IN VITRO EVALUATION OF *LEONOTIS LEONURUS* TO VALIDATE ITS POTENTIAL AS AN ALTERNATIVE ANTI-DIABETIC DRUG

UNIVERSITY *of the*
WESTERN CAPE

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A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science

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ABSTRACT

Africa is a rich source of medicinal plants with South Africa being known as a country with a strong history of traditional healing, which masses a variety of approximately 30 000 flowering plants and accounting for almost 10% of the world's higher plant species. *Leonotis leonurus*, a member of the Lamiaceae family, has shown potential biomedical applications in a great diversity of ailments and has shown to have antidiabetic activity, with the most common herbal active ingredients used in treating diabetes being flavonoids, tannins, phenolics, and alkaloids (Hussein, 2018). In the present study, *L. leonurus* was screened for possible bioactive chemicals.

The chemical screening of the methanolic extract of *L. leonurus* resulted in the isolation of five pure compounds viz Leoleorin C (**1**), Leoleorin H (**2**), Leoleorin I (**3**), Leoleorin A (**4**), and Marrubin (**5**). The compounds tested for glucose uptake activity, cytotoxicity effect, as well as alpha-glucosidase inhibition activity. The results suggested that amongst the five isolated compounds, only compound **4** showed a marked increase in glucose uptake, while compounds **1, 2, 3 and 4** showed significant signs of cytotoxicity. The results also demonstrate an overlap between glucose uptake and cytotoxicity for compound **4**. Compounds **2 and 5** both showed signs of cytotoxicity and no significant increase in glucose uptake activity. Therefore, it is concluded that compound **4** may be considered as a potential candidate for the management of type 2 diabetes. Compound **5** (Marrubin) has shown interesting activity against alpha-glucosidase. The results therefore suggest that the isolated compounds could become natural agents for the treatment of T2DM, with the prospect of being utilized in pharmaceutical products formulation upon further biological and clinical investigations.

Key words: Diabetes Mellitus, *Leonotis leonurus*, Biological activity, Anti-diabetic treatment, Marrubin,

DECLARATION

I, Yolanda Sigodi (Student number 3471318) hereby declare that "*In vitro* evaluation on *Leonotis leonurus* to validate its potential as an alternative anti-diabetic drug" is my original work and to the best of my knowledge, that it has not been submitted before for any degree or assessment in any other University, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

Date: 28 November 2021 **Signed:** ……

ACKNOWLEDGEMENTS

First and foremost, I want to thank God for giving me the strength and perseverance to see this work through despite the difficult challenges I faced during the completion of this dissertation. It was indeed not easy finalising this piece of work during a global pandemic, but "With God all things are possible" - **Matthew 19:26**.

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DEDICATION

To my family

Thozama, Sindiswa, and Solakha Sigodi

Whose presence in my life brings so much joy, courage, and motivation.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADR: Adverse Drug Reactions

13C-NMR: Carbon-13 nuclear magnetic resonance

1D-NMR: One-dimensional nuclear magnetic resonance

1H-NMR: Proton nuclear magnetic resonance

Cm: Centimetre

DCM: Dichloromethane

DMSO: Dimethyl sulfoxide

EDTA: Ethylenediaminetetraacetic acid

EtOAc: Ethyl acetate

Fig: Figure

G: Gram

G6P: Glucose-6-phosphate

G6PDH: Glucose-6-phosphate dehydrogenase

Hex: Hexane

HPLC: High-pressure liquid chromatography

IDF: International Diabetes Federation

L: Litre

MeOH: Methanol

Mg: Milligram

ML: Millilitre

Min: Minute

NADP+: Nicotinamide adenine dinucleotide phosphate

NADPH: Nicotinamide adenine dinucleotide phosphate hydrogen

Nm: Nanometre

NMR: Nuclear magnetic resonance

OCT: Organic Cation Transporter

SLC: Solute Carrier Transporter

Spp: Species

T2D: Type 2 Diabetes

T2DM: Type 2 diabetes Mellitus

TLC: Thin layer chromatography

UV: Ultraviolet

WHO: World Health Organization

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: LITERATURE REVIEW

1.1 Aim of the chapter

This chapter provides intensive literature on diabetes mellitus and type 2 diabetes mellitus which the study is focused on. It also summarizes the biological importance of the notable chemical constituents of *Leonotis leonurus* as applicable to this study. Previous studies that have been conducted on the use of available natural products from plants and their various applications are also summarized. Various benefits of medicinal and herbal plants and how they are used to treat different ailments in human beings are discussed. Finally, the chapter also highlights the traditional uses of *L. leonurus* including previous studies that have been reported on the extracts and various compounds that have been isolated from the plant.

1.2 Diabetes Mellitus

Diabetes mellitus (DM) is one of the four major non-communicable diseases worldwide (WHO, 2016). DM is a group of physiological dysfunctions characterized by hyper-glycemia resulting directly from insulin resistance, inadequate insulin secretion, or excessive glucagon secretion (Bilal *et al*., 2018; Goboza *et al.,* 2016). It is broadly classified into three categories, namely, Type 1, Insulin-dependent diabetes mellitus (IDDM), Type 2, Non-insulin-dependent diabetes mellitus (NIDDM) in which about 90 to 95% of diabetic patients suffer from, and Type 3; gestational diabetes which occurs during pregnancy because of dysglycemia (Mohammed *et al.,* 2015).

1.2.1 Diabetes mellitus and its prevalence worldwide

Diabetes Mellitus is an ailment that occurs when a person has high blood sugar levels in the body (Disanto *et al.,* 2015). This condition develops when the body fails to produce an adequate amount of insulin, thus resulting in the body being deprived of the energy it needs (Afolayan

& Sunmonu, 2019). Normally, a gland called the pancreas produces insulin which conveys sugar in the blood and into the cells; however, in diabetes, the pancreas fails to provide the body with enough insulin (Kangralka *et al.,* 2011). The increased blood sugar levels in the body for a prolonged period results in many symptoms which include amongst others; frequent urination, increased thirst and/or hunger, diabetic ketoacidosis, and hyperosmolar coma (Kitabchi *et al*., 2019). According to the World Health Organization (WHO), diabetes mellitus can be defined as a metabolic disease characterized by hyperglycemia, resulting from defects in insulin secretion (Kharroubi & Darwish, 2015). The pathogenesis of the disease also results from factors such as hyperlipidemia and enhanced oxidative stress (Kangralkar *et al.,* 2010). If not treated, diabetes may result in long term complications such as kidney failure, stroke, foot ulcers, diabetic heart disease, as well as damaged eye vision (Shoback, 2011).

Between the years 2012 and 2013, it was reported that about 1.5 to 5.1 million deaths occurred due to diabetes mellitus, thus making it the 8th leading cause of mortality worldwide (Pheiffer *et al*., 2018). The prevalence of diabetes was estimated to affect about 592 million people globally, by the year 2035 (Saeedi *et al*., 2019). The average age of diagnosis of diabetes was predictable to be 42 years and is believed to be due to the intake of high sugar and high calorie diet, lack of physical activity, lifestyle, and genetic predisposition (Tao *et al.*, 2015). A study by Wild *et al* (2014) estimated the prevalence of diabetes globally in the year 2000 and it was projected that by 2030 the number of persons with diabetes could rise from 171 million to 366 million worldwide as depicted in **Figure 1.1.** However, the increase in the occurrence of diabetes was estimated to be more conspicuous in developing countries; where the number of people with diabetes is expected to increase from 84 million to 228 million (Hossain *et al.,* 2009). This increase is believed to be due to environmental and lifestyle risk factors, such as

poor diet habits. Some of the risk factors which affect diabetes include obesity, family history, ethnicity, hypertension, or dyslipidemia, as well as physical inactivity (Alsafar *et al*., 2015).

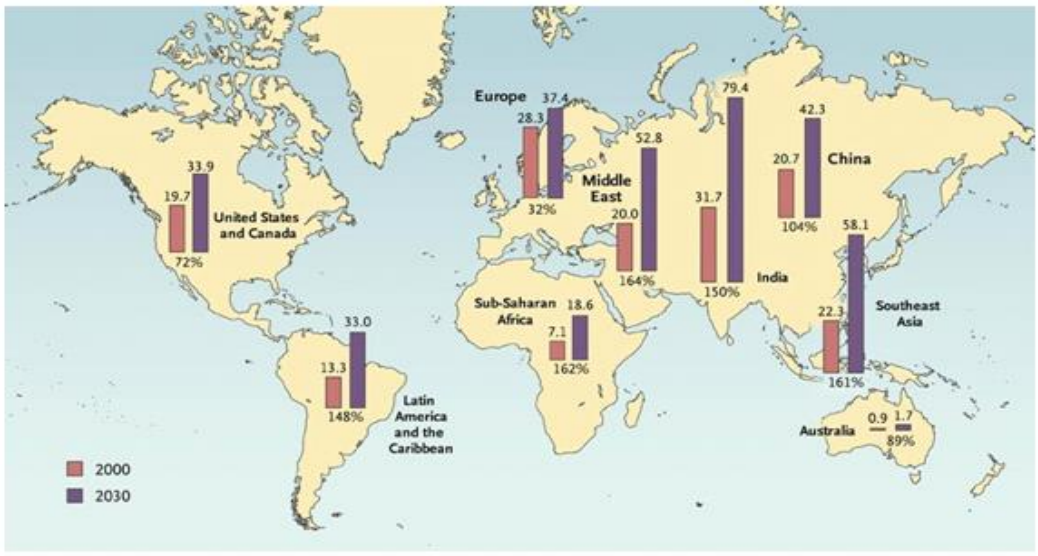


Figure 1.1: Cases of Diabetes in 2000 and projections for 2030, with estimated percent changes. Image retrieved from: https://www.nejm.org/doi/full/10.1056/nejmp068177

In 2013, a published report by the International Diabetes Federation Atlas (6th edition) stated that about 382 million people aged between 20 and 79 years globally were suffering from diabetes. Projections were made that this number may rise with a 10.1% global prevalence by the year 2035.

With diabetes being one of the growing clinical and public health problems globally (Herman, 2016); according to data released by the International Diabetes Federation (IDF) in 2015; approximately 415 million people had diabetes and this figure was projected to increase to 642 million by the year 2040. The burden of this disease has steadily increased over the past quarter century across the globe, with India contributing to a major part of the global burden (Pacanowski & Huang, 2016; Tandon, 2018). The overall diabetes burden estimates for the 1.3

billion population of India covers wide variations across the states of the country, many of which are comparable to large countries in terms of population (Tandon, 2018).

The Frequency of diabetes remains to rise worldwide and currently includes about half a billion people globally (Garg *et al.*, 2018). As depicted in **Figure 1.2**, the Middle East and North Africa region had the highest prevalence of diabetes; however, the Southeast Asia and Western Pacific regions were facing the cumulative prevalence of diabetes. It was also confirmed by WHO, that developing countries including those in Southeast Asia and Western Pacific region face cumulative prevalence of diabetes as compared to developed countries; with India and China facing the greatest challenges (Hossain *et al*., 2009; Ustulin *et al*., 2018). A study by Yang *et al* (2010) conducted in China concluded that diabetes is indeed a major health problem in the country and there are strategies aimed at preventing the occurrence and treating this disease.

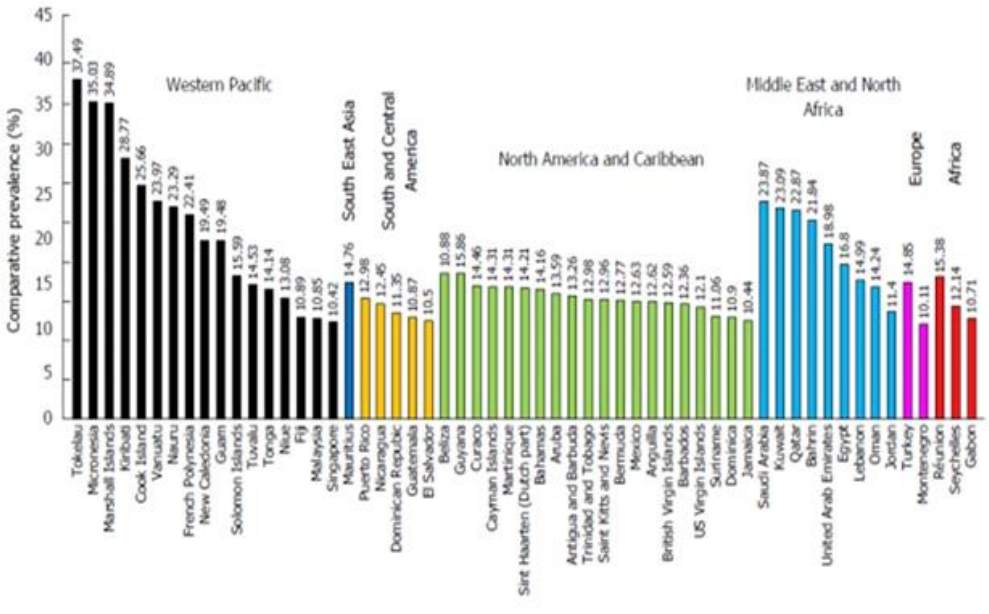


Figure 1.2: Comparative prevalence of diabetes in adults (aged between 20 and 79 years) in countries with high prevalence. (Data extracted from International Diabetes Federation Diabetes Atlas, 6th ed, 2013).

Data released by the Centers for Disease Control and Prevention (CDC) showed a slight increase in the prevalence of diabetes in the United States to be 9% with about 84 million prediabetic people (Bhutani, 2014). With the staggering increase in the number of people with diabetes, the financial burden of this disease is also huge (Zheng *et al.*, 2018); in 2015 it was estimated to be $673 billion globally and projected to increase to $802 billion by the year 2040 (Herman, 2016). In 2017, data released by IDF showed that diabetes was estimated to be 28% higher in people aged between 20 and 79 years old, than the IDF diabetes atlas publication from the year 2000 (Cho *et al.*, 2018).

The international diabetes federation also began to publish estimates of deaths caused by this disease. In Africa, westernization of lifestyles, environmental pollution etc., has resulted in diabetes becoming a significant cause of morbidity and mortality (Azevedo & Alla, 2008). In one of the published reports of five million deaths due to diabetes from 2015, over 321 100 occurred in the African region (**Figure 1.3**).

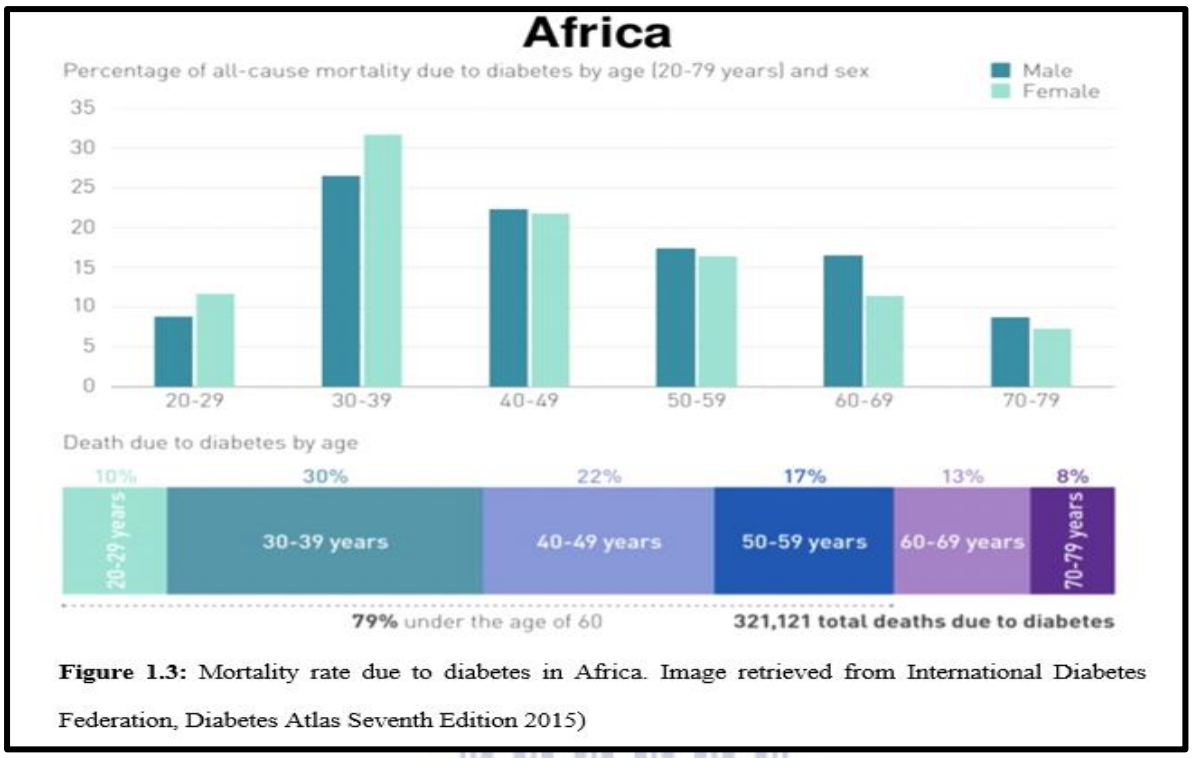


Figure 1.3: Mortality rate due to diabetes in Africa. Image retrieved from International Diabetes Federation, Diabetes Atlas Seventh Edition 2015)

The reports also revealed that 79% of these deaths in Africa occurred in persons under the age of 60 years, in sharp contrast to 47.3% globally (Foryoung *et al.*, 2017). In 2010, mortality rates attributable to diabetes in the sub-African region was estimated at 6%, with the highest rate occurring in persons aged between 20 and 39 years old (**Figure 1.4**).

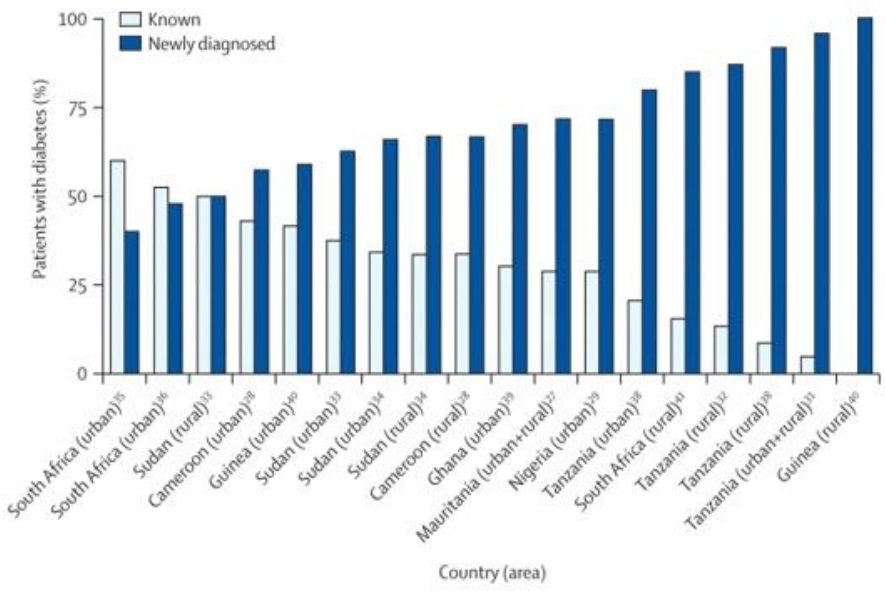


Figure 1.4: Prevalence of Diabetes in the Sub-African Region. Image retrieved from THE LANCET: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736 (10)60550-8/references

In a study by Mbanya *et al.* (2010), it was estimated that the number of people with diabetes in the African region would increase from 3 million to 8 million before the year 2025. This disease was found to be more frequent in urban areas than in rural areas of the African region (**Figure 1.4**), however, the risk factors for diabetes are like those in the other regions. Many Africans live in poverty, and therefore the cost of diabetes management is expensive in most countries. Africa has been reported to be the region where diabetes is growing fastest as compared to the rest of the globe (Pastakia *et al.*, 2017).

According to the World Health Organization, (2015); Diabetes in South Africa currently ranks second among the top ten leading natural causes of death, accounting for about 5.4% of deaths. In 2010, the Southern African Department stated that although only about 0.7% of the world's population resides in South Africa, the country has been reported to carry 6% of global diabetes cases. It is also estimated that another five million South Africans are prediabetes (Warnich *et*

al., 2011). With an adult population aged above 15 years old of close to 37 million, South Africa has an estimated number of 2.6 million people diagnosed with diabetes and a further 1.2 million people estimated to be living with undiagnosed diabetes. According to the International Diabetes Federation, in 2017 there were 1,826,100 diabetic cases in South Africa. The prevalence of diabetes in South Africa appears to be increasing over time; one recent study of urban black South Africans found that the prevalence increased from 8% in 1990 to 12.2% between 2008 and 2009. Urbanization and obesity were reported to be the main causes for this exaggerated increase in the prevalence of diabetes. In 2000, the burden of disease study group estimated that 5.5% of South Africans aged below 30 years had diabetes and that 4.3% of all deaths in South Africa were due to this disease (Webb *et al*., 2015). There are two types of diabetes mellitus, namely, type 1 and type 2 diabetes mellitus (Tuomi *et al.,* 2014).

1.2.2 Type 1 Diabetes Mellitus

Type 1 DM (**Figure 1.5**) is also known as Juvenile diabetes as it is most likely diagnosed in childhood, this type of diabetes is an autoimmune disorder leading to the destruction of pancreatic beta-cells (Katsarou *et al*., 2017). It occurs when the body's immune system attacks and kills the cells in the pancreas which produces insulin, thereby leaving the body without insulin and unable to regulate its blood sugar levels (Tuomi *et al*., 2014).

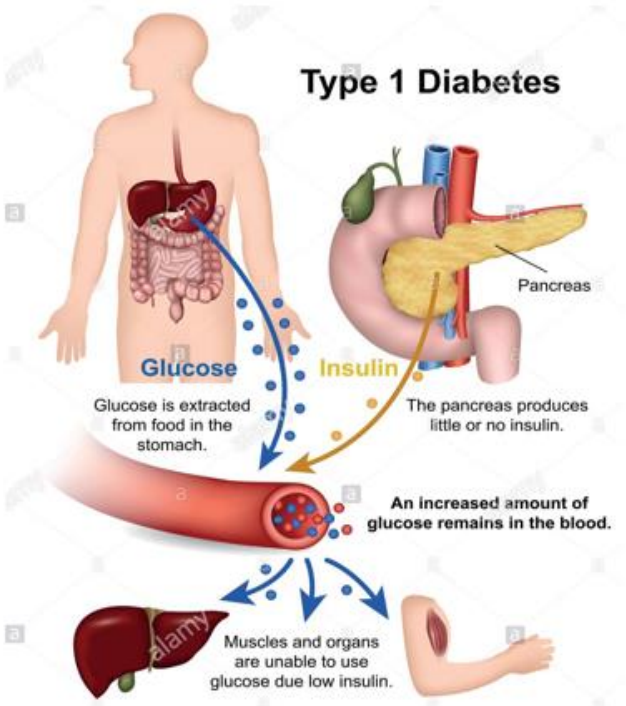


Figure 1.5: Illustration of the pathogenesis of Type 1 Diabetes Mellitus. Photo retrivied from: https://www.alamy.com/type-1-diabetes-medical-vector-illustration-with-english-description-image240966412.html

1.2.3 Type 2 Diabetes Mellitus

Type 2 diabetes (T2D), which is much more common, is primarily a problem of progressively impaired glucose regulation due to a combination of dysfunctional pancreatic beta cells and insulin resistance (**Figure 1.6**). In Type 2 DM, unlike type 1; insulin is produced by the body, it is just not used appropriately. Therefore, type 2 diabetes can be defined as a disease that occurs when the body cells are resistant to insulin (Di-Santo *et al.*, 2016). T2D affects the metabolism of carbohydrates, fats, and proteins due to a lack of insulin or insensitivity of target organs to insulin (Chatterjee & Davies, 2017; Odeyemi & Bradley, 2018).

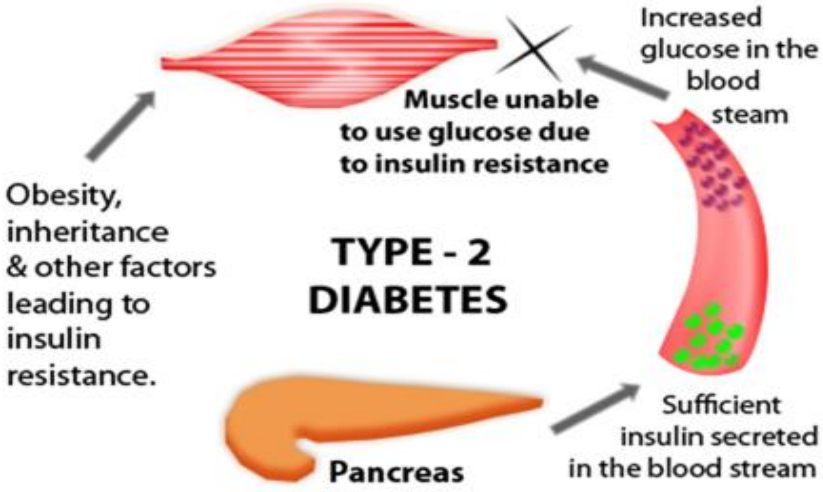


Figure 1. 6: Illustration of the pathogenesis of Type 2 Diabetes mellitus. Image retrieved from: http://www.health.am/db/more/risk-for-type-2-

There are many factors linked to insulin resistance that progresses to T2D, amongst others are obesity, ageing, b-cell dysfunction, tissue lipid accumulation, oxidative stress, endoplasmic reticulum stress (ER-stress) in beta cells, tissue inflammation, and physical inactivity (Akash *et al*., 2013). T2D is also correlated with high blood sugar levels, a condition where the body is incapable of managing the metabolism of glucose, which is the main energy source (Odeyemi & Bradley, 2018). Initially, there is a decline in glucose uptake by the liver, followed by an enhanced breakdown of fat as the body is starved from its energy source; thus, resulting in continual hyperglycemia, which eventually leads to beta-cell failure (Bankaru *et al*., 2016). Insulin is the primary hormone that controls the endorsement of glucose from the blood into most cells, including skeletal muscle cells and adipocytes. Insulin also serves as a major indicator for the transformation of glucose to glycogen for internal storage in the liver and skeletal muscle cells (Florez, 2017; Olokoba *et al.*, 2012). The pancreatic β-cells frequently produces insulin, irrespective of blood glucose levels. The insulin is then deposited within

vacuoles and released once triggered by an elevation of the blood glucose level. A decrease in the blood glucose level fallouts in a decline in the release of insulin from the β-cells and a rise in the release of glucagon from the α-cells, which encourages the transformation of glycogen to glucose. (Florez, 2017). Subsequent to a sudden fast, glucose is essentially produced by glycogenolysis and gluconeogenesis. Over time, high blood sugar levels lead to the appearance of symptoms and the risk of complications, such as microvascular and macrovascular complications (Vakhari *et al*., 2016).

T2DM and its complications have contributed tremendously to the burden of mortality and disability worldwide (Khan *et al*., 2020). The disease has reached epidemic levels and is increasingly becoming a global health problem (Zheng *et al.*, 2018). In the year 2015, the International Diabetes Federation (IDF) estimated that one (1) in eleven (11) adults between the age of 20 and 79 years (415 million adults) had diabetes mellitus globally (Cavan *et al*., 2017). By the year 2040, this figure is set to rise to 642 million people, with the largest increases coming from the regions experiencing economic shifts from low to middle-income levels (Ogurtsova *et al.*, 2017). Insulin resistance, a lack of insulin sensitivity, progressive pancreatic beta-cell failure, as well as hyperglycaemia have been known to be the underlying causes of T2DM (Odei-Addo *et al*., 2017). Additionally, long term consequences of T2DM include, among others, macrovascular and microvascular complications, when not treated. Macrovascular diseases include hypertension, hyperlipidaemia, heart attacks, coronary artery diseases, strokes, cerebral vascular diseases, and peripheral vascular diseases, while microvascular diseases include retinopathy and neuropathy (Chawla *et al.*, 2016; Papatheodorou *et al*., 2016; Chatterjee & Davies, 2017; Mohammedi *et al*., 2017). Currently, drugs such as sulfonylurea, meglitinides, metformin and thiazolidinedione are readily available in the market for the management of T2DM; however, they have shown to have unequal

efficacy amongst patients and have also been reported to have adverse drug reactions such as nausea, diarrhoea, abdominal pains, liver damage and heart failure (Florez, 2017). Considering the pathogenesis of T2DM, natural products have become the important resources of bioactive agents for drug discovery and have the potential to develop natural antidiabetic drugs with fewer side effects.

1.3 Management of Type 2 diabetes mellitus

To achieve good metabolic control in diabetes, changes in lifestyle and a combination of pharmacological treatment is essential. Achieving near normal glycated haemoglobin significantly decreases the risk of macrovascular and microvascular complications (Lipska *et al.,* 2013). Several drugs, both oral and injectable are available for the treatment of T2DM. With the treatment for patients with T2DM generally aimed at maintaining near-normal levels of glycaemic control, although diet and exercise are the first steps toward achieving treatment goals, 90% of patients with T2DM cannot maintain long-term glycaemic control with diet and exercise alone (Marín-Peñalver *et al.,* 2016). Therefore, anti-hyperglycaemic drugs are necessary for the management of T2DM. Several newer antidiabetic therapies such as meglitinides, α-glucosidase inhibitors, and insulin, target blood glucose spikes, and these agents should be considered increasingly in the long-term management of patients with T2DM.

1.3.1 Metformin

Metformin is the first line and most used drug to treat T2DM (Florez, 2017). This drug belongs to the group of biguanides; and functions by activating the adenosine monophosphate-activated protein kinase in the liver, causing hepatic uptake of glucose, and inhibiting gluconeogenesis through complex effects on the mitochondrial enzymes (Chaudhury *et al.,* 2017). The widespread use of metformin is attributed to the three fundamental qualities of good medicines

that it embodies; as it is safe, cheap, and effective as monotherapy, and in combination with other oral drugs (Burton *et al.,* 2015; Florez, 2017; Hostalek *et al*., 2015; Vakharia *et al*., 2016). The drug is beneficial to T2D patients as it promotes weight loss in an exercise-induced manner (Florez, 2017), it has also been shown to interrupt the development of T2D and decreases the risk of complications and mortality rates in patients by increasing hepatic glucose synthesis (Hostalek *et al*., 2015; Rines *et al.,* 2016; Zheng *et al.,* 2015). Additionally, the drug improves insulin sensitivity by activating the insulin receptor expression and enhancing tyrosine kinase activity (Umamaheswaran *et al.,* 2015). However, given its relative safety and beneficial effects on haemoglobin A_{1C}, weight, and cardiovascular mortality, the drug has been shown to be not equally effective in all patients, as some patients experience toxicities and/or adverse drug reactions (Du Plessis *et al.,* 2015, Thrasher, 2017). Studies have shown that only 40% of T2D patients benefit from its relative efficacy and achieve the optimal glycemic control (Florez, 2017); while other patients have been reported to experience gastrointestinal effects such as nausea, vomiting, diarrhoea, indigestion and abdominal cramps or bloating (McCreight *et al*., 2016). Due to these gastrointestinal side effects, nearly 5 to 10 % of patients cannot put up with metformin and end up discontinuing the drug (Bonnet & Scheen, 2017; Burton *et al.,* 2015; McCreight *et al*, 2016; Siavash *et al.,* 2017).

1.3.1.1 Mechanism and metabolism of metformin

Solute Carrier (SLC) transporters serve as facilitative transporters that mediate metformin uptake (Liang & Chen, 2015). It is known that metformin is a hydrophilic drug, and thus cannot easily traverse the lipid rich cell membranes of cells. To enter in and out of cells, it is thus transported via organic cation transporters in enterocytes, hepatocytes, and renal epithelial cells (**Figure 1.7**) (Florez, 2017; Vakharia *et al*., 2016). Organic cation transporters (OCTs) are active transporters that participate in the disposition of a variety of cationic substances

including endogenous amines and xenobiotics in tissues such as the liver, kidney, and placenta (Ahmed *et al*., 2016). Organic Cation Transporter 1 (OCT1), encoded by the *SLC22A1* gene, is responsible for the transport of metformin into the hepatic cells (Ulrike *et al*., 2015). OCT1 is extensively distributed in the kidney (Jacob *et al*., 2015).

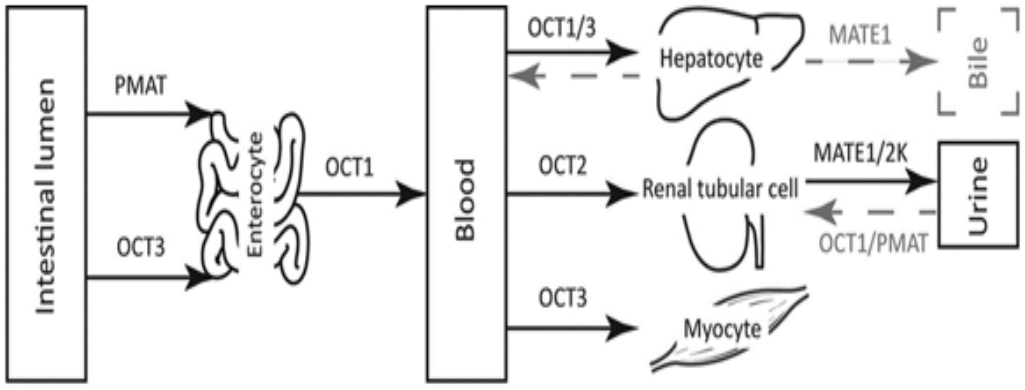


Figure 1.7: Representation of transporters that play a role in the absorption, hepatic uptake, and excretion of metformin. Image retrieved from https://journals.asm.org/doi/10.1128/AAC.01471-16

The physiological significance of the transporters depicted in grey is still unclear. PMAT is accountable for the uptake of metformin from the gastrointestinal tract, being far more abundant than OCT3. OCT1 and OCT3 together mediate the uptake of metformin in the liver, which is the most important site of action of metformin. The biliary excretion of metformin from the hepatocyte via MATE1 seems negligible in humans, and OCT1 and OCT3 probably also transport their substrates from the hepatocyte back into the blood. In the kidneys, OCT2 mediates the uptake of metformin into the proximal tubular cells, and MATE1 and MATE2K are responsible for the excretion of metformin into the urine (Van den Heuvel *et al.*, 2016).

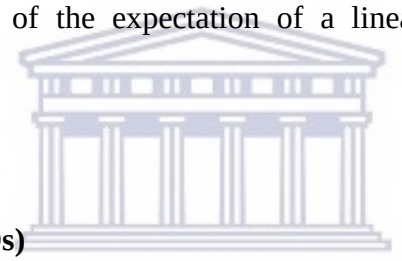
1.3.2 Sulfonylurea

Sulfonylureas are a classic first or second-line therapy for patients with T2DM, they have been utilized widely since their introduction to clinical practice in the 1950s. They are utilized as a reference to compare the efficacy and safety of other hypoglycaemic drugs excluding insulin. Gliclazide, Glimepiride and Glibenclamide are the main SUs currently used for T2D (Pollastro *et al,* 2015). These are hypoglycemia agents aimed at stimulating insulin release from pancreatic β- cells by binding to plasma membrane sulfonylurea receptor (SUR1). As a result, SUs persuade the closure of ATP-sensitive potassium (KATP) channel on the islet β-cell with the consequent depolarization of the *β*-cells membrane. This leads to the increased access of Ca^{2+} ions and a subsequent rise in intracellular calcium levels, causing an insulin release from *β*-cells (Rendel, 2014). Studies have reported that sulphonylureas have the ability to improve the defective insulin secretion in T2D patients. Nevertheless, long-term treatment has repeatedly shown a progressive decrease in SUs effectiveness, this could be a result of a progressive lack of the insulin-producing capacity of pancreatic *β*-cells. Additionally, SUs have proven to be particularly beneficial when combined with metformin, which decreases the extent of insulin resistance (Vella, 2019). Unfortunately, regardless of the wide use of these drugs in clinical practice, different side effects such as weight gain and increased risk of hypoglycemia, have been frequently observed in T2D patients.

1.3.2.1 Glibenclamide

Glibenclamide belongs to the second generation of sulfonylureas, which has shown to have a higher frequency of hypoglycemia than the other agents (Rambiritch *et al*., 2014). The drug stimulates insulin secretion from pancreatic beta cells and possesses an extensive or prolonged period of action and metabolites with the activity of hypoglycaemic (Sola *et al.,* 2015; Kalra & Gupta., 2015). Glibenclamide-induced hypoglycaemic occurs mainly in adults, this is due to

their unbalanced eating habits and renal damage. It has contentious cardiovascular effects, including effects on ischemic preconditioning and in acute coronary syndromes (Sola *et al*., 2015). Studies have provided evidence suggesting a non-linear relationship between sulfonylurea dose and glycaemic-lowering effects. Another study conducted by Groop *et al* (2010) concluded that glibenclamide is effective within a narrow range of plasma concentrations which can be reached with very low doses of the drug. The study was aimed at examining the association between plasma glibenclamide concentrations, response to insulin, as well as glucose metabolism during glycaemic and hyperglycaemic conditions. These observations suggest that increasing doses beyond a critical level in each patient may paradoxically worsen glycemic control. However, glibenclamide continues to be used in high doses, most probably because of the expectation of a linear dose-response relationship (Rambiritch *et al*., 2014).



1.3.3 Thiazolidinediones (TZDs)

Thiazolidinediones, also known as glitazones are anti-diabetic drugs intended to improve metabolic control in patients with T2D through the development of insulin sensitivity (Soccio *et al*., 2014). TZDs were reported as insulin sensitizing drugs in the 1980's, the common TZDs are Rosiglitazone, Troglitazone and Pioglitazone. Their mechanism involves improving the function of fat to safely store lipids thus resulting in decreased free serum fatty acids, decreased ectopic acids and less insulin resistance (Fiorenza *et al*., 2011). These drugs act by activating their molecular target, known as nuclear peroxisome proliferator-activated receptors (PPARs) in adipose tissue. These anti-diabetic agents have shown to have the potential to benefit the full insulin resistance syndrome associated with T2D, therefore, they also have potential benefits on the secondary complications of T2D such as cardiovascular disease (Hauner, 2012). Rosiglitazone and Troglitazone have been shown to improve glycaemic control and may act to

slow the progression of β-cell failure (Yakoi, 2010). However, these drugs have been frequently described to cause significant side effects including liver damage and also possess increased cardiovascular risk associated with their administration, therefore have been withdrawn from sale worldwide (Pollastro *et al*, 2015; Yakoi, 2010). Rosiglitazone has been found to increase the risk of congestive heart failure in T2D patients (Saraogi *et al*., 2011)

1.3.3.1 Mechanism of action of TZDs

The mechanism of TZDs involves binding to peroxisome proliferator-activated receptors (PPARs), a nuclear transcription factor found in humans, including adipocytes, to promote adipogenesis and fatty acid uptake. The concentration of PPAR gamma is increased in the skeletal muscle of obese and diabetic patients (Marín-Peñalver *et al*., 2016). Rosiglitazone is purely a PPAR gamma agonist, while pioglitazone has also some PPAR-alpha effects; therefore, they have different effects on lipids. Pioglitazone produces a more favourable lipid profile; the LDL-cholesterol remains constant during treatment while rosiglitazone raises them; in addition, decreased more triglyceride levels than rosiglitazone. HDL cholesterol increases 10% with both. TZD also may improve blood glucose levels by preserving pancreatic beta-cell function (Semiz *et al*, 2014). In efficacy, they are most probably like metformin in monotherapy, but they are not usually chosen due to their adverse effects and costs. By reducing circulating fatty acid concentrations and lipid availability in the liver and muscle, these drugs improve the patients' sensitivity to insulin and reduce hyperglycaemia. In addition, TZDs result in other therapeutic effects including anti-inflammatory effects and amelioration of hypertension, microalbuminuria and hepatic steatosis. TZDs are a normal target of PPARG because they are selective agonists for PPARG2 that is expressed in the adipose tissue. However, they have little activity with PPARG1 and PPARG3 (Topic, 2014, Pollastro *et al*, 2015).

1.3.4 Alpha-glucosidase Inhibitors

Alpha glucosidase inhibitors (AGIs) are a unique class of anti-diabetic drugs. These oral drugs are enzyme inhibitors that do not have a pancreato-centred mechanism of action (Karla, 2014). They are enzyme complexes located in the brush border membrane of the small intestine and hydrolyse oligosaccharides into monosaccharides. The inhibition of these enzymes postpones carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise. (Oboh *et al*., 2013). Acarbose, miglitol and voglibose are the three currently available agents under this group (Dirir *et al.,* 2021). They have different properties as compared to other antidiabetic therapies, due to their exclusive mode of action. Acarbose has been used as a treatment for hyperglycaemia for more than two decades (Rosak & Mertes., 2012). The alpha-glucosidase inhibitors reduce postprandial triglycerides but their effect on LDL and HDL cholesterol levels and fasting triglycerides is insignificant and inconsistent. Alpha-glucosidase inhibitors rarely induce hypoglycaemia because these agents do not stimulate insulin release, and do not significantly affect body weight. Acarbose has been demonstrated to have beneficial effects by reducing the risk of cardiovascular disease and slowing the progression to diabetes in patients with impaired glucose tolerance. (Standl *et al*., 2014).

1.3.5 Complications of T2D if not managed

The disease is related to the epidemic of obesity and sedentary lifestyles (Chatterjee & Davies, 2017). As it progresses, it may lead to long term consequences which include, among others, microvascular and/or macrovascular complications (Odei-Addo *et al.,* 2017). Macrovascular diseases include hypertension, hyperlipidemia, heart attacks, coronary artery disease, strokes, cerebral vascular disease, and peripheral vascular disease, while macrovascular diseases include Retinopathy and neuropathy (Chawla *et al*., 2016; Papatheodorou *et al*., 2016;

Chatterjee & Davies, 2017; Mohammedi *et al*., 2017). Elderly people with diabetes are more prone to the risk of developing macrovascular and microvascular complications, as compared to younger patients with diabetes. Other complications include kidney failure, leg amputation, visual loss, foot ulcers and cardiovascular diseases (Kleinberger and Pollin, 2015). These complications are responsible for increased morbidity, disability, and mortality, thus being a threat to the economies of many countries, particularly developing countries (Brownrigg *et al.*, 2016; Chatterjee & Davies, 2017). T2DM is also associated with cancer, as well as physical and mental disorders. In addition, it has been projected that more than 68% of adults diagnosed with diabetes over 65 years died of some form of coronary heart disease (CHD), whereas 16% died of a stroke. Therefore, diabetic patients are considered lifelong users of healthcare services once diagnosed with diabetes. This is due to the chronic and complex nature of diabetes.

1.4 Natural products and drug delivery

Natural products have gained approval worldwide for promoting healthcare, as well as disease prevention (Sofowora *et al*., 2013). The use of natural products as medicine has been defined in history as the form of traditional or herbal medicine, remedies, and potions, with many of these bioactive natural products still being unknown (Dias *et al*., 2012). To date, natural products have been the most successful source of potential drug leads due to their long-standing and critical role in drug discovery and development (Lahlou *et al.*, 2013). Plants have been used for medicinal purposes for thousands of years long prior to the introduction of chemical medicines, they have evolved to produce unique and structurally diverse secondary metabolites which have the potential to protect humankind from many diseases (Jamshid-Kia *et al*., 2018; Yuan *et al*, 2016). Their ethnopharmacological properties serve as the primary source of medicines for the early discovery of newly safe therapeutic agents. Many of the traditional herbal plants today are used by an estimated population of over 3.3 billion in developing

countries to treat a wide range of illnesses (Mahomoodally, 2013). Further evidence of the importance of natural products is provided by the investigations of medicinal plants as potential medicines which have led to the isolation of many phytochemicals that have become well known in pharmaceuticals (Dias *et al*., 2012). Herbal products, apart from their traditional and cultural significance, are generally regarded as safe, more accessible, and affordable (Ekor, 2014). Therefore, the trend to integrate traditional western medicine, especially in the primary health care setting is becoming increasingly important and will help to combat the global health challenges (Thomford *et al*., 2018).

1.5 General overview of the use of plants for disease treatment

Plants have been extensively used since ancient times as food, dietary supplements, and medicines due to their huge traditional and floral biodiversity health benefits (Yuan, 2016; Mirhoseini *et al*., 2013; Jamshidi-Kia *et al*., 2018,). Africa is a rich source of medicinal plants with South Africa being known as a country with a strong history of traditional healing (Tuasha *et al.,* 2018). South Africa is home to over 30 000 species of higher plants, out of which 10% of these plant species are used in traditional medicine to treat a variety of ailments (Van Wyk & Prinsloo, 2020). Medicinal plants can be used as crude extracts or standard, and enriched fractions in pharmaceutical preparations. These plants are known to possess therapeutic properties owing to various essential bioactive oils being present, and secondary metabolites such as phenolics, flavonoids, alkaloids, tannins, and terpenoids etc., (Mazimba, 2015; Yuan, 2016); which have guaranteed biological activity in the human body (Babiaka *et al*., 2015; Rehab & Amira, 2018). Among them, abietane diterpenes have been reported to demonstrate extensive promising biological activities, including diabetes (Etsassala *et al*., 2020). The isolation of plant secondary metabolites began in the nineteenth century, however at that time technology was the limiting factor in the structural elucidation of compounds (Atanasov *et al*.,

2015). The methods used were traditional and comprehensive degradative methods, and the derived structures were confirmed over the synthesis and biological activity determinations (Hanson, 2017). Following the history of the isolation of compounds, there have been several local and international initiatives actively exploring the plant resources of Southern Africa with the intention to screen indigenous plants for pharmacologically active compounds (Street & Prinsloo, 2013). Over the last 2500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practised more in the eastern continent. These traditions are still flourishing, since; approximately 80% of the people in developing countries rely on them for their primary health care needs (Mamun-Rashid *et al.*, 2014). With technological advancements, studies aimed at defining chemical profiles and compositions of medicinal plants to reveal the density of the varying compounds all contributing to the treatment of numerous ailments and diseases are being conducted (Altemimi *et al*., 2017).

In the 21st century, natural products represent more than 50% of all drugs in clinical use (Thomford *et al.*, 2018). One plant can be used for many purposes, for example, *Andrographis paniculate* also known as a king of bitter is recorded to be useful in the treatment of malaria, fever, diabetes, and as worm killers (Okhuarobo *et al*., 2014). On the other hand, many plants are also found to be useful for the treatment of specific diseases (Patel, 2015). Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusive of plant origin and a significant number are synthetic drugs obtained from natural products (Veeresham, 2012). Examples of important drugs obtained from plants are digoxin from *Digitalis spp*., quinine, and quinidine from *Cinchona spp*., vincristine, and vinblastine from *Catharanthus roseus*, atropine from Atropa belladonna and morphine and codeine from *Papaver somniferum* (Boy *et al.*, 2018). Malaria is the world's most important parasitic disease, a good example of anti-malarial treatment is quinine, isolated from Cinchona bark, which has

been used as a template for the synthesis of chloroquine and mefloquine (Rasool *et al*., 2020). Plant-derived substances are essential to identify to establish potential treatments and to help resolve the multi-drug resistant predicament (Altemimi *et al.,* 2017; Singh, 2015).

1.6 Medicinal plants with cardiovascular effects

Tectona grandis, for example, was investigated in a study by Ghaisas *et al* (2009) and was found to contain terpenoids amongst other compounds, thus supporting findings that the mechanism of action of terpenoids and steroids is to stimulate insulin secretion thereby increasing the sensitivity of islet to promote glucose uptake in pancreatic beta cells. **Table 1.1** below represents a list of documented medicinal plants with antidiabetic properties used in South Africa (Afolayan & Sunmonu, 2010).

Table 1.1: Documented antidiabetic plants used in South African herbal medicine

Species	Family	Parts used	References
Artemisia afra. Ex Wild	Asteraceae	Leaves, roots	Erasto *et al.*, 2005 Thring and Weitz, 2006 Van Wyk, 2008b
Brachylaena discolor DC	Asteraceae	Leaves, Leaves, roots, stem	Erasto *et al.*, 2005 Van de Venter *et al.*, 2008
Brachylaena elliptica Thumb	Asteraceae	Leaves	Van Wyk, 2008a
Bulbine natalensis Mill	Asphodelaceae	Roots	Erasto *et al*., 2005
Bulbine frutescens L.	Asphodelaceae	Roots	Erasto *et al.*, 2005
Cannabis sativa L.	Cannabaceae	Leaves	Van de Venter *et al.*, 2008
Catha edulis Forrsk. Ex Endl.	Celastraceae	Leaves, stems, roots	Van de Venter *et al.*, 2008
Catharanthus roseus (L) G.Don.	Apocynaceae	Leaves Leaves, twigs	Erasto *et al.*, 2005 Van de Venter *et al.*, 2008

Chilianthus olearaceus Burch.	Asteraceae	Leaves, twigs	Erasto *et al.*, 2005
Chironia baccifera L.	Gentianaceae	Whole plant	Van de Venter *et al.*, 2008
Cissampelos capensis L.F	Menispermaceae	Leaves	Van de Venter *et al.*, 2008
Conyza scabrida DC.	Asteraceae	Leaves	Thring and Weitz, 2006
Elytropapus rhinocerotis (L.F)	Asteraceae	Leaves	Thring and Weitz, 2006
Galium tomentosum Thunb.	Rubiaceae	Roots	Van Wyk *et al.*, 2008
Herichrysum nudifolium L.	Asteraceae	Leaves, roots	Erasto *et al.*, 2005
Herichrysum odoratissiumum L.	Asteraceae	Whole plant	Erasto *et al.*, 2005
Herichrysum petiolare H & B.L.	Asteraceae	Whole plant	Erasto *et al.*, 2005
Heteromorphica arborescens H.	Apiaceae	Leaves, roots	Erasto *et al.*, 2005
Hypoxis colchicifolia Bak.	Hypoxidaceae	Corms	Erasto *et al.*, 2005
Hypoxis hemerocallidea Fisch.	Hypoxidaceae	Corms	Erasto *et al.*, 2005
Leonotis Leonurus L.	Lamiaceae	Leaves, flowers	Thring and Weitz, 2006
Momordica balsamina L.	Cucurbitaceae	Steam, flowers	Van de Venter *et al.*, 2008
Momordica foetida Schumach.	Cucurbitaceae	Whole plant	Van de Venter *et al.*, 2008
Petroselenium crispum (Mill)	Apiaceae	Leaves	Thring and Weitz, 2006
Psidium guajava L.	Myrtaceae	Leaves, roots	Van de Venter *et al.*, 2008
Ricinus communis L.	Euphorbiaceae	Leaves Leaves	Thring and Weitz, 2006 Van Wyk , 2008a
Ruta graveolens L.	Rutaceae	Leaves	Thring and Weitz, 2006
Sclerocarya birrea Hochst.	Anacardiaceae	Stem, bark, roots Stem, bark	Van de Venter *et al.*, 2008 Van Wyk, 2008a
Sutherlandia frutescens L.	Fabaceae	Leaves	Van Wyk, 2008b
Vinca major L.	Apocynaceae	Leaves, roots, stem	Van de Venter *et al.*, 2008
Vernonia oligocephala Sch. Bip.	Asteraceae	Leaves, twigs, roots Leaves	Erasto *et al.*, 2005

Vernonia amygdalina Del.	Asteraceae	Leaves	Thring and Weitz, 2006 Erasto *et al*., 2005

1.7 Lamiaceae Family

Lamiaceae, formerly called Labiatae or the mint family is one of the most important herbal families which incorporates a wide variety of plants with biological and medical applications. It has 236 genera and more than 7000 species (Li *et al*., 2016). Plants in this family, are herbs or shrubs often with an aromatic smell. They are common in the Maltese Islands and other Mediterranean countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. Lamiaceae is distributed nearly worldwide, and many species are cultivated for their fragrant leaves and attractive flowers (Raja, 2012; Napoli *et al*., 2020). The family is particularly important to humans for herbal plants which are useful for flavours, fragrance, or medicinal properties. Some examples from this family include mints, thyme, Tulsa, spearmint, and coleus. Plants belonging to this family have been shown many biological activities such as antioxidant, cytotoxic, anti-inflammatory, antibacterial, antifungal, antiviral, analgesic, cardiovascular, hypoglycaemic, hypolipidemic, antispasmodic, antiepileptic, anti-anxiety, and anti-angiogenic (Hamed *et al.,* 2021).

1.8 *Leonotis leonurus* description and distribution

Leonotis leonurus is a shrub indigenous to tropical Africa and Southern India (Odei-Addo *et al*., 2017). In South Africa, it is commonly found at forest margins, on rocky hillsides and riverbanks, and in tall grasslands of the Eastern and Western Cape Provinces, Kwazulu-Natal, and Mpumalanga (**Figure 1.8 B**). It is easily identified as it rises above the shrubbery mass throughout the summer season (Mazimba 2015). *L. leonurus* is one of the most prominently

used traditional medicinal plants in South Africa and has been documented for use in treating numerous ailments. It is commonly known as “Lion’s ear” or “Klip dagga”, other common names are listed in **Table 1.2**. The plant has a thick wooden base, pale brown branches and grows from two to five meters in height (Nsuala *et al*., 2015). The leaves are hairy, long, and narrow with serrated upper edges, and are arranged opposite each other on the stems. The flowers are bright orange in colour and tubular in shape and are arranged in circles along branch ends. The hairy flowers bear a resemblance to lion’s ears, hence the name “leonurus”.

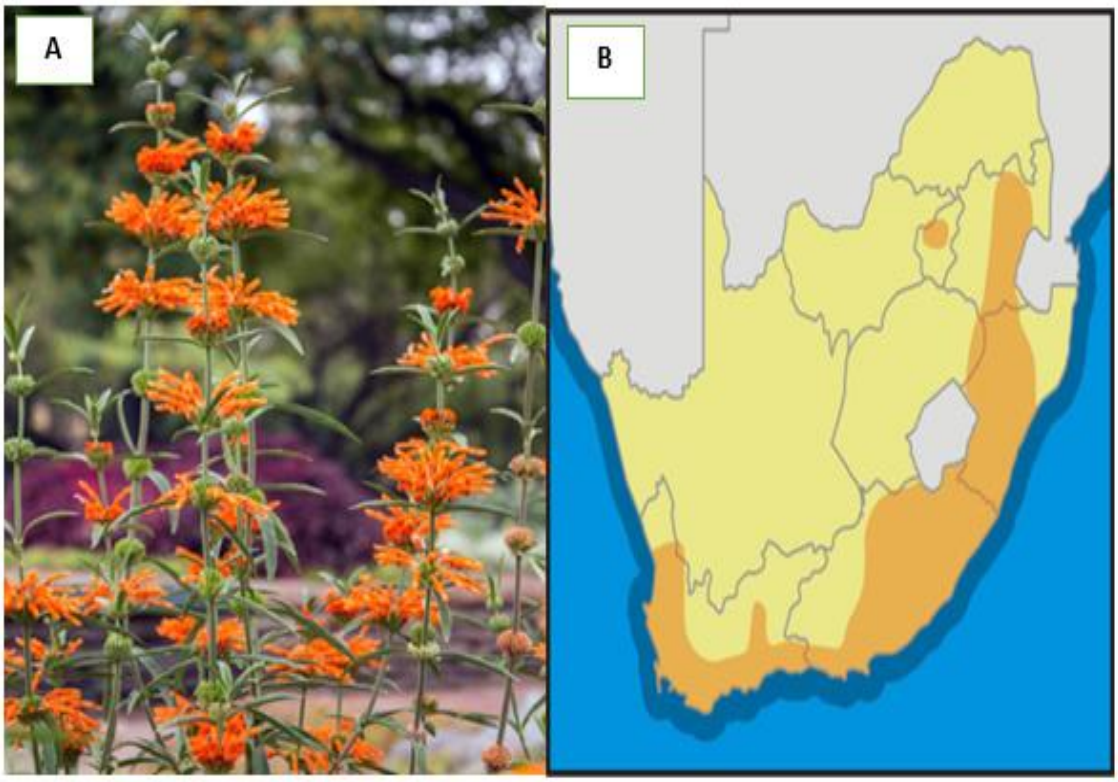


Figure 1.8: *Leonotis Leonurus* description **(A)** and distribution along South African costal area **(B)** (Image retrieved from Nsuala *et al*., 2015)

Table 1.2: *Leonotis leonurus* common names (Nsuala & Viljoen., 2015)

Language	Name(s)
English	"Lion's ear", Klip dagga
Afrikaans	Wilde dagga, Rooi dagga , duiwelstabak
IsiXhosa	Umfincamfincane
IsiZulu	Umunyane
Sesotho	Lebake

1.9 Why investigate *Leonotis leonurus*?

Leonotis leonurus; a member of the Lamiaceae family, is a shrub indigenous to Southern Africa and Southern India (Odei-Addo *et al.,* 2017). In South Africa, it is commonly found at forest margins, on rocky hillsides and riverbanks, and in tall grasslands of the Eastern and Western Cape Provinces, Kwazulu-Natal, and Mpumalanga. It is easily identified as it rises above the shrubbery mass throughout the summer season (Nsuala & Viljoen, 2015). This plant is commonly known as klip dagga or lion's ear and has been extensively studied (Mazimba 2015). It has a wide variety of medicinal uses, for example, it is used for the treatment of cold, bronchitis, tuberculosis, coughs, asthma, feverish headaches, dysentery, and chest infections. This plant is used in South Africa to treat diabetes (Odeyemi & Bradley, 2018). It has shown potential biomedical applications in a great diversity of ailments and has shown to have antidiabetic activity; with the most common herbal active ingredients used in treating diabetes being flavonoids, tannins, phenolic, and alkaloids (Hussein, 2018).

1.10 Medicinal uses of *Leonotis leonurus*

Leonotis leonurus claims many documented traditional uses. For many years, the plant has been widely used, both topically and orally, for the treatment of various ailments by southern African traditional healers (Hussein, 2018). The Aerial parts of the plant, both leaves and flowers, are administrated in various ways such as decoction, infusion or smoking. The Khoikhoi population was fond of smoking it instead of tobacco, the dried leaves and flowers are smoked and are believed to relieve epilepsy. They have been reported to produce a mild ecstatic effect when smoked and have been said to have a similar, although less potent, psychoactive effect to cannabis (Narukawa *et al*., 2015; Nsuala *et al.*, 2017).

A short review of *L. leonurus* as a herbal medicine has been published by Mazimba (2015), in which traditional uses of this plant species are stated concisely. These traditional uses include treatment of conditions such as eczema, skin rashes, boils, itching, muscular cramps, headache, epilepsy, chest infections, constipation, spider, and snake bites. Other studies have shown additional medicinal benefits of this plant species such as being a remedy for coughs, hypertension, jaundice, diarrhoea, tuberculosis, viral hepatitis, high blood pressure and diabetes mellitus. A decoction of *L. leonurus* is administered orally to treat coughs, colds, and bronchitis. It has also been used to relieve cardiac complaints and asthma. Powdered leaves are compounded into an ointment and applied topically for relief of pain above the eye. It has also been reported that a decoction of the powdered stem or seed is administered orally for the relief of haemorrhoids and used topically as a lotion for sores on the legs and head (Nsuala *et al*, 2015).

Previous studies have shown that the leaf extract of this plant contains hypoglycemic effects in a streptozotocin-induced diabetic rat model. The aqueous extracts from the leaves of *L.*

leonurus have been reported to prevent many free-radical-related diseases because of the presence of compounds such as phenolics, flavonoids, flavonols and proanthocyanidins in the extract (Oyedemi & Afolayan, 2011). A study by Ojewole (2003) reported that the aqueous leaf extract of *L. leonurus* possessed ant nociceptive, anti-inflammatory, as well as hypoglycaemic activity. Similarly, Oyedemi & Afolayan (2011) demonstrated that oral administration of aqueous extract of *L. leonurus* leaves has an anti-lipidemic and anti-hyperglycaemic effect which can reduce the blood glucose levels by potentiating insulin secretion. In addition to the traditional uses of *L. leonurus* mentioned above, other traditional uses of the plant species are highlighted in **Table 1.3** below.

Table 1.3: Traditional uses, preparation, and mode of administration of *Leonotis leonurus.*

Conditions	Plant material used/method	Administration route	References
Spider and snake bites, scorpion stings	Leaves and flowers prepared as an infusion	Oral administration	Bryant (1996) Jager *et al.* (1996) Van Wyk *et al.* (2000) Stafford *et al.* (2008)
Haemorrhoids	Powdered stem and/or seeds prepared as a decoction	Oral administration of the decoction	Watt and Breyer-Brandwijk (1962)
Respiratory conditions such as cough, colds, bronchitis, influenza, and asthma	Leaves prepared as a decoction or infusion	Oral administration or inhalation	Bryant (1966), Hutchings *et al*. (1996) ,Jäger *et al.* (1996), Duke (2001), Van Wyk *et al.* (2000) Stafford *et al.* (2008) Watt and Breyer-Brandwijk (1962)
Fever	Leaves and flowers prepared as a decoction	Oral administration	Jager *et al.* (1996) Van Wyk *et al.* (2000)
Tuberculosis	Leaves and flowers prepared as an infusion	Oral administration	Stafford *et al.* (2008) Hutchings *et al*. (1996)

Leprosy	Leaves and flowers prepared as a decoction	Oral administration and inhalation	Duke (2001)
Viral hepatitis	Leaves prepared as an infusion	Oral administration	Watt and Breyer-Brandwijk (1962)
Headache	Leaves and flowers prepared as an infusion	Oral administration	Jäger *et al*. (1996)
Headache with fever	Cold infusion of leaves	Nasal douche	Watt and Breyer-Brandwijk (1962)
Pain above the eye	As an ointment made from powdered leaves	Topical application of the ointment	Hutchings *et al.* (1996)
Epilepsy, partial paralysis	Leaves and flowers prepared as an infusion	Oral administration	Watt and Breyer-Brandwijk (1962), Jäger *et al*. (1996), Van Wyk *et al.* (2000) Stafford *et al.* (2008)
Cardiovascular system, Cardiac conditions, Hypertension	Leaves prepared as a decoction or infusion	Oral administration	Hutchings *et al.* (1996), Duke, (2001), Jäger *et al.* (1996) Van Wyk *et al.* (2000) , Stafford *et al.* (2008)
Muscular cramps	Leaves prepared as a decoction	Oral administration	Scott *et al.* (2004)
Gastrointestinal conditions, Dysentery	Leaves and flowers prepared as an infusion	Oral administration	Watt and Breyer-Brandwijk (1962), Jäger *et al.* (1996) Van Wyk *et al.* (2000) Stafford *et al.* (2008)
Anthelmintic: Vermifuge, intestinal worms	Leaves prepared as a decoction	Rectal administration as an enema, oral administration of an infusion	Watt and Breyer-Brandwijk (1962), Jäger *et al.* (1996), Van Wyk *et al.* (2000), Stafford *et al.* (2008)
Constipation	Leaves and flowers prepared as an infusion	Oral administration	Watt and Breyer-Brandwijk (1962)
Delayed menstruation, emmenagogue	Leaves and flowers prepared as an infusion	Oral administration	Jäger *et al.* (1996) Van Wyk *et al.* (2000) Stafford *et al.* (2008)
Jaundice/hepatitis	Leaves and flowers prepared as an infusion	Oral administration	Hutchings *et al.* (1996)
Diuretic	Leaves brewed as a decoction	Oral administration	Van Wyk *et al*. (2000) Hutchings *et al.* (1996) Watt and Breyer-Brandwijk (1962)

Obesity	Leaves brewed as a decoction	Oral administration	Van Wyk *et al*. (2000)

1.11 Active constituents of *Leonotis leonurus*

Many phytochemical studies have been conducted on *L. leonurus* and the curiosity about this plant continues to be high due to its numerous reported biological activities. The phytochemical studies performed on *L. leonurus* extracts shows that it contains tannins, flavonoids, sterols, diterpenoids, triterpenoids, alkaloids, quinines and saponins. Phytochemical studies have shown that terpenoids such as mono sequined diterpenoids, which are known to be biologically active are the main compounds found in this plant species (Narukawa *et al.*, 2015). It also contains a significant number of compounds including leonurine; a mildly psychoactive alkaloid isolated from *Leonotis. Sibricus*, as well as volatile oil, and several unusual diterpenoids (labdane type lactones) (Nsuala *et al.*, 2017). The essential oils have a high content of monoterpenoids and sesquiterpenoids, showing significant antimicrobial activities. *L. leonurus* is also known to contain marrubin which is a labdane type lactone also found in Marrubium vulgare, C-13 epimeric premarrubiin which is a diterpene spiro ether, and two labdane terpenoids known as compounds X and Y (Mazimba, 2015). The plant also contains different flavonoids, diterpenoids, polyphenolics, and other chemical constituents that may be involved in the antinociceptive, anti-inflammatory, and antidiabetic effects (Narukawa *et al*., 2015).

1.12 Study Rationale

T2DM is a major global health problem owing to its intensely cumulative occurrence, with over 400 billion people being affected worldwide (Pheiffer *et al.*, 2018). Currently, available therapies for diabetes include insulin and various oral hypoglycaemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. However, only 40% of

T2DM patients are reported to be benefiting from the relative effectiveness of the currently used antidiabetic drugs while other patients have been reported to experience adverse side effects such as liver problems and lactic acidosis; and gastrointestinal effects such as nausea, vomiting, diarrhoea, indigestion and abdominal cramps or bloating (McCreight *et al*, 2016). Due to these side effects, nearly 5 to 10 % of patients cannot put up with the drugs and end up discontinuing their treatment (Bonnet & Scheen, 2017; Siavash *et al*., 2017). The limitation of available antidiabetic agents in terms of efficacy or safety coupled with the emergence of the disease into the global epidemic has encouraged alternative therapy for the management of diabetes with more safety and efficiency. Reflecting on the pathogenesis of T2DM, natural products have become the important resources of bioactive agents for anti-T2DM drug discovery. *Leonotis leonurus* is used traditionally for the treatment of diabetes in SA and has been reported to have antidiabetic properties (Odei-Addo *et al*., 2017); with the most common herbal active constituents used in treating diabetes being flavonoids, tannins, phenolic, and alkaloids. The presence of these compounds implies the significance of screening the plant species to help contribute to the recovery of the function of pancreatic Beta-cells and an increase in insulin secretion. It is for this reason that the South African *Leonotis leonurus* species, widely distributed and readily available for human exploration for its potential application as an antidiabetic remedy was selected for this study.

1.13 Aim(s) of the study

The aim of this study is to 1) Isolate the chemical constituents of *Leonotis leonurus*, and 2) biologically evaluate the total extract and isolated compounds for their potential antidiabetic (glucose uptake activity and alpha glucosidase) and cytotoxic activities.

1.14 Objectives

The main objectives of the study are:

- Collection and identification of *L. leonurus*
- Extraction of the plant material with methanol
- Isolation of pure compounds using chromatography techniques.
- Elucidation of chemical structures of bioactive compounds isolated from plant species
- Investigation of glucose uptake activity and cytotoxicity, as well as alpha-glucosidase inhibitory activity of the crude extract and isolated compounds

CHAPTER 2: METHODOLOGY

CHEMICAL CHARACTERIZATION OF *L. LEONURUS* CONSTITUENTS

2.1 General experimental procedures

2.1.1 Reagents and equipment

Solvents, apparatus, and workspace were made available at the chemistry department of the Cape Peninsula University of Technology (CPUT). Organic solvents such as methanol, ethyl acetate, dichloromethane, hexane, acetone, and vanillin were supplied by Sigma-Aldrich (Cape Town, South Africa).

2.2 Chromatography

The isolation of pure compounds was carried out using three different chromatographic techniques, namely, Thin Layer Chromatography, Silica Gel Column Chromatography, and Preparative High Pressure Liquid Chromatography.

2.2.1 Thin layer chromatography

Thin-layer chromatography (TLC) is known as one of the most effective methods for the profiling of the different constituents of the plant extracts. The sample was spotted on silica gel TLC plates and developed in a suitable solvent system. Visualization of TLC plates was done by observing the bands spots after development under UV at λ254 nm and λ366 nm using a UV lamp. Chemical profiles of the fractions were identified based on the colour produced after viewing under UV and then spraying with the spray detecting reagent (vanillin).

2.2.2 Silica Gel Column chromatography

Column chromatography is an ideal method of chromatography that is used for the separation and purification of compounds. Column chromatography was performed using silica gel for

the separation of compounds found in the methanol extract of *L. leonurus*, whereby silica gel served as the stationary phase, used with different solvent systems, which served as the mobile phase. The sample extract was mixed with silica gel and allowed to dry out, then loaded into the column and added clean silica on top (**Figure 2.1**). The best solvent combinations based on increasing polarity were used to isolate the compounds.

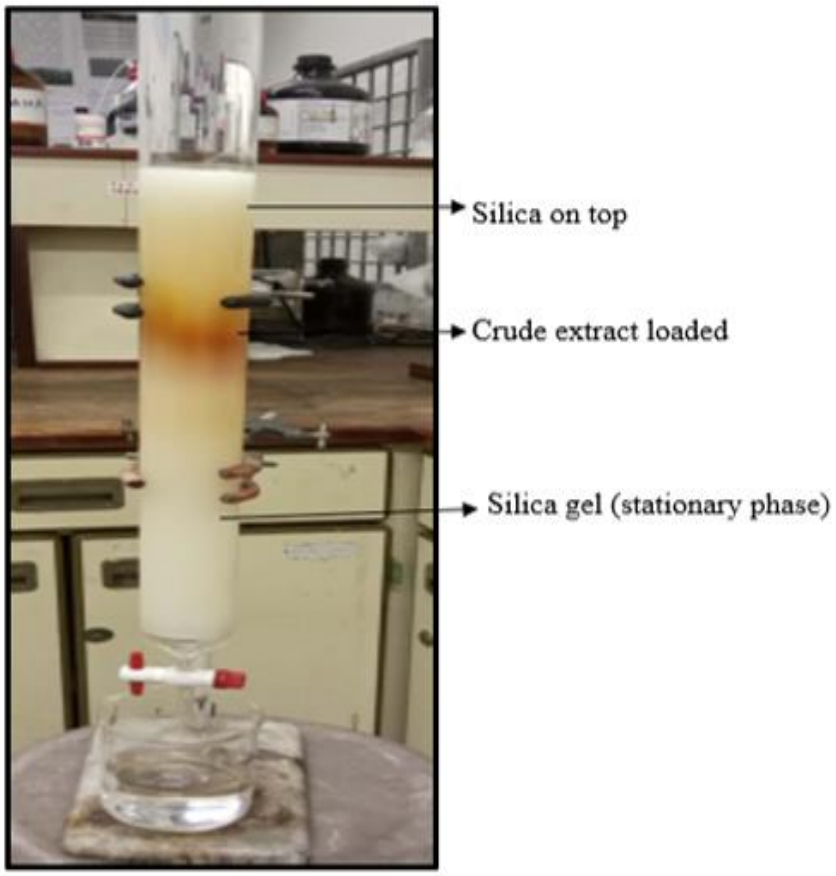


Figure 2.1: Representation of a packed column

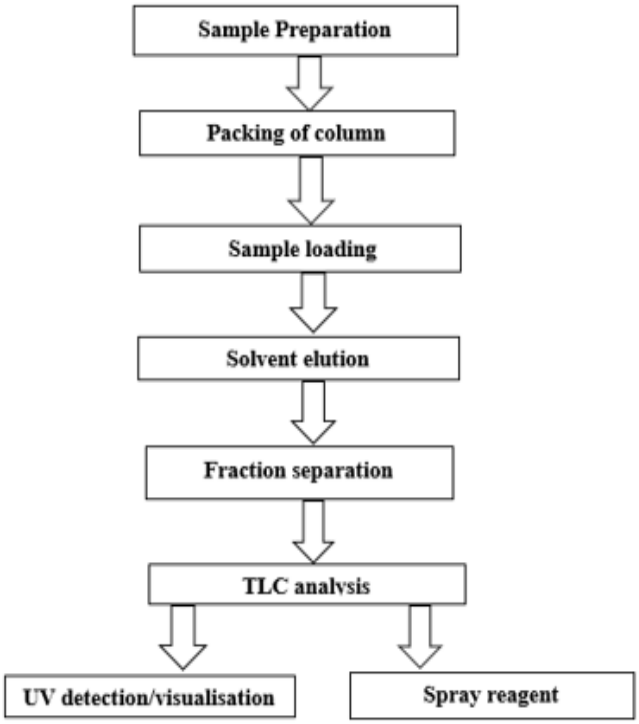


Figure 2.2: A summary of column chromatography used for the isolation procedure

2.2.3 Preparative High pressure liquid chromatography

High Pressure Liquid Chromatography (HPLC) is one of the best analytical methods for the separation of mixtures. It pumps a sample mixture or analyte in a solvent, which serves as the mobile phase at high pressure through a column with chromatographic packing material serving as a stationary phase.

2.3 Spectroscopy

2.3.1 Nuclear magnetic resonance

NMR spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. Nuclear magnetic resonance (NMR) spectroscopy was used to determine the purity of the isolated

compounds, providing information on the type of hydrogen and carbon to get the final chemical structure of the isolated compounds.

2.3.2 Ultraviolet spectroscopy

UV light spectrophotometer was used to visualize the TLC plates at 254 and 366 nm wavelengths.

2.4 Collection and identification of plant material

Fresh plant material of *Leonotis leonurus* was collected during March 2020 from the Cape flats nature reserve on the University of the Western Cape's campus, located in Bellville, a suburb of the City of Cape Town, South Africa. The plant material was taken to the chemistry building at the Cape Peninsula University of Technology (CPUT), Bellville campus for experimental work. The identification of the plant was done by Professor Ahmed A. Hussein Mohammed.

2.5 Extraction and fractionation of the total extract

The fresh plant material (stem and leaves; 100g) was blended and extracted with methanol (2.5 L). The filtrate was collected through a Buckner funnel connected to a pressure pump for suction and filter paper was used to pass through the infusion. The methanol extract was then evaporated to dryness using a rotary evaporator to yield 56g (56%). The methanol solvent which is polar was chosen because this research study aimed to focus on phenolic compounds/polar compounds which are well known for their alpha glucosidase and alpha amylase activities as well as glucose uptake.

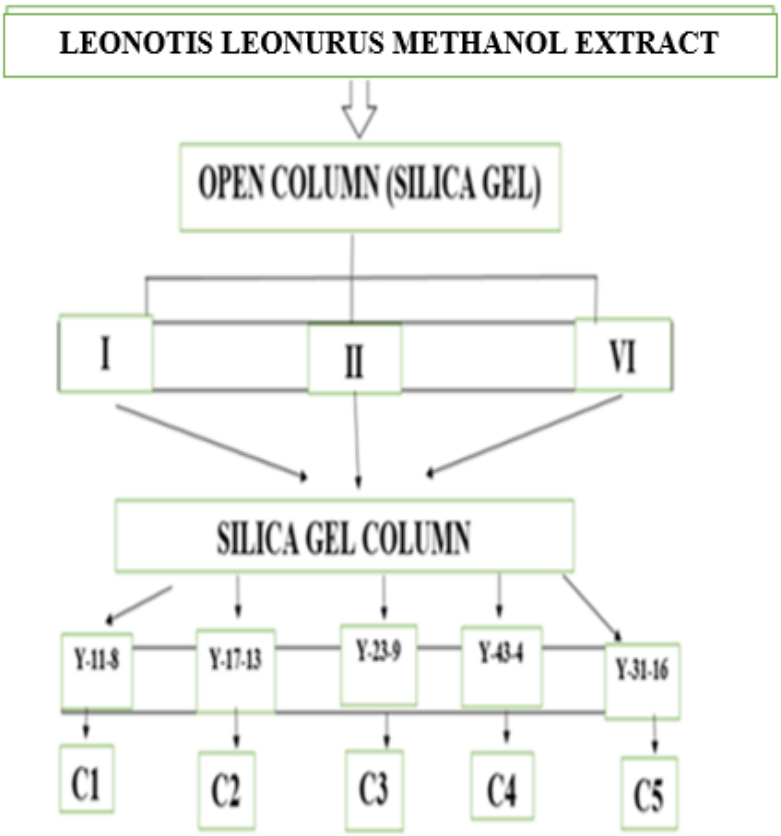


Figure 2.3: A flow diagram of experimental procedure for the isolation of *L. leonurus* constituents

Biological characterization of *Leonotis leonurus* constituents

2.6 Glucose uptake activity

Antidiabetic activity of the isolated compounds was evaluated by measuring the glucose uptake activity using kidney cells according to the standard procedure, listed in **Table 2.1**.

Table 2.1: Reagent preparation for glucose-uptake assay

Reagent	1 Reaction (µL)	50 Reactions (µL)	65 Reactions (µL)
Luciferase reagent	100	5	6.5
NADP+	1	50	60
G6PDH	2.5	125	163
Reductase	0.5	25	33
Reductase substrate	0.0625	13.2	4.1

The reaction mixtures were incubated at room temperature for 30 minutes. Subsequently, the cells were incubated with various concentrations of each compound, and the cells were washed with 100 µL PBS. A volume of 50 µL 2-Deoxy-D-glucose was added to each well and allowed to incubate for 10 minutes. A volume of 25 µL stop buffer was added to each well and shaken briefly. Thereafter, 25 µL of neutralization buffer was added to each well and shaken briefly. Finally, a volume of 100 µL of detection reagent was added and the plate was shaken briefly. The plate was then incubated at room temperature for 30 minutes and read on a plate reader.

2.7 Cytotoxicity Assay

The isolated constituents were screened for cytotoxicity, using the MTT cytotoxicity assay. The cytotoxic effect of each compound on human embryonic kidney (HEK293) cells was assessed following the well-established MTT protocol. MTT (3-(4,5- dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide), a yellow dye, is reduced to purple formazan in the mitochondria of living cells. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1% Penicillin-Streptomycin, and containing essential amino acids, sodium pyruvate, and L-glutamine at 37°C in 96-well microtiter plates (10,000 cells/well). The plates were exposed to a dose of 250 µg of each compound for 24 h. After treatment, the medium was separated, and cells were incubated with 200 µL of MTT in fresh medium at 37°C for 4 hours. DMSO was added to dissolve the resultant formazan crystals from the mitochondrial reduction in MTT. The absorbance of each sample was determined using a microplate absorbance reader at 570 nm, and the percentage of cell viability was calculated using the following equation: cell viability (%) = (absorbance of test – absorbance of background/absorbance of control – absorbance of background) × 100, according to manufacturer instructions (Dojindo, Maryland, MD, USA).

2.8 Alpha-Glucosidase Inhibition Assay

2.8.1 Stock preparation:

To prepare stock solutions, 10 mg of each isolated compound was carefully weighed and dissolved in 1 mL of DMSO. Hereafter, 20 µL of each stock solution was added to 980 µL of distilled water, to yield a final concentration of 200 µg/mL.

2.8.2 Glucosidase Assay:

Alpha glucosidase inhibitory activity of the isolated compounds was carried out according to the standard method (Pistia-Brueggeman & Hollingsworth, 2001), with a slight medication. In a 96-well plate, the reaction mixture containing 50 µL of phosphate buffer (100 mM, pH = 6.8), 10 µL alpha-glucosidase (1 U/mL) and 20 µL of varying concentrations of isolated compounds were pre-incubated further at 37°C for 20 minutes. Next, 20 µl of p-NPG (5 mM) was added as a substrate and incubated at 37°C for 15 minutes. The reaction was stopped by

adding 50 µL of sodium carbonate Na_2CO_3 (0.1M). The absorbance of the released p-nitro phenol was measured at 405 nm using a microplate reader (Multiskan Thermos Scientific, version 1.00.40, Vantaa, Finland). Acarbose at various concentrations (100, 150 and 200 µg) was included as a standard. Each experiment was performed in triplicate, and results were expressed as percentage inhibition that was calculated using the following formula:

$$\text{Inhibitory activity (\%)} = (1\text{-}A/B) \times 100$$

Where A is the absorbance in the presence of the test substance and B is the absorbance of the control.

2.8.3 Statistical Analysis:

Statistical analysis was performed using MedCalc Version 19.6.3. In short, data was tested for normality and subsequently parametric testing was followed, using the independent t-test, where $P<0.05$ was considered significant.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Summary

The chromatographic purification of the methanol extract of *L. leonurus* yielded five pure compounds viz Leoleorin C (**1**), Leoleorin H (**2**), Leoleorin I (**3**), Leoleorin A (**4**), and Marrubin (**5**). The structures elucidation of the isolated compounds was done by the interpretation of NMR spectral data, as well as comparison of the spectral data with those of closely related compounds previously reported from *L. leonurus.* The compounds were further evaluated for glucose uptake activity, cytotoxicity effect and alpha-glucosidase activity in HEK293 kidney cell lines.

3.2 Fractionation of the total extract

A portion of the total extract of *L. leonurus* (51 g) was applied to a silica gel column (30 x 18 cm) and eluted using a gradient of hexane and ethyl acetate following the order of increasing polarity as indicated in **Table 3.1**. Twenty-nine (29) fractions (500 mL each) were collected during the process and numbered 1 - 29.

Table 3.1 Fractionation of the methanol extract of *L. leonurus*

Solvent system	Solvent volume	Fractions collected
Hex	1 L	1-2
Hex- EtOAc 90:10	2 L	3-6
Hex- EtOAc 80:20	2 L	7-10
Hex- EtOAc 70:30	2 L	11-14
Hex- EtOAc 60:40	2L	15-18
Hex- EtOAc 50:50	2 L	19-23
Hex- EtOAc 30:70	2 L	24-27

Hex- EtOAc 10:90	1 L	28-29

The collected fractions (1- 29) were concentrated and combined according to their TLC profiles to yield 10 main fractions (**Figure 3.1**). The main fractions obtained were coded by roman numbers (I – X) and the results are summarized in **Table 3.2**.

Table 3.2: Main fractions obtained upon fractionation of the total extract.

Combined fractions	Designated number	Weight
1	I	3370 mg
2	II	3790 mg
3-4	III	2120 mg
5-6	IV	1940 mg
7-12	V	1000 mg
13-15	VI	3210 mg
16-20	VII	2620 mg
21-24	VIII	920 mg
25-27	IX	670 mg
28-29	X	1000 mg

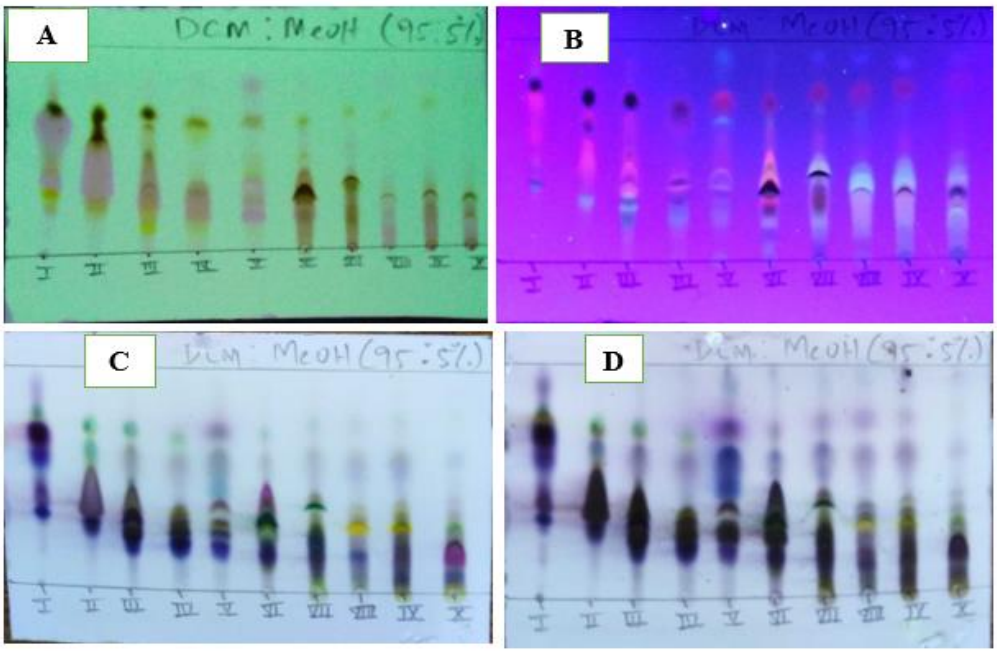


Figure 3.1: TLC profile of the main fractions under UV at 254 nm (A), 366 nm(B) and after spraying with vanillin and heating (C & D).

3.3 Isolation of pure compounds

3.3.1 Isolation of compound 4

Main fraction I (3370 mg) was chromatographed on silica gel using a gradient elution of Hexane-Ethyl acetate as indicated in **Table 3.3**. A volume of 200 mL of each fraction was collected and concentrated using the rotary evaporator.

Table 3.3: Fractionation of main fraction I

Solvent system	Solvent volume	Fractions collected
Hex	2 L	1-10
Hex- EtOAc 98:2	2 L	11-20
Hex-EtOAc 96:4	2 L	21-30

Fractions obtained were developed on TLC and fractions which displayed the same profiles were combined as indicated in **Table 3.4**.

Table 3.4: Sub fractions from main fraction I

Combined fractions	Assigned number	Weight
1-4	Y-11-1	30 mg
5-6	Y-11-2	40 mg
7-9	Y-11-3	60 mg
10-13	Y-11-4	150 mg
14-16	Y-11-5	130 mg
17-18	Y-11-6	150 mg
19	Y-11-7	120 mg
20-22	Y-11-8	1570 mg
23-27	Y-11-9	170 mg
28	Y-11-10	260 mg
29	Y-11-11	370 mg
30	Y-11-12	310 mg

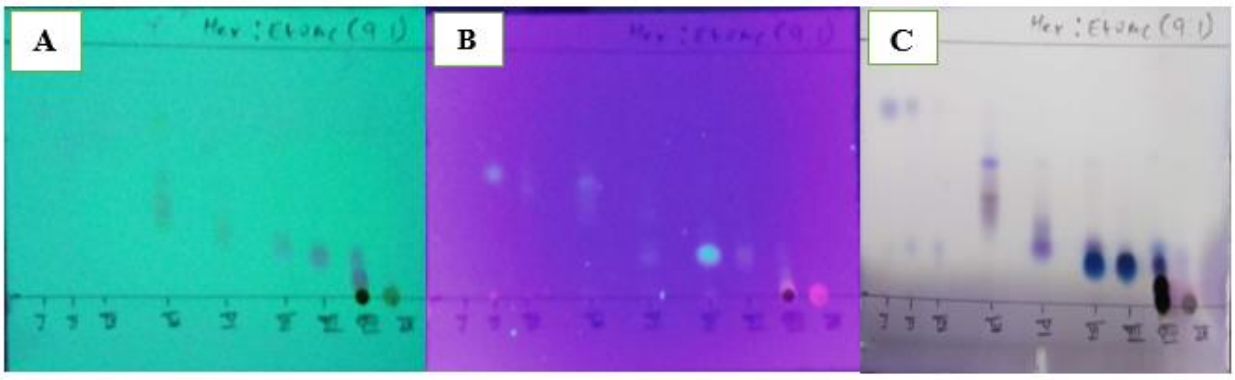


Figure 3.2: TLC profile of the combined fractions under UV at 254 nm (A), 366 nm (B) and after spraying with vanillin and heating (C).

The chromatography of subfraction Y-11-8 (1570 mg) on silica gel, using an isocratic gradient elution of Hex- EtOAc 97:3 afforded a pure compound (**C4**; 136.5 mg, **Figure 3.3**).

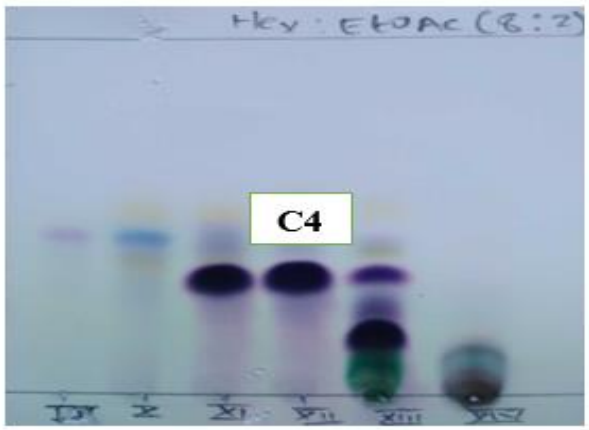


Figure 3.3: TLC profile of compound 4

3.3.2 Isolation of Compound 5

Sub-fraction Y-17-13 (674.7 g) was purified using an isocratic gradient elution of 5-10% Hex: EtOAc and yielded a pure compound (C5).

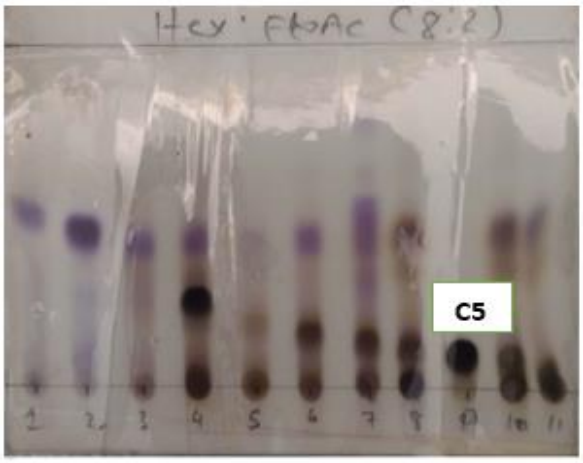


Figure 3.4: TLC profile of compound 5

3.3.3 Isolation of compound 1

Main fraction II (3790 mg) was chromatographed on silica gel and eluted using a gradient of hexane and ethyl acetate in the following order of increasing polarity as indicated in **Table 3.5**. Fifty-four (54) fractions (200 mL each) were collected during the process and numbered 1 - 54.

Table 3.5: Fractionation of main fraction II

Solvent system	Solvent Volume	Fractions collected
Hex-EtOAc 95:5	1L	1-5
Hex-EtOAc 93:7	2L	6-15
Hex-EtOAc 90:10	2L	16-25

The fractions were grouped into sub-fractions based on their TLC profiles and yielded 14 sub-fractions (**Table 3.6**).

Table 3.6: Sub fractions of Main fraction II

Combined fractions	Assigned number	Weight
1-7	Y-23-1	0.285g
8-18	Y-23-2	0.1844g
19-24	Y-23-3	0.096g
25-33	Y-23-4	0.1247g
34-39	Y-23-5	0.0192g
40-43	Y-23-6	0.4136g
44	Y-23-7	0.6183g
45	Y-23-8	1.0367g

46	Y-23-9	637.1 mg
47	Y-23-10	1.0557g
48	Y-23-11	0.7357g
49-50	Y-23-12	0.3354g
51-52	Y-23-13	0.361g
53-54	Y-23-14	0.2362g

Compound 1 was purified from subfraction (Y-23-9; 637.1 mg) using an isocratic gradient of 0.1% MeOH in DCM.

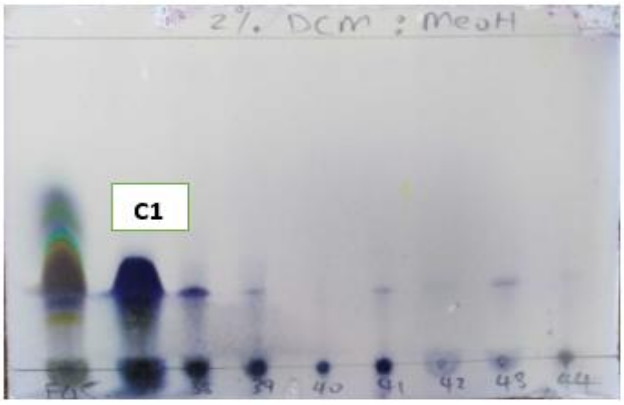


Figure 3.5: TLC profile of compound 1

3.3.4 Isolation of compound 2

Two sub-fractions (Y-23-(10+11)) of main fraction II were combined and chromatographed resulting in eight sub-fractions (Y-43-1 to 8). Compound 2 (**Figure 3.6**) was purified from subfraction Y-43-4 (177 mg) using an isocratic gradient elution of 0.1% MeOH in DCM.

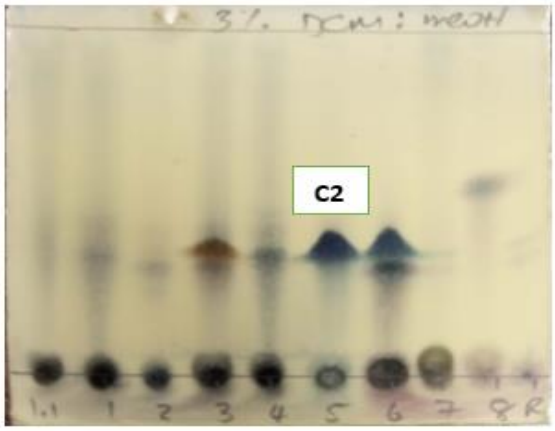


Figure 3.6: TLC profile of compound 2

3.3.5 Isolation of compound 3

Min fraction VI (3210 mg) was chromatographed on silica gel and eluted using a gradient of hexane and ethyl acetate in the following order of increasing polarity as indicated in T**able 3.7**. Seventy-six (76) fractions (200 mL each) were collected during the process and numbered 1 - 76.

Table 3.7: Fractionation of main fraction VI

Solvent system	Solvent Volume	Fractions collected
Hex	1L	1-5
Hex-EtOAc 80:20	2 L	6-15
Hex-EtOAc 70:30	2 L	16-25
Hex-EtOAc 60:40	2 L	26-35
Hex-EtOAc 50:50	8 L	36-76

The 76 fractions were developed on TLC and fractions which displayed the same profiles were combined as indicated in **Table 3.8** and yielded 18 sub-fractions.

Table 3.8: Sub-fractions of main fraction VI

Combined fractions	Assigned number	Weight
1-5	Y-31-1	71.1 mg
6-12	Y-31-2	452.5 mg
13-14	Y-31-3	150.3 mg
15-17	Y-31-4	95.5 mg
18-19	Y-31-5	119.3 mg
20-22	Y-31-6	172.8 mg
23-24	Y-31-7	68.6 mg
25-26	Y-31-8	39.8 mg
27-32	Y-31-9	479.1 mg
33-38	Y-31-10	197 mg
39-41	Y-31-11	131.5 mg
42-44	Y-31-12	125.1 mg
45-46	Y-31-13	27.1 mg
47-48	Y-31-14	190.5 mg
49	Y-31-15	193.2 mg
50-51	Y-31-16	216 mg
52-58	Y-31-17	407.4 mg
59-76	Y-31-18	152.6 mg
Wash	Y-31-19	25mg

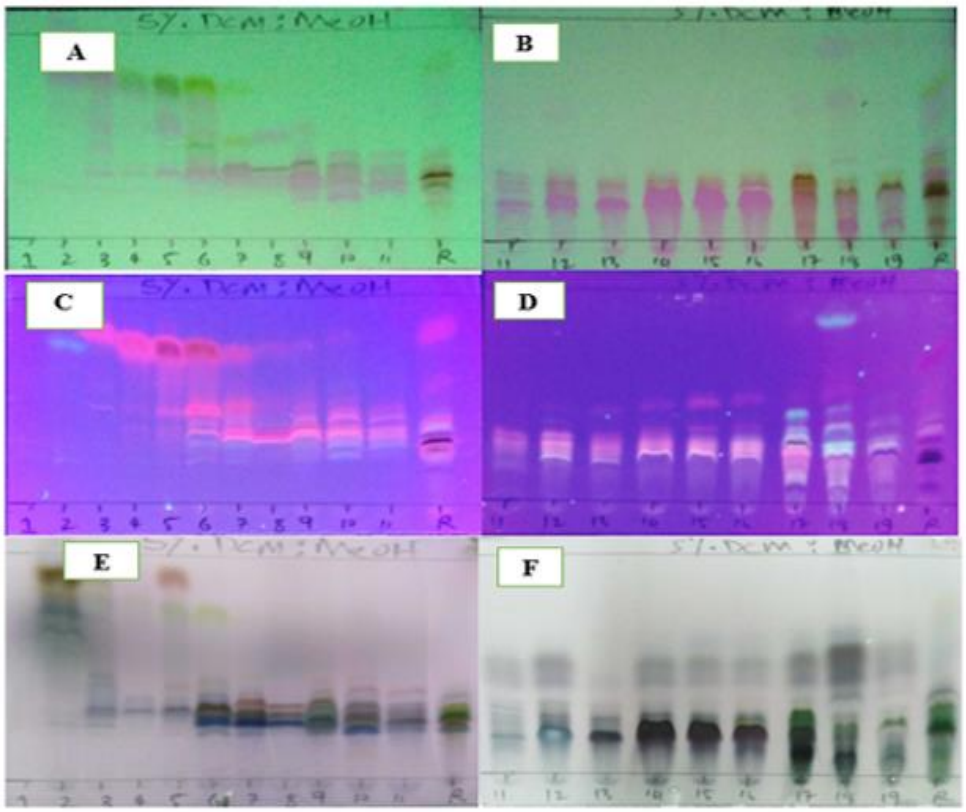


Figure 3.7: TLC profile of Sub-fractions (1-19) under UV at 254 nm (A-B), 366 nm (C-D) and after spraying with vanillin and heating (E-F).

Three sub-fractions (Y-31-(14, 15, 16); 500 mg) of main fraction VI, which showed similar TLC profile were combined and chromatographed using an isocratic gradient elution of Hex: EtOAc (6:4), collecting a volume of 100 mL, and yielded **C3** (**Figure 3.8**).

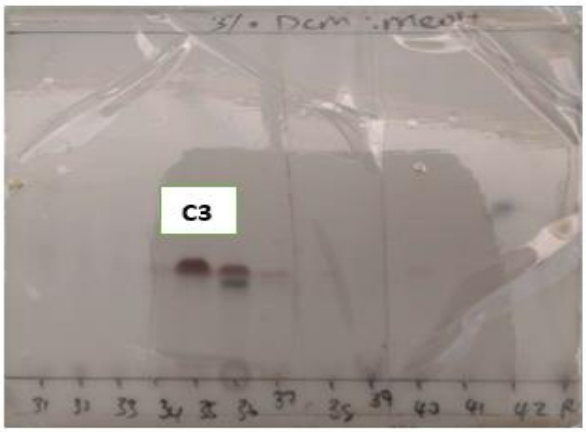
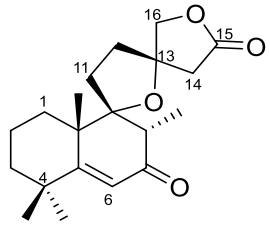
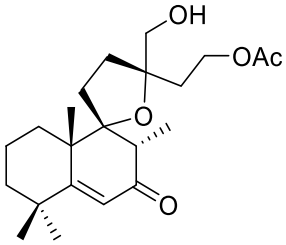
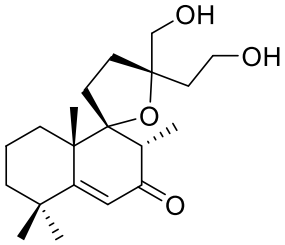
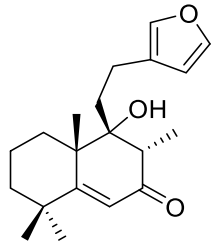
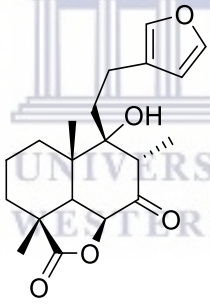


Figure 3.8: TLC profile of compound 3

3.4 Structure elucidation of the isolated compounds

The structures elucidation of the isolated compounds is demonstrated in **Table 3.9**. The structures were done by the interpretation of NMR spectral data (T**able 3.10**)

Table 3.9: Chemical structures of the isolated compounds

		
Compound 1	**Compound 2**	**Compound 3**
		
Compound 4	**Compound 5**	

Compound **1** was obtained as a colourless oil. The compound is identified as Leoleorin C (Wu *et al*., 2013) based on the NMR data analysis (**Table 3.10**). The 1H NMR spectrum showed singlets representing three tertiary methyl groups at δ_H 1.08 (Me-18), 1.11 (Me-19), and 1.27 (Me-20), and a doublet of one secondary methyl group at 1.08 (J = 6.8 Hz, Me-17), the olefinic proton at 5.93 (s, H-6), oxygenated methylene group at 4.14, 3.99 (d/each, J=8.8 Hz, CH_2-16), in addition to clusters of signals between 1.34 – 2.80. The ^{13}C NMR and DEPT spectra

exhibited 20 carbon resonances (7 × C, 2 × CH, 7 × CH2, and 4 × CH3) and indicate the diterpene skeleton. There were signals of two carbonyl carbons at δ_C 172.6 and 199.2, three oxymethylene groups at 77.8 (C-16) 99.1 (C-9) and 86.2 (C-13). The obtained data, when compared with literature (Wu *et al.*, 2013), perfectly matched Leoleorin C, which was previously isolated from the same source.

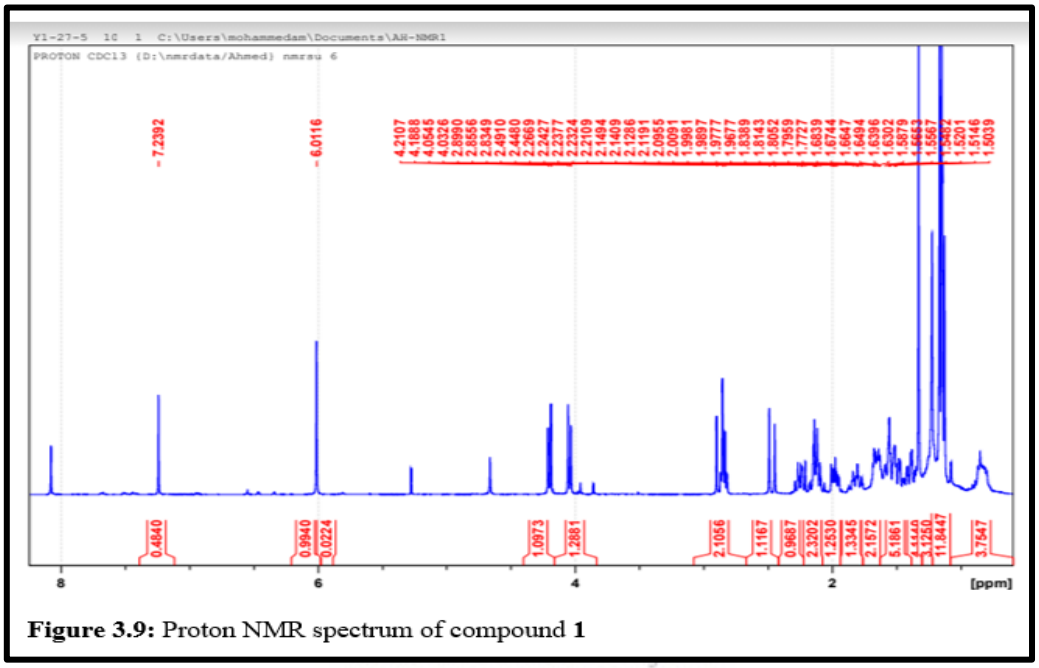


Figure 3.9: Proton NMR spectrum of compound **1**

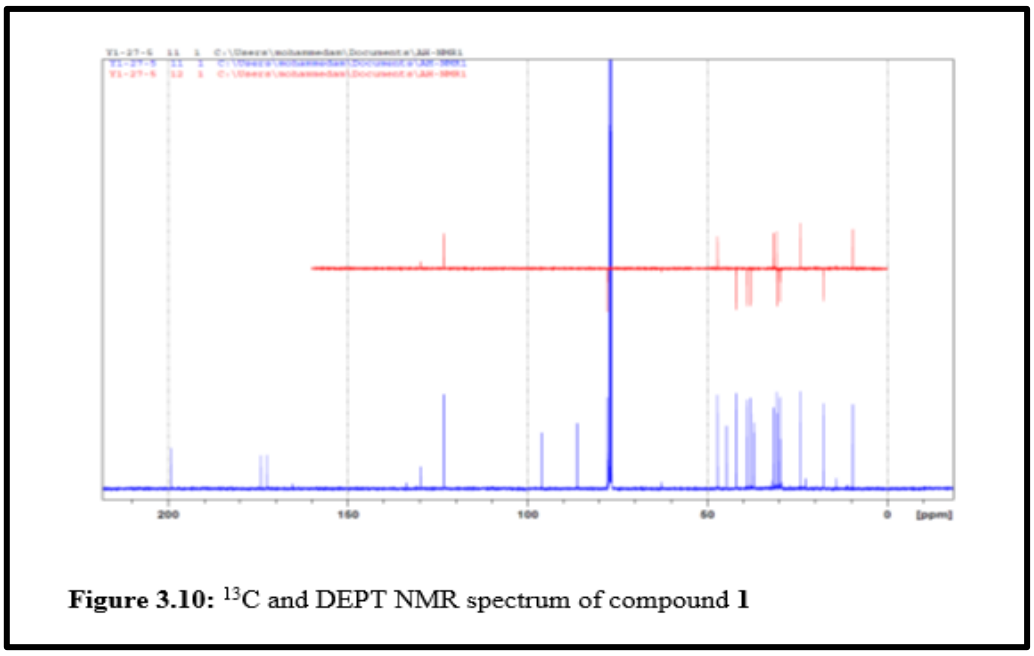


Figure 3.10: ^{13}C and DEPT NMR spectrum of compound **1**

Compound **2** was obtained as colourless crystals. The compound is identified as Leoleorin H (Wu *et al*., 2013) based on NMR data analysis (**Table 3.10**). The 1H NMR spectrum showed singlets representing four tertiary methyl groups at δ_H 1.17 (Me-18), 1.18 (Me-19), 1.25 (Me-20) and 2.00 (COCH3), a doublet of one secondary methyl group at 1.20 (J = 6.8 Hz, Me-17), the olefinic proton at 6.02 (s, H-6), two oxymethylene groups at 3.49, 3.39 (d/each, J=11.2 Hz, CH_2-16), 4.04/4.14 (m/each, CH2-15), in addition to clusters of signals between 1.43 – 2.78. The ^{13}C NMR and DEPT spectra exhibited 22 carbon resonances (8 × C, 2 × CH, 7 × CH2, and 5 × CH3) and indicate the diterpene skeleton with an acetate group. There were signals of two carbonyl carbons at δ_C 171.1 (COCH3) and 199.9 (C-7), three oxymethylene carbons at 65.8 (C-16), 85.2 (C-13), and 95.7 (C-9). The obtained data, when compared with literature (Wu *et al*., 2013), it perfectly matches with Leoleorin H, which was previously isolated from the same source.

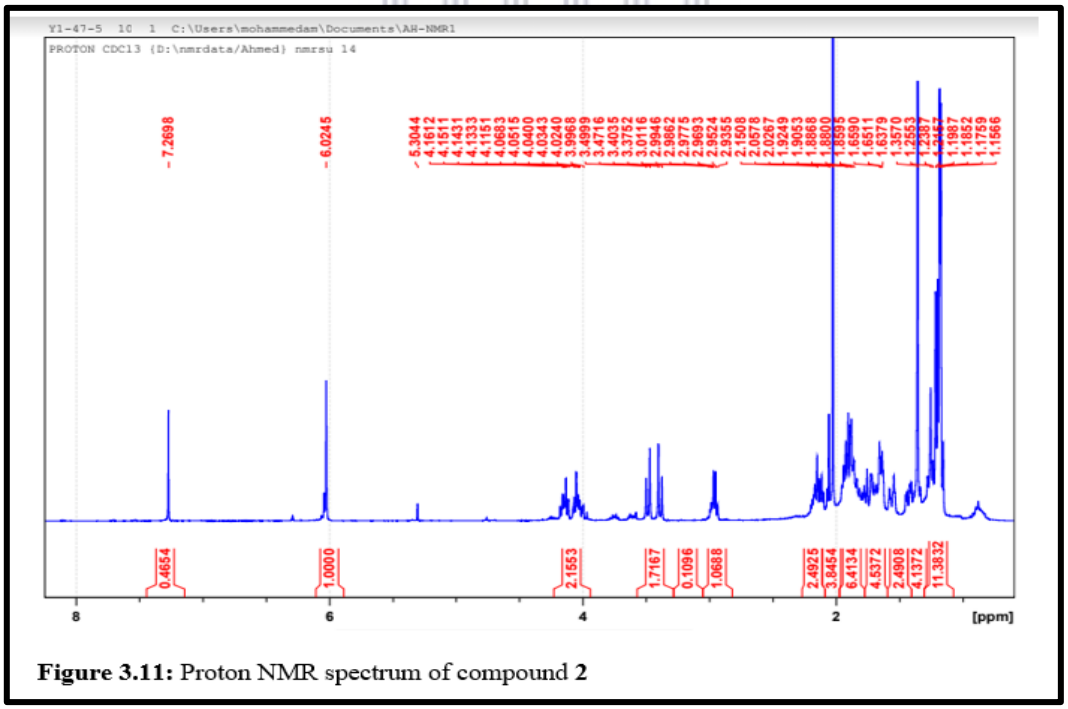


Figure 3.11: Proton NMR spectrum of compound **2**

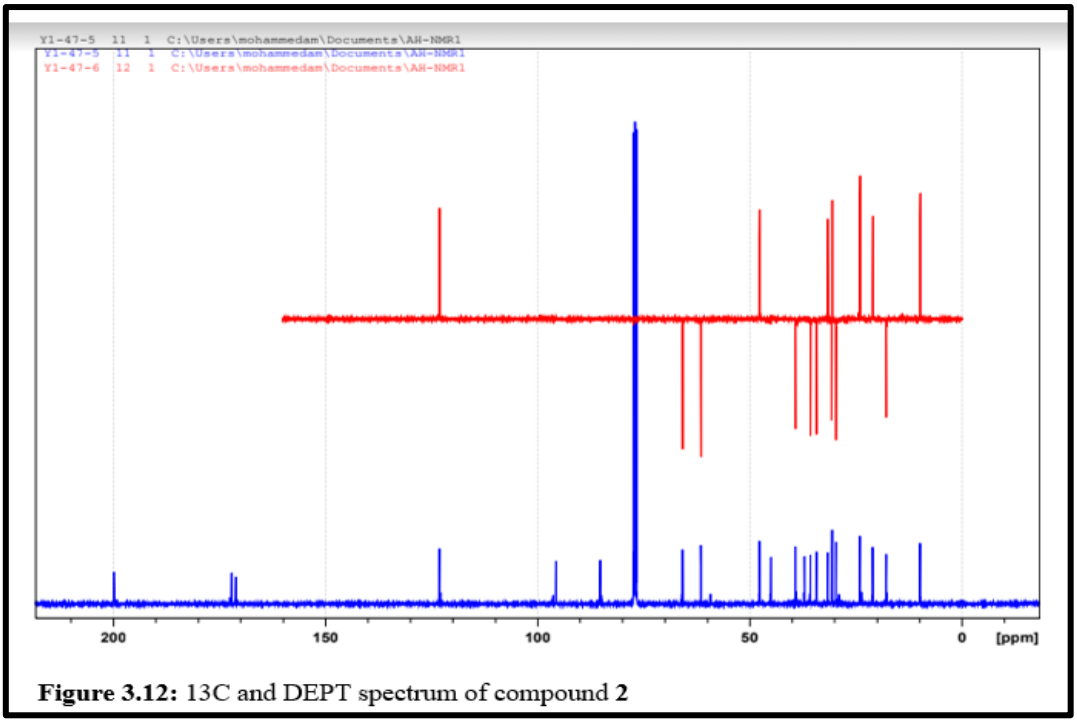


Figure 3.12: 13C and DEPT spectrum of compound **2**

Compound **3** was obtained as a colourless oil. The compounds identified as Leoleorin I (Wu *et al*., 2013) are based on NMR data analysis (**Table 3.10**).

The 1H NMR spectrum showed singlets representing three tertiary methyl groups at δ_H 1.06 (Me-18), 1.08 (Me-19), and 1.26 (Me-20), a doublet of one secondary methyl group at 1.07 (J = 6.8 Hz, Me-17), the olefinic proton at 5.89 (s, H-6), two oxymethylene groups at 3.41, 3.32 (d/each, J=11.2 Hz, CH_2-16), 3.15 (2H, m/each, CH2-15), in addition to clusters of signals between 1.43 – 2.87. The ^{13}C NMR and DEPT spectra exhibited 20 carbon resonances (7 × C, 2 × CH, 7 × CH2, and 4 × CH3) and indicate the diterpene skeleton. There were signals of a carbonyl carbon at δ_C 200.5 (C-7), olefinic double bond at 173.0 (C-5), 122.7 (C-6); three oxymethylene carbons at 66.3 (C-16), 86.0 (C-13), and 95.5 (C-9). The compound showed similar NMR data with those of compound 2 except for the absence of the acetyl group, and when compared with literature (Wu *et al*., 2013), it perfectly matches with Leoleorin I, which was previously isolated from the same source.

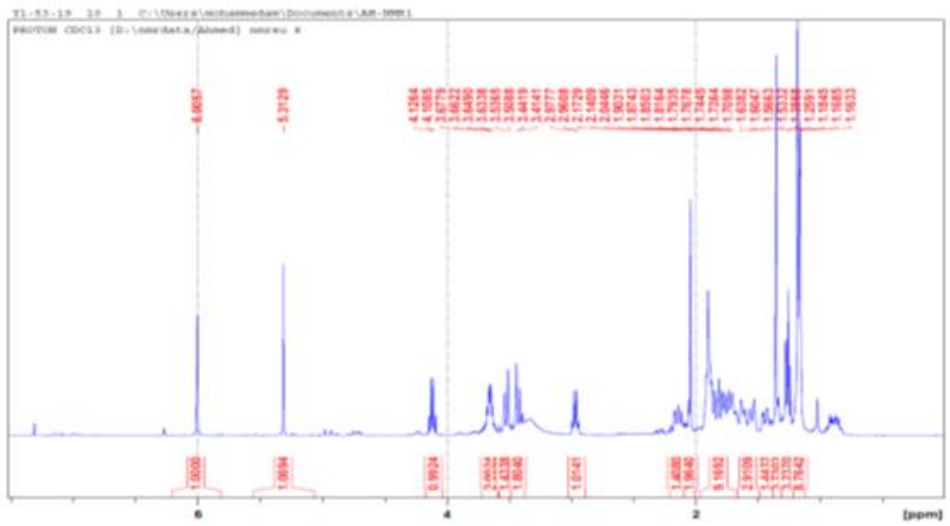


Figure 3.13: Proton NMR spectrum of compound **3**

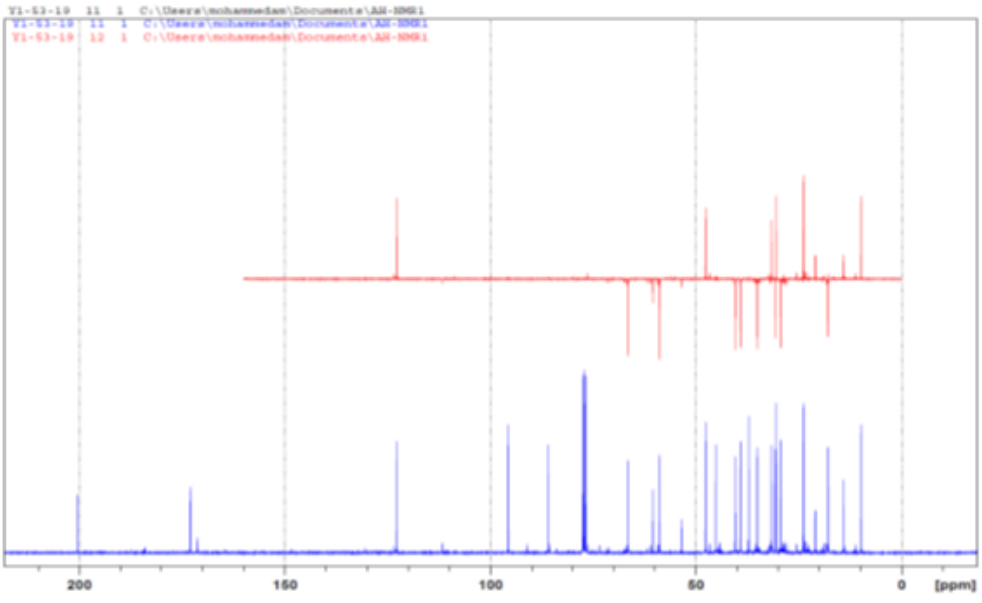


Figure 3.14:13C and DEPT spectrum of compound **3**

Compound **4** was obtained as colourless crystals. The compounds identified as Leoleorin A (was identified initially as compound Y) (Wu *et al.*, 2013) based on NMR data analysis (T**able 3.10**).

The 1H NMR spectrum showed singlets representing three tertiary methyl groups at δH 1.18 (Me-18), 1.23 (Me-19), and 1.36 (Me-20), a doublet of one secondary methyl group at 1.28 (J = 6.8 Hz, Me-17), three olefinic protons at 6.12 (s, H-6), 6.28 (H-14), 7.35 (H-15); in addition to clusters of signals between 1.43 – 2.78. The 13C NMR and DEPT spectra exhibited 20 carbon resonances (7 × C, 2 × CH, 7 × CH2, and 4 × CH3) and indicate the diterpene skeleton. There were signals of a carbonyl carbon at δC 200.5 (C-7), three double bonds at 173.0, 122.7 (C-5/6), 125.0, 110.7 (C13/14), 142.8; 138.5 (C-15/16); an oxymethylene carbons at 79.2 (C-9). The compound is one of the oldest compounds isolated from this species in 1965, recently was given the name Leoloerin A and the old name was compound Y (Wu *et al.*, 2013).

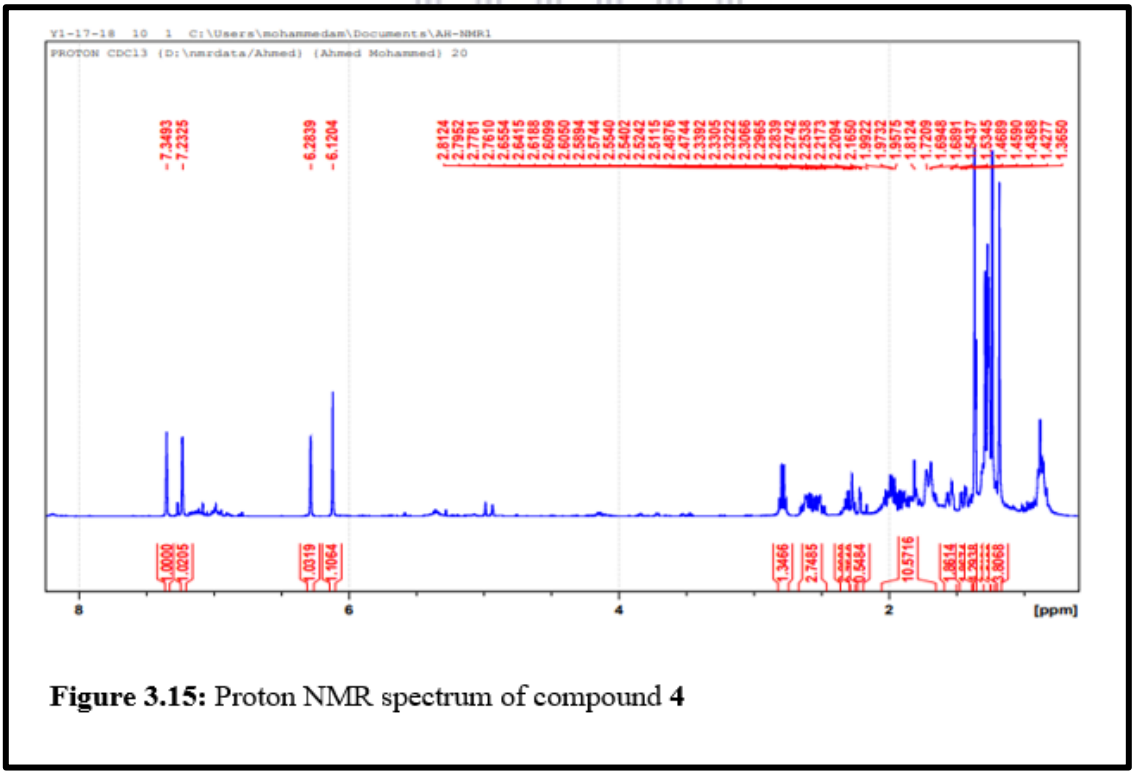


Figure 3.15: Proton NMR spectrum of compound **4**

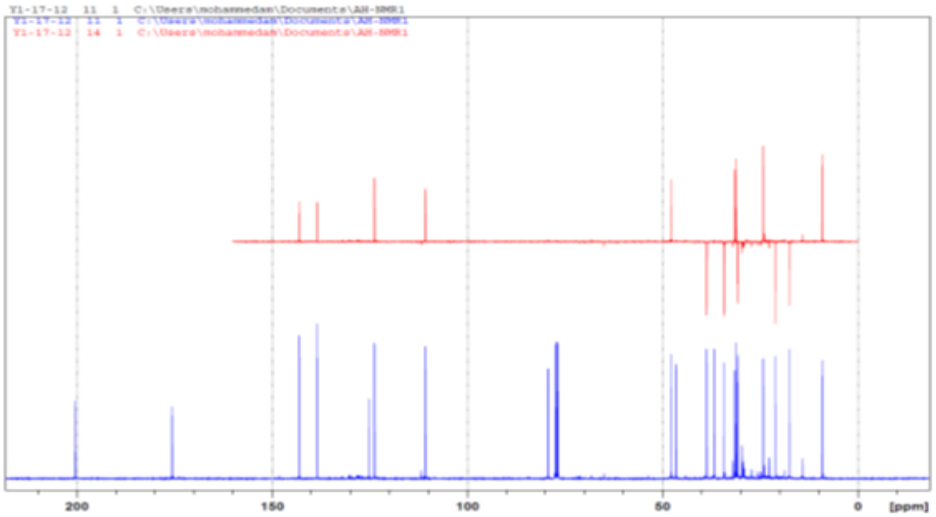


Figure 3.16: 13C and DEPT spectrum of compound **4**

Compound **5** was obtained as colourless crystals. The compound was identified as marrubiin (Popoola *et al*., 2013) based on NMR data analysis (**Table 3.10**). The 1H NMR spectrum showed singlets representing two tertiary methyl groups at δ_H 1.28 (Me-18), and 1.05 (Me-20), a doublet of one secondary methyl group at 0.96 (J = 6.8 Hz, Me-17), three olefinic protons at 6.12 (s, H-6), 6.28 (H-14), 7.35 (H-15); in addition to clusters of signals between 1.43 – 2.78. The ^{13}C NMR and DEPT spectra exhibited 20 carbon resonances (5 × C, 6 × CH, 6 × CH2, and 3 × CH3) and indicate the diterpene skeleton. There were signals of a lactonic carbonyl carbon at δC 183.8 (C-20), two double bonds at 125.0, 110.7 (C13/14), 143.1; 138.6 (C-15/16); oxymethylene carbon at 75.8 (C-9). The compound is well described in the literature with broad-spectrum biological activities and isolated previously from the same source (Wu *et al*., 2013).

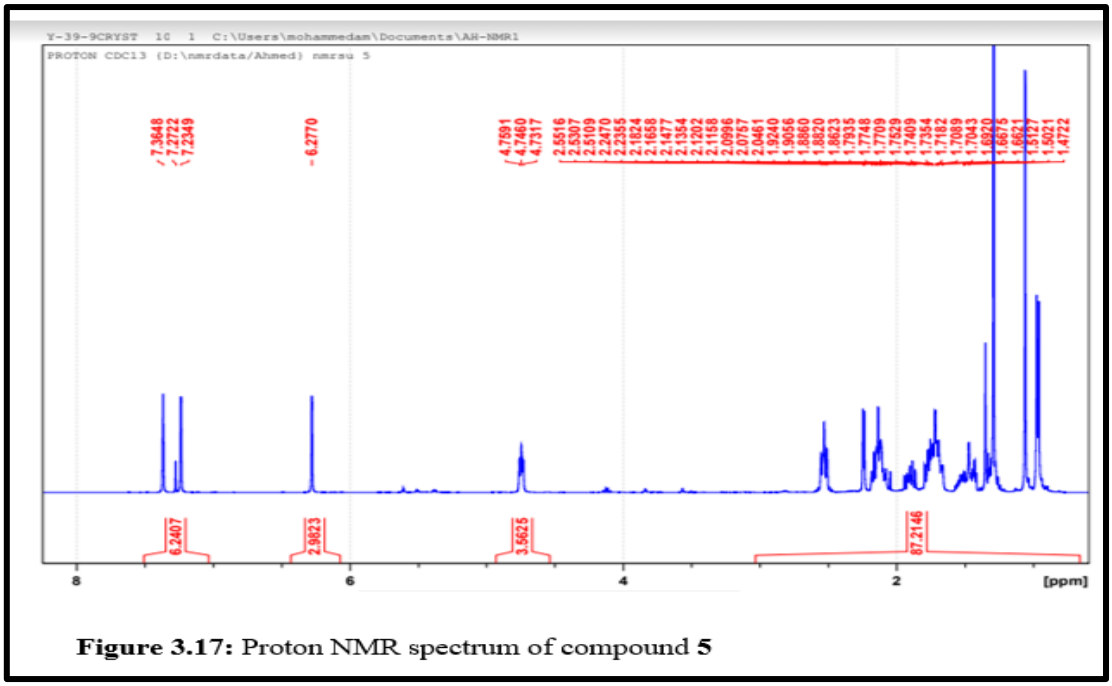


Figure 3.17: Proton NMR spectrum of compound **5**

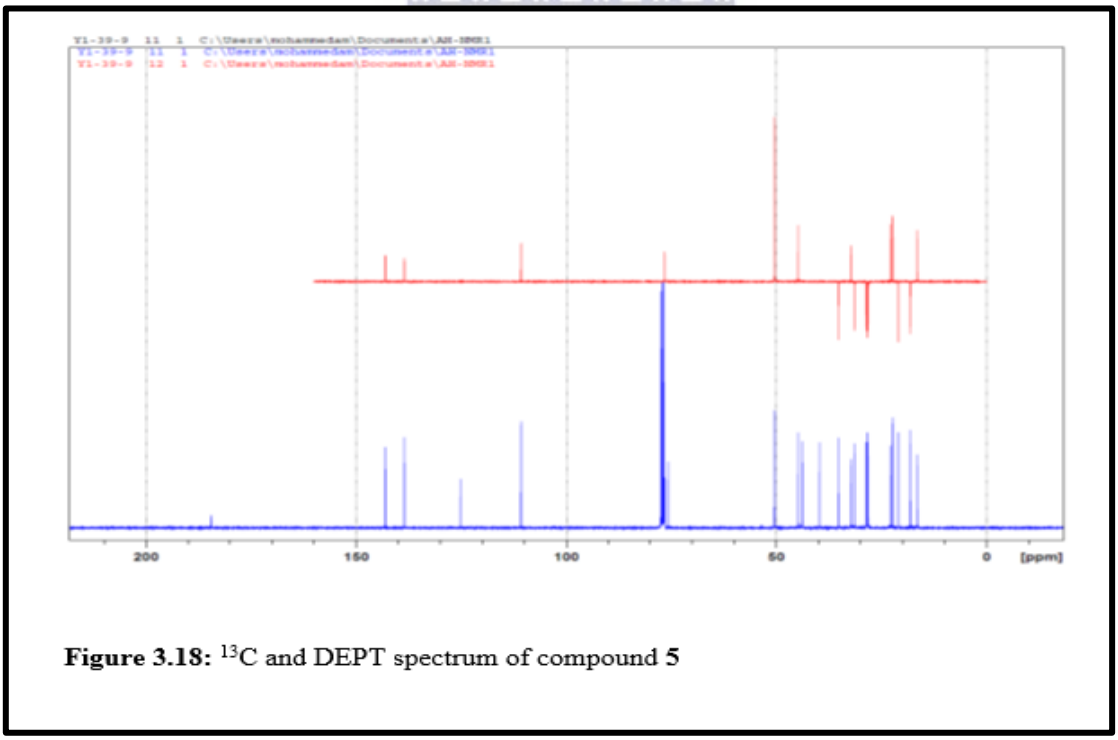


Figure 3.18: ^{13}C and DEPT spectrum of compound **5**

Table 3.10: ^{1}H NMR data of compounds 1–5 ($CDCl_3$, δ ppm, J in Hz, 400 MHz).

No.	27-5 (1)		47-5 (2)		53-19 (3)		17-12 (4)		39-9 (5)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	30.6 *t*	1.54, *m*	30.6 *t*	1.54, *m*	30.5 *t*	1.84, *m*	30.7 *t*	1.98, *m*	28.6 *t*	1.66 m
		1.44, *m*		1.45, *m*		1.67, *m*		1.88, *m*		1.30, *dt*(14.4, 3.6)
2	17.6 *t*	1.92, *m*	17.9 *t*	1.73, *m*	17.8 *t*	1.73, *m*	17.4 *t*	1.82, *m*	18.2 *t*	1.71 m
		1.65, *m*		1.59, *m*		1.57, *m*		1.67, *m*		1.49, *ddt*(13.6, 13.2, 3.6)
3	39.0 *t*	1.50, *m*	39.3 *t*	1.45, *m*	39.1 *t*	1.80, *m*	38.6 *t*	1.55, *m*	28.4 *t*	2.10 m
		1.34, *m*		1.34, *m*				1.43, *m*		1.44, *ddd*(14.4, 13.6, 3.6)
4	37.1 *s*		37.1 *s*		37.0 *s*		36.7 *s*		43.8 *s*	
5	172.5 *s*		172.1 *s*		173.0 *s*		175.3 *s*		44.9 *d*	2.22, *d*(4.7)
6	123.2 *d*	5.93, *s*	123.2 *d*	6.02, *s*	122.7 *d*	5.89, *s*	123.7 *d*	6.12, *s*	76.2 *d*	4.73, *ddd*(6.4, 4.7, 1.6)
7	199.2 *s*		199.8 *s*		200.5 *s*		200.1 *s*		31.5 *t*	2.15, *ddd*(14.0, 6.4, 1.6)
										1.67
8	47.1 *d*	2.84, *q* (6.8)	47.7 *d*	2.78 *q* (6.8)	47.6 *d*	2.87, *q* (6.8)	47.6 *d*	2.78, *q* (6.8)	32.3 *d*	2.10
9	96.1 *s*		95.7 *s*		95.5 *s*		79.2 *s*		75.8 *s*	
10	44.7 *s*		45.0 *s*		45.1 *s*		46.5 *s*		39.7 *s*	
11	29.8 *t*	2.23, *m*	29.7 *t*	2.16, *m*	29.3 *t*	2.06, *m*	34.3 *t*	1.99, *m*	35.1 *t*	1.89, *ddd* (14.6, 10.4, 7.2)
		1.98, *m*		1.98, *m*		1.78, *m*		1.71, *m*		1.75, *ddd* (14.6, 10.4, 7.2)
12	37.9 *t*	2.10, *m*	35.7 *t*	1.92, *m*	34.9 *t*	1.83, *m*	21.0 *t*	2.62, *m*	21.0 *t*	2.52 *m*
				2.14, *m*						
13	86.2 *s*	2.05, *m*	85.2 *s*	1.85, *m*	86.0 *s*	1.68, *m*	125.0 *s*	2.53, *m*	125.0 *s*	
14	41.9 *t*	2.87, *d* (17.2)	34.3 *t*	1.88, *m*	40.3 *t*	1.80, *m*	110.7 *d*	6.28, *s*	110.7 *d*	6.22, *dd*(1.7, 0.8)
		2.47, *d* (17.2)		1.75, *m*						
15	174.2 *s*		61.5 *t*	4.14, *m*	58.7 *t*	3.53, *m*	142.8 *d*	7.35, *s*	143.1 *d*	7.35 *t* (1.7)
				4.04, *m*						
16	77.8 *t*	4.20, *d* (8.8)	65.8 *t*	3.49, *d* (11.2)	66.3 *t*	3.41, *d* (11.2)	138.5 *d*	7.23, *s*	138.6 *d*	7.22 *ddt*(1.7, 1.0, 0.8)
		4.04, *d* (8.8)		3.39, *d* (11.2)		3.32, *d* (11.2)				
17	9.5 *q*	1.08, *d* (6.8)	9.8 *q*	1.20, *d* (6.8)	9.8 *q*	1.07, *d* (6.8)	9.0 *q*	1.28, *d* (6.8)	16.6 q	0.96 *s*
18	31.5 *q*	1.08, *s*	31.7 *q*	1.17, *s*	31.5 *q*	1.06, *s*	31.3 *q*	1.18, *s*	23.0 q	1.28, *s*
19	30.7 *q*	1.11, *s*	30.7 *q*	1.18, *s*	30.6 *q*	1.08, *s*	31.0 *q*	1.23, *s*	183.8 *s*	
20	24.1 *q*	1.27, *s*	24.0 *q*	1.35, *s*	23.7 *q*	1.26, *s*	24.1 *q*	1.36, *s*	22.2 q	1.05, *s*
22			171.1 *s*	2.00, *s*						
			21.0 *q*							

3.5 Biological evaluations

An *in vitro* investigation was carried out to determine the glucose uptake activity, cytotoxicity, and alpha-glucosidase activity of the methanolic extract of *L. leonurus* and its five isolated constituents.

3.5.1 Glucose uptake activity

Facilitative glucose uptake transport systems are abundant in animal cells and are accountable for moving glucose across cell surface membranes (Navale & Paranjape, 2016). The evaluation of glucose uptake is critical in the study of many diseases and metabolic disorders such as myocardial ischemia, diabetes mellitus, and cancer. The kidney contributes to glucose homeostasis through processes of gluconeogenesis, glucose filtration, glucose reabsorption, and glucose consumption. Each of these processes can be altered in (T2DM), thus providing potential targets for novel therapies (Mather & Pollock, 2011). Recent studies have indicated that the kidney is responsible for up to 20% of all glucose production via gluconeogenesis. In patients with T2DM, overall glucose production increases by as much as 30%, with equal contributions from hepatic and renal sources (Dokken, 2012; Mather & Pollock, 2011). This increased production contributes not only to increased fasting glucose in T2DM patients but also to raised postprandial glucose since, in contrast to the liver, glucose ingestion increases renal gluconeogenesis (DeFronzo *et al*., 2012; Mather & Pollock, 2011).

L. leonurus has been reported to be traditionally used to cure a wide range of ailments including diabetes (Rattray & Van Wyk, 2021). The major chemical constituents of the plant extract are diterpenoid labdane lactones such as premarrubiin and marrubin, which have been reported to possess antidiabetic properties (Mnonopi *et al.,* 2012).

The glucose uptake ability of the isolated compounds was evaluated on human embryonic kidney (HEK293) cells using 2-deoxyglucose-6-phosphate. **Figure 3.19** presents the relative

glucose uptake for the given compounds, compared to the control. The results obtained in this study showed that all compounds increased glucose uptake in the cells, however, only **Compound 4** exhibited prominent glucose uptake activity when compared to the control (P = 0.004). It is suspected that this compound increases glucose sensitivity through stimulation of glucose metabolism, therefore, it may be concluded that it could potentially aid in the treatment of type 2 diabetes mellitus.

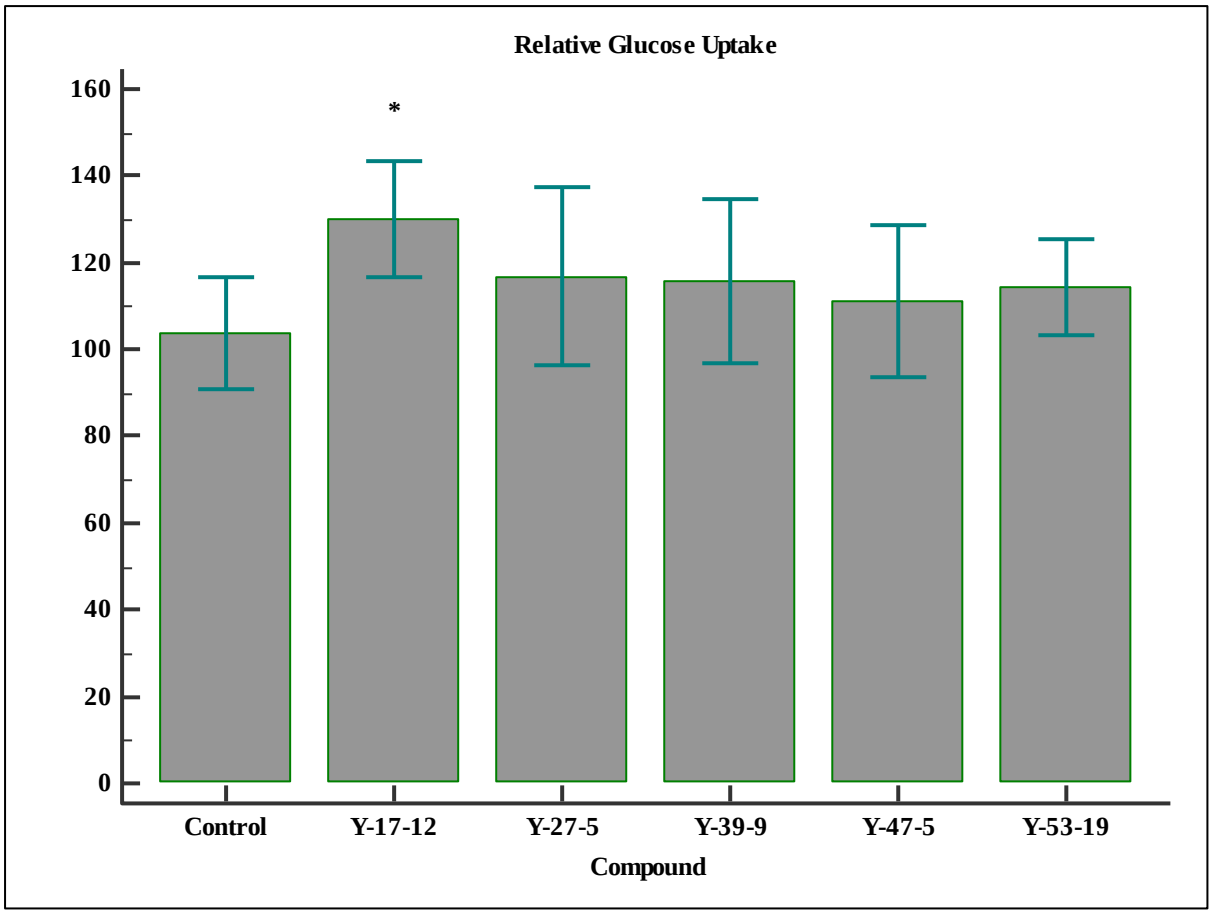


Figure 3.19: Relative glucose uptake of isolated compounds; where the p-value is indicative of the statistical significance versus the control, using an independent T-test to generate two tailed probability * ($p<0.05$).

Table 3.11: p-values of the compounds calculated using an Independent T-test.

T-test	P-value
Control vs Y_27_5	0.221
Control vs Y_47_5	0.428
Control vs Y_53_19	0.159
Control vs Y_17_12	0.004
Control vs Y_39_9	0.238

Marrubin (**C5**) with a P-value of 0.238, exhibited no glucose uptake activity. However, several studies have shown that *L. leonurus* extract and its constituent, marrubin, has an increased insulin sensitivity in 1NS-1 cells. *In vitro* analysis carried out on marrubin confirmed the stimulatory index of INS-1 cells cultured under hyperglycaemic conditions, and this was significantly increased in cells exposed to them (Mnonopi *et al*., 2012). Furthermore, the insulin and glucose transporter-2 gene expressions were significantly increased by marrubin (Mnonopi *et al*., 2012). There are no reports on glucose uptake activity evaluation for the other four compounds that have been previously reported. However, there are studies indicating the isolation of these compounds, for example, a study by Wu *et al* (2013).

3.5.2 Cytotoxicity assessment

Several studies have elucidated that some of the plants used in traditional medicine have cytotoxic and genotoxic effects (Okaiyeto & Oguntibeju, 2021; McGaw *et al*., 2014; Yuet Ping *et al*., 2012). The cytotoxicity assessment is a very critical aspect of drug discovery from plant origin. This is mainly because it reveals information about the safety of drugs, as well as precautionary measures that should be considered. In the current study, the cytotoxic effect of the isolated compounds expressed as cell viability on kidney (HEK293) cells was determined

using the MTT assay. As illustrated in **Figure 3.20**, compounds 2, 4, and 5 versus the control showed the greatest impact on cell viability, accounting for a 20% reduction, while compounds 1 and 3 yielded no clearly observable reduction in cell viability. However, despite compounds compounds Y-27-5 and Y-53-19 only yielded an approximate 20% reduction at best, a degree of caution should be exercised if they are to be used as therapeutic agents, particularly over extended periods of time. Nevertheless, these isolated compounds exhibited a low overall degree of toxicity, and therefore remain promising therapeutic agents.

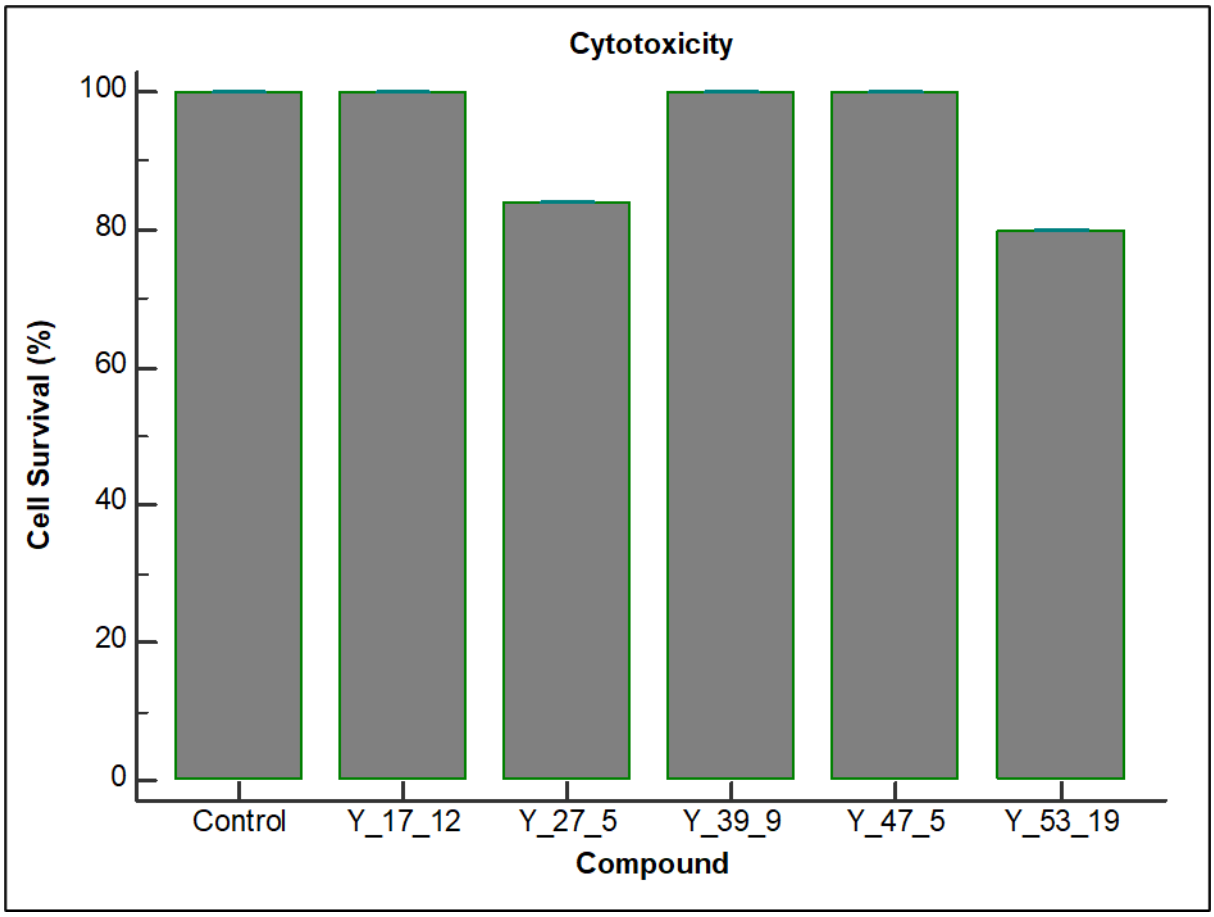


Figure 3.20: Cell viability (Cytotoxicity) of isolated compounds

3.5.3 Alpha-Glucosidase Inhibition

Anti-hypoglycaemic drugs generally used to treat T2DM include alpha-glucosidase inhibitors such as acarbose. Acarbose is a nitrogen-containing pseudo-tetrasaccharide, forming part of the alpha-glucosidase inhibitor group (Shibao, 2012). Medicinal plants and herbal therapies have been indicated as effective methods to reduce the level of blood glucose after a meal (Ademiluyi & Oboh, 2013; Konate *et al*., 2014). As part of their defence mechanisms, plants make a wide range of compounds, some of which are glucosidase inhibitors and may be successful in the inhibition of hyperglycaemia (Kavimani *et al*., 2014). Managing blood glucose levels is a critical strategy in the control of diabetic complications. Inhibitors of saccharide hydrolysing enzymes such as alpha-amylase and alpha-glucosidase have been useful as oral hypoglycaemic agents for the control of hyperglycaemia especially in patients with T2DM (Oboh *et al*., 2013). The inhibition of these enzymes postpones carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise. Alpha-glucosidase inhibitors are one of the most effective classes of antidiabetic therapeutics capable of improving hyperglycemia especially postprandial hyperglycaemia over alpha-amylase inhibitors (Bhatia *et al.*, 2019). There are several studies reported for such inhibitors being beneficial to prevent or delay impaired glucose tolerances in diabetes. For example, a study by Rotsoma, (2019) revealed a strong inhibitory activity of leaf extracts of *L. leonurus* to α-glucosidase.

Though being effective, these drugs have been reported to cause severe side effects such as gastrointestinal discomfort, including diarrhoea and flatulence. Even though drug treatment for T2DM has been improved to some extent during the last decade, drug resistance is still a big concern that needs to be dealt with. Thus, natural products of great structural diversity are a good source for searching for such inhibitors (El-Mohsen *et al.* 2014).

The methanolic extracts of *L. leonurus* and its five isolated constituents were evaluated for their inhibitory effect on α-glucosidase enzymes by the in-vitro method. Statistically significant (P=0.0005, P=0.0195, P<0.0001, and P=0.0064) inhibition of alpha-glucosidase was observed for compounds 4, 5, 1, and 2, respectively, when compared to the control.

One compound (C3) out of the five did not show significant alpha-glucosidase inhibitory activity when compared to the control.

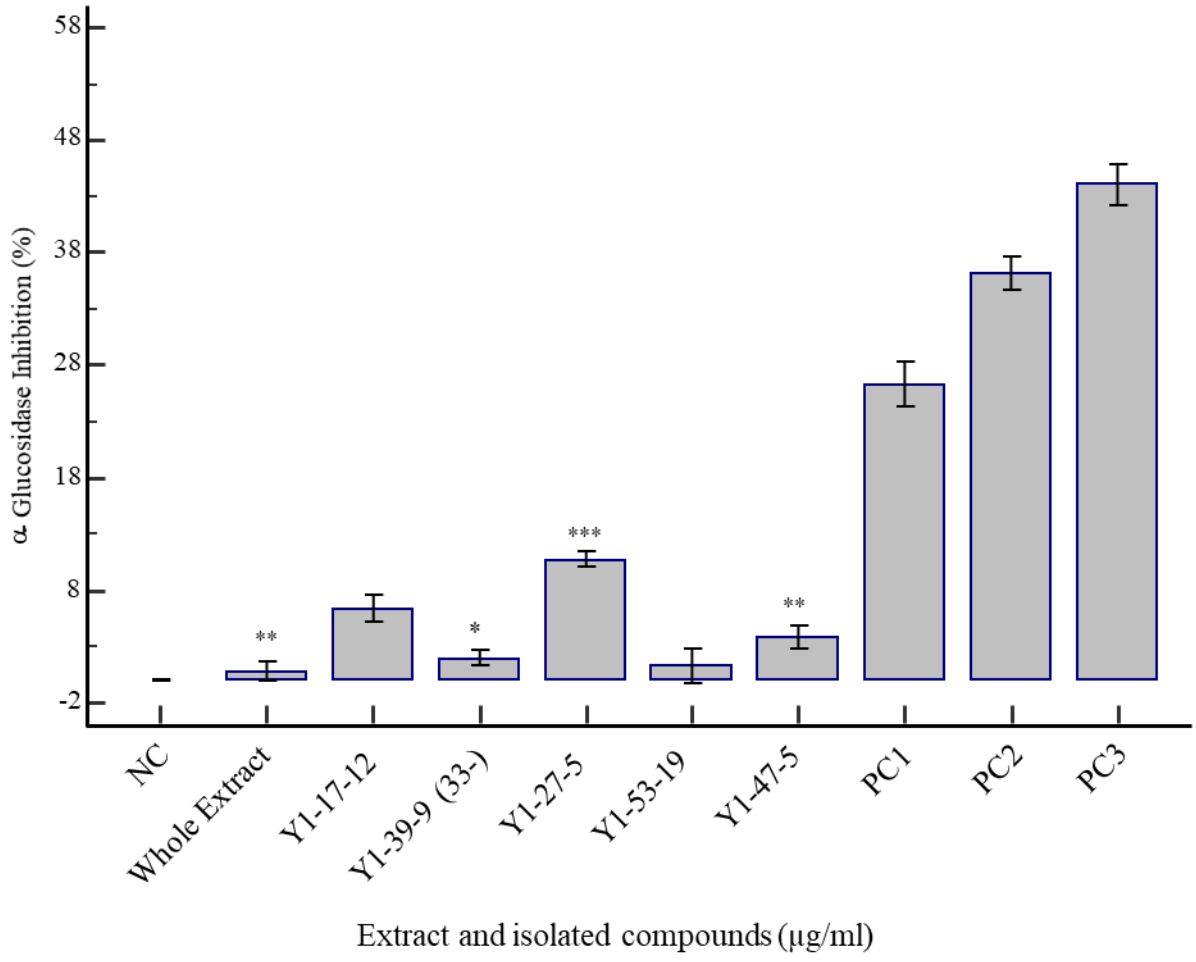


Figure 3.21: Comparative representation of α-glucosidase inhibitory activity. NC: Negative control, PC1: Positive control 1, PC2: Positive control 2, PC3: Positive control 3.

In the current study, Marrubin (**C5**) with a p-value of P=0.0195 has significant alpha-glucosidase inhibition. Relatively, in a study by El-Mohsen *et al*. (2014), aimed at investigating the effect of marrubin, a major constituent of many medicinal plants including marrubium alysson, as an alpha-glucosidase inhibitor. The bioassay-guided isolation led to the identification of three known labdane diterpenes; from which marrubin showed strong inhibition. The results, therefore, suggested that marrubin has an inhibitory effect on alpha-glucosidase and these findings provide insight into the traditional uses of Marrubium species for the treatment of diabetes. Accordingly, the speculations that marrubin has an inhibitory effect on alpha-glucosidase activity are confirmed. This could be helpful to develop medicinal preparations or nutraceutical and functional foods for diabetes and related symptoms.

CHAPTER 4: CONCLUSION

Diabetes mellitus remains one of the major health problems and a leading cause of death worldwide. The number of people living with diabetes is increasing exponentially day after day. Many of the current antidiabetic drugs are based on synthetic compounds which are most likely to have side effects. Although a limited number of effective antidiabetic drugs from plant sources are currently in use, there is still a need for developing effective, safe, and cheap antidiabetic drugs. The aim of this thesis was to investigate *Leonotis leonurus*, a plant used traditionally for the treatment of diabetes with the specific objectives to confirm the antidiabetic activity of the methanolic extract of the leaves of *L. leonurus*, and to evaluate the glucose uptake activity, cytotoxicity effect, as well as alpha-glucosidase of the isolated compounds. The present study demonstrated the medicinal potential of *L. leonurus* constituents to manage type 2 diabetes mellitus. The current findings indicated the antidiabetic and anticytotoxic properties of some of the isolated constituents using *in vitro* assays. The current findings provide a rationale for the use of this plant species in traditional medicine. Extracts of these plants can be developed into functional food for the amelioration or prevention of chronic diseases such as diabetes and chronic inflammation. However, the use of animal models or in vitro assays are needed to establish the safety and efficacy of these species in traditional medicine. The results suggested that amongst the five isolated compounds, only compound **4** showed a marked increase in glucose uptake, while compounds **1,2,3 and 4** showed significant signs of cytotoxicity. The results also demonstrate an overlap between glucose uptake and cytotoxicity for compound **4**. Compounds **2 and 5** both showed signs of cytotoxicity and no significant increase in glucose uptake activity. Therefore, it may be concluded that compound **4** can be considered as a potential candidate for the management of type 2 diabetes.

Marrubin (**C5**) is a furanic diterpene and represents a major constituent of many medicinal plants including *M. alysson*. It has shown interesting activity against alpha-glucosidase.

Therefore, marrubin and plant extracts containing a high quantity of marrubin can be considered as a new potential source of T2DM diabetes treatment due to their safety and efficiency as indicated in the literature.

Although these results provide important findings and confirm the antidiabetic properties of the leaf extract, without a doubt this research has some limitations which call for future studies. Therefore, the focus of future research could be

- Further isolation of more compounds found in this plant, also an investigation on other parts of this plant species, such as the branches and roots are proposed.
- The assays on both the extracts and pure compounds could be stretched to the evaluation of the isolated compounds on muscle cell lines to determine which compounds have the best antidiabetic properties.
- Alpha-amylase inhibitory activity

References

Ahmed, S., Zhou, Z., Zhou, J., and Chen S., 2016. Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine. Genomics Proteomics Bioinformatics. Vol 14 :298-313

Akash, M.S.H., Rehman, K. and Chen, S., 2013. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. *Journal of cellular biochemistry*, *114*(3), pp.525-531.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A., 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6(4), p.42.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H. and Rollinger, J.M., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology advances, 33(8), pp.1582-1614.

Atlas, D., 2015. International diabetes federation. *IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation*.

Babiaka, S.B., Ntie-Kang, F., Lifongo, L.L., Ndingkokhar, B., Mbah, J.A. and Yong, J.N., 2015. The chemistry and bioactivity of Southern African flora I: a bioactivity versus ethnobotanical survey of alkaloid and terpenoid classes. RSC Advances, 5(54), pp.43242-43267.

Bilal, M, Iqbal, M.S., Shah, S.B., Rasheed, T. and Iqbal, H., 2018. Diabetic complications and insight into antidiabetic potentialities of ethno-medicinal plants: a review. Recent Patents on Inflammation and Allergy Drug Discovery, 12, 7-23.

Bonnet, F. and Scheen, A., 2017. Understanding and overcoming metformin gastrointestinal intolerance. Diabetes, Obesity and Metabolism, 19(4), pp.473-481.

Bhatia, A., Singh, B., Arora, R. and Arora, S., 2019. In vitro evaluation of the α-glucosidase inhibitory potential of methanolic extracts of traditionally used antidiabetic plants. BMC complementary and alternative medicine, 19(1), pp.1-9.

Bhutani, J. and Bhutani, S., 2014. Worldwide burden of diabetes. *Indian journal of endocrinology and metabolism*, *18*(6), p.868

Brownrigg, J.R., Hughes, C.O., Burleigh, D., Karthikesalingam, A., Patterson, B.O., Holt, P.J., Thompson, M.M., de Lusignan, S., Ray, K.K. and Hinchliffe, R.J., 2016. Microvascular disease and risk of cardiovascular events among individuals with type 2 diabetes: a population-level cohort study. *The Lancet Diabetes & Endocrinology*, *4*(7), pp.588-597.

Burton, J.H., Johnson, M., Johnson, J., Hsia, D.S., Greenway, F.L. and Heiman, M.L., 2015. Addition of a gastrointestinal microbiome modulator to metformin improves metformin tolerance and fasting glucose levels. *Journal of diabetes science and technology*, *9*(4), pp.808-814.

Boy, H.I.A., Rutilla, A.J.H., Santos, K.A., Ty, A.M.T., Alicia, I.Y., Mahboob, T., Tangpoong, J. and Nissapatorn, V., 2018. Recommended medicinal plants as source of natural products: a review. Digital Chinese Medicine, 1(2), pp.131-142.

Cavan, D., Makaroff, L., da Rocha Fernandes, J., Sylvanowicz, M., Ackland, P., Conlon, J., Chaney, D., Malhi, A. and Barratt, J., 2017. The diabetic retinopathy barometer study: global perspectives on access to and experiences of diabetic retinopathy screening and treatment. Diabetes research and clinical practice, 129, pp.16-24.

Chatterjee, S., Khunti, K. and Davies, M.J., 2017. Type 2 diabetes. *The Lancet*, *389*(10085), pp.2239-2251

Chaudhury, A., Duvoor, C., Dendi, R., Sena, V., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N.S., Montales, M.T. and Kuriakose, K., 2017. Clinical review of

antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Frontiers in Endocrinology, 8*, p.6.

Chawla, A., Chawla, R. and Jaggi, S., 2016. Microvasular and macrovascular complications in diabetes mellitus: distinct or continuum? *Indian journal of endocrinology and metabolism*, *20*(4), p.546.

Cho, N.H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J.D., Ohlrogge, A.W. and Malanda, B., 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes research and clinical practice*, *138*, pp.271-281.

DeFronzo, R.A., Davidson, J.A. and Del Prato, S., 2012. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. *Diabetes, Obesity and Metabolism*, *14*(1), pp.5-14.

Dias, D.A., Urban, S. and Roessner, U., 2012. A historical overview of natural products in drug discovery. Metabolites, 2(2), pp.303-336.

Dirir, A.M., Daou, M., Yousef, A.F. and Yousef, L.F., 2021. A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews*, pp.1-31.

Dokken, B., 2012. The kidney as a treatment target for type 2 diabetes. *Diabetes Spectrum*, *25*(1), pp.29-36.

Du Plessis M, Pearce B, Jacobs C, Hoosain N, and Benjeddou M. (2015). "Genetic polymorphisms of the organic cation transporter 1 gene (SLC22A1) within the Cape Admixed population of South Africa". *Molecular Biology Reports, 42*(3), p.665-672

Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology, 4, p.177.

El-Mohsen, A., Rabeh, M.A., Abou-Setta, L., El-Rashedy, A.A. and Hussein, A.A., 2014. Marrubiin: a potent α-glucosidase inhibitor from Marrubium alysson. International Journal of Applied Research in Natural Products, 7(1), pp.21-27.

Etsassala, N.G., Cupido, C.N., Iwuoha, E.I. and Hussein, A.A., 2020. Abietane Diterpenes as Potential Candidates for the Management of Type 2 Diabetes. *Current pharmaceutical design*, *26*(24), pp.2885-2891.

Florez JC. 2017. The Pharmacogenomics of metformin. Diabetologia. Vol 60:1648-1655

Florez JC. 2017. The Pharmacogenomics of metformin. *Diabetologia*. (60), p.1648-1655

Jamshidi-Kia, F., Lorigooini, Z. and Amini-Khoei, H., 2018. Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1).

Goboza, M., Aboua, Y.G., Meyer, S. and Oguntibeju, O.O., 2016. Diabetes mellitus: economic and health burden, treatment, and the therapeutical effects of Hypoxis hemerrocallidea plant. Medical Technology South Africa, 30, 39-46.

Hanson, J.R., 2017. A hundred years in the elucidation of the structures of natural products. Science progress, 100(1), pp.63-79.

Hamed, A.N., Attia, E. and Desoukey, S.Y., 2021. A review on various classes of secondary metabolites and biological activities of Lamiaceae (Labiatae)(2002-2018). *Journal of advanced Biomedical and Pharmaceutical Sciences*, *4*(1), pp.16-31.

Hostalek U, Gwilt M, Hildemann S. (2015). Therapeutic Use of Metformin in Prediabetes and Diabetes Prevention. Drugs. Vol 75:1071–1094

Hussein, A.A., 2018. Chemistry of South African Lamiaceae: Structures and biological activity of terpenoids. Terpenes and Terpenoids.

Jacob C, Pearce B, Du Plesses M, Hoosain N. and Benjeddou, M., 2015. Single nucleotide polymorphisms of the SLC22A2 gene within Xhosa population of South Africa. *Drug metabolism and Pharmacokinectics*. Vol 30:457-460

Jamshidi-Kia, F., Lorigooini, Z. and Amini-Khoei, H., 2018. Medicinal plants: Past history and future perspective. Journal of herbmed pharmacology, 7(1).

Kalra, S., 2014. Alpha glucosidase inhibitors. *JPMA. The Journal of the Pakistan Medical Association*, *64*(4), pp.474-476.

Kalra, S. and Gupta, Y., 2015. Sulfonylureas. *JPMA. The Journal of the Pakistan Medical Association*, *65*(1), pp.101-104.

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B.J., Jacobsen, L.M., Schatz, D.A. and Lernmark, Å., 2017. Type 1 diabetes mellitus. *Nature reviews Disease primers*, *3*(1), pp.1-17.

Khan, M.A.B., Hashim, M.J., King, J.K., Govender, R.D., Mustafa, H. and Al Kaabi, J., 2020. Epidemiology of type 2 diabetes–global burden of disease and forecasted trends. Journal of epidemiology and global health, 10(1), p.107.

Kharroubi AT, Darwish HM. 2015. Diabetes mellitus: The epidemic of the century. World J Diabetes 2015; 6(6): 850-867 [PMID: 26131326 DOI: 10.4239/wjd.v6.i6.850]

Kleinberger, J.W. and Pollin, T.I., 2015. Personalized medicine in diabetes mellitus: current opportunities and future prospects. *Annals of the New York Academy of Sciences*, *1346*(1), pp.45-56.

Lahlou, M., 2013. The success of natural products in drug discovery.

Liang Y, Li S, and Chen L., 2015. The physiological role of drug transporters. Protein & Cell. Vol 6(5):334–350

Li, B., Cantino, P.D., Olmstead, R.G., Bramley, G.L., Xiang, C.L., Ma, Z.H., Tan, Y.H. and Zhang, D.X., 2016. A large-scale chloroplast phylogeny of the *Lamiaceae* sheds new light on its subfamilial classification. Scientific reports, 6(1), pp.1-18.

Lipska, K.J., Warton, E.M., Huang, E.S., Moffet, H.H., Inzucchi, S.E., Krumholz, H.M. and Karter, A.J., 2013. HbA1c and risk of severe hypoglycemia in type 2 diabetes: the Diabetes and Aging Study. *Diabetes care*, *36*(11), pp.3535-3542.

Mather, A. and Pollock, C., 2011. Glucose handling by the kidney. *Kidney international*, *79*, pp. S1-S6.

Mahomoodally, M.F., 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evidence-Based Complementary and Alternative Medicine, 2013.

Marín-Peñalver, J.J., Martín-Timón, I., Sevillano-Collantes, C. and del Cañizo-Gómez, F.J., 2016. Update on the treatment of type 2 diabetes mellitus. *World journal of diabetes*, *7*(17), p.354.

Mazimba, O., 2015. Leonotis nepetifolia: A herbal medicine review. Journal of pharmacognosy and phytochemistry, 3(6).

Mbanya, J.C.N., Motala, A.A., Sobngwi, E., Assah, F.K. and Enoru, S.T., 2010. Diabetes in sub-saharan africa. The lancet, 375(9733), pp.2254-2266.

McCreight, L.J., Bailey, C.J., and Pearson, E.R. 2016. Metformin and the gastrointestinal tract. *Diabetologia*. 59, p. 426-435

McCreight, L.J., Bailey, C.J. and Pearson, E.R., 2016. Metformin and the gastrointestinal tract. Diabetologia, 59(3), pp.426-435.

McGaw, L.J., Elgorashi, E.E. and Eloff, J.N., 2014. Cytotoxicity of African medicinal plants against normal animal and human cells. In Toxicological Survey of African Medicinal Plants (pp. 181-233). Elsevier.

Mirhoseini, M., Baradaran, A. and Rafieian-Kopaei, M., 2013. Medicinal plants, diabetes mellitus and urgent needs. Journal of HerbMed Pharmacology, 2(2).

Mohammedi, K., Woodward, M., Marre, M., Colagiuri, S., Cooper, M., Harrap, S., Mancia, G., Poulter, N., Williams, B., Zoungas, S. and Chalmers, J., 2017. Comparative effects of microvascular and macrovascular disease on the risk of major outcomes in patients with type 2 diabetes. *Cardiovascular diabetology*, *16*(1), p.95.

Mnonopi, N., Levendal, R.A., Mzilikazi, N. and Frost, C.L., 2012. Marrubiin, a constituent of Leonotis nepetifolia, alleviates diabetic symptoms. Phytomedicine, 19(6), pp.488-493

Navale, A.M. and Paranjape, A.N., 2016. Glucose transporters: physiological and pathological roles. *Biophysical reviews*, *8*(1), pp.5-9.

Napoli, E., Siracusa, L. and Ruberto, G., 2020. New tricks for old guys: recent developments in the chemistry, biochemistry, applications and exploitation of selected species from the Lamiaceae family. *Chemistry & biodiversity*, *17*(3), p.e1900677.

Narukawa, Y., Komori, M., Niimura, A., Noguchi, H. and Kiuchi, F., 2015. Two new diterpenoids from Leonotis nepetifolia R. Br. *Journal of natural medicines*, *69*(1), pp.130-134.

Nsuala, B.N., Enslin, G. and Viljoen, A., 2015. "Wild cannabis": A review of the traditional use and phytochemistry of Leonotis nepetifolia. *Journal of ethnopharmacology*, *174*, pp.520-539.

Nsuala, B.N., Kamatou, G.P., Sandasi, M., Enslin, G. and Viljoen, A., 2017. Variation in essential oil composition of Leonotis nepetifolia, an important medicinal plant in South Africa. *Biochemical systematics and ecology*, *70*, pp.155-161.

Nsuala, B.N., Enslin, G. and Viljoen, A., 2015. "Wild cannabis": a review of the traditional use and phytochemistry of Leonotis nepetifolia. Journal of ethnopharmacology, 174, pp.520-539

Oboh G, Akinyemi AJ, Ademiluyi AO, Bello FO., 2013. Inhibition of alpha-amylase and alpha-glucosidase activities by ethanolic extract of Amaranthus cruentus leaf as affected by blanching. Afr J Pharm Pharmacol.;7(17):1026-32.

Odei-Addo, F., Shegokar, R., Müller, R.H., Levendal, R.A. and Frost, C., 2017. Nanoformulation of Leonotis nepetifolia to improve its bioavailability as a potential antidiabetic drug. 3 Biotech, 7(5), pp.1-9.

Odeyemi, S. and Bradley, G., 2018. Medicinal Plants Used for the Traditional Management of Diabetes in the Eastern Cape, South Africa: Pharmacology and Toxicology. *Molecules*, *23*(11), p.2759.

Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., Cavan, D., Shaw, J.E. and Makaroff, L.E., 2017. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes research and clinical practice, 128, pp.40-50.

Okhuarobo, A., Falodun, J.E., Erharuyi, O., Imieje, V., Falodun, A. and Langer, P., 2014. Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pacific journal of tropical disease*, *4*(3), pp.213-222.

Okaiyeto, K. and Oguntibeju, O.O., 2021. African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications. International Journal of Environmental Research and Public Health, 18(11), p.5988.

Olokoba, A.B., Obateru, O.A. and Olokoba, L.B., 2012. Type 2 diabetes mellitus: a review of current trends. *Oman medical journal*, *27*(4), p.269.

Papatheodorou, K., Papanas, N., Banach, M., Papazoglou, D., and Edmonds M., 2016. Complications of Diabetes. *Journal of Diabetes Research*

Papatheodorou, K., Banach, M., Bekiari, E., Rizzo, M. and Edmonds, M., 2018. Complications of Diabetes 2017. *Journal of diabetes research*, *2018*.

Pistia-Brueggeman, G. and Hollingsworth, R.I., 2001. A preparation and screening strategy for glycosidase inhibitors. *Tetrahedron*, *57*(42), pp.8773-8778.

Pheiffer, C., Pillay-van Wyk, V., Joubert, J.D., Levitt, N., Nglazi, M.D. and Bradshaw, D., 2018. The prevalence of type 2 diabetes in South Africa: a systematic review protocol. BMJ open, 8(7), p.e021029.

Pollastro, C., Ziviello, C., Costa, V. and Ciccodicola, A., 2015. Pharmacogenomics of drug response in type 2 diabetes: toward the definition of tailored therapies?. *PPAR research*, *2015*.

Raja, R.R., 2012. Medicinally potential plants of Labiatae (Lamiaceae) family: an overview. *Research journal of medicinal plant*, *6*(3), pp.203-213.

Rambiritch V, Maharaj B and Ramberitch PN., 2014. glibenclamide in patients with poorly controlled type 2 diabetes: a 12-week, prospective, single-center, open-label, doseescalation study. Clinical pharmacology: advances and applications. Vol 6: 63-6

Rattray, R.D. and Van Wyk, B.E., 2021. The Botanical, Chemical and Ethnobotanical Diversity of Southern African Lamiaceae. Molecules, 26(12), p.3712.

Rasool, A., Bhat, K.M., Sheikh, A.A., Jan, A. and Hassan, S., 2020. Medicinal plants: Role, distribution and future. Journal of Pharmacognosy and Phytochemistry, 9(2), pp.2111-2114.

Rendell, M., 2004. The role of sulphonylureas in the management of type 2 diabetes mellitus. *Drugs*, *64*(12), pp.1339-1358.

Rehab, A.H. and Amira, A.E., 2018. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. Inbook: Herbal Medicine, doi, 10.

Rines, A. K., Sharabi, K., Tavares, C. D., & Puigserver, P., 2016. Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. *Nature reviews. Drug discovery*, *15*(11), 786-804.

Rosak, C. and Mertes, G., 2012. Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes, metabolic syndrome and obesity: targets and therapy*, *5*, p.357.

Soccio, R.E., Chen, E.R. and Lazar, M.A., 2014. Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. *Cell metabolism*, *20*(4), pp.573-591.

Sofowora, A., Ogunbodede, E. and Onayade, A., 2013. The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary and alternative medicines, 10(5), pp.210-229.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K. and Shaw, J.E., 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice*, *157*, p.107843.

Siavash, M., Tabbakhian, M., Sabzghabaee, A.M. and Razavi, N., 2017. Severity of gastrointestinal side effects of metformin tablet compared to metformin capsule in type 2 diabetes mellitus patients. *Journal of research in pharmacy practice*, *6*(2), p.73.

Sola, D., Rossi, L., Schianca, G.P.C., Maffioli, P., Bigliocca, M., Mella, R., Corlianò, F., Fra, G.P., Bartoli, E. and Derosa, G., 2015. Sulfonylureas and their use in clinical practice. *Archives of medical science: AMS*, *11*(4), p.840.

Standl, E., Theodorakis, M.J., Erbach, M., Schnell, O. and Tuomilehto, J., 2014. On the potential of acarbose to reduce cardiovascular disease. *Cardiovascular Diabetology*, *13*(1), pp.1-12.

Street, R.A. and Prinsloo, G., 2013. Commercially important medicinal plants of South Africa: a review. Journal of chemistry, 2013.

Thrasher, J., 2017. Pharmacologic management of type 2 diabetes mellitus: available therapies. *The American journal of cardiology*, *120*(1), pp.S4-S16.

Thomford, N.E., Senthebane, D.A., Rowe, A., Munro, D., Seele, P., Maroyi, A. and Dzobo, K., 2018. Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. International journal of molecular sciences, 19(6), p.1578.

Tuasha, N., Petros, B. and Asfaw, Z., 2018. Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle District, Sidama Zone, Ethiopia. *Journal of ethnobiology and ethnomedicine*, *14*(1), p.15.

Tuomi, T., Santoro, N., Caprio, S., Cai, M., Weng, J. and Groop, L., 2014. The many faces of diabetes: a disease with increasing heterogeneity. *The Lancet*, *383*(9922), pp.1084-1094.

Umamaheswaran, G., Praveen, R.G., Damodaran, S.E., Das, A.K. and Adithan, C., 2015. Influence of SLC22A1 rs622342 genetic polymorphism on metformin response in South Indian type 2 diabetes mellitus patients. *Clinical and experimental medicine*, *15*(4), pp.511-517.

Van Wyk, A.S. and Prinsloo, G., 2020. Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. South African Journal of Botany, 133, pp.54-62.

Veeresham, C., 2012. Natural products derived from plants as a source of drugs. *Journal of advanced pharmaceutical technology & research*, *3*(4), p.200.

Vella, A., 2019. Pharmacogenetics for type 2 diabetes: practical considerations for study design. J Diabetes Sci Technol. Vol 1;3(4):705-9.

Vhakaria, M.P., Zad, V.R., Mankar, N.N., Wadivkar, P.P., Shah, K.U., 2016. Pharmacogenomics of Metformin: A way to personalised medicine. *Interanational Journal of Phramacology and Clinical Sciences*. *5*(2), p.55-61

Webb, E.M., Rheeder, P. and Van Zyl, D.G., 2015. Diabetes care and complications in primary care in the Tshwane district of South Africa. Primary care diabetes, 9(2), pp.147-154.

Whiting , D.R., Guariguata, L., Weil, C. and Shaw, J., 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes researcg and clinical practice, 94,311-321.

Yuan, H., Ma, Q., Ye, L. and Piao, G., 2016. The traditional medicine and modern medicine from natural products. Molecules, 21(5), p.559.

Yuet Ping, K., Darah, I., Yusuf, U.K., Yeng, C. and Sasidharan, S., 2012. Genotoxicity of Euphorbia hirta: an Allium cepa assay. Molecules, 17(7), pp.7782-7791.

Zheng, J., Woo, S.L., Hu, X., Botchlett, R., Chen, L., Huo, Y. and Wu, C., 2015. Metformin and metabolic diseases: a focus on hepatic aspects. *Frontiers of medicine*, *9*(2), pp.173-186.

Zheng, Y., Ley, S.H. and Hu, F.B., 2018. Global etiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*, *14*(2), p.88