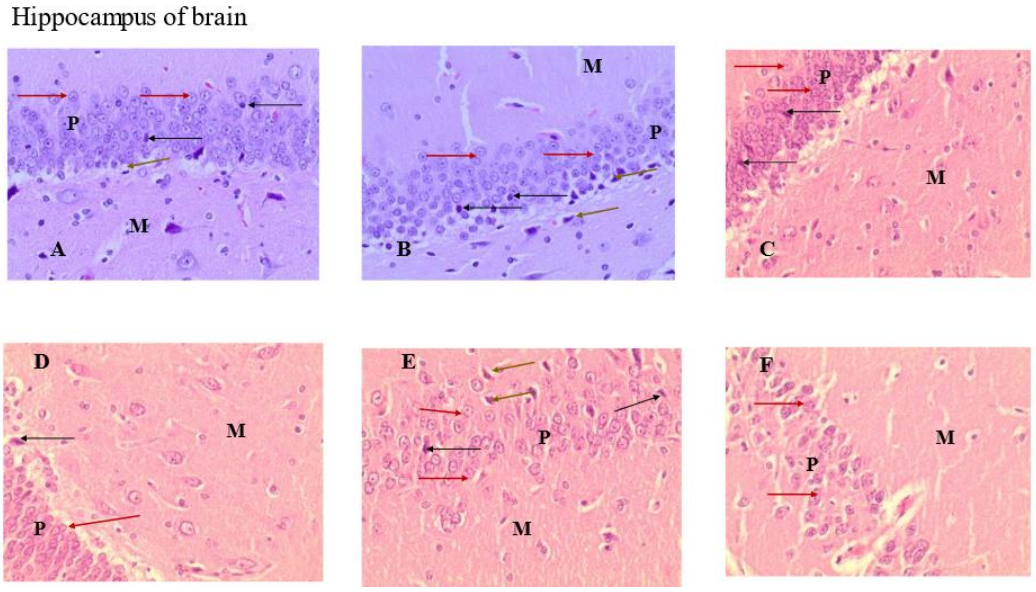


Figure 4.9: Histology of the hippocampus of the brain following treatment. H &E stain X 400 magnification

P- pyramidal layer characterised by densely packed pyramidal neurons; M- Molecular layer characterised by fewer pyramidal cells

*A –Butanol fraction; B- Ethyl acetate fraction; C- Chloroform fraction; D- Glibenclamide (positive control); E- Aqueous crude extract; F- Normal saline (negative control); *Red arrows show necrotic cells; * Black arrows show normal cells; * Brown arrows show regenerating cells.*

4.4. I*n vitro* effects of the ethyl acetate fraction on enzymes involved in carbohydrate absorption in the gastrointestinal tract.

The ethyl acetate fraction, which had the best blood glucose lowering activity, was subjected to tests for post-prandial antidiabetic activities, which included the alpha-amylase inhibitory activity, alpha-glucosidase inhibitory activity, as well as the effects on glucose uptake and utilization assay in order to determine bioactivity. These studies helped

to elucidate the mechanism of action of the aqueous extract and its ethyl acetate fraction.

4.4.1. Inhibition of alpha-amylase activity

The aqueous crude extract did not display any α-amylase inhibitory activity. However, the ethyl acetate fraction of the aqueous extract displayed very low dose-dependent inhibitory activity against alpha-amylase. The IC_{50} of the ethyl acetate fraction *(240.6 ±0.0 µg/m)* was slightly lower than that of the aqueous crude extract (*258.4 ±0.5 µg/mL)*. Moreover, the activity of both the extract and its fraction was significantly lower than that of the positive control, acarbose ($p < 0.0001$). (See Table 4.4.1).

Table 4.4.1. ***Percentage (%) inhibition of Kigelia aqueous extract and ethyl acetate fraction vs Acarbose (positive control) on alpha-amylase***

Drug	**Acarbose**	**Aqueous Crude µg/mL**				**EtOAc (Fraction) µg/mL**			
Conc	500µM	**62.5**	**125**	**250**	**500**	**62.5**	**125**	**250**	**500**
% inhib*	**95.057 ± 1.52**	0.121 ±0.41	0.105 ±0.03	0.212 ±0.09	0.408 ± 0.09	2.10 ±1.08	3.23 ±0.91	5.93 ±1.17	8.464 ±0.46
P<0.0001, determined via ANOVA									

4.4.2. Inhibition of alpha-glucosidase activity

The aqueous extract demonstrated a dose-dependent increase in α-glucosidase inhibitory activity. At the highest concentration of 500 µg/mL, there was 64.10 ± 2.67% inhibition. The IC_{50} for the aqueous crude extract was 193.7 ± 0.08 µg/mL. In contrast, a statistically significant increase in α-glucosidase inhibitory activity was observed in the ethyl acetate fraction ($p<0.0001$). At 500 µg/mL, there was an 89.82 ± 0.76% inhibition. The IC50 for the ethyl acetate fraction was 10.4 ± 0.08 µg/mL, (See table 4.4.2). These results indicate

that the ethyl acetate fraction is a more potent alpha-glucosidase inhibitor than the crude aqueous extract. Therefore, further testing of the ethyl acetate subfractions was warranted.

Table 4.4.2. ***% Inhibition of Kigelia aqueous crude extract and Ethyl acetate fraction on alpha glucosidase activity***

Concentration	**EGCG (% inhibition)**	**Aqueous Crude (% inhibition)**	**EtOAc (fraction) (% inhibition)***
200 µM	91.82 ± 0.28		
62.5 µg/mL		26.73 ± 8.44	74.75 ± 5.09
125 µg/mL		47.56± 7.36	85.20 ± 2.35
250 µg/mL		53.80± 2.01	89.88 ± 0.88
500 µg/mL		64.10± 2.67	89.82 ± 0.76
*(p<0.0001, determined via two-way ANOVA).			

EGCG- Epigallocatechin

Thirteen subfractions collected from the ethyl acetate fraction were tested for alpha-glucosidase inhibitory activity. Subfractions F, G, H, N, P, and J exhibited notable activity. Subfraction G exhibited the highest inhibitory activity (85.10 ± 0.68 %). There was a statistically significant difference among the *%* inhibition among fractions ($p<0.0001$). A statistically significant difference was observed between the positive control EGCG [200ug/mL] and subfraction G[100ug/mL] ($p = 0.02$). Refer to Table 4.4.3.

Table 4.4.3. ***% Inhibition of alpha glucosidase enzyme by fractions of the Ethyl acetate fraction***

Drug	**Concentration**	**% inhibition***
EGCG	200uM	94.88 ± 0.12
A	10ug/ml	-5.42 ± 1.64
	100ug/ml	-1.12± 7.58

B	10ug/ml	1.04 ± 4.51
	100ug/ml	5.71 ± 5.71
D	10ug/ml	3.50 ± 6.31
	100ug/ml	3.28 ± 7.24
E	10ug/ml	5.93 ± 7.21
	100ug/ml	6.56 ± 5.90
F	10ug/ml	11.94 ± 7.08
	100ug/ml	57.97 ± 2.30
G	**10ug/ml**	**34.10 ± 6.24**
	100ug/ml	**85.10 ± 0.68**
H	10ug/ml	9.28 ± 1.37
	100ug/ml	63.62 ± 1.27
I	10ug/ml	6.34 ± 5.62
	100ug/ml	29.46 ± 5.03
J	10ug/ml	17.80 ± 5.02
	100ug/ml	71.99 ± 4.66
L	10ug/ml	10.45 ± 1.61
	100ug/ml	28.07 ± 0.64
O	10ug/ml	10.58 ± 4.59
	100ug/ml	25.67 ± 2.61
N	10ug/ml	9.38 ± 2.79
	100ug/ml	39.44 ± 8.21
P	10ug/ml	-10.64 ± 6.88
	100ug/ml	36.26 ±3.02
Significant difference between EGCG & subfraction G ($p = 0.02$)		
Significant difference among % inhibition of sub fractions ($p<0.0001$)		

4.4.3. Glucose utilization and uptake

The effects of the aqueous crude extract and ethyl acetate fraction did not increase the percentage of glucose uptake in cells exposed to the aqueous crude extract ($p=0.85$) and ethyl acetate fraction ($p=0.36$), despite an increase in the concentration of the extracts. This suggested that no glucose reuptake was observed in the colorectal cells used in this study. Similarly, there was no change in glucose utilization despite an increase in concentration,

and these results were comparable to those of untreated cells (p= 0.35). Refer to figure 4.10 and 4.11. There was no observed cytotoxicity using the Hoechst 33342 nuclear dye staining method, thus eliminating bias from the results observed in this study.

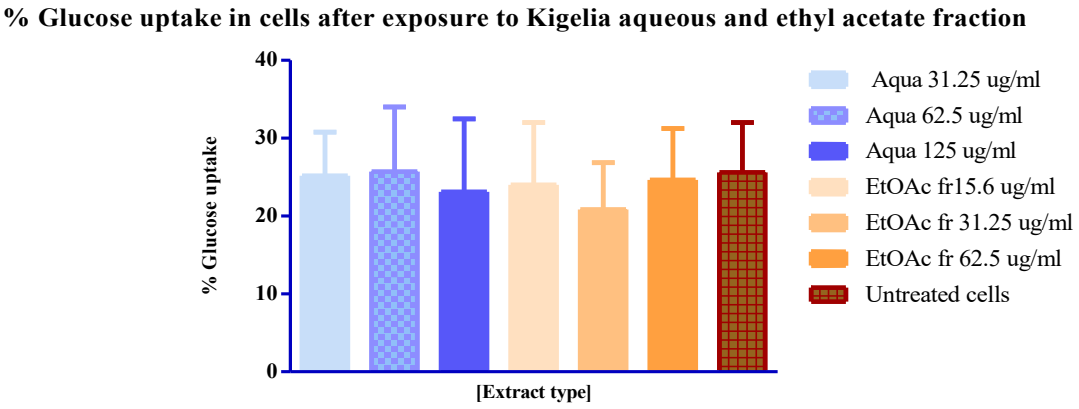


Figure 4.10: Glucose uptake in colorectal cells exposed to Kigelia aqueous extract and ethyl acetate fraction.

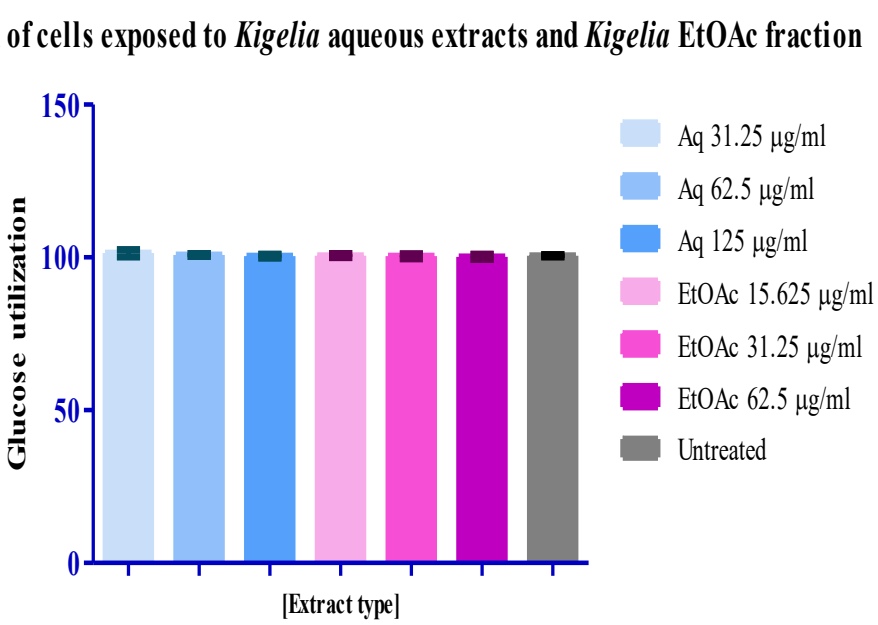


Figure 4.11: Glucose utilization in cells exposed to Kigelia aqueous crude extract and Ethyl acetate fraction. *Results were normalised to cell viability as determined using the Hoechst staining method.*

4.4.4. DPPH radical scavenging activity of *Kigelia* fractions (for *in vitro* antioxidant effect)

There was a dose-dependent DPPH radical scavenging activity increase with respect to the standard drug ascorbic acid, as indicated in Figure 4.12. The IC50 value of ascorbic acid was determined to be 7.244 ± 3.89 µg/ml. The chloroform fraction and crude ethyl acetate fraction demonstrated the most effective radical-scavenging activity.

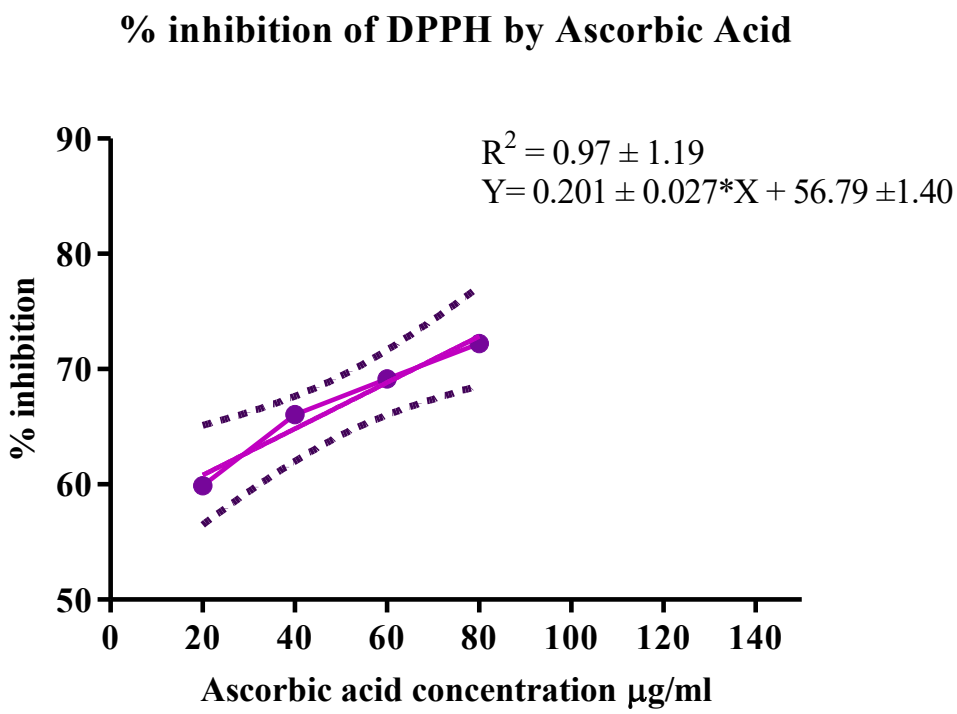


Figure 4.12: Percentage (%) inhibition of DPPH by standard drug Ascorbic acid

The DPPH IC50 values were the highest for the ethyl acetate crude extract and lowest for the chloroform fraction. Thus, the chloroform fraction was the most potent inhibitor of DPPH with an IC50 of 53 mg/ml, which meant it had the most free radical scavenging activity. However, the aqueous extract and its fractions were not as potent free radical scavengers as the standard drug ascorbic acid, which had an IC50 of 7.244 ± 3.89 µg/ml.

See Figure 4.12 above and Table 4.4.4, which shows the IC50 of *Kigelia* extracts and fruit fractions against DPPH.

Table 4.4.4. ***IC50 Kigelia extracts and fractions against DPPH***

Drug	Butanol fraction	Chloroform fraction	EtOAc fraction	Aqueous crude	EtOAc crude
IC50	401. 3 ± 1.75 mg/ml	53.3 ± 6.12 mg/ml	53.5 ± 2.13 mg/ml	183.0 ± 1.75 mg/ml	711.1 ± 2.10 mg/ml

4.5. Cytotoxicity, Mutagenicity and Genotoxicity study

4.5.1. Ames' test for mutagenicity

The cytotoxicity of *Kigelia africana* against TA98 cells was evaluated. The impact of *Kigelia africana* fruit extracts on the viability of *S. typhimurium* tester strains (TA 98), which were employed in mutagenicity assays, indicated no significant toxic effects. Table 4.5.1 presents the results.

Table 4.5.1. Salmonella typhimurium TA98 cell viability after exposure to both crude fruit extract samples

Sample	Number of CFU/plate (mean +/- SD)	Viability %
Ethyl acetate crude extract	25.60 ± 3.50	68.30
Aqueous crude extract	23.80 ± 3.06	69.45
Untreated cells	40.78 ± 3.1	99

Assessment of the mutagenicity of the extracts of *Kigelia africana* fruit powder revealed that there was no statistically significant difference in the number of His+ revertant colonies induced by both the aqueous and ethyl acetate extracts of *Kigelia africana* for *S. typhimurium strains TA97, TA98*, and *TA100* (p=0.7). However, the frequencies of His+ revertants, induced by the positive control mutagens, demonstrated a significant increase in the spontaneous mutation rate across all three strains of *S. typhimurium*. This resulted in

a statistically significant difference between the experimental and positive control groups (p= 0.004), indicating that the fruit extracts did not exhibit mutagenic properties. For further details, refer to Table 4.5.2.

Table 4.5.2. ***The mutagenic potential of Kigelia africana crude extracts on Salmonella typhimurium strains TA100, TA97 and TA98***

Sample	**Number of His + /plate**		
	TA 97	**TA 98**	**TA 100**
Negative control	22.00 ± 6.00	26.00±5.00	**145.82 ± 22.50**
Positive control (sodium azidee)	**680.10 ± 44.00**	**720.00 ± 32.00**	**1300.90 ±112.20**
Ethyl acetate crude extract (100 µg/mL)	21.20 ±0.30	25.40 ±0.50	150.70 ± 10.50
Aqueous crude extract (100 µg/mL)	20.08±0.60	24.70 ± 0.78	**145.80 ± 1.30**
P= 0.7 No significant difference between extracts			
P=0.004 significant difference between positive control and the extracts			

4.5.2. In vitro test for hepatotoxicity

The viability of cells exposed to *Kigelia africana* aqueous extract ranged between 102.1 and 103.9% compared to the viability of untreated cells which was set at 100%. These differences were not statistically significant ($p > 0.05$). Nevertheless, when cells were exposed to the ethyl acetate extract, there was a noticeable decrease in cell viability, which was directly proportional to the increase in the dosage. A statistically significant difference in cell viability was observed between the aqueous and ethyl acetate crude extracts at 250 µg/mL ($p = 0.019$), and between the aqueous extract and melphalan ($p < 0.0001$). See Figure 4.13 and 4.14. However, even at the highest dose of 250 µg/mL, cell viability was

still 59%. The ethyl acetate fraction had an IC of 414.8 ± 8.69 µg/mL. Therefore, only a relatively high concentration of ethyl acetate would cause hepatotoxicity.

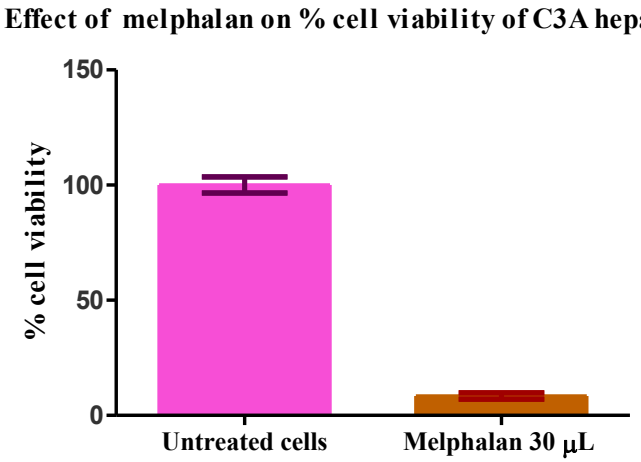


Figure 4.13. Effect of the control (Melphalan) on % cell viability of C3A hepatocyte after 48 hours of exposure

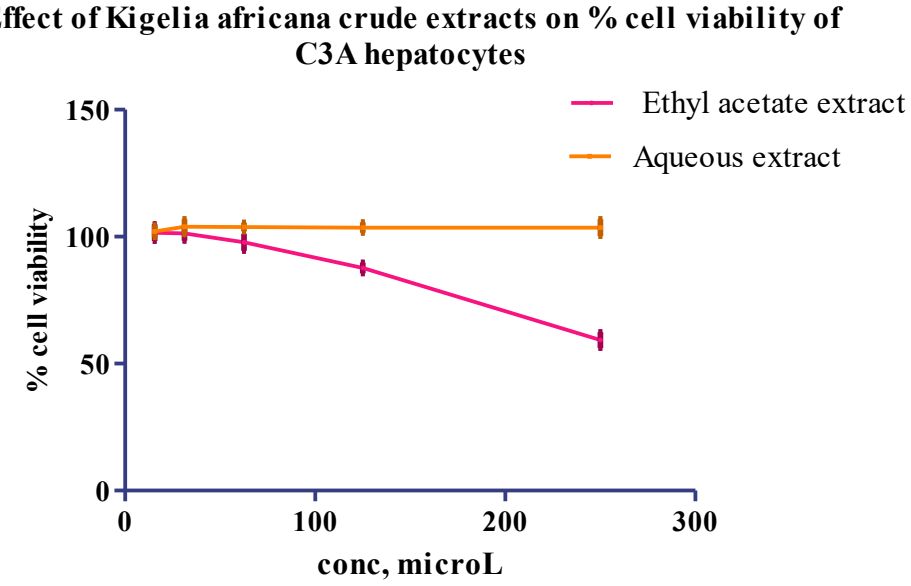


Figure 4.14: Effect of Kigelia crude extracts on % cell viability of C3A hepatocytes after 48 hours of exposure

4.5.3. Genotoxic evaluation using Vero cells.

The ethyl acetate (EtOAc) crude extract demonstrated cytotoxic effects against Vero cells

at doses of 250 and 500 µg/mL, as depicted in Figure 4.15 with an IC50 of *338.6 ± 1.058* µg/mL. Similar observations were made for the elevated concentrations of the positive control (7.5, 15, and 30 µM), with an IC 50 of 8.248 ± 1.459 whereas the aqueous extract did not affect the total cell number.

The aqueous extract exhibited a marginal although statistically significant elevation in the percentage of micro-nucleated cells at the three most concentrated levels (Figure 4.16 and 4.17) with an EC50 of 46.22 ±35.89µg/mL compared to that of Griseofulvin which was pinned at 8.67 ± 0.67 µM. The highest concentration resulted in a significant increase in percentage of micro-nucleated cells, from 2.68% to 3.8%. The observed phenomenon did not substantially increase multinucleation (Figure 4.18) or the mean nuclear size (Figure 4.19 and 4.20). The positive control, known to be an aneugen, exhibited a considerably more pronounced increase in the percentage of micro-nucleated cells, accompanied by substantial and statistically significant increases in both ploidy and nuclear size with an EC50 of 11.34 ± 0.11 µM.

The ethyl acetate (EtOAc) extract did not result in a notable increase in the proportion of micro- or multinucleated cells, as depicted in Figures 4.17 and 4.18. However, a statistically significant increase in the average nuclear area was observed, as illustrated in Figure 4.20 with an EC50 of 601.173 ± 3.899 µg/mL. The observed alteration is likely attributable to the cytotoxic properties of the extract at elevated concentrations.

Note: The error bars in the figures represent the standard deviation of quadruplicate measurements obtained from a single experimental trial. Statistical significance was assessed using two-tailed Student's t-test, with significance levels denoted as **$p<0.05$ and ***$p<0.001$ when compared to the control group.

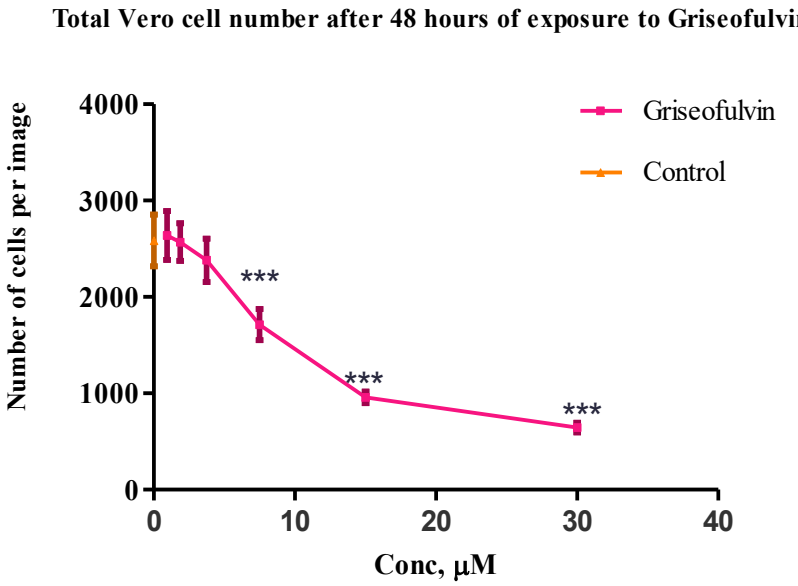


Figure 4.15: Cytotoxic effect of Griseofulvin (positive control) at different doses on the number of Vero cells following a 48-hour exposure.

****p<0.001 when compared to the control group*

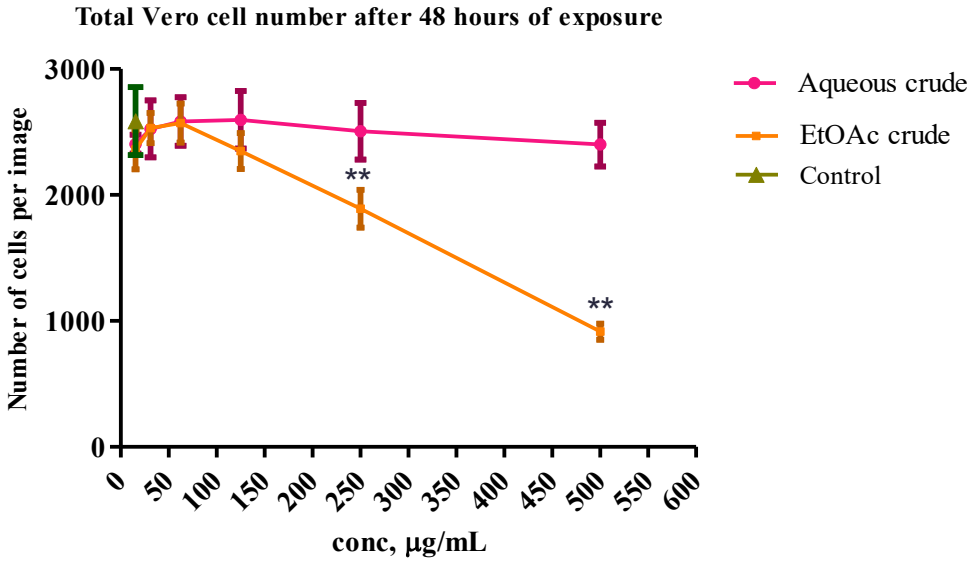


Figure 4.16: Cytotoxic effect of Kigelia fruit extracts at different doses on the number of Vero cells following a 48-hour exposure.

***p<0.005 when compared to the control group*

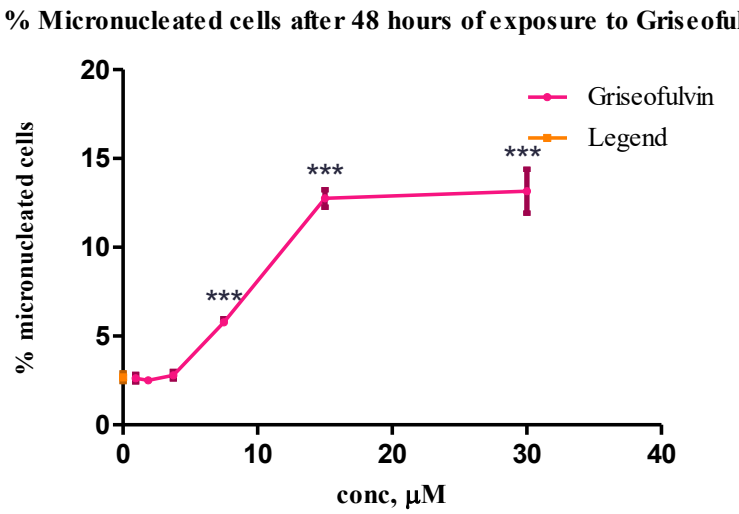


Figure 4.17: Percentage of micro-nucleated Vero cells following a 48-hour exposure to griseofulvin as positive control.

****p<0.001 when compared to the control group*

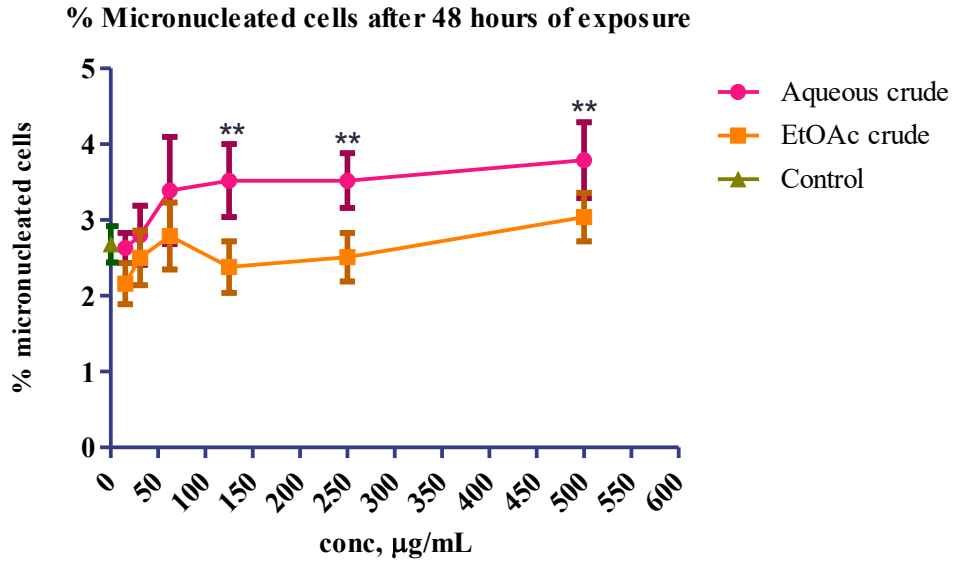


Figure 4.18: Percentage of micro-nucleated Vero cells following a 48-hour exposure to two Kigelia africana fruit extracts and control.

***p<0.05 when compared to the control group*

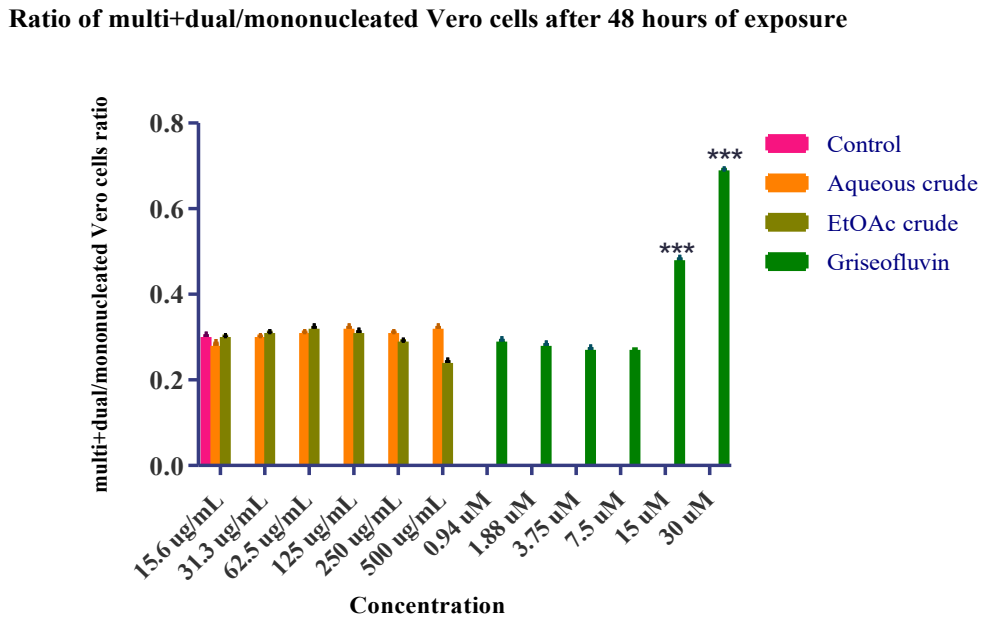


Figure 4.19: Effect of Kigelia fruit extracts at different concentrations on multi+dual/mononucleated cells ratio following a 48-hour exposure.

**** p<0.001 when compared to the control group.*

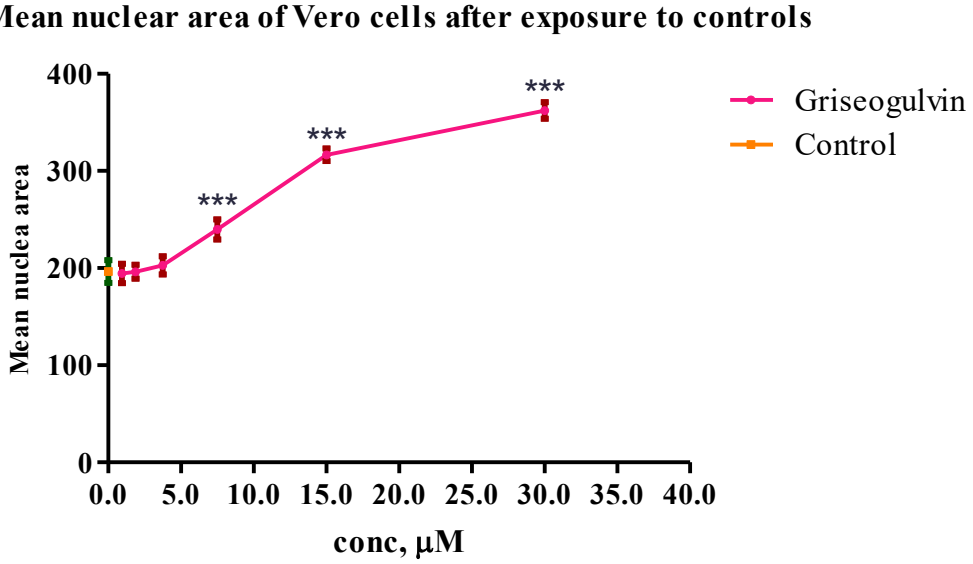


Figure 4.20: Effect of Griseofulvin (positive control) at different concentrations on the mean nuclear area of Vero cells following a 48-hour exposure.

****p<0.001 when compared to the control group*

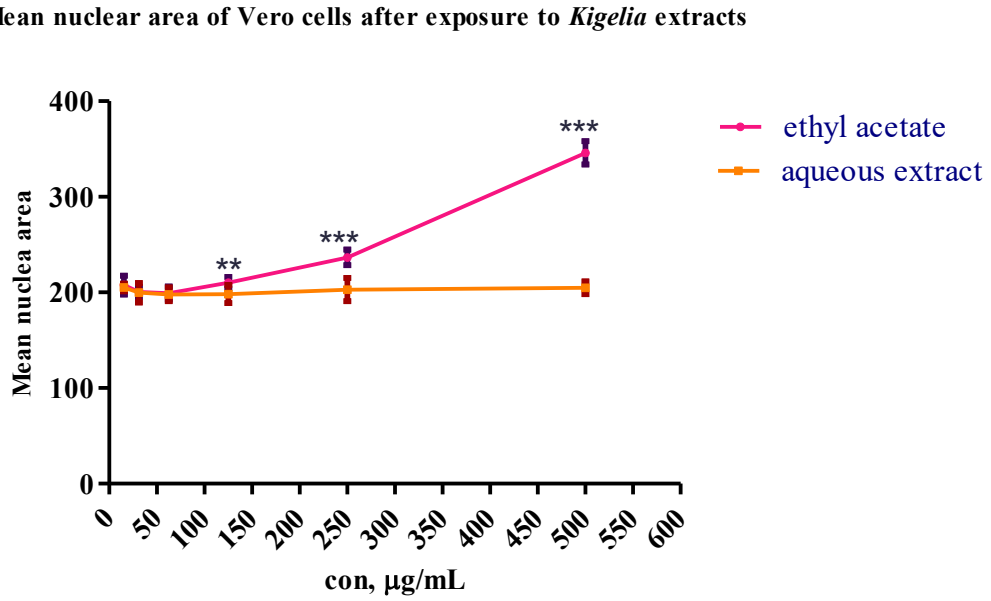


Figure 4.21: Effect of Kigelia fruit extracts at different concentrations on the mean nuclear area of Vero cells following a 48-hour exposure.

***p<0.05 and ***p<0.001 when compared to the control group.*

CHAPTER FIVE: DISCUSSION

5.1. Key Findings.

This study uncovered important information regarding the impact of *Kigelia on* hyperglycemia, body weight, lipid profile and mechanism of bioactivity in diabetes induced rats and identified fractions that may contribute to its hypoglycemic effects. Water had a higher extraction value than ethyl acetate. Animals treated with aqueous crude extract, ethyl acetate crude extract, and ethyl acetate fraction lost weight. All animals treated with fruit extracts and fractions showed cell regeneration and renoprotective effects in vital organs. Additionally, the study found that animals treated with the aqueous crude extract and ethyl acetate fraction had the most favorable lipid profiles after 28 days of treatment in comparison to the positive control (glibenclamide). *Kigelia* fruit extract did not inhibit α-amylase activity, whereas the ethyl acetate fraction showed minimal dose-dependent inhibitory activity. However, both the aqueous crude extract and the ethyl acetate fraction displayed significant α-glucosidase inhibitory activity ($p<0.05$). Through GC-MS, we observed that the fractions that demonstrated significant bioactivity contained the following phytocompounds: undecanoic acid, 11-cyclopentane undecanoic acid, n-hexadecenoic acid, akuammilan-17-ol, 10 methoxy, hydroxylamine, O" (2-methyl pentyl propyl), oxirane, and epoxyhexanol 2,3. The aqueous crude extract did not impair cell viability and was not mutagenic or genotoxic within the therapeutic dosage range.

5.2. Phytochemical analysis of and extractive values

Water continues to play a vital role as a solvent for the extraction of phytochemicals for traditional medicinal practices. In this investigation, it was observed that the aqueous extract exhibited a higher extractive value compared to the ethyl acetate extract.

Furthermore, the phytochemical analysis of both extracts revealed no substantial difference in the phytochemicals present, except for the ethyl acetate fraction not containing proteins and amino acids, saponnins, tannins, and fats. Conversely, the aqueous extract contained a considerable quantity of proteins and carbohydrates, which may have contributed to the increased weight of the aqueous extract. A comparison of the total flavonoid and phenolic contents between the two crude extracts revealed no significant disparities ($p>0.05$). Notably, the aqueous extract possessed a higher total flavonoid content than the crude ethyl acetate extract, although the crude ethyl acetate extract had a higher total phenolic content than the crude aqueous extract.

The use of a variety of solvents enabled us to isolate phytochemicals based on their polarity. Although chloroform provided the highest extraction value, it did not result in elevated levels of phenols, flavonoids, or fatty acids. However, the GC-MS results indicated that a greater quantity of fatty acids was present in the chloroform fraction than in other fractions. This may have accounted for the observed extractive values.

These extractive values are consistent with the findings reported by Dzomba *et al*., (2021), who also observed a lower extractive value for the ethyl acetate extract than for the ethanol extract in the extraction of *Kigelia* fruit.

Solvent polarity plays a crucial role in phytochemical extraction (Tatipamula *et al*.,2021). In this study, the use of water and ethyl acetate provided two polarities, resulting in slightly different phytoconstituents in the aqueous extract. Babbar *et al*., (2014) demonstrated that changes in polarity impact the phenolic content of plant extracts. Further, Zombe *et al.,* (2022) also demonstrated that solvent use often affects the total phenolic content (TPC) and total flavonoid content (TFC). Although organic solvents are generally more

advantageous in laboratory settings than water, this study demonstrated that water can yield a substantial number of phytochemicals. This suggests that many biologically significant phytoconstituents of *Kigelia africana* for diabetes management are inherently hydrophilic. Although Muyenga *et al.,* (2018) reported maceration as a traditional extraction method, the use of the Soxhlet apparatus in this study aligns with the approach reported by Bello *et al.,* (2016), wherein a decoction is traditionally employed for extraction, showing that *Kigelia* phytocompounds are not thermal liable. This study supports the traditional use of aqueous extracts from *Kigelia* fruit to manage diabetes and related metabolic syndromes. It also indicates that oral hydrophilic dosage forms are effective for delivering the desired phytoconstituents.

The *K. africana* fractions were separated based on varying polarities using chloroform, ethyl acetate, and butanol. The ethyl acetate fraction demonstrated superior antidiabetic activity, although it contained a few fatty acids, including n- and cis-cis-hexadecenoic acid. In contrast, the chloroform fraction contained stearic acid, oleic acid, hexadecenoic acid, and undecanoic acid. Additionally, the ethyl acetate fraction contained phenols, such as propenoic acid derivatives, ferulic and trans-ferulic acids, and hydroxymethylfurfural. The butanol fraction was richer in stearic and palmitic acids and a few phenols. In this study, we observed that fractions rich in fatty acids like 11- cyclopentanoic acids, udecanoic acids, and hexadecenoic acids showed antidiabetic effects. The subfractions of *Kigelia* that demonstrated *in vitro* antidiabetic activity mainly consisted of fatty acids like hexadecenoic acid, undecanoic acid, decanoic acid, and a significant presence of rare cyclic fatty acids like 11"(2-cyclopenten-1-yl) undecanoic acid, (+)- and cyclopentane undecanoic acid.

Fatty acids may vary according to their chemical composition. Some are oxygenated while

others are not, some of them are conjugated while others are not this variation attributes to their vast therapeutic applications ranging from cosmetology to disease management such as diabetes (Avato & Tava, 2022). Some fatty acids have been associated with antioxidant activity. For example, several studies have demonstrated that extracts rich in fatty acids such as palmitic acid, oleic acid nonadecanoic acid, arachidonic and eicosapentaenoic among others were able to effectively inhibit DPPH and demonstrate antioxidant activity (Beber *et al.,* 2014; Alencer *et al.,* 2018; Gawron-Skarbek *et al.,* 2023). In this study, it was observed that all fractions had fatty acids which may have varied in combination and intensity although, fatty acids were not the only contents.

Studies have indicated that there is a strong correlation between total phenolic content (TPC) and total flavonoid content (TFC) and DPPH scavenging activities of plant extracts (Mustafa *et al*., 2010; Aryl *et al.,* 2019). Our results also showed the presence of phenolic acids such as methyl furfural Kojic acid derivates, vanillic acid, benzoic acid derivatives to mention but a few as well as pyridine and pyrimidine derivatives as well as other nitrogen containing compounds such as hydroxylamine. These phytocompounds contributed to the observed TPC and TFC of the fruit extracts and fractions. There distribution across all fractions and extracts could be responsible for the lack statistically significant difference in the TPC ($p=0.32$) and the TFC ($p=0.06$). Consequently, it was also observed that there was no significant difference in the DPPH scavenging activity among the fractions and samples ($p=0.059$).

5.3. Diabetes ameliorative properties of *Kigelia fruit* extracts and fractions in relation to fruit phytochemistry

A significant reduction in blood glucose levels was observed between the 3rd and 28th days of treatment ($p = 0.03$) in all animals administered the extracts and fractions. Additionally, a notable difference was evident between animals treated with *Kigelia* extracts and fractions and those administered normal saline ($p=0.002$). These results are consistent with previous studies in diabetes-induced rats and *in vitro* (Muyenga *et al.,* 2015; Njogu *et al.,* 2018; Uhuo *et al.,* 2018; Fagbohun *et al*., 2020). In this study, post-induction sugars ranged between 11.3 ± 4.5 mmol/l and 31.4 ± 1.9 mmol/l. These ranges are similar to those reported by Fagbohun *et al*. who reported post-induction sugars that ranged between 14.5 ± 0.17 mmol/l and 25.57 ± 1.77 mmol/l, and Uhuo *et al*.who reported post-induction sugars ranging between 17.49 ± 8.84 mmol/l and 31.02 ± 0.78 mmol/l. It was found that animals treated with the crude extract experienced greater reductions in blood glucose levels than those treated with the ethyl acetate fraction, which showed the highest activity among the tested fractions. These results indicate that the aqueous crude extract may have produced a stronger effect, potentially due to its broader range of phytoconstituents compared to those present in the fraction. Additionally, the observed bioactivity may be attributed to mechanisms beyond the alpha glucosidase and alpha amylase inhibitory activities identified in the in vitro assays performed in this study.

To avoid bias in this study, random block sampling was employed, and animals of similar weight and age were used. A research assistant administered the doses to the animals. However, variances in treatment groups may have been due to the stress experienced by the animals, which was beyond the researcher's control (Bailey, 2017). Similar to this

study, which was conducted over 4 weeks, Uhuo *et al.* and Fagbohun *et al.* also conducted their research over 4 weeks. While Uhuo *et al*. used ethanol as a solvent, Fagbohun *et al.* used hexane and ethyl acetate fractions. Consistent with the results of the present study, the ethyl acetate fraction demonstrated significant antidiabetic activity. However, the dosage used in this study was twice that reported in the previous studies, even though in a study conducted by Muyenga *et al.,* (2015), the maximum dose used was 1000mg/kg. Despite this dosage used, the animals did not show signs of toxicity while Oyebanji *et al.,* (2015); Farah *et al.,* (2018); Fagbohun *et al*., (2020) have reported that the extracts were safe at 5000 mg/kg in rodents.

Although studies have determined the basic phytochemistry of *Kigelia* in relation to diabetes, it has often been associated with the presence of alkaloids, iridoids and phenolic compounds (Khan *et al*., 2012; Kumar, Kumar and Prakash, 2012; O. Fagbohun *et al*., 2020). In this study, the subfractions of *Kigelia that* demonstrated in-vitro antidiabetic activity mainly constituted of fatty acids like hexadecenoic acid, undecanoic acid, decanoic acid, and a significant presence of rare cyclic fatty acids like 11"(2-cyclopenten-1-yl) undecanoic acid, (+)- and cyclopentane undecanoic acid. The GC-MS also revealed nitrogen-containing molecules like Hydroxylamine, O"(2-methylpropyl}-; and tridecane, 4-methyl- butyl nitril.

This study observed stearic acid in all fractions with different intensities. Other than stearic acid, palmitic acid was also observed in the chloroform fraction. While stearic acid has been reported to be associated with good antioxidant properties (Choi *et al*., 2021), there is a school of thought that doubts the benefits of palmitic acids in diabetes, as there are suggestions that the presence of palmitic acid increases glucose intolerance and promotes

obesity (Kochikuzhyil *et al.*, 2010; Mancini *et al*., 2015). Therefore, while stearic acid may ameliorate diabetes symptoms when consumed (Reeves, 2012; Zhang *et al.,* 2012), the presence of more palmitic acid in the chloroform fractions may have been responsible for the poor glycaemic control observed in animals treated with this fraction.

While most studies have demonstrated that these fatty acids may inhibit alpha-amylase activity (Go *et al.,* 2019; Mitri *et al*., 2021), scanty information is available to demonstrate their alpha-glucosidase inhibitory activity (Nguyen & Kim, 2015). From the results observed in this study, the sub fractions that were rich in 11"(2-cyclopenten-1-yl) undecanoic acid, (+)- and cyclopentane undecanoic acid, as well as hexadecenoic acid, undecanoic acid, decanoic acid were able to ameliorate diabetes by significantly inhibiting the alpha-glucosidase activity with an IC_{50} – 10.41 ± 0.08 µg/mL for the ethyl acetate fraction. The observed bioactivity of *Kigelia* fractions implies that the fruit extracts can be beneficial to both diabetic and prediabetic patients in a quest to prevent the development of diabetes. Studies have shown that alpha-glucosidase inhibitors can prevent the development of diabetes in prediabetics and can also prevent disease progression and the emergence of complications of diabetes (Moelands *et al.,* 2018). The findings of this study suggest that *Kigelia* aqueous fruit extract has great potential in preventing the development of diabetes among patients at risk of T2DM and the development of complications of diabetes, including death, which was observed in this study for animals that did not receive any intervention. This suggests a possible alpha-glucosidase inhibitor that is readily available to Southern African patients, considering that conventional alpha-glucosidase inhibitors are not readily available to diabetic patients in most Southern African countries (MOH, 2013; Rossiter,2014).

The study also observed a difference in weight between the first and last day of treatment. Animals treated with extracts lost more weight than those treated with the positive control glibenclamide. Moreover, the observed minimal weight loss in animals treated with *Kigelia* compared with the negative control ($p<0.05$) suggests that the reversal of diabetic effects by the plant prevents wasting in diabetes-induced animals. These findings are consistent with those reported by Kumar *et al*. (2012), who demonstrated that the flowers of Kigelia pinnata, a species of Kigelia native to India, reduced blood glucose levels and weight in streptozotocin-induced diabetic rats. In contrast, Fagbohun *et al*., (2020) reported that *Kigelia* fruit fractions extracted from plants growing in Nigeria increased animal weight. Our study demonstrated that *Kigelia* ameliorates diabetes by reducing body weight. This implies that *Kigelia* reduces complications that result in weight gain in diabetes, especially insulin resistance in subjects with type II diabetes, and thus reduces disease progression. These results further support our biological activity tests, which demonstrated the α-glucosidase inhibitory activity of the aqueous extract and its fractions, suggesting that the animals may have experienced satiety and reduced food consumption. The presence of fatty acids, such as decanoic, undecanoic, hexadecenoic, octadecanoic, steric, and linoleic acids, may be responsible for reduced food consumption, as some studies on plants rich in them have demonstrated their potential use for weight management (Kim *et al*., 2014). Furthermore, the observed presence of 4H-pyran-4-one,5 hydroxy -2-(hydroxymethyl)-[kojic acid] derivatives in this study may have contributed to this result. A reduction in weight by the fruit extracts suggests their possible use for managing metabolic disorders, including obesity. The differences in weight observed in animals treated with extracts and fractions remained within the established 10% threshold for weight change, which is used

as an indicator of toxicity (van Berlo *et al.,* 2022).

Studies have demonstrated that methylfurfural is effective in glycaemic control and provides protective effects in cardiovascular-related conditions. Furthermore, methyl furfural has been associated with cytoprotective effects in hyperglycaemia-injured cells (Cao *et al*., 2013; Das *et al*., 2021; Essien *et al*., 2021). In this study, animals treated with the extract benefited from its cardioprotective effect in comparison to those treated with normal saline, as it was observed that the treated animals had fewer cardio-degenerated cells than normal cardiomyocytes. The presence of oleic acid also suggests ameliorative properties in diabetes and cancels the negative effects when combined with palmitic acid (Palomer *et al*., 2018). The study above agrees with the findings in this study that show that while the chloroform fraction, which contained a combination of both, did not provide adequate glycemic control, the rats had a good survival rate as they benefited from the antioxidant and cytoprotective effects because of their presence. The presence of oleic acid could also help with glycaemic control observed in the ethyl acetate fraction because it offers protection against insulin resistance and helps in the management of metabolic syndrome (Pastor *et al.,* 2021).

The presence of 4H-pyran-4-one,5 hydroxy -2-(hydroxymethyl)- (kojic acid) in the ethyl acetate fraction may also have contributed to glycaemic control. Recent research suggests that kojic acid derivatives might have beneficial effects on diabetes by promoting GLUT4 translocation (Sharma *et al*., 2014). However, this study did not evaluate the fruit extract's influence on glucose transport via GLUT4, which is primarily found in muscle and adipose tissues. The current study focused on glucose uptake in colorectal cells. Unlike muscle

tissue, glucose translocation in healthy colorectal cells is facilitated by either GLUT1 transporters for basal glucose uptake or GLUT2 transporters, along with the sodium-glucose co-transporter (SGLT1), which serves as the main glucose transporter in the GIT (Pragallapati & Manyam, 2019). The minimal effect observed on glucose uptake and utilization in this study may suggest that the drug had little impact on these transporters and achieved its glucose-lowering effects through a different mechanism of action. Since the cells involved in this study were tested for viability before the assay was conducted and found to be viable, any confounding issues in relation to the viability of the cells involved in this study were removed.

Kojic acid derivatives may also help in the management of obesity as reported by El-Korany and colleagues, who observed that animals treated with extracts rich in kojic acid had a reduced per cent weight gain, significantly reduced food intake and serum triglyceride levels in high-fat diet-fed Sprague–Dawley rats (Wei & Yang, 2012; Sharma *et al*., 2014; El-Korany *et al*., 2020). The results obtained in this study agree with those reported in the literature, as we observed a significant difference in weight between the first and last days of treatment ($p=0.016$) among animals treated with *Kigelia* extract and those treated with the positive control.

Research has shown that oxidative stress reduces pancreatic cell mass, impairs insulin sensitivity, decreases insulin receptor activity, and causes cellular damage, ultimately contributing to diabetes mellitus and its complications (Darenskaya *et al*., 2021). As a result, using antioxidants in diabetes treatment is beneficial. Studies have found that phenols and flavonoids are associated with high antioxidant capacity (Ofosu *et al*., 2020) and possess anti-diabetic properties (Babbar *et al.,* 2014; Tatipamula & Kukavica, 2021).

Particularly, AL-Ishaq *et al.,* (2019) and Vu *et al.,* (2020) reported that plant extracts rich in flavonoids can improve diabetes complications and outcomes using different mechanisms (AL-Ishaq *et al*., 2019; Vu *et al*., 2020). Ferulic acid may exist in both cis- and trans-forms. The trans form was identified in this plant. Ferulic acid exists in many plants in nature and has been associated with many therapeutic effects, including antioxidant activity, hepatoprotective activity, and antidiabetic activity. Zhao *et al.,* (2020) and Moloto *et al.,* (2020) reported that phenolic compounds such as ferulic acid can improve outcomes in diabetic rats. Kumar & Pruthi (2014) reviewed several studies that suggest that ferulic acid alleviates oxidative stress in diabetes-induced rat pancreatic cells and improves insulin secretion. Zhao and colleagues reported that ferulic acid protects the beta cells of the pancreas in induced diabetes mellitus in the rat species (Zhao *et al.,* 2020). Additionally, ferulic acid exhibits antioxidant properties by scavenging free radicals. Furthermore, studies have demonstrated the renoprotective effects of *Kigelia* extracts in rats due to the presence of flavonoids (Josiah *et al*., 2020). Our research also revealed that *Kigelia* extracts and fractions contained a substantial amount of total flavonoid content, with the highest levels found in the aqueous crude extract and its ethyl acetate fraction.

In this study, we also found that animals treated with *Kigelia* extracts exhibited better histopathological outcomes across most organs. Specifically, we noted hepatoprotective effects, evidenced by a reduced presence of necrotic cells compared to those treated with the negative control (saline), as well as an improved pancreatic cell mass in rats treated with the fractions. Although the ethyl acetate fraction had the lowest blood glucose levels at the end of the study, all fractions lowered the blood glucose levels after diabetes induction. We also observed that animals treated with the fruit extract and fractions had

less necrotic tissue in the islets of Langerhans. Thus, we may conclude that this free radical scavenging may have reduced necrosis of the islets of Langerhans and contributed to a reduction in blood glucose levels by the fractions. This was in tandem with the observed antioxidant activity via DPPH inhibitory activity and the TPC and TFC of the fractions and crude extracts.

Histopathological analysis revealed that the fruit extracts and their fractions were successful in regenerating cells damaged by diabetes compared with the negative control. For instance, in the liver, rats treated with the aqueous extract and ethyl acetate fraction had the highest number of regenerated cells, as indicated in Figure 4.6 of this paper. Additionally, histopathology of the hippocampus demonstrated the cytoprotective properties of the fruit extracts and fractions, which may be due to the presence of fatty acids such as oleic acid, which have been reported to possess neuroprotective effects (Song *et al*., 2019). We observed that the histoarchitecture of the kidney improved in animals treated with extracts and fractions, with a narrowed urine space around the glomerulus. These histopathological results agree with the biochemical results, which showed reduced ALT and serum creatinine levels ($p < 0.001$), as well as a decreased glycaemic index in rats. The reported presence of pyridine derivatives, propanoic acid derivatives, hydroxymethyl furfural, pyrimidine derivatives, phenols, trans-ferulic and ferulic acids, and stearic and oleic acids could have contributed to the observed glycaemic control as well as improved histopathological outcomes.

In our study, we observed a statistically significant reduction in lipid levels in animals treated with *Kigelia fractions* and crude extracts compared to those treated with the positive control glibenclamide. These results are consistent with those reported by Kumar *et al*.

(2012) and Fagbohun *et al*. (2020), who documented the hypolipidemic effects of *Kigelia flower* and fruit extracts, respectively. Elevated lipid levels are commonly associated with diabetes mellitus, which poses a significant risk of cardiovascular comorbidities. While all extracts demonstrated a general hypolipidemic effect, the rats treated with the chloroform fraction had the least reduction in the lipid profile compared with the other groups. The Chloroform fraction contained the highest quantity and variety of fatty acids, including stearic and palmitic acids. Although stearic acid has been reported to possess good antioxidant properties (Choi *et al*., 2021), there are differing opinions regarding the benefits of palmitic acid. Some studies have suggested that its presence may promote obesity and worsen glucose intolerance (Kochikuzhyil *et al*. 2010; Mancini *et al*. 2015). Therefore, although stearic acid may help ameliorate diabetes symptoms when consumed (Reeves, 2012; Zhang *et al*., 2012), the presence of palmitic acid in chloroform fractions may have contributed to poor glycemic control. However, hypolipidemic properties observed in animals treated with these fractions suggest the use of *Kigelia* fruit not only to manage metabolic conditions but also to prevent complications of both diabetes and cardiovascular diseases. The observed TPC, TFC and DPPH inhibitory activities and phytochemical combinations in the three fractions influenced the observed glycemic levels in diabetes-induced rats.

Although the doses used in this experiment were higher than previously reported doses, conversion factors involved when translating doses between humans and animals (Nair & Jacob, 2016) suggest that a lower drug dose would be required in human subjects. However, it should be acknowledged that this calculation is not adequate, considering that pharmacokinetic studies play a role in transitioning doses from preclinical studies to

clinical studies (Arunachalam & Sasidharan, 2021). Further, since this extract would be consumed using an oral route of administration, important pharmacokinetic effects that affect drug absorption such as presence or absence of food in the stomach, biotransformation due to intestinal microflora and first pass metabolism would likely affect the plasma concentration of the fruit extracts (Li *et al.,* 2015). Also, it is important to consider that crude extracts of medicines are complex systems with multiple compounds which may affect their pharmacokinetics and pharmacodynamic effects (Alolga *et al.,* 2015). We can still assume that there is a good possibility of using *Kigelia* fruits as a potential herbal medicine with regard to the management of diabetes, considering their current use as traditional medicine.

5.4. Genotoxicity and mutagenicity potential of *Kigelia crude* extracts

A preliminary cytotoxicity assessment of *Kigelia africana* fruit extracts on *S. typhimurium* strain TA98 indicated that neither of the fruit extract samples exhibited any noticeable toxic effects that could be considered substantial or indicative of possible harm. Given that the survival rate of *S. typhimurium* TA98 cells exposed to both samples exceeded sixty per cent, it was plausible to assess the mutagenic potential of the fruit extracts in *S. typhimurium* strains TA97 and TA100 without encountering the possibility of false-negative or inconsistent outcomes (as indicated in table 4.5.2). *Salmonella* strains TA 98 and TA 100 were selected because of their reported 93 per cent sensitivity in identifying plant extracts that had mutagenic potential (Dantas *et al*.,2020). Using the Ames test, the study demonstrated the effect of the extract on both the frame-shifting mutagenicity of TA97 and TA98 strains, as well as base-pair substitution mutagenicity with TA98 (Maron & Ames, 1982; Bugda *et al*.,2022). We concluded that the extracts did not possess

mutagenic or clastogenic properties in bacteria or cultured mammalian cells, regardless of the presence or absence of S9 activation. No significant difference was observed in the counts of revertants induced by the ethyl acetate and aqueous extracts compared to the negative control.

Thus, in this study, we have made a pioneering contribution by demonstrating that neither extract induces base pair replacement nor frameshift gene alterations in the bacterial cells utilised within the scope of this study. The results presented here bear resemblance to the findings published previously by Abdul-Hafeez *et al*. (2018) and Eldeen *et al*. (2007). Notably, these earlier investigations focused on the stem bark extracts of *Kigelia africana* originating from Egypt and leaf extracts from the same plant grown in Sudan. It is noteworthy that, in contrast, our study is the first to specifically explore the mutagenic profile of fruit extracts of *Kigelia africana*, which patients in Zambia frequently employ. In contrast to the studies conducted by Eldeen and Van Staden, who employed a single strain of *Salmonella* (TA 98), and Abdul-Hafeez *et al*., who used both TA 98 and TA 100, this study incorporated three strains of *Salmonella* (TA 97, TA 98, and TA 100). In summary, based on the findings obtained from the Ames test, it can be inferred that neither frameshifting nor point mutations played a significant role. This conclusion was drawn from the absence of any noteworthy disparity between the outcomes observed with the fruit extracts and the negative control.

The findings of this investigation indicate that the ethyl acetate extract of *Kigelia* fruit exhibited a decline in cell viability of C3A hepatocytes, which was proportional to the dosage administered. Notably, the highest concentration of 250 µg/L resulted in a cell viability of 59%. Conversely, the aqueous extract showed substantial cell viability

throughout the experiment. Nevertheless, the observation of a cell viability of over 50% solely at the highest dose (IC50 > 250 µg/mL) still indicates that the phytochemicals extracted by the ethyl acetate solvent and their metabolites pose minimal danger of inducing hepatotoxicity. The use of herbal medications and nutritional supplements has been linked to hepatotoxicity, as evidenced by studies conducted by Andrade (2019) and Gómez-Lechón *et al*., (2010). Consequently, based on the results of this study, it is improbable that aqueous and ethyl acetate extracts would induce hepatotoxicity in individuals. Moreover, implementing a dose adjustment to the ethyl acetate extract during treatment would also decrease potential hepatotoxicity.

The genotoxicity of *Kigelia* fruit extracts was assessed using the MTT assay, and the results were compared with the Ames results obtained in this study. The MTT test conducted on Vero cells is a reliable method that can provide valuable insights into the potential clinical implications of the plant extracts investigated in this study (Anywar *et al*.,2021; Winikoff *et al*.,2004). According to Rampa *et al*., (2022), if the detected cytotoxicity testing result exceeds 55%, it would be appropriate to proceed with a genotoxicity test. However, in this case, even at the highest dose, the ethyl acetate extract showed 59% cell viability. The ethyl acetate extract had a cytotoxic effect on Vero cells, similar to that observed on C3A hepatocytes at high concentrations. Similar observations were found for the elevated concentrations of the positive control (7.5, 15, and 30 µM). However, the observed hepatotoxicity was avoided when lower concentrations were used. Further, studies have reported that even at average doses of 250 mg/kg to 500 mg/kg in animals, the efficacy of fruit extracts in managing diabetes has been recorded (Muyenga *et al*., 2015).

The aqueous extract exhibited a minimal but statistically significant increase in the

proportion of micro-nucleated cells at the three highest doses, as shown in Figure 4.18. Nevertheless, there was no substantial rise in multinucleation (Figure 4.19) or enlargement of the nucleus (Figure 4.21) observed in conjunction with this. The observation of an augmented nuclear size may indicate the possibility of genotoxicity associated with the extracts at very high doses. However, the positive control, known as an aneugen, exhibited a considerably more notable increase in the percentage of micro-nucleated cells, accompanied by substantial and statistically significant increases in the ploidy and nuclear size. Thus, we may conclude that the findings of this investigation indicate that the extract does not exhibit an aneugenic impact on the cells. In contrast, the EtOAc fruit extract did not significantly affect the percentage of micro- or multinucleated cells, as shown in Figures 4.17 and 4.18. However, a notable increase in the mean nuclear area was observed (Figure 4.21). The observed alteration can be attributed to the cytotoxic properties of the extract at elevated concentrations. Additionally, as the aqueous extract exhibited an IC50 of 193.7 ± 0.08 µg/mL for therapeutic in vitro effects, it indicates that the extract is not toxic within the observed therapeutic range. Similar studies conducted on antidiabetic drugs such as sitagliptin have demonstrated genotoxicity at high doses of approximately 500 µg/mL (Majeed *et al*., 2021). However, the genotoxicity test of the drug in human subjects only showed genotoxicity at the highest treatment dose, which was approximately 1000 µg/mL (Yuzbasioglu *et al*., 2018). Thus, there is a need to conduct genotoxicity studies in patients exposed to aqueous fruit extracts for extended periods of time, bearing in consideration that the observed *in vitro* cytotoxic effect at higher doses of the fruit extract requires extrapolation to determine genotoxicity in human subjects.

5.5. Novelty of this research

This investigation demonstrates the in vitro alpha-glucosidase inhibitory activity of *Kigelia* fruit extract and its fractions, with estimated IC50 values of 193.7 µg/mL for the aqueous crude extract and 10.41 µg/mL for the ethyl acetate fraction. The mechanistic effects of *Kigelia* fruit extract have rarely been documented in the reviewed literature. To the best of our knowledge, this is the first report of this mechanism of action in Southern Africa.

Furthermore, this study identified rare cyclic fatty acids, including 11"(2-cyclopenten-1-yl) undecanoic acid, (+)- and cyclopentane undecanoic acid; indole alkaloids such as akuammilan-17-ol- 10-methoxy, N-nitroso-2-methyl-oxazolidine and epoxide oxirane2.2" -(1.4-butanediol) bis-, and a naturally occurring phthalic acid ester in the bioactive subfractions. This study presents a potential nutritional supplement that has a positive impact on glucose metabolism in the body. Additionally, it has elucidated pharmacophores with potential anti-diabetic properties.

This study is the first to report that *Kigelia* fruit extracts are neither mutagenic, genotoxic, nor cytotoxic when administered within the therapeutic range.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1. Conclusion

This final chapter provides a summary of the research findings and their correlation with the research objectives. This chapter also highlights the significance of this study and potential avenues for future research.

This study aimed to investigate the bioactivity and phytochemical profile of *Kigelia africana* fruit fractions, their effect on diabetes diagnostic markers in alloxan-induced diabetic rats, and their mutagenic properties. These results suggest that the ethyl acetate fraction improves diabetes outcomes by inhibiting α-glucosidase, which is facilitated by the presence of fatty acid combinations of 5-Hexenoic acid, 2-Octenoic acid, and (E)-, including unique fatty acids such as 11-(2-cyciopenten-1-yl) undecanoic acid, (+)-, n-decanoic acid, and phenolic compounds such as oxirane, pentyl- and Akuammilan-17-ol, 10-methoxy-, butane nitrile, 2,3- dioxo-, dioxime, O, O'-diacetyl-, N-Nitroso-2-methyl-oxazoiidine and hydroxylamine.

We observed reduced plasma blood glucose and lipid levels, and weight loss, potentially linked to the in vitro α-glucosidase inhibitory bioactivity and DPPH scavenging activity of the extracts and fractions. This research elucidated the compounds associated with the antidiabetic properties of fruit extracts. The study also demonstrated that the fractions provided neuroprotective, cardioprotective, renoprotective, and hepatoprotective effects, and observed pancreatic mass regeneration post-treatment.

This study also demonstrated the safety profile of the fruit at cellular and genetic levels, as the aqueous and ethyl acetate extracts of *Kigelia* did not exhibit mutagenic or genotoxic effects at therapeutic doses.

6.2. Recommendations

We recommend developing pharmaceutical formulations for cheaper alternative treatments, as *Kigelia* fruit shows potential as a nutritional supplement or pharmaceutical agent to mitigate diabetes symptoms and complications. Its diverse phytochemical composition and established biological effects may serve as a foundation for developing anti-diabetic medications and lead compounds for alpha-glucosidase enzyme inhibition. The high cost of some recent antidiabetic drugs, such as alpha-glucosidase inhibitors, makes *Kigelia* fruit extract a potentially cost-effective and accessible treatment option for individuals in Southern Africa when appropriate dosage forms are formulated.

The implications of this study for communities where herbal medicine use among diabetics is prevalent are that *Kigelia* fruit can be used as an affordable alternative to expensive and scarce α-glucosidase inhibitors. The fruit and its dosage form may also benefit patients with metabolic syndromes owing to the abundance of beneficial fatty acids, thus reducing complications associated with metabolic disorders. More efficacious pharmaceutical formulations providing optimised antidiabetic and antioxidant dosage forms, based on these fruit extract compounds, should be developed.

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APPENDICES

APPENDIX I- UNAM ETHICAL APPROVAL

UNAM

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: HG-/ 612/ 2021 Date: 29 July, 2021

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: An investigation of the effect of *Kigelia africana* fruit fractions on diabetes bio-markers in alloxan monohydrate induced diabetes Wistar rat model

Student: TUMELO MUYENGA

Student Number: 202065871

Supervisor(s): *Dr S.K.D. Bamitale (Main); Prof C.C. Ezeala; Dr D. Kibuule (Co)*

Hage Geingob Campus

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the HREC-H. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the HREC-H
3. The Principal Researcher must report issues of ethical compliance to the HREC-H (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by HREC-H
4. The HREC-H retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

HREC-H wishes you the best in your research.

Prof. C Wilders(Chairperson)

Pamela Claassen (Secretary)

APPENDIX II- MULUNGUSHI UNIVERSITY ETHICS APPROVAL AND ZAMBIAN NATIONAL HEALTH RESEACH AUTHORITY (NHRA) APPROVAL

Mulungushi University

School of Medicine and Health Sciences

Ethics Review Committee

IRB: 00012281 *FWA: 0002888* *Email: somhsethics@mu.ac.zm*

SMHS-MU3-2020-12

22nd December, 2020

Tumelo M. Akapelwa

Mulungushi University

Livingstone.

Dear Mrs Akapelwa,

RE: ETHICAL CLEARANCE OF THE STUDY PROTOCOL

Reference is made to your protocol entitled, "*An investigation of the effect of Kigelia africana fruit fractions on diabetes bio-markers in alloxan monohydrate induced diabetes Wistar rat model*" that was submitted on 29th September, 2020.

On behalf of the Research Ethics Committee (REC) Chairperson, I wish to inform you that your protocol has been successfully reviewed and according to the reviewer's recommendations your protocol has been granted Ethical clearance based on the following conditions:

Should there be need to modify or amend the approved protocol, you are required to notify the REC and submit protocol amendments for approval by quoting your REC reference number. You are further required to submit progress reports to the REC twice a year and a final report at the end of your study.

You are now required to submit your protocol to National Health Research Authority (NHRA) for authorization following the link: https://www.nhra.org.zm/

This approval is valid for a period 22nd December, 2020 to 22nd December, 2021.

The Committee wishes you success in the execution of your study.

Yours sincerely,

MULUNGUSHI UNIVERSITY - SOMHS _ RESEARCH ETHICS COMMITTEE

Warren Chanda

SECRETARY – MUSOMHS_REC

Cc: Chairperson – MUSOMHS_REC

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No:..... **Date: 25th January, 2021**

The Principal Investigator,
Mrs. Tumelo M. Akapelwa
Mulungushi University
Livingstone.

Dear Mrs Akapelwa,

Re: Request for Authority to Conduct Research

The National Health Research Ethics Board (NHREB) is in receipt of your request for authority to conduct research titled **"AN INVESTIGATION OF THE EFFECT OF KIGELIA AFRICANA FRUIT FRACTIONS ON DIABETES BIO-MARKERS IN ALLOXAN MONOHYDRATE INDUCED DIABETES WISTAR RAT MODEL."**

I wish to inform you that following submission of your request to the Board, the review of the same and in view of the ethical clearance, this study been given **Conditional Approval.**
Therefore, you are required to address the following issues before final authority is granted.

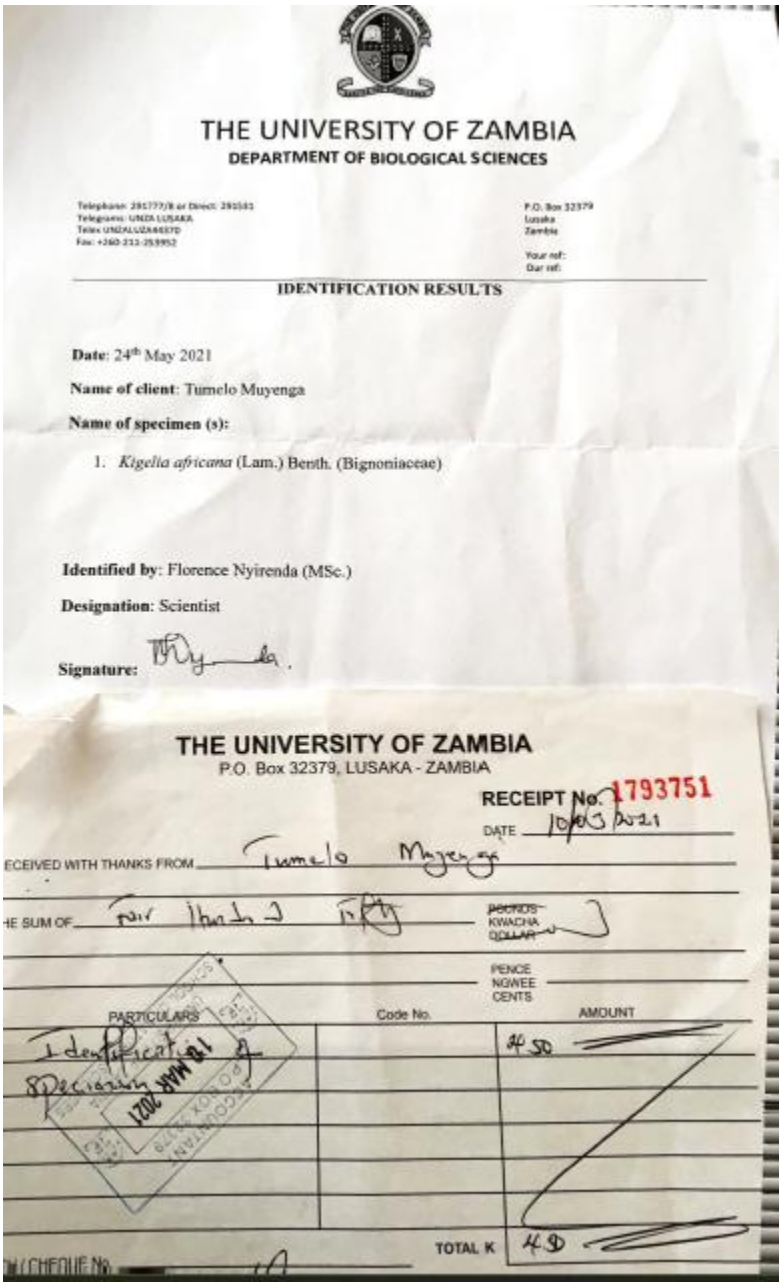
1. Kindly provide the data collection tools for your study.
2. Provide clarification on the health assessment criteria for the animals during the induction of diabetes and required responses.

Yours sincerely,

Fusya Goma

Dr. Fusya Goma
Vice Chairperson

APPENDIX III- PLANT IDENTIFICATION CERTIFICATE



THE UNIVERSITY OF ZAMBIA
DEPARTMENT OF BIOLOGICAL SCIENCES

Telephone: 291777/8 or Direct: 290581
Telegrams: UNZA LUSAKA
Telex UNZALUZA44370
Fax: +260-211-253952

P.O. Box 32379
Lusaka
Zambia

Your ref:
Our ref:

IDENTIFICATION RESULTS

Date: 24th May 2021

Name of client: Tumelo Muyenga

Name of specimen (s):

1. *Kigelia africana* (Lam.) Benth. (Bignoniaceae)

Identified by: Florence Nyirenda (MSc.)

Designation: Scientist

Signature:

THE UNIVERSITY OF ZAMBIA
P.O. Box 32379, LUSAKA - ZAMBIA

RECEIPT No. 1793751

DATE 10/05/2021

RECEIVED WITH THANKS FROM Tumelo Muyenga

THE SUM OF Fifty ~~POUNDS~~ KWACHA DOLLAR

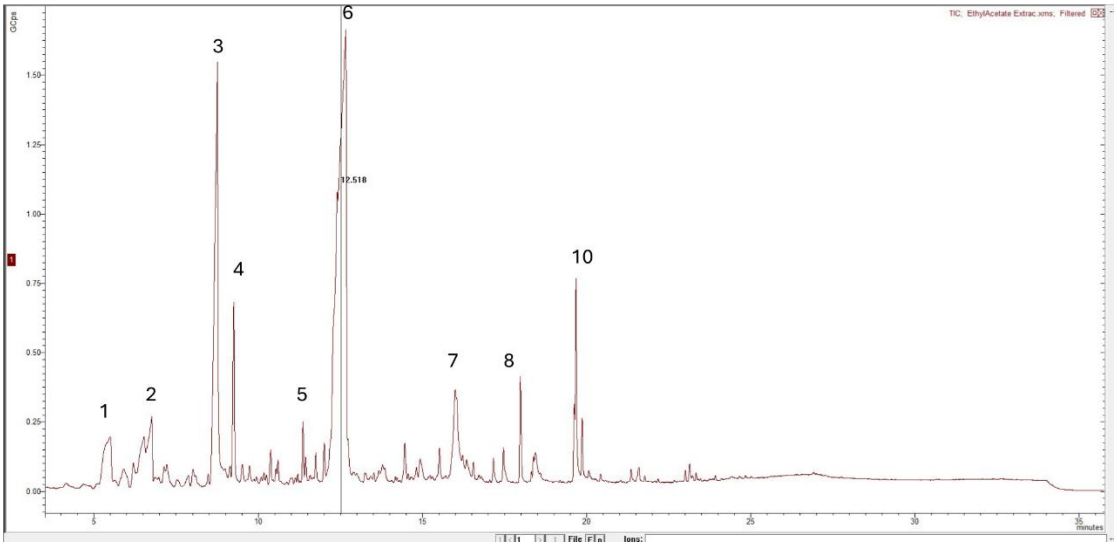
PENCE NGWEE CENTS

PARTICULARS	Code No.	AMOUNT
Identification of Specimen		50
	TOTAL K	50

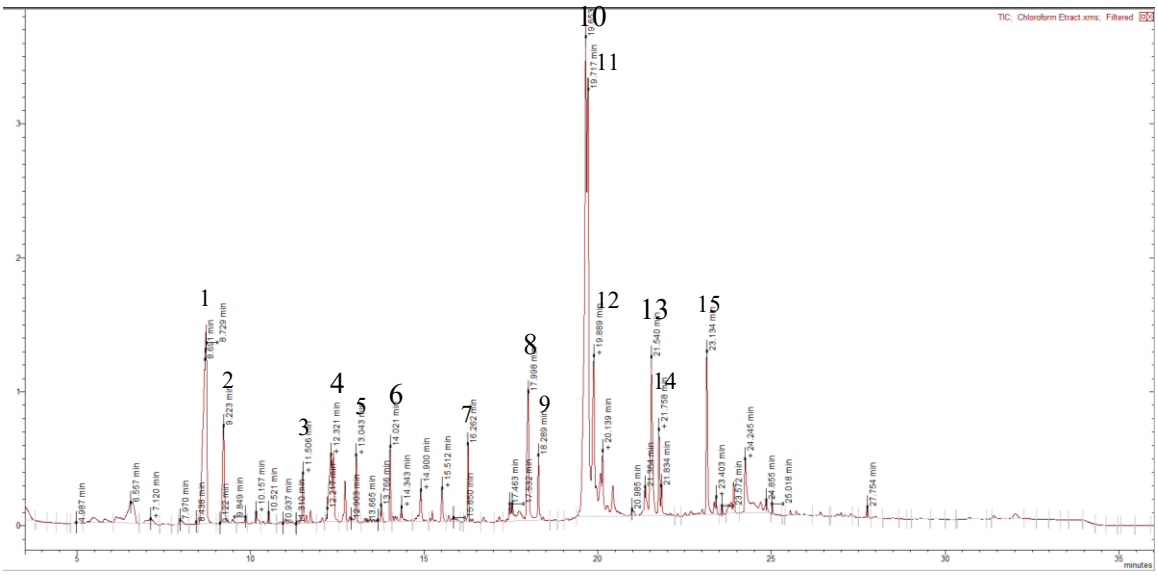
BY CHEQUE No.

APPENDIX IV- GC-MS FINGERPRINTS

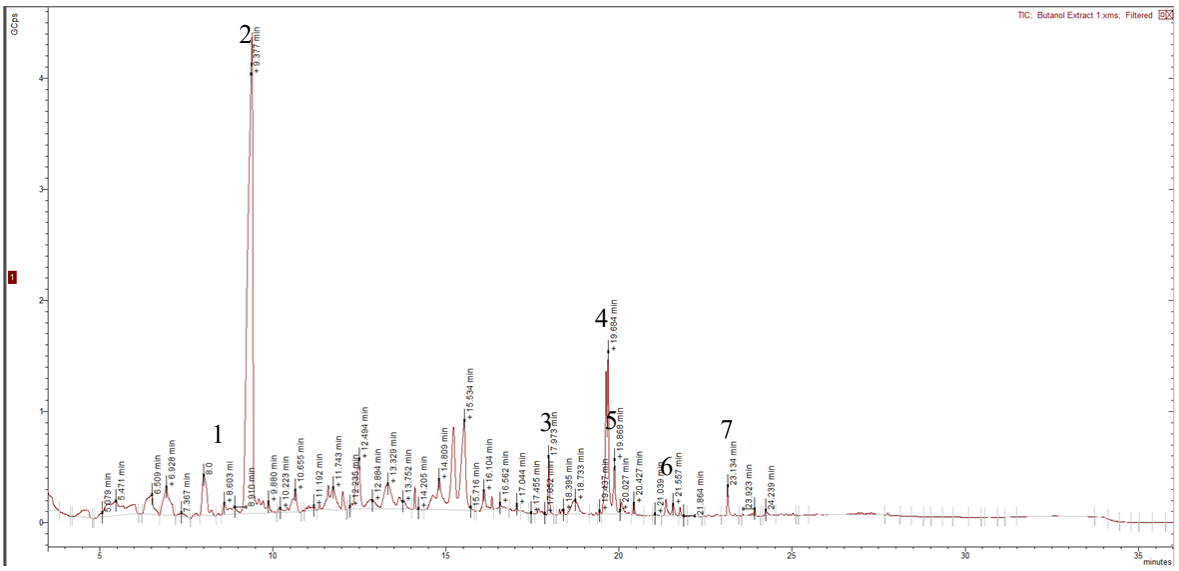
Appendix 4-1 Ethyl acetate fraction GC-MS peaks



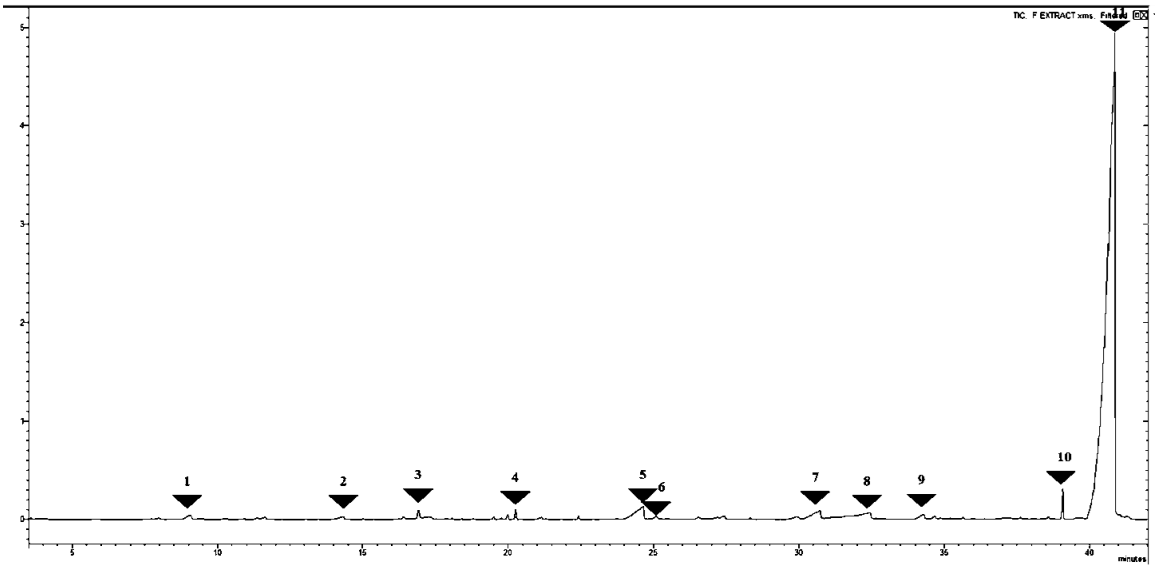
Appendix 4-2 Chloroform fraction GC-MS peaks



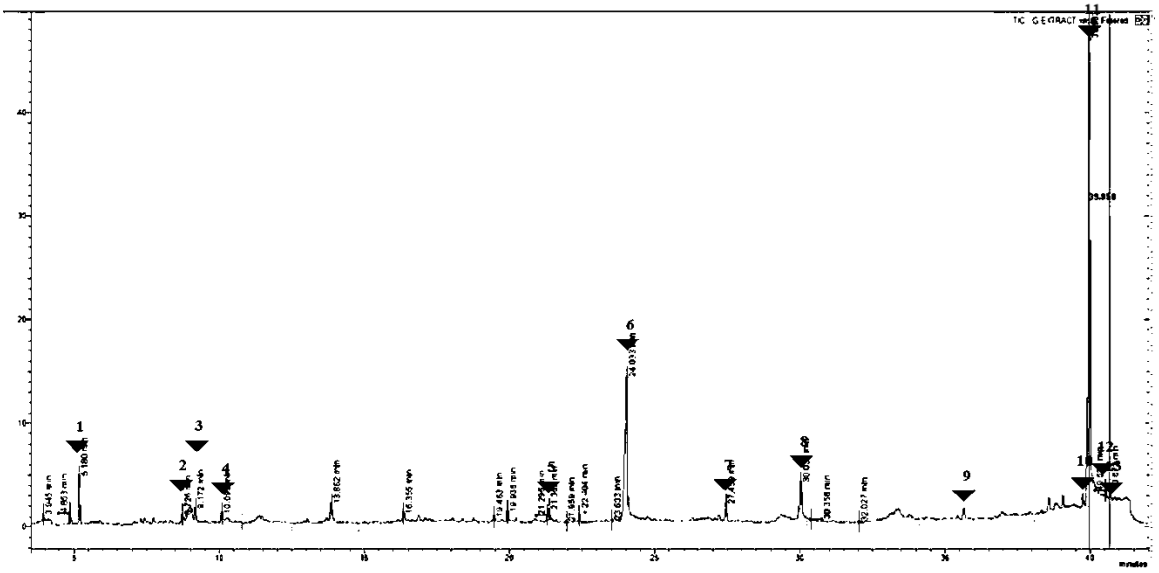
Appendix 4-3 Butanol fraction GC-MS peaks



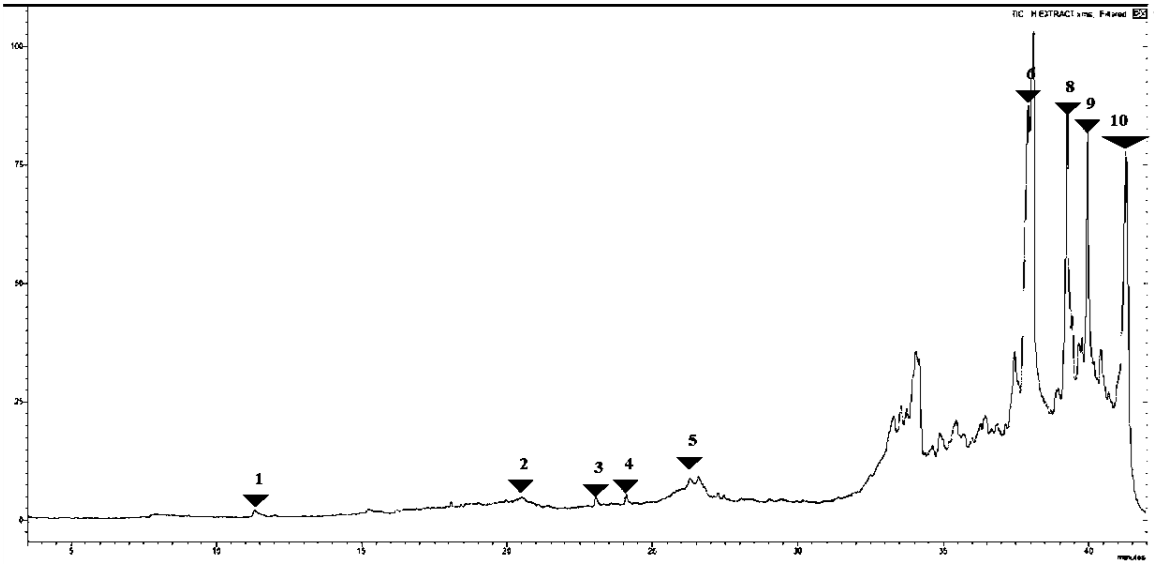
Appendix 4-4 Sub fraction F GC-MS peaks



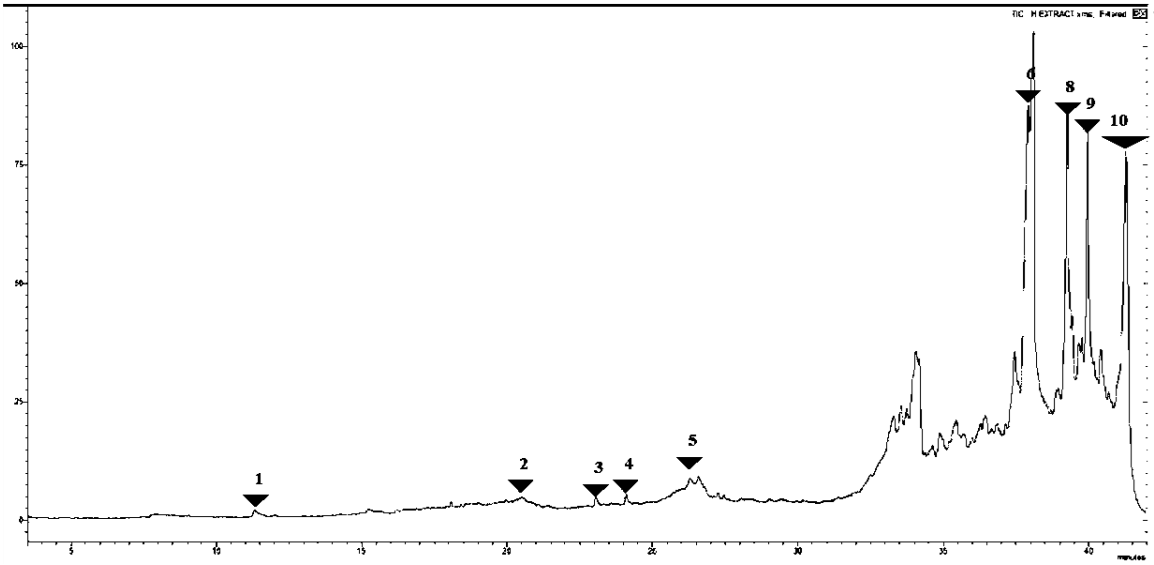
Appendix 4-6 Subfraction G GC-MS peaks



Appendix 4-7 Subfraction H GC-MS peaks



Appendix 4-7 Subfraction H GC-MS peaks



Appendix 4-7 Subfraction J GC-MS peaks

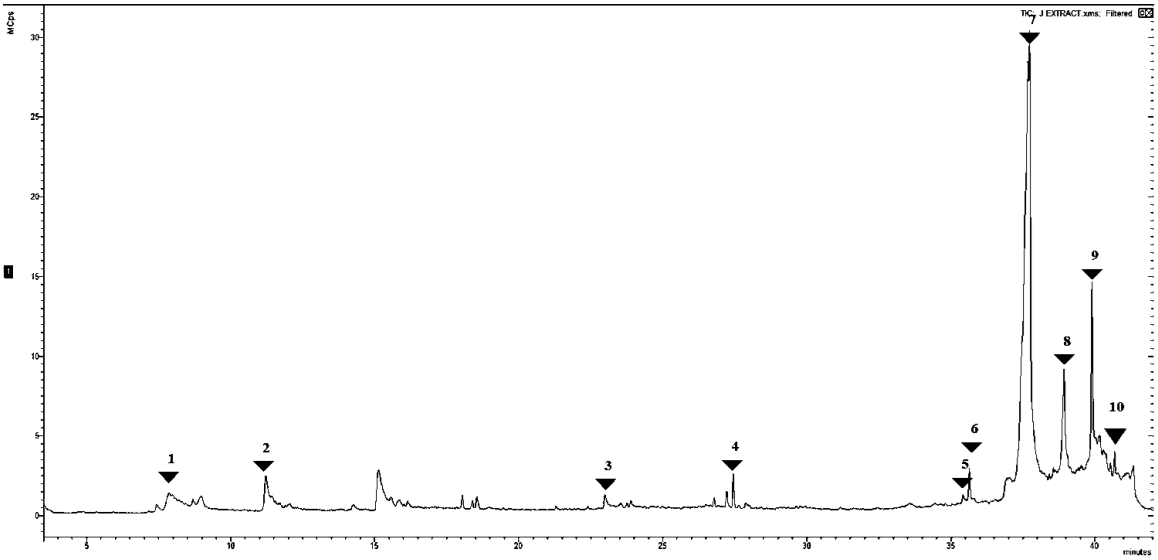
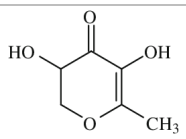
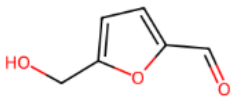
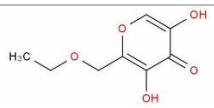
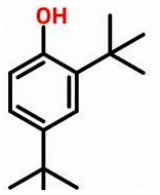
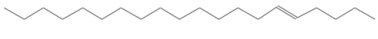
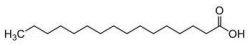
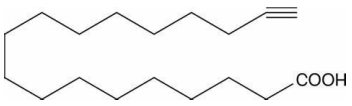
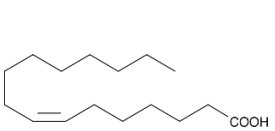
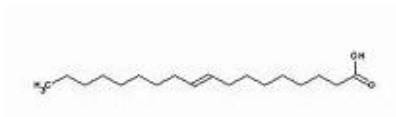


Table A4.1

The phytochemicals for the Chloroform fraction peaks are shown in appendix 4-2

RT	Chemical structure	Name	MW	Formula
8.670		4H-Pyran-4-one, 3, 5-dihydroxy-2-methyl-	142	$C_6H_6O_4$
9.233		5-Hydroxymethylfurfural	126	C6H6O3
11.505		7-Hexadecene, {Z}-	224	$C_{16}H_{32}$
12.311		4H-Pyran-4-one, 5-hydroxy-2-{hydroxymethyl}-	142	$C_6H_6O_4$
13.044		2,4Di-tert-butylphenol	206	$C_{14}H_{22}O$
14.019		5-Octadecene,	252	$C_{18}H_{36}$
16.257		5-Eicosene, (E}-	280	$C_{20}H_{40}$
17.9		n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
18.291		5-Eicosene, (E}-	280	$C_{20}H_{40}$
RT	**Chemical structure**	**Name**	**MW**	**Formula**
19.650		17-Octadecynoic acid	280	$C_{18}H_{32}O_2$
19.714		cis-7-Hexadecenoic acid	254	$C_{16}H_{30}O_2$
19.887		Octadecanoic acid	284	$C_{18}H_{36}O_2$

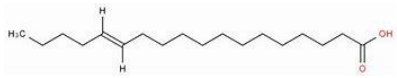
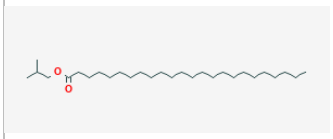
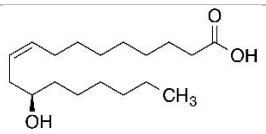
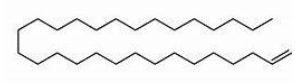
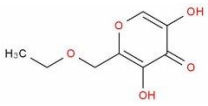
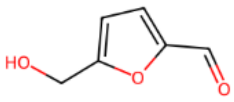
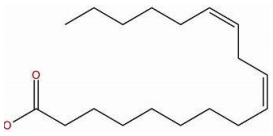
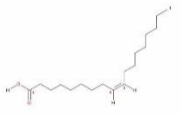
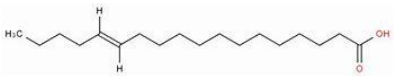
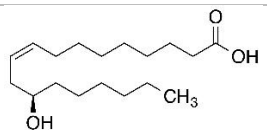
21.541		trans-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$
21.761		Tetracosanoic acid, isobutyl ester	424	$C_{28}H_{56}O_2$
23.138		Ricinoleic acid	298	$C_{28}H_{34}O_3$
24.852		Nonacos-1-ene	406	$C_{29}H_{58}$

Table A4.2

The phytochemicals for the Butanol fraction peaks are shown in Appendix 4-3.

RT	Chemical structure	Name	MW	formula
8.603		4H-Pyran-4-one, 3,5-dihydroxy-2-methyI-	*142*	$C_6H_6O_4$
9.376		5-Hydroxymethyl furfuraI	*126*	$C_6H_6O_3$
17.973		n-Hexadecanoic acid	*256*	$C_{16}H_{32}O_2$
RT	**Chemical structure**	**Name**	**MW**	**formula**
19.638		9,12-Octadecadienoic acid {Z,Z}-	280	$C_{18}H_{32}O_2$
19.855		Oleic Acid	*282*	$C_{18}H_{34}O_2$
21.567		trans-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$

23.123		Ricinoleic acid	*298*	$C_{18}H_{34}O_3$