

ANTI-HYPERGLYCEMIC, ANTI-OXIDANT, AND CYTOTOXICITY
ACTIVITY OF SELECTED ETHNO MEDICINAL PLANTS FROM THE
HARDAP REGION OF NAMIBIA.

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ABSTRACT

Diabetes mellitus is a multifactorial non-communicable metabolic disorder, characterized by the perpetual manifestation of hyperglycemia as a consequence of the disturbed metabolism of carbohydrates, fat, and protein, due to defects in insulin secretion and/or its effectiveness. Currently prescribed medication such as the derivatives of Biguanides, have excellent therapeutic benefits but cause impaired liver and kidney due to lactic acidosis. Based on the historic success of medicinal plants as remedies for many ailments and the increasing need for alternative medication for diabetes, plants from the Hardap region, in Namibia, were evaluated as potential Complementary and Alternative Medicine (CAM) for diabetes. In Namibia, medicinal plants are used as primary health care to manage diabetes, especially by those in resource-poor settings. However, there is a paucity of data supporting the use of these plants for this purpose. Scientific data must be generated to support the use of Namibian plants as complementary and alternative medicines for managing diabetes. To this end, a survey on the use of medicinal plants for the management of diabetes in the Hardap region of Southern Namibia was conducted. The survey revealed eleven plants belonging to nine different families, that is Asphodelaceae, Malvaceae, Pedaliaceae, Apocynaceae, Lamiaceae, Geraniaceae, Zygophyllaceae, Tiliaceae, and Fabaceae as authenticated by the National Herbarium. However, of the eleven plants, only five were available for collection for laboratory analysis, namely *Corchorus tridens*, *Sarcocaulon salmoniflorum*, *Zygophyllum decumbens*, *Hermannia fruticulosa*, and *Hoodia gordonii*.

The plant materials were air-dried at room temperature before being ground to a powder for extraction using ethanol, methanol, and water. The plant extracts were

subjected to Thin Layer Chromatography (TLC) screening, followed by quantification of total flavonoid content (TFC) and total phenolic content (TPC). The biological properties of the extracts; antioxidant activity involving DPPH and reducing power; antihyperglycemic activity using α amylase and α glucosidase assay; and *in vitro* cytotoxicity assay using MTT assay, were evaluated.

The study demonstrated the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids in plants. Quantification of phytochemicals showed high TPC content in *C. tridens*, methanol extracts (23.58 ± 0.41) mgQE/g, and ethanol extracts (22.79 ± 0.16). High TFC was observed in ethanol extracts of *C. tridens* (96.90 ± 7.04) mgGAE/g, followed by *Z. decumbens* (49.98 ± 2.97) mg GAE/g then *S. salmoniflorum* (44.55 ± 0.44) mg GAE/ g. Data showed a statistically significant difference between TFC and TPC ($p < 0.05$).

Plants showed free radical scavenging potential, with *C. tridens* ethanol extracts exhibiting the highest scavenging activity with IC_{50} 0.0312 ± 21.05 mg/ml followed by methanol extracts of *H. fruticulosa* with IC_{50} of 0.0339 ± 24.64 mg/ml when compared with the positive control (ascorbic acid) with IC_{50} of 0.0279 ± 17.09 mg/ml. The different solvents used for plant extraction significantly influenced the free radical scavenging potential of plants ($p < 0.05$) while no significant difference was demonstrated across the different concentrations ($p > 0.05$). The reducing power of extracts showed no significant concentration dependence ($p > 0.05$). High reducing activity was recorded in methanol extracts of *S. salmoniflorum* 1.84 ± 0.025726834 and *C. tridens* 1.5 ± 0.014854405 when compared with the ascorbic acid of 3.16 ± 0.026394 .

Antihyperglycemic potency of plant extracts was evident with the inhibition of α -amylase recorded for aqueous extracts of *Z. decumbens* and *H. gordonii*, 96.3% and 93.9 % respectively when compared with the positive control (acarbose) inhibition capacity of 64.2 % at 1 mg/ml. The inhibition of α -amylase is not significantly influenced by the concentration ($p > 0.05$), though showed significant reliance on the solvent used for extraction ($p < 0.05$). Aqueous extracts of *H. gordonii* revealed potency with the lowest IC₅₀ of 0.1667 mg/ ml. A qualitative α -glucosidase inhibition potential was demonstrated by all the tested plant extracts, through the ability to prevent the degradation of starch in the presence of α -glucosidase and starch that was evident by the stain that was produced on the agar. The cell line of 3T3 proliferated 90 % and above in the presence of the studied plant extracts. The Kruskal- Wallis test revealed that the growth of 3T3 cell lines was significantly influenced by the difference in extract concentrations ($p < 0.05$). Moreover, these cells proliferate differently in the presence of the different studied plants ($p < 0.05$). The safety data of cytotoxicity indicate less toxicity to none except for *C. tridens* with IC₅₀ 0.2014 \pm 5.491 μ g / μ l.

The demonstrated anti-hyperglycemic activity of the studied plants, especially aqueous extracts of *Z. decumbens* and *H. gordonii* can be attributed to the presence of the tested phytochemicals and may infer that they have potential anti-hyperglycemic agents as well as validate their use in the traditional setting. Further *in vivo* and detailed phytochemical analysis is recommended to identify active components of the plants and their mechanism of action.

Keywords: Anti-hyperglycemic, Diabetes mellitus, anti-oxidants, DPPH, TLC, 3T3 cell line

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LIST OF ABBREVIATIONS AND OR ACRONYMS

AGEs	Advanced glycation end products
AMPK	Activated protein kinase
ATM	African traditional medicine
BAT	Brown adipose tissue
CAD	Coronary artery disease
CNS	Central nervous system
DAG	Diacylglycerol
DKA	Diabetes ketoacidosis
DM	Diabetes Mellitus
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNSA	Dinitrosalicylic acid
DPP-4	Dipeptidyl-peptidase-4
DPPH	2,2-diphenyl-1-picrylhydrazyl
FFAs	Free fatty acids
FPG	Fasting plasma glucose
GAE	Gallic acid equivalent
GDM	Gestational diabetes mellitus
GI	Glycemic index
GIBEX	Global institute for bio exploration
GIP	Glucose-dependent insulintropic polypeptide
GL	Glycemic load
GLUT4	Glucose transporter-4
HbA1c	Glycated hemoglobin
HGP	Hepatic glucose production
IC ₅₀	Half maximal inhibitory concentration
TIIDDM	TIIDM (Insulin depended diabetes mellitus)
IGT	Impaired glucose tolerance
IR	Insulin resistivity
LA	Lactic acidosis
LDL	Low-density lipoprotein
MTT	MTT (3-(4,5-Dimethylthiazol-2-yl)
NADPH	Nicotinamide adenine dinucleotide phosphate
NEFAs	Non-esterified fatty acids
NOX	NADPH oxidase
O ₂ ⁻	Superoxide
OGTT	Oral glucose tolerance test
PAD	Peripheral artery disease
PBS	Phosphate buffer solution
PKC	Protein kinase

ROS	Reactive oxygen species
RPG	Random (casual) plasma glucose
SAT	Subcutaneous WAT
SD	Standard Deviation
SGLT2	Sodium glucose cotransporter 2
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TCM	Traditional Chinese medicine
TFC	Total Flavonoid content
TGs	Triglycerides
TKM	Traditional Korean medicine
TLC	Thin Layer Chromatography
TM	Traditional medicine
TPC	Total phenolic content
TZDs	Thioazolidinediones
WAT	White adipose tissue
WHO	World Health Organization

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DEDICATION

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DECLARATIONS

I, Kaveire Kaitjizemine, declare that this study is a true reflection of my research and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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CHAPTER ONE: INTRODUCTION

1.1 Orientation of the proposed study

The World Health Organization (WHO) defines traditional medicine (TM) as ‘the sum total of knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, that are used to maintain health, as well as to prevent, diagnose, improve or treat physical or mental illness’ (World Health Organization, 2013). This treatment has been the primary means of treating illnesses due to the lack of access to modern medication and its assumed efficacy (Dan, Mchombu and Mosimane, 2010). Plants have been an integral part of traditional medicine for generations and at present, are also used as precursors for drug discovery and development (Jain *et al.*, 2019). Researchers around the globe have reported on how plants contribute to the healthcare systems, especially to those in the rural community. Mussarat *et al.* (2014) reported that out of 422,000 flowering plants globally, 50,000 of those plants are used for medicinal purposes. Moreover, in Namibia of the 3159 plant species recorded only 615 (19.5 %) plant species are reported for their medicinal significance (Cheikhoussef *et al.*, 2011). Despite the prevailing therapeutic significance displayed by many medicinal plants, there is still a lack of scientific validation and their use is either poorly recorded or just not recorded (Ekor, 2014). A survey of the literature has revealed, no information on Namibian medicinal plants that are scientifically validated for their antihyperglycemic effect.

The therapeutic properties demonstrated by plants are based on the presence of naturally occurring phytochemicals usually secondary metabolites (DAS *et al.*, 2018). These phytochemicals provide defense against herbivores, enable interaction with other microorganisms, and assist in survival during adverse growth conditions, such as hot and drought conditions, and various other environmental stressors. (Jain *et al.*,

2019). Amongst the many classes of secondary phytochemicals, alkaloids, flavonoids, and saponins are documented for their anti-hyperglycemic effect (Saxena *et al.*, 2013; Yoo *et al.*, 2018).

1.2 Diabetes Mellitus (DM)

Diabetes mellitus is a complex, non-communicable metabolic disorder characterized by persistent high blood sugar levels due to impaired carbohydrate, fat, and protein metabolism as a result of insulin secretion and/or action defects (Beseni *et al.*, 2019; Muriira, 2014). Hyperglycemia may progress into Chronic hyperglycemia with both microvascular and macrovascular consequences (cardiovascular disorder, neuropathy, and retinopathy) (Balogun *et al.*, 2016; Afroz *et al.*, 2019).

DM is associated with significant progressive morbidity and early mortality worldwide, resulting in reduced life expectancy (Adekanmbi *et al.*, 2019). In 2012 Africa had 14 million cases of diabetes and 401 276 deaths (Etsassala *et al.*, 2019), and it is further projected to increase to 34.2 million by 2040 (World Health Organization, 2016). According to Adekanmbi *et al.* (2019), the prevalence of diabetes in Namibia is 5.1 % and it is expected to continue increasing. This emphasizes the urgent need to educate Namibians about the burden of diabetes and prediabetes.

Diabetes mellitus is classified into three most prominent types, namely, Insulin-dependent diabetes mellitus (type I DM), Insulin-independent diabetes (type II DM), and gestational diabetes mellitus (GDM), type III. Type I is an auto-immune-induced diabetes that results from defects (inability to produce insulin) in the pancreatic beta cells. Type II is the most common form of diabetes which is caused by reduced insulin secretion and or insulin resistance. Type III is common during pregnancy, it is

characterized by insulin resistance and may progress to type II diabetes (International Diabetes Federation, 2015).

The complexity of the human body and the pathways involved in the pathogenesis of diseases make it impossible to manage diseases with a single target compound. In the same way, the management of DM involves several pathways (Peron, Ogbonna and Donohoe, 2015) and treatment with a single glucose-lowering agent only provides limited glucose control (Lavernia, Adkins and Shubrook, 2015). Consequently, two enzymes (alpha-amylase and alpha-glucosidase) are used to regulate the digestion of carbohydrates and postprandial glucose levels in diabetic patients (Poovitha and Parani, 2016; Alqahtani *et al.*, 2019). Additionally, managing diabetes includes modifying diets, making lifestyle changes, and using multiple glucose-lowering medications (Forouhi *et al.*, 2018).

The available synthetic drugs such as metformin, sulfonylurea, thiazolidinediones, acarbose, and miglitol have their efficiency limited by their inaccessibility, high pricing, and in some cases, life-threatening side effects. These include the prolonged conditions of hyperglycemia which damage targeted organs and causes neuropathy (Karthikeyan, 2017), flatulence, diarrhea hypoglycemia, liver damage, weight gain, drug resistance, heart failure, and induced lactic acid which may lead to death (Etsassala *et al.*, 2019).

Plant-based medicine has been utilized for generations and is considered to be safer, more accessible, and more affordable, especially for those in resource-poor settings. This is because of the synergistic effects provided by their multiple constituents, resulting in multiple targets (Caesar and Cech, 2019). There is a need to find safer natural anti-hyperglycemic compounds with minimum side effects.

1.3 Problem statement

Diabetes is an increasingly important cause of morbidity and mortality worldwide, including in Namibia. While the synthetic medications available are effective, they are associated with long-term, life-threatening side effects; they are not widely accessible to all communities; and their pricing regimen makes them unaffordable to those in resource-poor settings (Balogun *et al.*, 2016). In Namibia communities such as those in the Hardap region use medicinal plants to manage diabetes, however, there is no scientific evidence on their efficacy, chemical composition, and safety to support this practice. It is thus essential to document the use of these and promote their mainstream use based on their biological activity and phytochemical properties.

1.4 Significance of the study

The study will provide information on the *in vitro* anti-hyperglycemic activity and safety of the plants of interest used by the Hardap community to manage diabetes, consequently validating their use. Therefore, warrant their use in the development of effective and safe herbal products while further motivating future research capacity on other plants, contributing towards value addition of natural resources, and generating bases for access and benefit sharing agreements.

1.5 Objectives of the study

- To identify and document medicinal plants used to treat symptoms of diabetes in the Hardap region, Namibia.
- To screen for classes of phytochemical constituents of the identified medicinal plants using Thin Layer Chromatography (TLC).
- To examine the antioxidant activity of the plant extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (stable free radical) and reducing power assay.

- To determine the anti-hyperglycemic activity of different plant extracts using Enzyme-based assays (alpha-amylase and alpha-glucosidase).
- To evaluate the cytotoxicity effects of the medicinal plant extracts using embryonic fibroblast cell lines 3T3 cell line.

1.6 Research Hypothesis

Ethno medicinal plants, from the Hardap region used to treat symptoms of DM, have anti-hyperglycemic activity, due to their potential to lower blood glucose levels which are attributed to the presence of plant secondary metabolites.

1.7 Limitations of the Study

The plants used in this study were based on their availability during the period of collection, the study could have included more plants had they been available. Furthermore, secondary metabolites that are responsible for activity are dependent on adverse conditions including climatic conditions under which plants grow. Hence, a generalization about these plants collected during other periods is difficult to qualify. Screening of phytochemicals was done for only seven classes of compounds known for anti-hyperglycemic activity therefore new uses for other classes of compounds may not be identified. A muscle cell line was used for cytotoxic studies and in vitro screening for anti-hyperglycemic activity which may not be a true representation of a clinical situation. Lastly, only one mode of action focusing on the prevention of diabetes was studied, other modes of action such as the conversion of glucose into fat and increased absorption of glucose by muscles were not studied.

1.8 Delimitation of the study

The research focal area was the Hardap region but was not exhaustive focusing on two districts. The delimiting factor for this study is that it only focused on the Hardap region in Southern Namibia and only medicinal plants used in this community and on which the community was knowledgeable were included.

CHAPTER 2: LITERATURE REVIEW

2.1 Status of diabetes mellitus

2.1.1 Morbidity and mortality prevalence of diabetes mellitus

The global prevalence of diabetes mellitus remains a leading cause of progressive morbidity and mortality (Adekanmbi *et al.*, 2019). Due to factors such as being overweight, physical inactivity, unhealthy diets, and sedentary lifestyles (WHO, 2016; IDF, 2015). According to the World Health Organization (2019), the global prevalence increased from 4.7 % in 1980 to 8.5% in 2014, which is the greatest rise recorded in low to middle-income countries. Consequently, increasing global deaths estimates in 2019 to 4.2 million (Saeedi *et al.*, 2019).

2.1.2 Prevalence of diabetes in Africa

In 2017, Africa reported 15.5 million diabetic adults aged 20-79 and over 69.2% undiagnosed (prediabetes) (IDF, 2017). Undiagnosed diabetes is when individuals have plasma glucose levels that meet the testing criteria for diabetes but have not been diagnosed by a physician (An, 2016). This group of individuals accounts for over 50 % of diabetic cases globally, impacting both the economy and health sector due to delayed monitoring of secondary complications that diabetes may pose (Forouzanfar *et al.*, 2016). Alarming 80% of diabetic cases in Africa are undiagnosed (Kasole, Martin and Kimiywe, 2019).

According to IDF (2017) diabetes in Africa is projected to escalate to 41 million in 2045 from 16 million. This increase is fairly low compared to other regions but disturbingly high as it translates to a 156 % increase which is the highest increase across all regions (Figure 1).

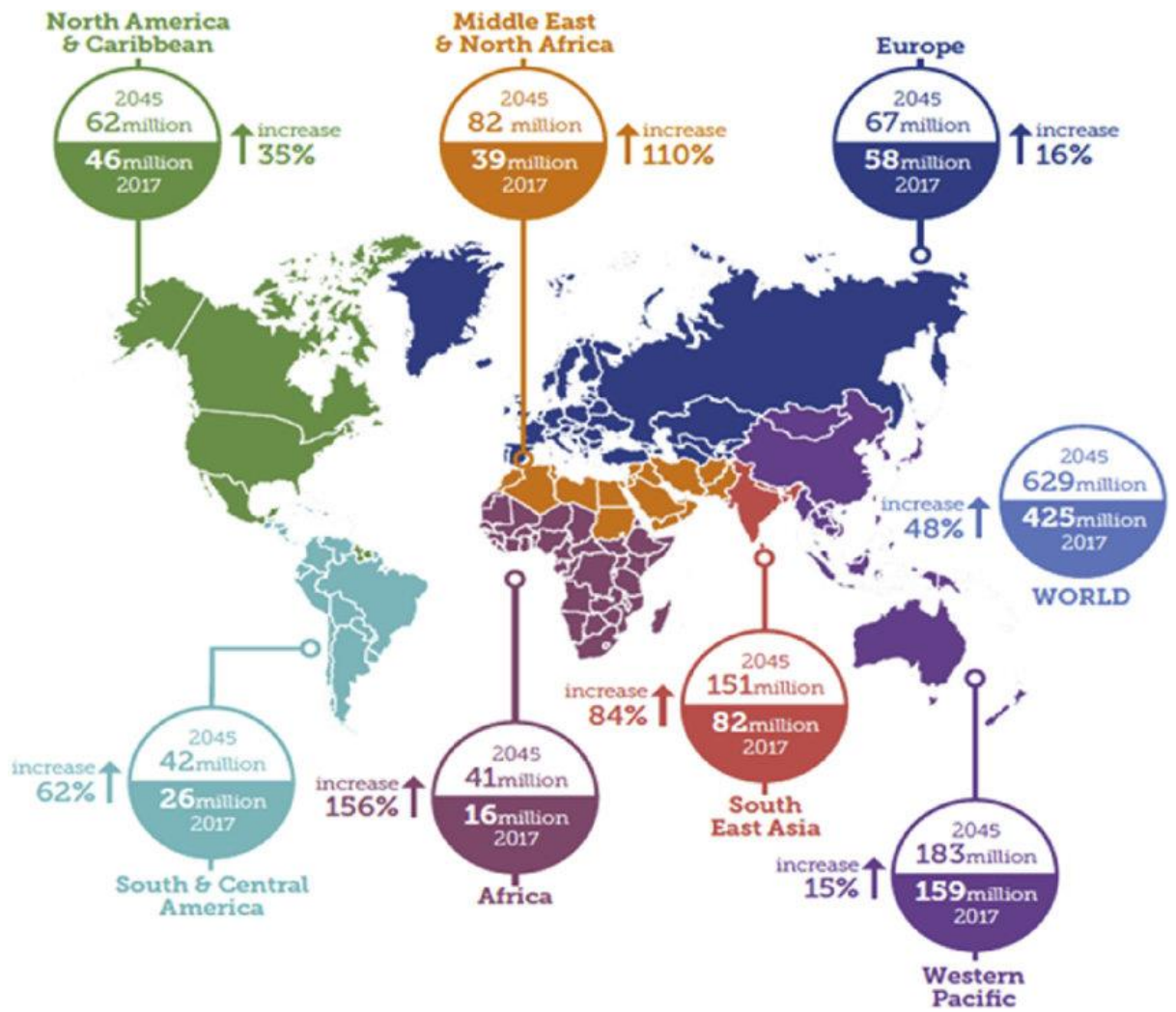


Figure 1. Estimates of DM prevalence worldwide and in regions, in 2017-2040 for years 20-79 (IDF Atlas, 2017).

Furthermore, the reported deaths due to diabetes in Africa were 366 200, and 73.1 % of these deaths were people below the age of 60 years, accounting for the highest proportion in the world when compared to Europe with 31.4 % (Saeedi *et al.*, 2019).

In Namibia, the prevalence of DM steadily increased from 3883 to 6337 cases over five years from 2008 to 2013, with the highest cases reported in the cities (Ministry of Health and Social Services, 2014). A Namibian-focused study by Adekanmbi *et al.* (2019) reported a 6.7 % age-adjusted prevalence of diabetes, with no significant

difference found between prevalence and prediabetes amongst both genders suggesting a future of high diabetes cases in the country.

2. 2. Classes of Diabetes Mellitus

DM is classified based on the cause and presented symptoms of the disease. The most prevalent types of diabetes are type I, type II, and type III.

2.2.1 Type I diabetes

Type I diabetes mellitus also referred to as insulin-dependent diabetes (T1DM) was previously associated with juvenile or childhood-onset, however, it is now common in all age groups (International Diabetes Federation, 2015; WHO, 2019). T1DM is a chronic auto-immune-induced diabetes attacking insulin-generative pancreatic beta cells of the islet of Langerhans, which results in insulin deficiency (IDF, 2015). Genetic susceptibility, environmental (viral infection), epigenetic, and immunologic (cellular immunity) are some of the common contributing factors of T1DM (Figure 2) (Paschou *et al.*, 2018).

Symptoms of T1DM include polyphagia (constant hunger), polydipsia (persistent thirst), polyuria (continuous urination), and constant high blood glucose (hyperglycemia) (Kahanovitz, Sluss and Russell, 2017). This type is managed with a daily injection of insulin and lack thereof may result in ketone build-up, also known as diabetes ketoacidosis (DKA) which is life-threatening (Kamau, 2018). T1DM accounts for 10 % of diabetes cases worldwide (Diabetes Federation International, 2019). Patients with this type of diabetes in economically disadvantaged countries succumb to early death and disability due to limited access to self-management education and insulin (Patterson *et al.*, 2019). Unfortunately, the cure for T1DM is still

being sought. At present, there is no effective and safe treatment available to prevent or delay the progression of type I diabetes (IDF, 2019).

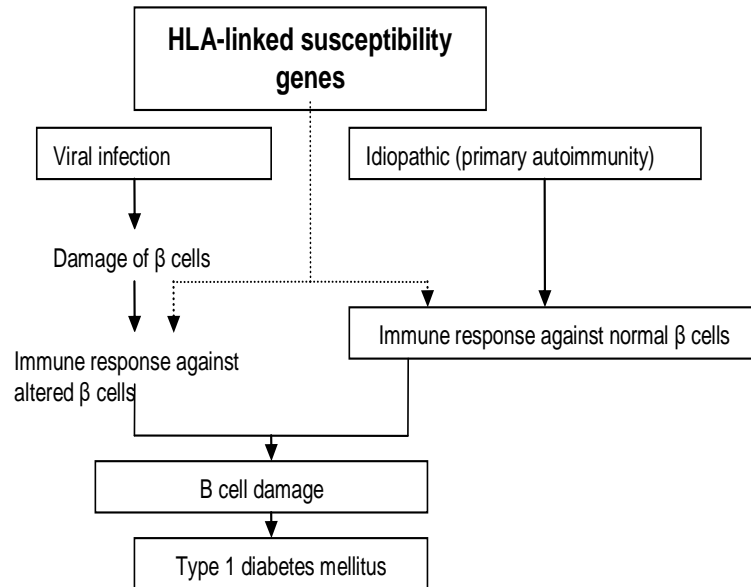


Figure 2. Pathogenesis of type I diabetes mellitus (Thovhogi, 2009).

2.2.2 Type II diabetes

Type II diabetes mellitus which is referred to as insulin-independent diabetes (TIIDM), was in the past common among adults but now seems high in children and adolescents (Kamau, 2018). TIIDM is characterized by two factors; a reduction in insulin secretion Bharti *et al.* (2018) and a failure of insulin-sensitive tissue to respond to the insulin produced (WHO,2016). This type of diabetes accounts for 90-95 % of diabetes cases worldwide and is most prevalent in middle to low-income countries (IDF, 2019).

As shown in Figure 3, factors such as urbanization, gluttony, poor diet, physical inactivity obesity contribute to the development and progression of type 2 diabetes (IDF, 2017). And results in excess nutrients which end up being stored as fat and accumulate around organs, causing increased adipocytes and further reducing cellular

insulin sensitivity, decrease in glucose transportation, and subsequently inducing hyperglycemia, especially in those pre-dispositioned by their genetics (Malone and Hansen, 2019).

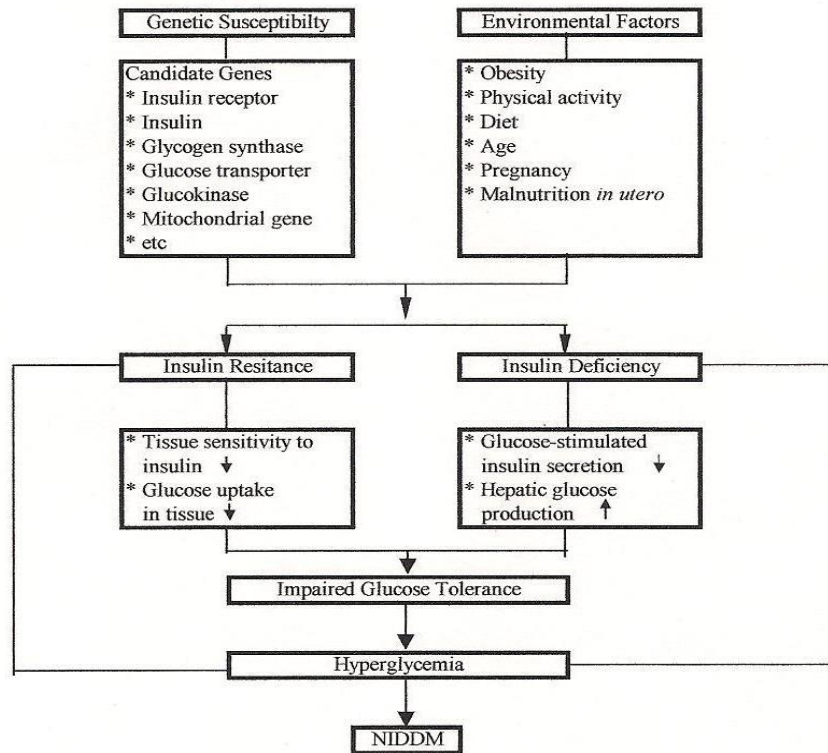


Figure 3. Pathogenesis of type II diabetes mellitus (Thovhogi, 2009).

The reduced transport of glucose consequently encourages oxidation-induction of glucose transporter (GLUT4) which further induces oxidative stress (Malone and Hansen, 2019). This induced oxidative stress contributes significantly to the progression of IIDM and insulin resistivity (IR) through the activation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase (NOX) and superoxide (O_2^-) which further triggers five major pathways involved in diabetes complications. These pathways are polyol pathways, increased formation of advanced glycation end products (AGEs) increased expression of AGEs receptors, activation of ligand,

activation of protein kinase (PKC) isoforms, and overreaction of hexosamine pathway (Galicia-garcia *et al.*, 2020; Masschelin *et al.*, 2020). AGEs cause macro and microvascular complications through increased cross-linking in vascular walls (atherosclerosis) and thrombosis due to the accumulation of low-density lipoprotein (LDL) particles as a result of nonfunctional endothelial cells (Kamau, 2018).

Despite the alarming figures, information and education on diabetes are lacking in sub-Saharan African countries Zimmermann *et al.* (2018) and Namibia in particular (Adekanmbi *et al.*, 2019). Adekanmbi *et al.* (2019), further acknowledged that acquiring substantial knowledge on diabetes would allow for early detection and improve management, lessening the impact it would have on the patient, especially for T1DM patients whose symptoms are often delayed.

2.2.3 Gestational diabetes

Type III diabetes mellitus or Gestational diabetes mellitus (GDM) is a short-term glucose intolerance condition detected during pregnancy as a result of hormones produced by the placenta that affect glucose metabolism (IDF, 2017; Plows *et al.*, 2018). Placental hormone lactogen triggers an increase in blood glucose by reducing the body's insulin sensitivity, leading to high blood glucose which in most instances progresses to GDM (Muche, Olayemi and Gete, 2019). Visceral adiposity, advanced age, polycystic ovarian syndrome, and hereditary hypertension are factors that contribute to the development of GDM (Oyebode, 2018). According to the WHO (2016), GDM may be transient but pose serious complications as it may progress into either type I or type II for the mother or the fetus and even result in childbirth complications.

The prevalence of GDM is 15 % globally, and 87.6 % of this is from low to middle-income countries (Muche, Olayemi and Gete, 2019). GDM contributes to 90 % of perinatal complications when compared to non-diabetic pregnancy (Oyebode, 2018). Management of GDM includes the use of insulin and or oral antidiabetic drugs, whose safety and effectiveness remain a concern (Plows *et al.*, 2018).

2.3 Mechanism of Insulin action and resistance

Insulin is an endocrine peptide hormone secreted by the pancreatic beta-cells of the islet of Langerhans, to regulate the metabolism of carbohydrates, fat, and protein (Qaid and Abdelrahman, 2016). Its role in the metabolism of glucose is defined by the effect of insulin on one or a combination of the main three organs namely the skeletal muscle, liver, and adipocytes (Figure 4) (Petersen and Shulman, 2018).

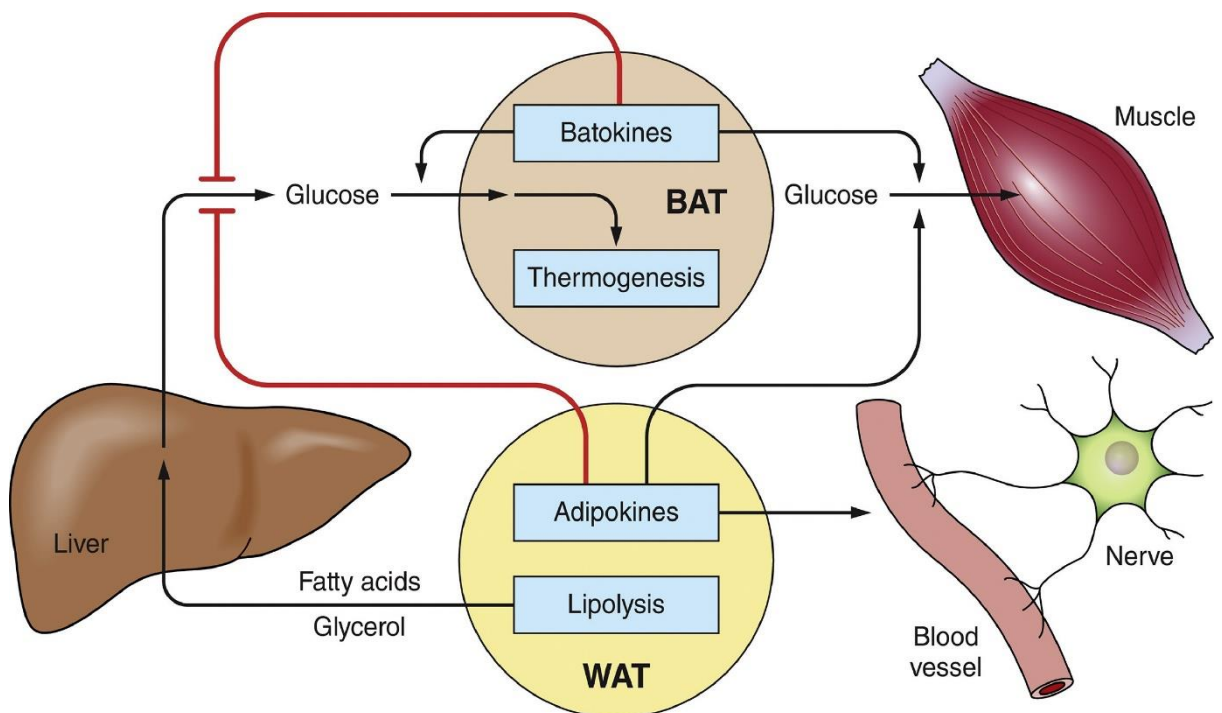


Figure 4. Major pathways used in regulating systematic metabolism. (Czech, 2020).

Skeletal muscle contributes to the metabolism of glucose by regulating the disposal and transportation of insulin-stimulated glucose. It accounts for over 80% of insulin-stimulated glucose uptake and is thus a major site for peripheral insulin resistance (Mazibuko-Mbeje *et al.*, 2018). Glucose is first phosphorylated by hexokinase to glucose-6-phosphate before being transported to skeletal muscle and the rest of the body (Evans *et al.*, 2019). Failure to successfully dispose of glucose leads to impaired glucose uptake in the entire body, increasing blood glucose levels, which subsequently contribute to the development and progression of Type 2 diabetes mellitus (Chadt and Al-Hasani, 2020).

Whereas the liver is involved in the metabolism of glucose, through the amalgamated pathways to produce, store, and regulate glucose homeostasis (Jiang *et al.*, 2020). During low blood glucose levels, the pancreatic alpha cells activate the peptide hormone glucagon to facilitate the breakdown of glycogen to glucose molecules (hepatic glycogenolysis) and the production of glucose from non-carbohydrate precursors anew (hepatic gluconeogenesis) in the liver (Chadt and Al-Hasani, 2020). Glucose production induced by hepatic gluconeogenesis accounts for approximately 90 % of the circulating blood glucose as cited by Szablewski (2017) and is pivotal for maintaining systemic glucose homeostasis (Petersen and Shulman, 2018). However, under diabetic conditions, the overproduction thereof may induce impaired insulin-dependent suppression of hepatic glucose production (HGP) and subsequently stimulate elevated blood glucose levels (Zhang *et al.*, 2019). According to Jiang *et al.* (2020), the impaired insulin resistance of hepatic cells is triggered by gluconeogenesis, increasing levels of glucagon which translates to high levels of glucose, fatty acids, and as well as the excess of free fatty acids circulating (FFAs).

Thirdly, adipose tissue is the third organ involved in the metabolism of glucose, Adipose tissue encompasses three types of adipose tissues, namely the white adipose tissue (WAT) the main triglyceride storage, the beige within the WAT and lastly the brown adipose tissue (BAT) which are responsible for regulating respiration in the mitochondria (Czech, 2020a). WAT is further subdivided into subcutaneous WAT (SAT) and Visceral WAT (VAT) concentrated around internal organs (Choe *et al.*, 2016). Adipose tissues are generally known for their metabolic influence on glucose and lipids, while also regulating insulin sensitivity (Chadt and Al-Hasani, 2020; Gastaldelli *et al.* 2017). They are the chief regulators of metabolism and cell signaling in the liver and skeletal muscle (Czech, 2020). The insulin released during metabolism acts on adipocytes to perform the following actions; the uptake of glucose as the main energy reservoir, manufacturing of triglyceride, and release of FFA and glycerol in addition to the attenuation of triglyceride hydrolysis (Gastaldelli *et al.*, 2017). When neutral triglycerides (TGs) the energy stored in adipocytes is in excess, it leads to the expansion of adipose and consequently obesity (Luo and Liu, 2016). The expansion of adipocytes leads to the release of non-esterified fatty acids (NEFAs) glycerol, hormones, and pro-inflammatory cytokines that induce insulin resistance and subsequently the progression of type 2 diabetes (Wondmkun, 2020). A study suggested that the initiation of insulin resistance is a counter-response to the increase in plasma NEFA levels (Algoblan, Alalfi and Khan, 2014). Similar to the revelation by Algoblan *et al.* (2014) numerous studies on this topic suggest many hypotheses on the molecular and cellular disruption that contributes to insulin resistance. Nevertheless, noteworthy uncertainty remains on the mechanisms of its initiation and long-term maintenance (Czech, 2020b).

2.4 Complications of diabetes mellitus

Poor management of hyperglycemia may progress to either acute or chronic complications, (IDF, 2017). Complications of type I include disability or early death due to ketone buildup in the body, also known as DKA (IDF, 2019). Whereas complications of type II are categorized into two, namely microvascular and macrovascular complications (Chawla et al., 2016). Microvascular complications encompass nephropathy, neuropathy, and retinopathy. Those associated with macrovascular complications include coronary artery disease (CAD), angina / myocardial infarction, peripheral artery disease (PAD) stroke, diabetic encephalopathy, seizures, and diabetic foot (Kosiborod *et al.*, 2018). Differentiating between the pathogenic mechanism of these two types of diabetes and their response to therapy remains controversial and unclear (Zhao *et al.*, 2019).

2.5 Oxidative stress in T1DM complications

Hyperglycemia and many other age/lifestyle-related diseases stimulate mitochondrial oxidative stress through the production of reactive oxygen species (ROS) production (Forrester *et al.*, 2018). ROS are byproducts of enzymatic activities and are essential for normal cellular functions including that of beta-cells (Elksnis *et al.*, 2019). However excessive production thereof, such as that induced by hyperglycemia through the mitochondrial respiratory chain, triggers the advancement of diabetes secondary complications (Volpe *et al.*, 2018). Due to low oxidative enzymes in the beta-cells, they subsequently succumb to oxidative damage affecting cell functionality and even resulting in cell death (Haque *et al.*, 2016; Fakhruddin *et al.*, 2017). As a consequence, mitochondrial diacylglycerol (DAG), polyol, and hexosamine pathways are activated as indicated by Volpe *et al.* (2018) and Thovhogi, (2009) (Figure 5), validating the

suggestion by MAH *et al.* (2016) to include anti-oxidant agents in the regime of diabetes. As antioxidants help with the deleterious effects of hyperglycaemia as well as enhance glucose metabolism and uptake (Sarian *et al.*, 2017).

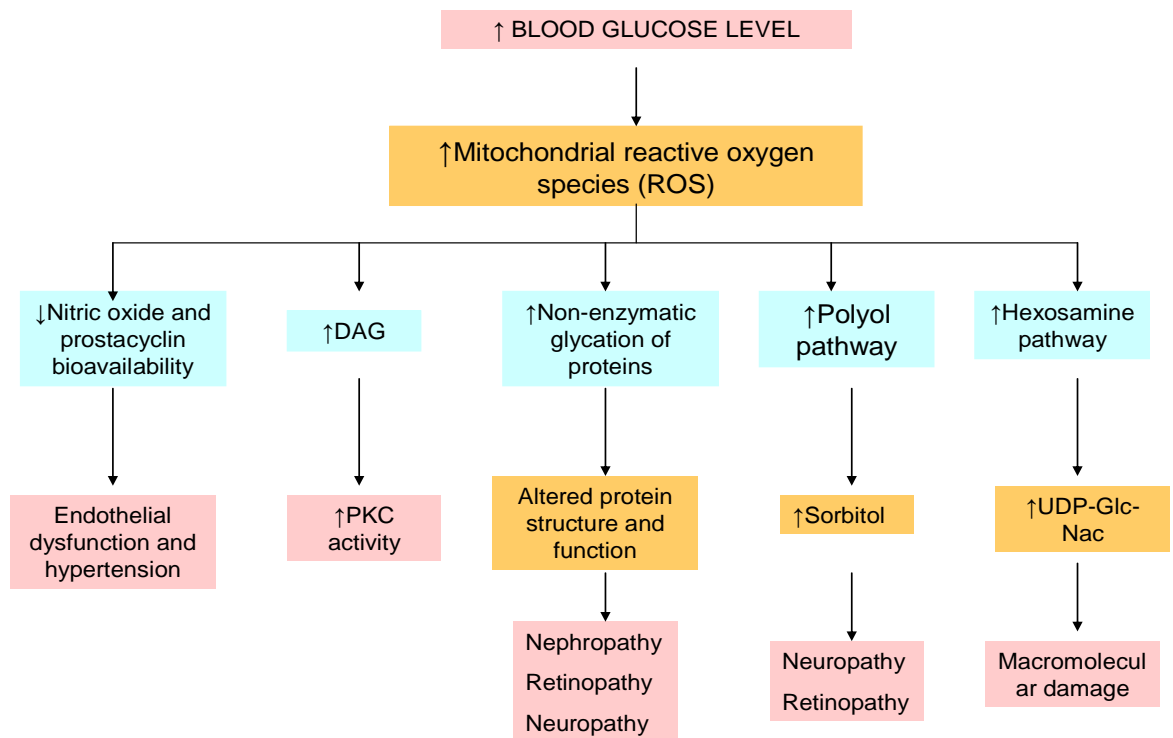


Figure 5. Metabolic pathways triggered by hyperglycemia-induced ROS and resulting complications (Thovhogi, 2009).

2.6 Diagnosis of diabetes mellitus

The diagnosis of DM is based on glucose plasma levels. A healthy person's glucose plasma level ranges between 100 mg / dL fasting and 160 mg / dL postprandial state, levels above the indicated scenarios are considered hyperglycemia or diabetes (WHO, 2019). The following are the certified diagnostic criteria.

2.6.1 Fasting plasma glucose test (FPG)

FPG is a type of testing that requires fasting for at least eight hours before the test, without the uptake of calories. Any time after the eighth hour, if plasma glucose values

are ≥ 126 mg/ dL or ≥ 7.0 mmol/L, this indicates a positive for diabetes mellitus (ADA, 2019; WHO, 2019).

2.6.2 Oral glucose tolerance test (OGTT)

There are two types of oral glucose tests, namely one-hour and two-hour plasma glucose tests (IDF, 2019). This test requires prior consumption of 75 grams of glucose preceding the test, either one or two hours prior. If the plasma glucose level is ≥ 200 mg / dL or ≥ 11.1 mmol / L it is an indication of a positive diabetes test. Among the two, a one-hour plasma glucose test is the most recommended, owing to its sensitivity and ability to identify early intermediate hyperglycemia (ADA, 2019).

2.6.3 Glycated hemoglobin (HbA_{1c})

The glycated hemoglobin test detects long-term hyperglycemia as far back as over 90 90-day period (Cheneke *et al.*, 2016). This method of testing is convenient in that no prior consumption of glucose is required and has greater pre-analytical stability with a cutoff point for diagnosing diabetes of ≥ 6.5 % or 48 mmol / mol (IDF, 2019).

2.6.4 Random (casual) plasma glucose (RPG)

As per the name, this test can be performed at any given time, randomly. If plasma glucose is ≥ 200 mg/dL the patient is diabetic (ADA, 2019; WHO, 2019). RPG is endorsed for its feasibility and inexpensiveness (Ain *et al.*, 2017). This test may provide a lead for further diagnosis and early detection, narrowing the gap between undiagnosed and diagnosed patients.

WHO and IDF recommend the use of one-hour OGTT for the detection of impaired glucose tolerance (IGT) and fasting glucose tolerance (FGT). One-hour OGTT is the most sensitive method of the four, capable of detecting intermediate hyperglycemia earlier on, which would mean early intervention. The second recommendation is given

to Glycated hemoglobin (HbA1c), but this method is only endorsed for the use of HbA1c > 6.5 % diabetic diagnosis and not for intermediate hyperglycemia because of the quality assurance measurements that are not readily available worldwide (IDF, 2019).

2.7 Management of DM

As indicated above, DM has life-threatening complications and its advancement may be delayed with tightened glycemic controls. These controls may be achieved through the use of the non-pharmacological mainstay of diabetes treatment, which is diet and physical activity, however when the glycemic treatment goal is not achieved other treatments are considered (Patel *et al.*, 2012). These treatments include the use of pharmacological oral hypoglycemic drugs, acupuncture, hydrotherapy, and mineral supplements (Muriira, 2014). Although controlled glycemic levels are achieved with oral hypoglycemic drugs, after prolonged use the efficacy of the drugs is reduced coupled with long-term side effects (Ganesan, 2022). As a result, the pursuit of less toxic drugs originated.

2.7.1 Modifiable management of DM

2.7.1.1 Exercise and Physical Activity

Exercise and physical activity are modifiable diabetic risk factors. The two are of great health benefits in the progression and management of most ailments. They are both voluntary body movements with the ability to delay the progression and manage DM (Colberg *et al.*, 2016). The former includes any structured and specific activity purposed to improve fitness, while the latter involves any skeletal body movement with no clear objective (Critch, 2017). Skeletal muscle is the principal tissue in regulating blood glucose homeostasis. It does this by controlling the disposal and transportation of insulin-stimulated glucose (Mazibuko-Mbeje *et al.*, 2018). Skeletal

muscle reacts distinctively to muscle contraction or exercise with increased sensitivity to subsequent insulin stimulation while regulating the metabolism of glucose. This is orchestrated by the complex and highly regulated signaling cascades that prompt diverse and unique effects on skeletal muscle (Sylow *et al.*, 2021). Furthermore, the skeletal movement also helps regulate the distribution of intra-abdominal fat, thus reducing the amount of fat stored by the body (Sami *et al.*, 2015).

As the prevalence of diabetes is on the rise globally, exercising is appreciated even more. Similarly, a study by Stanford and Goodyear (2014) showed partial to complete remission of type II diabetes with increased physical activeness. However, factors such as the duration of exercise, the intensity of exercise, assessment of skeletal muscle, and the effect of glucose uptake by muscles are different across patients and be examined accordingly for effective results (Evans *et al.*, 2019).

2.7.1.2 Dietary modification

The other modifiable factor used in managing DM is the modification of diets alongside a well-maintained normal body weight (Critch, 2017). Nutritional management is a pivotal component of metabolic controls, thus delaying the progression of chronic complications (Alberti, 2010). The concept of Glycemic index (GI) and Glycemic load (GL) is key in diet modification and has reported a direct correlation to increased risks of diabetes (Greenwood *et al.*, 2013). GI measures the change in blood glucose after ingestion of carbohydrate-containing foods, whereas (GL) measures glucose response and insulin demand produced by the total amount of carbohydrates ingested. They presented a 40 % and 27 % correlation respectively (Farvid *et al.*, 2014). Evidence strongly suggests that high GI and GL diets predispose patients to higher postprandial blood glucose and insulin concentration which

consequently results in glucose intolerance hyperglycemia and increases the risk of progressing into type II diabetes (Greenwood *et al.*, 2013; Farvid *et al.*, 2014).

2.7.2 Pharmacological management of Diabetes mellitus

2.7.2.1 Sulfonylureas

Sulfonylureas is an insulin secretagogue, one of the oldest oral medications for DM, dating back to 1950 (Costello, Nicola, Shivkumar 2021). The drugs under this group all have a phenyl-sulfonyl-urea sequence which gives them hypoglycemic properties, the R and R₁ radicals change according to the pharmacokinetic and pharmacotoxicological characteristics (Figure 6) (Confederat *et al.*, 2015). This class includes the likes of glipizide, glyburide, glydazide, and glimepiride (Ganesan *et al.*, 2020). They function to directly stimulate insulin secretion, by binding to sulfonylureas receptors (inhibiting ATP-sensitive potassium channels in the pancreatic beta cells), leading to the release of potassium and subsequent cellular depolarization. The depolarization triggers the release of calcium which further results in the stimulation of insulin exocytosis (Wajid *et al.*, 2019).

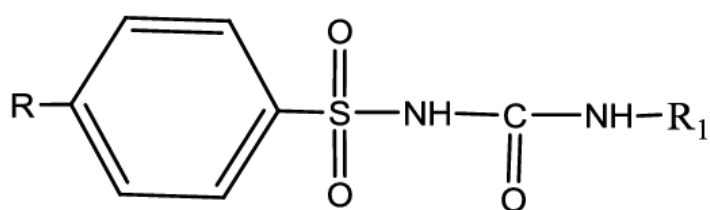


Figure 6. Sulfonylurea's general chemical structure (Confederat *et al.*, 2015).

Sulfonylureas are one of the most preferred oral drugs, due to their ability to rapidly achieve adequate glycemic control when used in combination with metformin (Sola *et*

al., 2015). Moreover, sulfonylureas are an excellent antioxidant that can neutralize ROS produced by diabetes (MAH *et al.*, 2016)

However, since it acts directly on beta cells for the stimulation of insulin release, Sulfonylureas require some functioning beta cells, thus only indicated for type II patients (Muriira, 2014). Due to direct involvement with beta cells, sulfonylureas over time lead to the progressive dysfunction of beta cells and worsen insulin secretion (Sola *et al.*, 2015). Further research on sulfonylureas reported induced hypoglycemia and cardiovascular complications through increased insulin receptors for both glucose and non-glucose secretagogues, implying the excess release of insulin (Riddle, 2017). In uncontrolled cases, hypoglycemia may result in loss of consciousness, seizure, coma, or demise (Costello, Nicola, Shivkumar 2021).

2.7.2.2 Meglitinides

Meglitinides (Figure 7) and sulfonylureas are both insulin secretagogues. Just like sulfonylureas, meglitinide drugs are well documented for their ability to instantly lower blood glucose through rapid insulin secretion. Their mechanism of action is similar to that of Sulfonylureas, even though their binding site is distinct. The group of medication under meglitinides include repaglinide and nateglinide (Wajid *et al.*, 2019; Patil *et al.*, 2021).

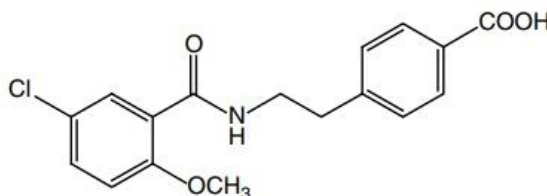


Figure 7. Meglitinide's chemical structure (Patil *et al.*, 2021).

As much as the mechanism of action of meglitinides is similar to that of sulfonylureas and even in requiring at least a few functioning beta cells, their level of hypoglycemia intensity is less in contrast to that of sulfonylureas (Marín-Peñalver *et al.*, 2016).

Milner and Akhondi (2021) reported hypoglycemia and cardiovascular complications as the major consequences resulting from the use of meglitinides, with minor risks including weight gain, diarrhea, joint pain, and peripheral edema, especially when used in combination with Thiazolidinediones (TZDs).

2.7.2.3 Biguanides

Biguanides (Figure 8) are a class of oral glucose-lowering drugs that include metformin (dimethyl biguanide), a worldwide recommended first-line pharmacological treatment for type 2 diabetes (T2D) (Song, 2016). The success of metformin as the first-line treatment of choice is due to its relatively low levels of hypoglycemia and its excellent use as either monotherapy or combination therapy with other oral glucose-lowering drugs (Giaccari *et al.*, 2021; LaMoia and Shulman, 2021). Despite being recognized as the first line of treatment for type II DM for over 50 years, the molecular mechanism of metformin is not fully understood (Giaccari *et al.*, 2021; Minamii *et al.*, 2018; Deepika *et al.*, 2015).

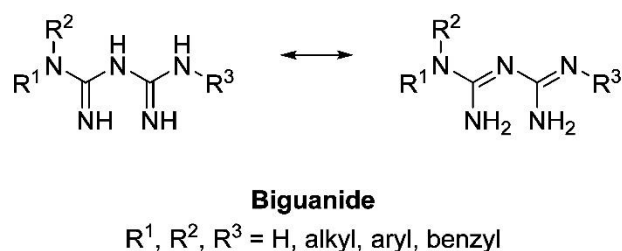


Figure 8. Biguanide chemical structure (Deepika Kathuria, Apoorva A. Bankar, 2015).

Metformin achieves its functions as a hyperglycemic treatment through the suppression of hepatic glucose production and hepatic gluconeogenesis, whilst improving insulin sensitivity and further activating peripheral glucose absorption and consumption (Wajid *et al.*, 2019). Furthermore, metformin is not absorbed in the body but expelled directly through urine without its activity being affected by metabolic enzymes (Becker *et al.*, 2013). Since it is not affected by enzymatic metabolisms, its successful elimination depends on renal function (Giaccari *et al.*, 2021).

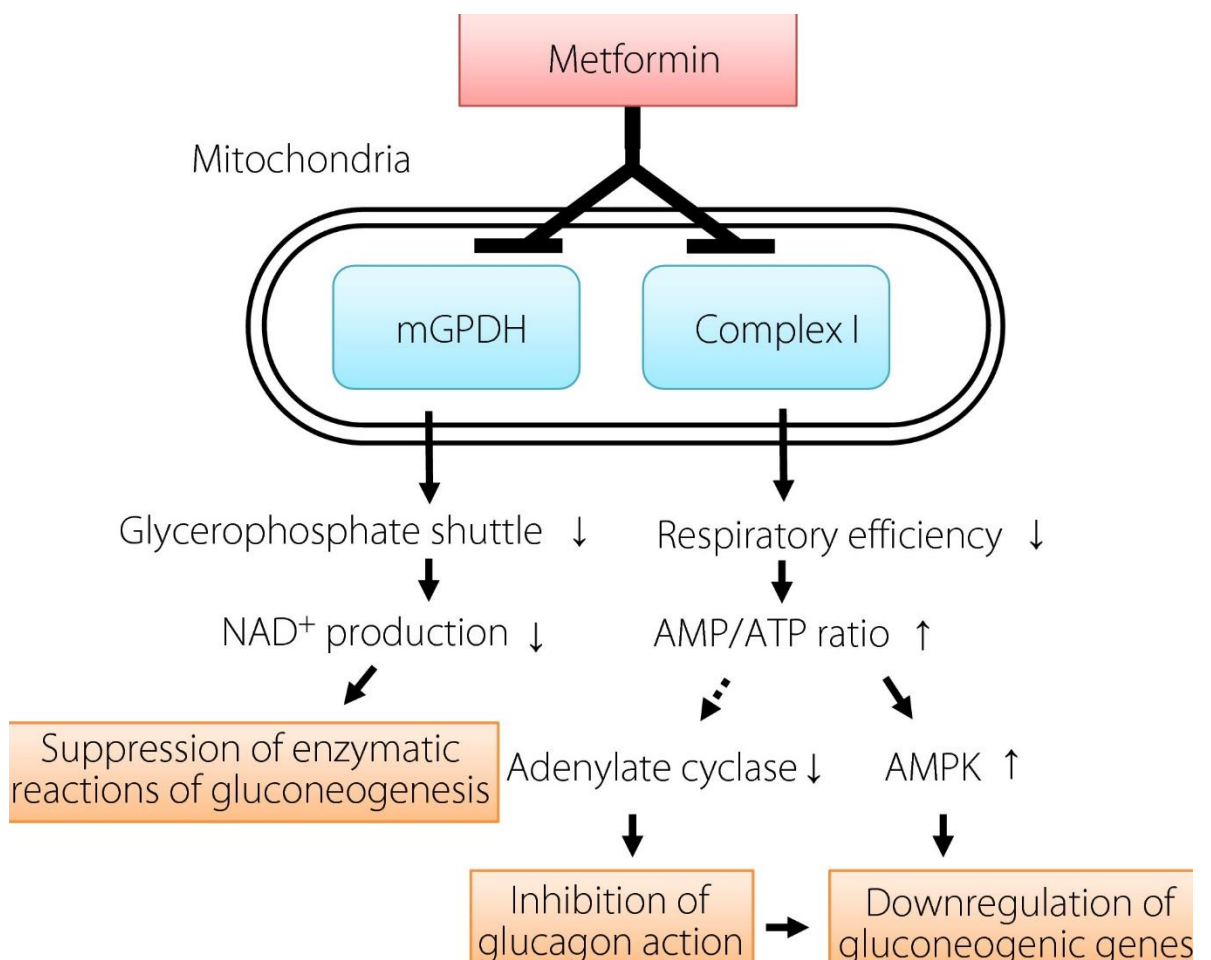


Figure 9. Metformin mechanisms involved in the inhibition of gluconeogenesis (Minamii et al., 2018).

The main issue with the use of metformin is lactic acidosis (LA) which results from impaired liver and kidney function (Riddle, 2017). According to Ghosal and Ghosal (2019), LA can be life-threatening. Symptoms associated with LA include lethargy,

irregular breathing, irregular heart rate, diarrhea, nausea, abdominal pain, muscle pain, and fatigue (Ghosal and Ghosal, 2019; Nasri and Rafieian-Kopaei, 2014).

2.7.2.4 Thiazolidinediones (TZDs)

According to Wajid *et al.* (2019) and Lebovitz (2019a), the use and popularity of TZDs (Figure 10) was discovered in 1997 and have since then been considered the only treatment for insulin resistance. Rosiglitazone and pioglitazone are the only two classes of TZDs, available in the United States. These drugs are agonists of the peroxisome proliferator-activated receptor (PPAR- γ) (Eggleton and Jialal, 2021; Wajid *et al.*, 2019).

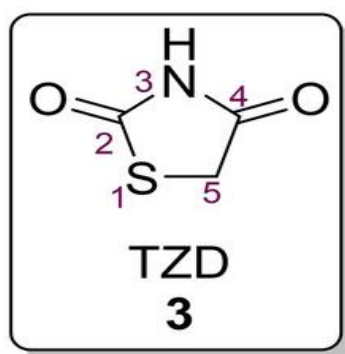


Figure 10. Thiazolidinediones (TZDs) chemical structure (Long, Gresley and Wren, 2021).

TZDs control of insulin sensitivity is achieved through the regulation of gene expression by binding to the nuclear transcription regulator, which activates paroxysmal proliferator receptor gamma (PPAR-gamma) (Eggleton and Jialal, 2021). Binding to the nuclear paroxysmal proliferator-activated receptor induces the activation of genes that encode proteins responsible for the metabolism of glucose and lipids. Their activation translates into increased glucose uptake by the skeletal muscle and adipose tissue, reduced hepatic glucose output, and lastly, an increase in free fatty acid uptake and consequently reduced glucose levels in the blood over time (Wajid *et*

al., 2019). Muriira (2014) suggested that the increase in insulin sensitivity is achieved through the suppression of adipokine expression involved in insulin resistance.

TZDs drugs are efficient and provide long-term glycemic controls, with no hypoglycemia reported (Hurren and Dunham, 2021). However the main issue is with long-term use of TZDs, patients are faced with side effects such as peripheral edema, congestive heart failure, and bone fractures (Lebovitz, 2019). As well as weight gain, bladder cancer, hepatotoxicity, diabetic macular edema, increased ovulation, and teratogenic effects (Eggleton and Jialal, 2021).

2.7.2.5 Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors are the most effective drugs for post-prandial hyperglycemia (Wajid *et al.*, 2019). Alpha-glucosidase inhibitor competitively inhibits α -glucosidase, the enzyme that converts complex non-absorbable carbohydrates into simple absorbable carbohydrates. This happens at the brush border of the small intestines (Figure 11). The action of alpha-glucosidase inhibitor causes a delay in the digestion of complex carbohydrates and intestinal absorption of glucose. This delay consequently delays the digestion of complex carbohydrates and intestinal absorption of glucose. By suspending carbohydrate absorption, it subsequently reduces postprandial blood glucose concentrations (Akmal & Wadhwa, 2021; Patil *et al.*, 2015).

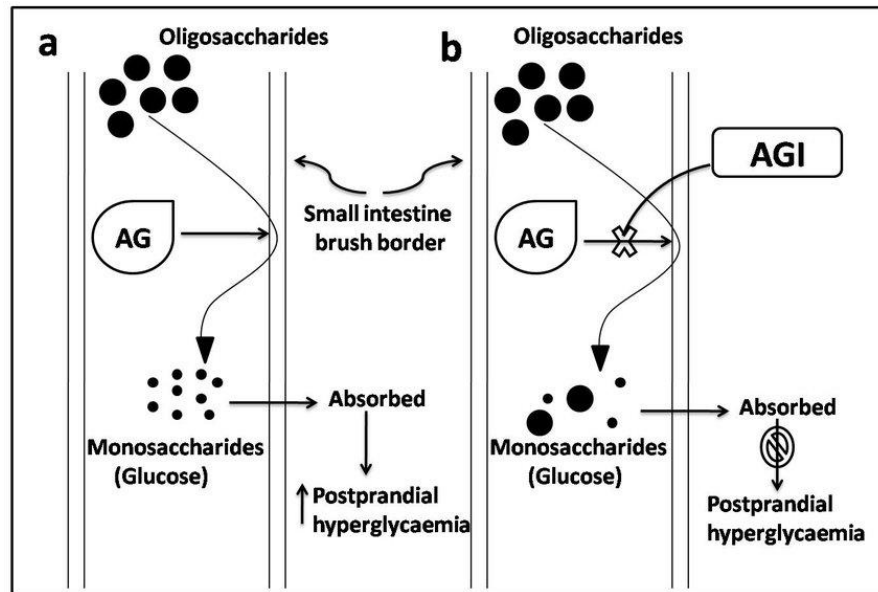


Figure 11. Mechanism of alpha glucosidase (a: in the absence of alpha glucosidase) (b: in the presence of alpha glucosidase) AGI (alpha glucosidase inhibitors) AG (alpha glucosidase) (Patil et al., 2015).

The use of alpha-glucosidase inhibitors alone has no reported hypoglycemia but only when in combination with Sulfonylureas (Rehani, 2019).

2.7.2.6 Dipeptidyl-peptidase-4 inhibitors (DPP-4)

DPP-4 inhibitors or gliptins (Figure 12) are a group of medications used for type II DM (Munir and Lamos, 2017). According to Wajid *et al.* (2019), five common DPP-4 inhibitor drugs are known, namely sitagliptin, saxagliptin, vildagliptin, linagliptin, teneligliptin, and alogliptin. These groups of drugs act through incretin, which are hormones secreted to regulate the metabolism of glucose (Gallwitz, 2019). Their role in the regulation of glucose levels in the blood is achieved by inhibiting the degradation of incretin gastrointestinal hormones mainly glucagon-like peptide-1 (GLP-) and glucose-dependent insulinotropic polypeptide (GIP) (Thornberry and Gallwitz, 2009). Inhibiting GLP-1 and GIP, leads to a rise in beta-cell insulin secretion in the pancreas,

in this manner reducing postprandial and fasting hyperglycemia (Kasina and Baradhi, 2021).

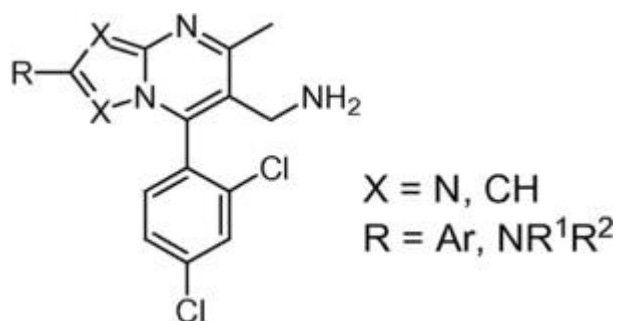


Figure 12. General chemical structure of Dipeptidyl-peptidase-4 inhibitor (Brigance *et al.*, 2010).

Nasopharyngitis, skin lesions (Gallwitz, 2019), heart failure, headache, acute pancreatitis, nausea, and ulcerative colitis were the reported side effects of the continued use of DPP-4 inhibitors (Wajid *et al.*, 2019).

2.7.2.7 Sodium-glucose cotransporter 2 (SGLT2) inhibitors

SGLT-2 inhibitors differ from other anti-diabetic drugs, in that they offer insulin-independent mechanisms of action. The classes under this group of medication include dapagliflozin and canagliflozin (Figure 13) (Ganesan *et al.*, 2020). These classes of drugs require functional kidneys (Simes and Macgregor, 2019). Their antihyperglycemic action is achieved by the elimination of excess glucose through glycosuria and natriuresis, as a result of glucose reabsorption inhibition at the proximal tubule of the kidneys (Wajid *et al.*, 2019). The effectiveness of SGLT2 is hindered by the following side effects, genital tract infection, lower leg amputation, bone fractures, electrolyte imbalance, uric acid/ chronic kidney disease, and diabetes ketoacidosis (Hsia *et al.*, 2017).

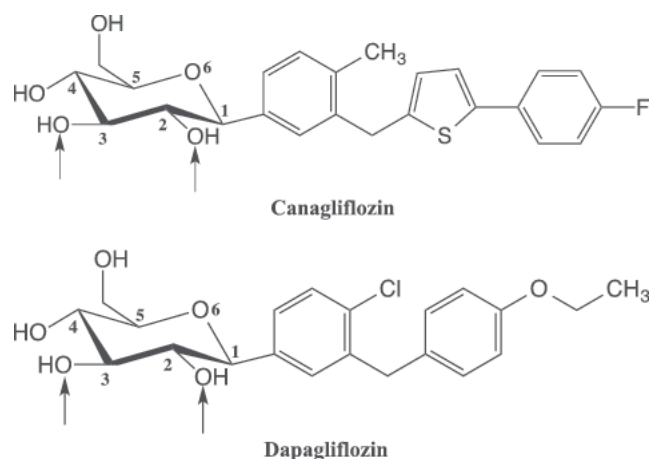


Figure 13. Chemical structure of SGLT inhibitors (Pattanawongsa et al., 2015).

2.7.2.8 Cycloset

What differentiates this type of oral anti-hyperglycemic drug is its mechanism of action that is facilitated via the central nervous system (CNS) (DeFronzo, 2011). According to Ganesan et al. (2020), cycloset is a sympatholytic dopamine D2 receptor that regulates blood glucose levels by enhancing insulin sensitivity and reducing glucose synthesis as directed by the CNS. Its effectiveness is hindered by side effects such as headache, dizziness, fatigue gastrointestinal complications, fibrosis, Psychosis, and cardiac failure (Via et al., 2010).

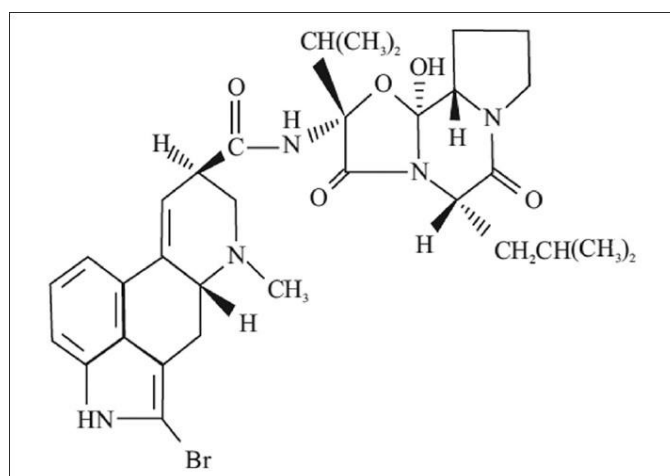


Figure 14. Chemical structure of Cycloset (Shivaprasad and Kalra, 2011).

2.8 Herbal medicine

2.8.1 History of herbal medicine

Despite its poor documentation, traditional herbal medicine remains the oldest and most diverse type of medicine system (Javadzadeh and Hamedeyaz, 2014). The use of traditional medicine is backdated to 60,000 years (Jamshidi-Kia *et al.*, 2018). Their use included the use of plant seeds, leaves, flowers, fruits, bark, and herbs as primary health care for managing numerous ailments (Yuan *et al.*, 2016). This is attributed to their assumed efficacy and availability (Balogun *et al.*, 2016).

The first man used plants and herbs for medicinal purposes (Petrovska, 2012). The Sumerian clay slab from Nagpur dating back nearly 5000 years, recorded the use of laurel, caraway, and thyme by the Sumerians of Mesopotamia (Jamshidi-Kia *et al.*, 2018). Their use continues globally either as medicine or food flavoring. Another record revealed the use of Ebers' papyrus by the Egyptians (1550 BC) (Land and Boeck, 2020). The Bible's Old Testament also mentions the medicinal use of plants (2 Kings 20:57) (Dafni and Böck, 2019). Likewise, scholars reported schools that were founded on the history of medicinal plant use, such as that of Greek medicine founded by the Greek scientist Theophrastus (Jamshidi-Kia *et al.*, 2018).

Similarly, the *De Materia Medica* handbook by a Greek philosopher compiled over 600 species of medicinal plants and remained a standard mainstay for medicine in Europe for more than 1500 years (Thovhogi, 2009). Traditional medicine gave rise to numerous medicine systems such as traditional Chinese medicine (TCM), Ayurveda, Kambo, traditional Korean medicine (TKM), and Unani (Yuan *et al.*, 2016). Some of these systems may lack scientific footing in the medical world, but they are still vital sources of human knowledge and may provide a basis for further research. Among the different medical systems globally, the most popular are TCM and Indian (Ayurveda)

(Eigenschink *et al.*, 2020). According to Eigenschink *et al.* (2020), TCM is one of the well-established medicine systems, with documented use of plants and a series of diagnostic approaches for 4000 years back. TCM is based on years of multiple scientific investigations, system development, and clinical trials which warrants its effectiveness and efficacy (Yuan *et al.*, 2016).

However the same cannot be said about African traditional medicine (ATM), which is the oldest in Africa and native to the many diverse African cultures yet the paucity of documentation is a major concern (Javadzadeh and Hamedeyaz, 2014). According to McFarlane (2015), the advancement of ATM was delayed by colonialism and in the end paved the way for Europeans to achieve dominion over Africans by imposing their values, morals, culture, and way of life. Traditional medicine was regarded as mediocre and apart from a lack of understanding, Europeans thought it would pose a threat to their authority over Africa and consequently never tested to investigate its legitimacy. Regardless of the suppression, the use of ATMs continued owing to their accessibility and affordably for both rural and urban communities (Antwi-Baffour *et al.*, 2014).

Due to side effects reported with the use of anti-hyperglycemic drugs, there is a great need for alternative medicine with fewer life-impacting side effects. One of the most recommended alternatives is the use of medicinal plants. The advantage of medicinal plants is that they have already gained appreciation globally (Howes *et al.*, 2020; Hussein and El-Anssary, 2019). Hussein and El-Anssary (2018), attribute their therapeutic action to their bioactive phytochemical compounds which allow their use as a source of new drugs, new drug leads, and new chemical entities. Consequently WHO advocates their advanced recognition, acceptance, and integration into health care systems (Javadzadeh and Hamedeyaz, 2014).

Studies are underway to screen for plants with anti-hyperglycemic activity amongst many other ailments. Similarly, Alene *et al.* (2020) discovered over a thousand plants used to manage DM. However, only less than half of these plants have scientific backing. This justifies the urgent need to screen more plants for scientific validation with the intent to use them as alternative medicine.

Plant processing is crucial in ensuring the scientific validation of plants. According to Abubakar and Haque (2020), the primary parameters such as the use of fresh or dry and ground or powdered material for processing plant material are generally the same. Abubakar and Haque deem dried and powdered material to be the most preferred form of use because fresh plant material is fragile and prone to decay. A study by Azwanida (2015) revealed that powdered plant materials have an increased surface area, which subsequently increases particle-to-solvent contact. However, when particles are too fine may also lead to excessive solvent absorption, which further affects the filtration outcome (Zhang *et al.*, 2019). Therefore, a balance needs to be reached in determining the right size of plant material.

Other parameters such as the use of a plant or plant parts alongside the choice of solvent, extraction methods, phytochemical screening procedures, fractionation methods, and compound identification techniques, all determine the nature and quality of bioactive molecules extracted (Olusegun *et al.*, 2019; Azwanida, 2015). The plant(s) or plant parts used in an experiment are determined by the desired bioactive compound to be extracted, thus different parts are used either in combination or separately (Olusegun *et al.*, 2019). According to several studies, the use of roots and or leaves precedes the use of other plant parts (Tugume and Nyakoojo, 2019; Jima and Megersa, 2018).

According to Zhang, Lin, and Ye (2018), the choice of solvent for extraction is based on the law of similarity and impermissibility. Solvents are selected based on the complementary properties of the target compounds (Poole, 2020). Based on the nature of the compounds, either polar, intermediate polar, or nonpolar, the suitable solvent is thus used to extract the active compounds or secondary metabolites of choice (Abubakar and Haque, 2020).

There are several inexpensive methods of extraction such as maceration, digestion, decoction, infusion, percolation, soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions (Abubakar and Haque, 2020). Yet, maceration is the most recommended, widely adopted, most applicable, convenient, and cost-friendly method of extraction (Azwanida, 2015). Maceration involves the soaking of mass with either cold or hot solvent in a stoppered container at room temperature with frequent agitation, followed by filtration and concentration of extracts (Olusegun *et al.*, 2019).

As ATMs gradually gain appreciation in Western medicine, industries are encouraged to invest in research to pursue promising medicinal plants and novel chemical compounds (Antwi-Baffour *et al.*, 2014). In the same way, WHO has recognized ATM's contribution claiming effective health care would not have been solely the role of orthodox medicine (Oguntibeju, 2019). Moreover, Oguntibeju (2019) reports that 25% of the drugs prescribed worldwide and 121 clinically active compounds have been of plant origin, moreover, 60% of anti-tumor and anti-infectious drugs are derived from plants. Amongst the drugs derived from medicinal plants is metformin. Metformin is currently used as a first-line drug for managing type II diabetes. This drug was originally derived from *Galega officinalis* (goat's rue), an herbaceous plant

rich in guanidine. Guanidine is the active compound used in the manufacturing of metformin (Bailey, 2017).

Therefore, recommendations are to treat diseases including diabetes using medicinal plants, because of the various phytochemical constituents plants possess such as terpenoids, flavonoids, saponins, carotenoids, alkaloids, and glycosides which are assumed to have antidiabetic activity (Salehi *et al.*, 2019).

2.9 Secondary metabolites with reported anti-hyperglycemic properties

According to Jain *et al.* (2019), metabolites are intermediate products of metabolism. Metabolites are categorized into two, primary and secondary metabolites despite their indistinctiveness due to the overlapping intermediates. Primary metabolites are present in all cells and are paramount for cell survival (Roopan and Madhumitha, 2018). Secondary metabolites, on the other hand, are a group of heterogeneous natural products, that are not vital for vegetative growth and are only found in certain plants with medicinal properties (Yang, Vijayakumar and Kahn, 2018). The role of secondary metabolites is diverse, some may function as defensive compounds against herbivores and pathogens. Others may function by signaling molecules for ecological interactions, symbiosis, metal transport, and competition (Thirumurugan *et al.*, 2018). Among the many secondary metabolites only a handful number of compounds have displayed anti-hyperglycemic properties, such compounds include but not limited to alkaloids, carotenoids, flavonoids, glycosides, terpenoids, saponins (Salehi *et al.*, 2019).

2.9.1 Alkaloids

Alkaloids are a class of nitrogen-containing heterocyclic compounds, with pronounced wide pharmacological properties. The potency of alkaloids enables the plant's defense

against herbivores and pathogens (Jain *et al.*, 2019). Naturally occurring alkaloids have displayed pharmacological activity against DM and its complications (Bharti *et al.*, 2018). Research has revealed that the best approach to managing high blood glucose levels is through controlling postprandial glucose, by inhibiting alpha-amylase and alpha-glucosidase enzymes (Ghadyale *et al.*, 2012). A study by Kumar *et al.* (2019) illustrated this through two naturally occurring alkaloids namely Chelerythrine and sanguinarine extracted from *Anguinaria canadensis*, *Chelidonium majus*, and *Macleaya cordata*, that displayed complete (100 %) porcine pancreatic alpha-amylase inhibition. Likewise, Assefa *et al.* (2020) isolated alkaloids Nojirimycin and fagomine with excellent alpha-glucosidase inhibiting activity, affording it the potential to compete with those already in the market.

One of the well-studied alkaloids is berberine (PubChem CID: 2353), it is a pronounced hypoglycemic compound derived from *Berberis vulgaris*, *aquifolium* (Berberidaceae), *Coptis chinensis* (Ranunculaceae), *Hydrastis Canadensis*, (Ranunculaceae) and *Tinospora cordifolia* (Willd) Miers (Menispermaceae). Berberine functions by improving the action of insulin, through the stimulation of activated protein kinase (AMPK) which helps in regulating the cellular uptake of glucose, the oxidation of fatty acids and, the synthesis of GLUT4. Furthermore, berberine regulates skeletal glucose carrier and cardiac muscle that is responsible for transporting glucose from the bloodstream to cells as well as enhancing insulin sensitivity (Sharma *et al.*, 2018).

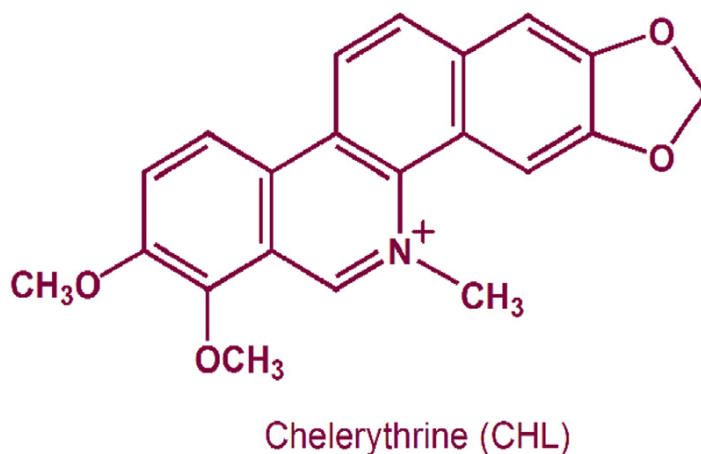


Figure 15. Alkaloid structure of Chelerythrine.

2.9.2 Flavonoids

Flavonoids are polyphenolic compounds with two moieties, benzopyran (A and C ring) and phenyl (B ring). They are classified into subgroups based on the C and B rings attached, as well as the saturation rate of the C ring. The different subgroups are flavones, flavonols, flavanones, flavanonols, flavanols (catechins) anthocyanins, and chalcones (Panche et al., 2016). Flavonoids are known for their excellent anti-oxidant properties (Assefa *et al.*, 2020). The hydroxylated phenolic substances of flavonoids are synthesized by plants in response to microbial infection (Kumar and Pandey, 2013). Hyperglycemia triggers the overproduction of reactive oxygen species. Flavonoids being excellent anti-oxidants counter the deleterious effects induced by hyperglycemia and further protect against oxidative stress. Apart from their oxidative properties, flavonoids have displayed anti-diabetic properties by inhibiting alpha-amylase (Jain and Joshi, 2019), enhancing insulin secretion via regeneration of pancreatic β -cells, enhancing insulin-mediated glucose uptake by target cells, inhibiting aldose reductase and increasing Ca^{2+} uptake (Marella, 2017).

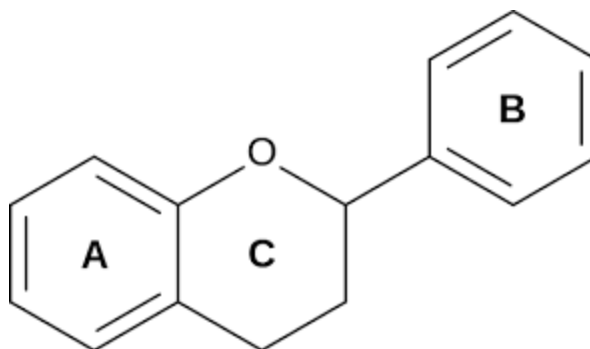


Figure 16. Flavonoid basic structure.

2.9.3 Saponins

Saponins are a group of natural glycosides, made up of a complex structure with a sugar moiety linked to a hydrophobic aglycone called sapogenin (Marrelli *et al.*, 2016). Studies have revealed saponins' ability to regulate blood glucose levels. Saponins derived from *Astragalus membranaceus* have demonstrated various therapeutic effects including anti-hyperglycemic effects. It does this by stimulating the release of insulin and delaying the progression of DM complications (Barky and Hussein, 2017).

Diosgenin [25R-spirost-5-en-3 β -ol] is a class of saponin derived from *Dioscorea rotundata* through the hydrolysis of dioscin contained in the rootstock of yam. It is known for its biological activity including hypoglycemic effects, it is reported to have reduced intestinal disaccharidase extensively (Elekofehinti, 2015). Furthermore, records of Diosgenin activities indicate a significant reduction of intestinal Na⁺- K⁺-ATPase activity, which consequently reduced the active transport of glucose into the intestinal epithelial cells (Gan *et al.*, 2020). Continuous extensive use of Diosgenin as a precursor for various synthetic drugs in the pharmaceutical industry was reported (Gan *et al.*, 2020; Raju and Rao, 2012).

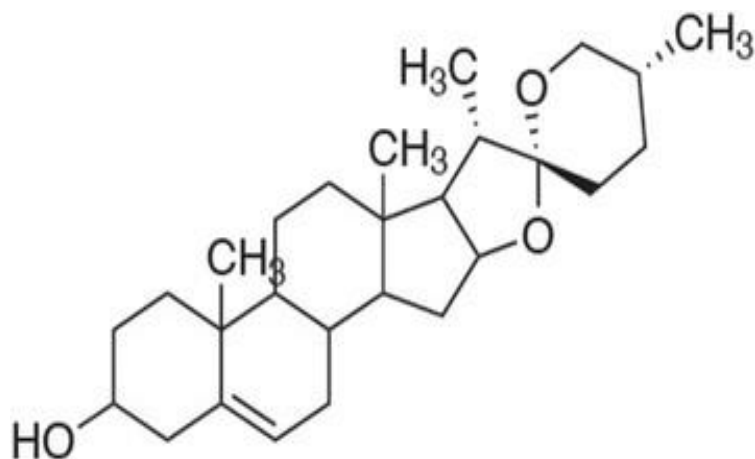


Figure 17. Diosgenin [25R-spirost-5-en-3 β -ol].

2.10 Use of Medical plants to manage Diabetes in Africa

In a comprehensive literature survey of South African medicinal plants used for managing diabetes in the Eastern Cape, 48 plants were identified for their potential in diabetes management. However, only 8 of these plants have been scientifically validated (Sagbo and Hussein, 2022). One of those scientifically validated plants is the use of *Sutherlandia frutescens* which belongs to Fabaceae family (Von Koenen, 2007). It is a small genus, found mainly in Southern Africa with representatives in South Africa, Botswana, and southern parts of Namibia. The leaves of this plant were reported for their anti-hyperglycemic activity due to the presence of pinitol (Deutschländer *et al.*, 2009). D-pinitol exerts insulin-like anti-hyperglycemic effects through its interaction with the insulin post-receptor signaling pathway, resulting in improved glucose uptake in L6 muscle cells (Sánchez-Hidalgo *et al.*, 2021).

Namibia also relies on medicinal plants to manage various ailments, although many of these plants lack scientific validation. One similar study is that of Maroyi and Cheikyoussef (2015) which identified 16 medicinal plants used in the Oshikoto region of Namibia and Zimbabwe for the treatment of human ailments. Their study recorded

the use *Berchemia discolor*, *Diospyros lycioides*, *Diospyros mespiliformis*, *Euclea divinorum*, *Peltophorum africanum*, *Pterocarpus angolensis*, *Ximenia americana*, *Ximenia caffra* and *Ziziphus mucronata* for human ailments in both Namibia and Zimbabwe, however, these plants have not been scientifically validated (Maroyi and Cheikhoussef, 2015). In another Namibian-focused study, *Euclea divinorum* and *Dichapetalum cymosum* leaves were reported for their use in treating mental illness in the Kavango region of Namibia (Shirungu and Cheikhoussef, 2018). *Bulbine frutescens* and *Gomphocarpus fruticosus* are believed to have the potential to treat diabetes but lack scientific evidence to support their efficacy (Von Koenen, 2007). Shirungu and Cheikhoussef (2018), emphasize the importance of government involvement in creating the capacity for in-depth scientific analysis. This analysis is crucial for identifying and validating the ethnomedicinal and pharmacological compounds of interest, which may lead to the discovery of new and improved alternative medicine.

Research done on some of the current plants of interest includes work done by Zhang *et al.* (2014) and Vermaak *et al.* (2011) who highlighted that *Hoodia gordonii* (Apocynaceae) is used as an appetite suppressant and as a thirst quencher. Since appetite is influenced by decreased blood glucose levels, there may be a connection between the appetite-suppressing effects of *Hoodia gordonii* and diabetes (Pereira, Pereira and Corrêa, 2010).

The second plant of interest comes from the genus *Corchorus* (Tiliaceae) which includes *Corchorus olitorius*. *C. olitorius* has been reported to have analgesic and antioxidant activity (Ipav, Moronkola and Aiyelaagbe, 2018). These properties have been attributed to the presence of flavonoids, tannins, alkaloids, and steroids (Adjatin

et al., 2018). Another species from the same genus, *Corchorus trilocularis*, has shown significant antioxidant and anti-hyperglycemic activity, with alkaloids, flavonoids, steroids, and tannins detected in the chloroform extracts (Gupta and Pradesh, 2017).

The third plant is from the Malvaceae family, *Hermannia pinnata* which is used traditionally by the Basotho Tribe of Eastern Free State to manage diabetes mellitus, despite a lack of scientific evidence supporting its efficacy (Balogun *et al.*, 2016). Another study on the same family of plants includes *Hermannia geniculata* which revealed the presence of flavonoids with antioxidant and anti-hyperglycemic properties (Lawal and Onoja, 2020).

The fifth plant of interest is *Sarcocaulon salmoniflorum*, *S. salmoniflorum* is previously known as *Monsonia salmoniflora*, which belongs to the Geraniaceae family and is known for the production of high-value essential oils made possible by the classes of terpenes (Kremer *et al.*, 2013). Terpenoids have shown anti-hyperglycemic potential (Panigrahy, Bhatt, and Kumar, 2021). Moreover, *Pelargonium graveolens*, also in the Geraniaceae family, exhibited in vitro antimicrobial, anti-inflammatory, antioxidant, and in vivo antidiabetic, antioxidant properties (Narnoliya, Jadaun, and Singh, 2019).

Lastly, *Zygophyllum decumbens*, a genus in the Zygophyllaceae family, which is well-documented for its traditional use in managing diabetes, as well as its anti-inflammatory properties, and the presence of zygophyllin, quinovic acid, and glycosides, (Mnafgui *et al.*, 2012).

CHAPTER THREE: MATERIAL AND METHODS

3. 1 Study area and population

Hardap region was the study site for the present study, it is located in the southern part of Namibia. This region covers 110 km² and constitutes six constituencies namely Gibeon, Mariental Rural, Mariental Urban, Rehoboth Rural, Rehoboth Urban East, and Rehoboth Urban West (Mundia, 2013). Hardap is arid to semi-arid with a temperature of as low as 2 °C and as high as 36 °C (IECN, 2011). A home to about 79 000 people (Mundia, 2013).

3. 2 Research design

This study employed both qualitative and quantitative approaches. A qualitative approach was used for the presence or absence of a phytochemical in thin-layer chromatography and the activity of alpha-glucosidase inhibition activity was visually verified by stain-formation of the spotted solution. A quantitative approach was used in the quantification of selected phytochemical compound classes (phenols and flavonoids), alpha-amylase inhibition, IC₅₀ values, and cell variability. The study was designed in a manner in which chemical preliminary assays are used as a basis for further biological assays.

3. 3 Plant identification, selection, and collection

A permit was obtained from the Ministry of Environment and Tourism for the collection of plants from the Hardap region (Appendix D). Ethnobotanical knowledge holders (the interviewees) were identified based on recommendations of the community leaders and knowledge holders. The study was explained to the respondents and their consent was sought before engaging them in a semi-structured interview (Appendix F). Fifteen respondents were interviewed and further employed

in the identification and collection of plants used to manage diabetes in the field. Information on the plants and parts used, vernacular names of the plants, and mode(s) of preparation were documented. The frequency of use was determined by the sum of total usage reports for a particular species.

The voucher specimens were collected onsite (in the field), allocated an identification number, and preserved using a plant press before being deposited with the National Herbarium at the National Botanical Research Institute (NBRI) of Namibia for scientific identification.

3.4 Preparation of plant extracts

The collected plants were air-dried at room temperature for six weeks, except for *Corchorus tridens* which dried for two weeks as a result of low moisture content compared to the others. Dried plant material was subsequently pulverized using an electrical blender, Fritsh Pulverisette, No. 4387713. Powdered plant material (100g) was macerated separately, using one liter of ethanol, methanol, and distilled water respectively for 24 hours. The extracts were filtered using Whatman Filter Paper No. 540 further concentrated using a Rotary Evaporator and then stored in an airtight container at 4 °C for further use, (Muriira, 2014).

3.5 Phytochemical screening using, Thin Layer Chromatography (TLC)

The extracts were subjected to phytochemical screening using a method adapted from Harborne (1998) with some modifications. Thin layer chromatography (TLC, silica gel coated 60 F₂₅₄, 20X20 cm, Merck) was used. TLC plates were spotted with different plant extracts and visualized under UV light (wavelength at 254 nm). One cm line was drawn from the bottom of the plate ensuring it was 2 cm away from the edge of the plate. The different extracts were spotted on the 1 cm line using capillary tubes.

Spotting was done with several applications while ensuring spots dry completely in between applications. Different solvent systems were prepared (Table 1), before running the plate as stipulated in the table below and transferred to the chromatographic tank below the 1 cm depth. The tank was allowed to saturate with the solvent while preparing the plates. The plates were then lowered into the tank with tweezers and covered until the solvent reached the stationary phase. The plates were removed, and the solvent front was marked followed by spraying of TLC plates with the chromogenic reagent to confirm the presence or absence of the compound. Their presence or absence was scored based on the color observed after spraying.

Table 1. The solvent systems, chromogenic reagents, and standards used in the TLC analysis.

Phytochemical compounds	Positive control	Solvent/ Mobile phase	Chromogenic solvent
Phenols	Gallic acid	Benzene:Methanol: acetic acid 90:16:08	FeCl ₃ and potassium ferricyanide 2M HCl
Flavonoids	Quercetin	Butanol: Acetic acid: water 100:07:13	Dragendorff reagent
Alkaloids	Quinine	Methanol: Concentrated nitric acid 200:3	Dragendorff reagent
Saponins	Saponin	Chloroform: Glacial acetic acid: methanol: water 60:3.5:0.5	1% Ethanolic vanillin and 5 % ethanolic sulphuric acid
Tannins	β -sitosterol	Chloroform: methanol: water 65:35:10	0.5 % vanillin and 4 % HCl ethanol solution
Steroids	β -sitosterol	Chloroform: Glacial acetic acid: methanol: water 64:34:12:8	Folin Ciocalteu's reagent
Terpenoids	β -sitosterol	Hexane: Ethyl acetate 17:03	Lieberman reagent

3.6 Quantification of Phytochemical compounds

3.6.1 Total phenolic content (TPC)

The amount of phenol in the plant extracts was determined using the Folin-Ciocalteus reagent (Folin-Ciocalteus reagent, Merck) method (Saeed, Khan and Shabbir, 2012). A reaction mixture was prepared by mixing 1 mg /ml of plant extracts of aqueous, ethanol, and methanol respectively, with 1 ml of Folin-Ciocalteus reagent, 10 ml of 7 % sodium carbonate, and 13 ml of distilled water. The resulting mixture was mixed thoroughly before incubation at room temperature in the dark for 1 hour and 30 minutes. The absorbance reading was measured at 750 nm. All tests were performed in triplicate. Gallic acid was used as a standard at 1 mg/ml. The concentrations of the extracts were obtained using the standard curve. Total phenolic content was determined using the formula below:

$$\text{Total phenol (mg. g)} = \frac{\mathbf{R \times D.F \times V \times 1}}{\mathbf{W}}$$

Where R =Results from the standard curve, D.F. =Dilution factor, V = Volume of stock solution, 1 =Gram of dried plant, and W =Weight of the plant used in the experiment

3.6.2 Total Flavonoid content (TFC)

The Flavonoid content of the plant extracts was studied spectrophotometrically using the Aluminium trichloride method as described by (Josipovic *et al.*, 2016). One ml of plant extracts at 1 mg / ml was mixed with 4 ml of distilled water and 0.3 ml of 5 % sodium nitrite. After 5 minutes, 0.3 ml of 10 % aluminium trichloride (aluminium trichloride, Merck) was added to the mixture and left at room temperature for 6 minutes followed by further addition of 2 ml of 1 M sodium hydroxide and adjusted to 10 ml total volume with distilled water. All tests were done in triplicate and

absorbance was measured at 510 nm. Quercetin (Alfa Aesar) was used as a positive control at 1 mg / ml and a mixture of the above excluding the plant extracts was used as a negative control. The flavonoid concentration of the plant extracts was determined using the quercetin standard curve and the flavonoid content of the extracts was expressed as a quercetin equivalent mg/g using the formula above.

$$\text{Total flavonoid (mg. g)} = \frac{\mathbf{R \times D.F \times V \times 1}}{\mathbf{W}}$$

W

Where R = Results from the standard curve, D.F. = Dilution factor, V = Volume of stock solution, 1 = Gram of dried plant, and W = Weight of the plant used in the experiment.

3.7 Anti-oxidant activity

3.7.1 DPPH Scavenging Activity Assay

Free radical scavenging activity of plants of choice was determined using DPPH, a method adopted (Chanda and Dave, 2009). A mixture of 1.0 ml plant extracts (0.0781-1mg / ml), 1.0 ml of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH, Sigma Aldrich) (0.3mM in methanol) and 1.0 ml methanol was left to incubate in the dark for 10 minutes. Thereafter absorbance was measured at 517 nm using a spectrophotometer (SpectraMax, M2). All test was performed in triplicates. Ascorbic acid was used as a positive control. Percentage inhibition was calculated as below:

$$\text{Inhibition (\%)} = \frac{\mathbf{A_0 - A_1}}{\mathbf{A_0}} \times \mathbf{100}$$

Where; A₀ is the absorbance of the control and A₁ is the absorbance of the test.

3.7.2 Reducing power assay

The ability of extracts to reduce ferric ions (Fe^{3+}) was determined (Chanda and Dave, 2009). One ml of plant extracts (0.0781-1mg / ml) was mixed with 2.5 ml of phosphate buffer (200mM) and 2.5 ml of potassium ferricyanide (30 mM), the mixture was subsequently incubated for 20 min at 50 °C before adding 2.5 ml of trichloroacetic acid (600 mM) to the mixture. Thereafter the mixture was centrifuged at 3000 rpm for 10 minutes, and 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl_3 (6 mM). Absorbance was measured at 700 nm using a spectrophotometer (SpectraMax, M2). The experiment was done in triplicates. Ascorbic acid (Merck) was used as a positive control. An increase in absorbance of the extracts indicated increased reducing power.

3.8 Anti-hyperglycemic activity

3.8.1 Alpha-amylase

This assay was conducted using a modification of the protocol by (Sangeetha and Vedesree, 2012). Five microlitres of various plant extract concentrations reconstituted in methanol (1-0.03125 mg / ml) was allowed to react with 50 μl of alpha-amylase, (Sigma Aldrich) enzyme solution (0.5 mg / ml in 0.02 M sodium phosphate buffer at pH 6.9) in a tube at 30 °C for 10 minutes. After incubation, 50 μl of 1 % starch in 0.02M sodium phosphate buffer was added and further incubated for 10 minutes at 30 °C. This was followed by the addition of 200 μl stop reagent 96mM dinitrosalicylic acid (DNS) (Merck). The tubes were then incubated in boiling water for 5 minutes before being allowed to cool to room temperature. The reaction was diluted with 1.25 ml of distilled water. The absorbance was recorded at 540 nm using a spectrophotometer. A control was prepared the same way by replacing the extracts

with buffer. The percentage inhibition of alpha-amylase was calculated using the formula below

$$\% \text{ inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

Where; Abs control is the absorbance of the control and Abs test is the absorbance of the extracts.

3.8.2 Alpha-glucosidase

This assay was done using the GIBEX Screen-to-Nature manual for Namibia (Screen-to-Nature manual for Namibia, 2012). Potato starch of 1.5 g was added to 20 ml of water and allowed to dissolve. In a different container, 1.5 g of bacteriological agar was added to 80 ml of water and left to boil for 1 minute. The two mixtures were combined before being poured into Petri dishes to solidify. The solidified agar plates were marked on the edges to test for 1-8 extracts at a time, with the center marked with (-) and (+) for positive and negative controls. Ten microliters of acarbose solution 2 mg / ml (positive control) was placed on the (+) mark and 10 ul of methanol (-) and 10 µl of various extracts (1 mg / ml). Ten microliters of alpha-glucosidase (Sigma) were added to each drop of acarbose, extracts, and methanol. It was left to incubate on the bench for 10 minutes before excess alpha-glucosidase was rinsed off from the surface. Diluted Lugol's solution was poured over the surface of the plate and excess Lugol's solution was discarded. The inhibition activity was visually verified by the stain-formation of the spotted solution. If the dot did not stain for at least 30 minutes the sample was considered negative for alpha-glucosidase inhibition.

3.9 Cytotoxicity

3.9.1 Maintaining cell culture

Mouse embryonic fibroblasts cell line, 3T3 was used for this experiment. The cells were maintained at 37 ° C under 5 % CO₂ and 100 % humidity in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10 % fetal bovine serum, 1 % penicillin-streptomycin, 2250 mg glucose, and 750 mg sodium bicarbonate. The media was changed every other day. Once cells were confluent, they were prepared for cytotoxicity assay.

3.9.2 MTT -(3-(4,5-Dimethylthiazol-2-yl) assay

Plant cytotoxic effect was determined using a method by Bahuguna *et al.* (2017) with some minor modifications. Once the cells were confluent, growth media was aspirated and cells were washed with 12 ml of sterile phosphate buffer solution (PBS) by gently tilting the flask back and forth. The PBS was aspirated carefully without touching the cells. Two ml of trypsin-PBS (1:10 v / w) was added to the cells and incubated for 3 minutes at 37 ° C under 5 % carbon dioxide (CO₂) and 100 % humidity to detach the cells from the flask. This was followed by scrapping off cells from the flasks and adding 8 ml of fresh growth media. The cell seeding density was determined using a haemocytometer based on the number of viable cells. The cells were then seeded at 1×10^5 for 48 hours in a sterile flat-bottom 96 well plate, each well contained 100 µl of cell suspension with drug-free media. The working stock for plant extracts and triton X was prepared in dimethyl sulfoxide (DMSO) and further diluted using growth media. The old media in the 96-well plate was gently removed after 48 hours and replaced with 100 µl of various plant extracts at different concentrations (100 µg/ µl - 3.125 µg/µl) and incubated for a further 48 hours. Each drug concentration and controls (5 % triton, 1 % DMSO, and cells without drug treatment) were done in

triplicate. After 48 hours of incubation, 30 μ l MTT (5 mg / ml) was added to each well in the dark and incubated at 37 ° C for 4 hours. The formazan crystals were thereafter solubilized with 50 μ l of DMSO per well for 30 minutes at 37 ° C in a CO₂ incubator and quantified spectrophotometrically at 570 nm. The cell viability was calculated as per the formula below

$$\% \text{ cell viability} = (\text{absorbance of experiment} / \text{absorbance of the control (untreated cells)}) \times 100$$

3.10. Data analysis

Microsoft Excel® was used to capture data on inhibitions at different concentrations. Tables were developed using Microsoft Excel®. Graphs, averages, standard deviation, and standard error were calculated using GraphPad Prism 6.01. The analysis of quantitative data obtained from the phytochemical quantification, antioxidant activity, alpha-amylase percentage inhibition, and percentage cell viability of the MTT assay were analyzed using One-way Student's t-test, at 95% confidence level on IBM SPSS Statistics 28.0. For each Student's t-test performed, the decision criteria were $p < 0.05$ for a significant difference while $p > 0.05$ was considered as no significant difference.

3.11. Research ethics

Ethical approval for this study was sought and obtained from the University of Namibia Research Ethics Committee, and a research permit was applied for and obtained from the NCRST. These documents were used to apply for a plant collection permit from the Ministry of Environment and Tourism of Namibia for the collection of plant material for research experiments and the preparation of voucher specimens. All voucher specimens were submitted to the National Botanical Research Institute of Namibia for authentication.

CHAPTER FOUR: RESULTS

4.1 Socio-demographic characteristics of study participants

In this study, a total of fifteen knowledge holders were interviewed. The respondents were all residing in the Hardap rural area. The information was made available upon engaging them at no fee. The profile of the respondents is provided in Table 2. Eighty-seven percent of the respondents were female and 13 % were male. Only 6.7 % of the respondents were under the age of 40.

Table 2. Ethnographical information on indigenous knowledge holders interviewed in the rural areas of the Hardap region.

	Characteristics	Frequency	Percentage
Gender	Male	2	13
	Female	13	87
	Total	15	100
Age	30-39	1	6.7
	40-49	4	27
	50-59	2	13
	60-69	3	20
	Above 70	2	13

4.2 Plants used to manage diabetes in the Hardap Region of Namibia

Table 3. List of plants used to manage diabetes mellitus in the Hardap region of Namibia

Scientific name	Local name	Family name
<i>Aloe hereroensis</i>	Aukore	Asphodelaceae
<i>Aloe zebrina</i>	Bitter alwyn	Asphodelaceae
<i>Bulbine frutescens</i>	Lym vyggie	Asphodelaceae
<i>Corchorus tridens</i>	-	Malvaceae
<i>Harpogophytum procumbens</i>	Gamagu	Pedaliaceae
<i>Hermannia fruticulosa</i>	Jackals boom	Malvaceae
<i>Hoodia gordonii</i>	!Hoba	Apocynaceae
<i>Leonotis ocymifolia</i>	Wilde dagga	Lamiaceae
<i>Sarcocaulon salmoniflorum</i>	Baarbos	Geraniaceae
<i>Zygophyllum decumbens</i>	#u#oe	Zygophyllaceae
<i>Sutherlandia frutescens</i>	Kankerbos	Fabaceae

Ten plants were identified scientifically (Appendix E), of which *Sutherlandia frutescens* was not one of them; as it was not available at the time for voucher specimen preparation. Furthermore, plant material was only collected for five of the plant species for laboratory analysis due to the unavailability of the others. This included *Hoodia gordonii*, *Corchorus tridens*, *Hermannia fruticulosa*, *Sarcocaulon salmoniflorum*, and *Zygophyllum decumbens*. The distribution of the specimens collected for authentication was concentrated around Mariental Urban and Rural constituency of the Hardap region of Namibia (Figure 18).

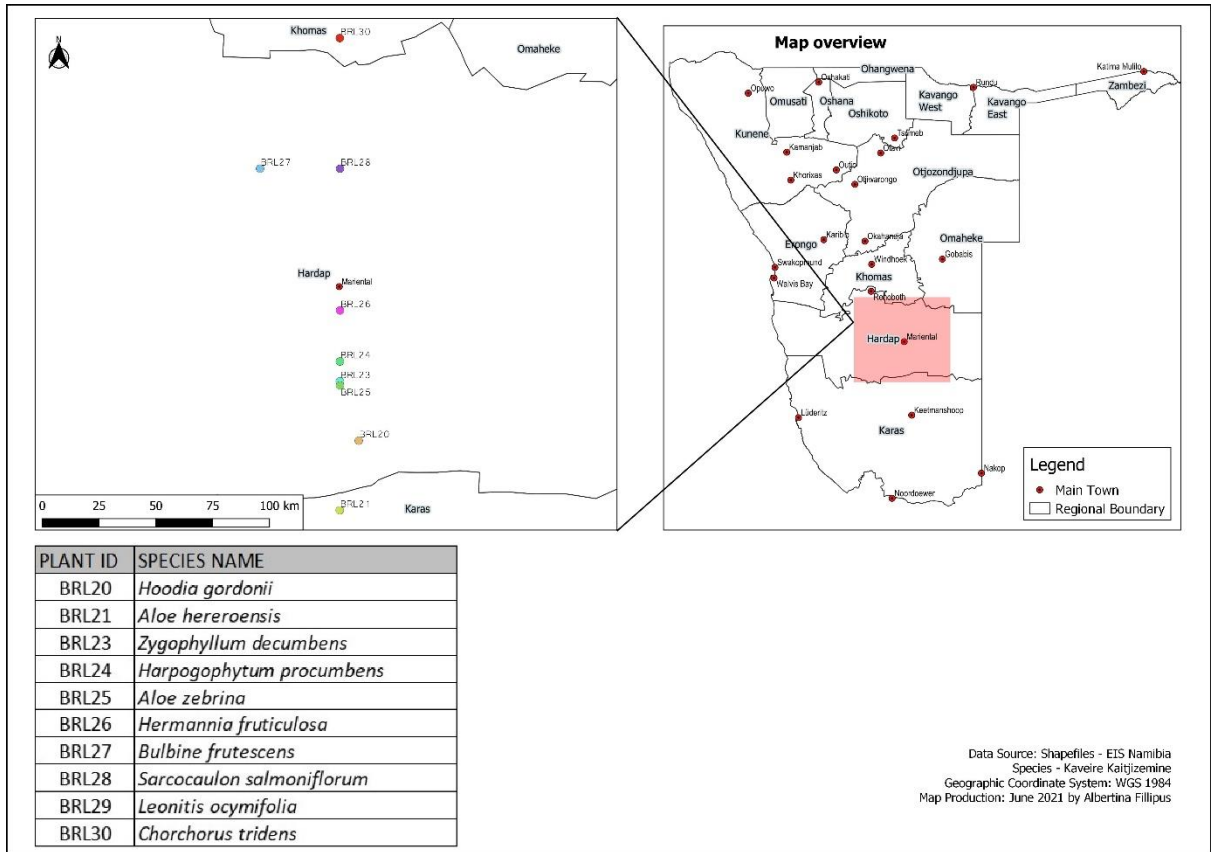


Figure 18. Distribution of some of the plants used to manage diabetes in the Hardap region with their voucher specimen numbers.

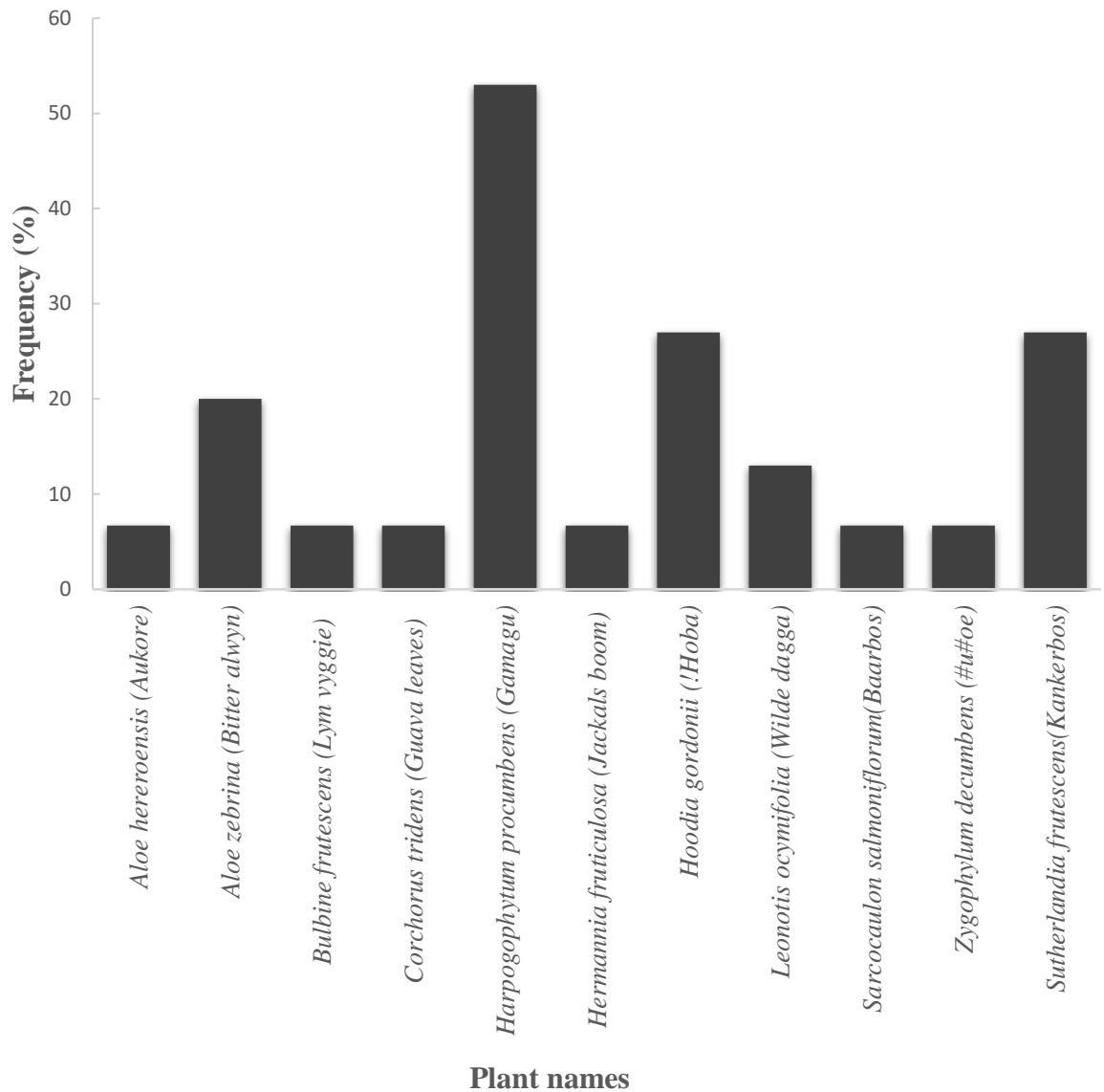


Figure 19. Percentage frequency of mention of some of the plants used for managing DM in the Hardap region.

4.3 Plant parts used

The plant parts used for antihyperglycemic remedies were the leaves, roots, stems, tuber, and twigs individually or combined with other plant parts. The most frequently used plant parts were the leaves (28 %) followed by the stems or twigs (18 %) and then tubers or roots separately or in combination with other plant parts 9 % (Figure 20).

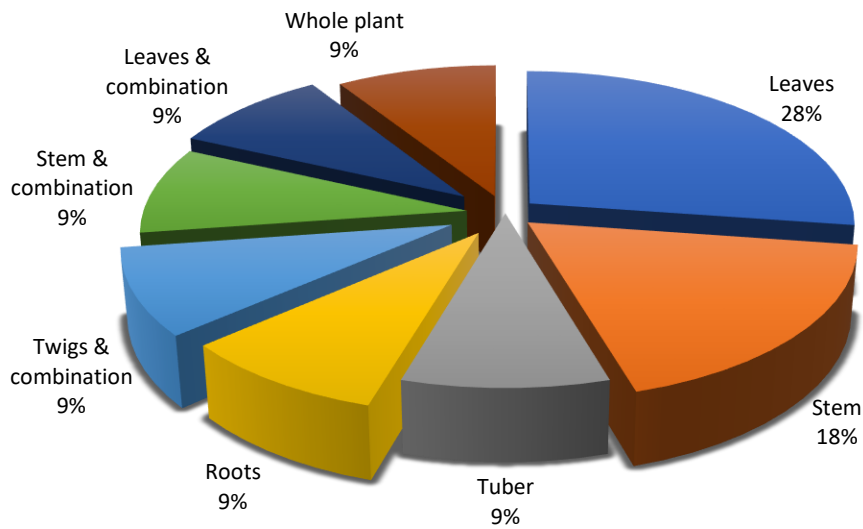


Figure 20. Frequency of plant parts used of some of the plants used in managing diabetes in the Hardap region.

4.4 Mode of preparation

According to the respondents, the plant(s) are administered orally for clinical symptoms such as chronic fatigue, polydipsia, polyuria, and excessive weight loss observed over a period, which according to them are symptoms of DM, until improvement is evident. The plant is either chewed on or a decoction or an infusion/ or powder is prepared from the plant material (Figure 21), using one plant species or a combination of plant species or parts based on the severity of the condition. Among the different methods of preparation, decoction was the most common (47 %). The dose for the patient is determined by the one who prescribes the treatment, no standardized measure is in place.

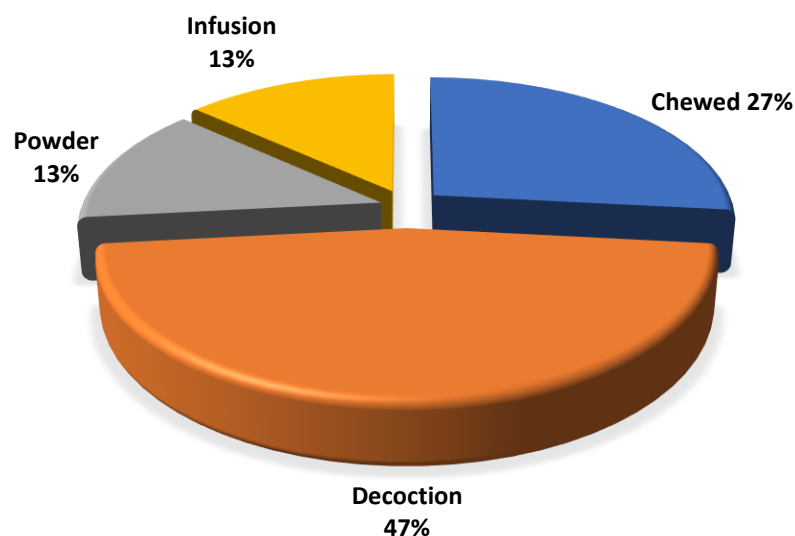


Figure 21. Method of preparation and/or administration of medicinal plants used to treat diabetes.

4.5 Phytochemical screening

Aqueous extracts showed no to low presence of the tested compounds (alkaloid, flavonoids, terpenoids, saponins, phenols, tannins, and steroids), and moderate to high levels of saponins, steroids, and tannins when compared to methanol and ethanol extracts (Table 4). In the ethanol extracts all seven compounds (alkaloids, flavonoids, terpenoids, saponins, phenols, tannins, and steroids) for *H.gordonii*, *C. tridens*, *H. fruticulosa* were detected except for *Z. decumbens* and *S. salmoniflorum* extracts with no alkaloids detected (Table 5). In the methanol extracts high levels of flavonoids, phenols, saponins, steroids, tannins, and terpenoids, in the four plant extracts except for *C.tridens* were present (Table 6). Only *H.fruticulosa* displayed the presence of all the tested compounds in both methanol and ethanol extracts.

Table 4. Qualitative phytochemical analysis of aqueous extracts of some of the medicinal plants used to treat DM.

Test compounds-Aqueous extracts							
Plants	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Tannins	Terpenoids
<i>H. gordonii</i>	-	-	-	++++	+	+	-
<i>C. tridens</i>	-	-	-	+	+	-	-
<i>Z. decumbens</i>	-	-	-	++++	++++	-	-
<i>H. fruticulosa</i>	-	-	-	++++	+++	+++	-
<i>S. salmoniflorum</i>	-	++	-	++++	+	+++	-

Key: ++++very high presence, ++ moderate presence, +presence, -absence

Table 5. Qualitative phytochemical analysis of ethanol extracts of some of the medicinal plants used to treat DM.

Test compounds-Ethanol extracts							
Plants	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Tannins	Terpenoids
<i>H. gordonii</i>	+	++	+	++++	++++	+	+++
<i>C. tridens</i>	+	++	+	++++	++++	+	++++
<i>Z. decumbens</i>	-	+	++	++++	++++	+	++++
<i>H. fruticulosa</i>	++++	++++	++++	++++	++++	++	+++
<i>S. salmoniflorum</i>	-	+++	++	++++	++++	+	++++

Key: +++high presence, ++ moderate presence, +presence, -absence

Table 6. Qualitative phytochemical analysis of methanol extracts of some of the medicinal plants used to treat DM.

Test compounds-Methanol extracts							
Plants	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Tannins	Terpenoids
<i>H. gordonii</i>	-	+++	++	++++	++++	+++	+++
<i>C. tridens</i>	-	-	+	++++	++++	-	++++
<i>Z. decumbens</i>	-	++++	+++	++++	++++	+	++++
<i>H. fruticulosa</i>	++	++++	++++	++++	++++	+++	++++
<i>S. salmoniflorum</i>	-	+++	++	++++	++++	++	++++

Key: ++++very high presence, ++ moderate presence, +presence, -absence

4.6 Total phenolic content (TPC)

Quantification of the phenolic content of the different plant extracts *H. gordonii*, *C. tridens*, *Z. decumbens*, *S. salmoniflorum*, and *H. fruticulosa* was determined using a calibration curve generated from the absorbance of different gallic acid concentrations. A regression equation ($Y = 0.5443X - 0.4864$, $R^2 = 0.9551$) obtained from the calibration curve was then used to calculate the total phenol content (Appendix B). The value was expressed as mg Gallic acid equivalent (GAE) per gram of dry sample weight (mg / g) (Table 6). The highest phenolic content was recorded in methanol extracts of *C. tridens* (23.58 ± 0.41) mg GAE / g followed by *S. salmoniflorum* (17.06 ± 0.04) mg GA / g. An overall high TPC was observed in the methanol and ethanol extracts of *C. tridens* in comparison to the other tested plant extracts. Data analysis found a very strong statistically significant difference in the phenol content of all plant extracts, ($p < 0.05$), with a significant difference between extracts of aqueous-ethanol ($p < 0.05$) and aqueous-ethanol ($p < 0.05$) but no significant difference in the total phenol content when comparing methanol to ethanol ($p > 0.05$).

Table 7. Total phenolic content as Gallic acid equivalent (mg GAE/g) in plant extracts.

Plants	Aqueous	Methanol	Ethanol
<i>H.gordonii</i>	11.71 ± 0.13	11.4 ± 0.03	10.65 ± 0.00
<i>C.tridens</i>	8.94 ± 0.00	23.58 ± 0.41	22.79 ± 0.16
<i>Z.decumbens</i>	13.9 ± 0.20	13.45 ± 0.11	15.35 ± 0.03
<i>S.salmoniflorum</i>	14.14 ± 0.17	17.06 ± 0.04	16.07 ± 0.07
<i>H.fruticulosa</i>	12.13 ± 0.03	15.49 ± 0.04	15.61 ± 0.05

Values are expressed as mean \pm standard deviation (n=15), $p < 0.05$ TPC.

4.7 Total flavonoid content (TFC)

The total phenol content of the different plant extracts *H. gordonii*, *C. tridens*, *Z. decumbens*, *S. salmoniflorum*, and *H. fruticulosa* was determined using a calibration curve generated from the absorbance of different quercetin concentrations. A regression equation ($Y = 0.0674X - 0.0201$, $R^2 = 0.9954$) obtained from the calibration curve was then used to calculate the total flavonoid content (Appendix B). The value was expressed as mg quercetin equivalent (QE) per gram of dry sample weight (mg / g) (Table 8). *C. tridens* recorded a high TFC of (96.90 ± 7.04), followed by *Z. decumbens* (49.98 ± 2.97) then *S. salmoniflorum* (44.55 ± 0.44) across all three extracts, and with the highest recorded in ethanol extracts. *H. gordonii* showed the least TFC across all solvents. Data analysis found a significant difference in the flavonoid content of all plant extracts, ($p < 0.05$). Furthermore, a solvent comparison found a statistically significant difference between extracts of aqueous to ethanol ($p < 0.05$). The different studied plants showed a significant difference in total phenolic content and total flavonoid content ($p < 0.005$). With aqueous-methanol and aqueous ethanol significantly contributed to TPC and TFC, $p < 0.05$ and $p < 0.05$ respectively. Yet no significant difference in the TPC and TFC content with ethanol to methanol extracts ($p > 0.05$).

Table 8. Total flavonoid content as quercetin equivalent (mg QE/g).

Plants	Aqueous	Methanol	Ethanol
<i>H. gordonii</i>	13.09±0.31	16.92±0.88	16.29±0.47
<i>C. tridens</i>	50.64±0.59	80.33±0.58	96.90±7.04
<i>Z. decumbens</i>	19.13±0.45	27.32±0.09	49.98±2.97
<i>S. salmoniflorum</i>	17.62±0.24	29.29±0.39	44.55±0.44
<i>H. fruticulosa</i>	15.43±1.64	18.19±0.57	23.43±1.11

Values are expressed as mean \pm standard deviation (n=15), $p < 0.05$ TFC.

4.8 Antioxidant

4.8.1 DPPH scavenging activity assay

The extracts of *H. gordonii*, *C. tridens*, *Z. decumbens*, *S. salmoniflorum*, and *H. fruticulosa* were tested for their ability to scavenge DPPH, a stable free radical which in the presence of antioxidants accept electron or hydrogen (Frezzini *et al.*, 2019). The highest scavenging activity of 95.1 % was observed in *H. fruticulosa* methanol extracts followed by ethanol extracts of *H. fruticulosa* with 94.3% (Figure 26) in comparison to ascorbic acid with 92.3 %. The inhibitory activity of all the plant extracts tested showed potency, with the highest inhibitory activity exhibited by *C. tridens* with an IC₅₀ value of 0.0312 mg/mL when compared with the positive control, Ascorbic acid with an IC₅₀ value of 0.02794 mg/mL (Table 9). The data showed that the plant's ability to scavenge free radicals is affected by the concentration of the extracts ($p < 0.05$) and not the solvent used for extraction, with no significant difference across the plant samples ($p > 0.05$).

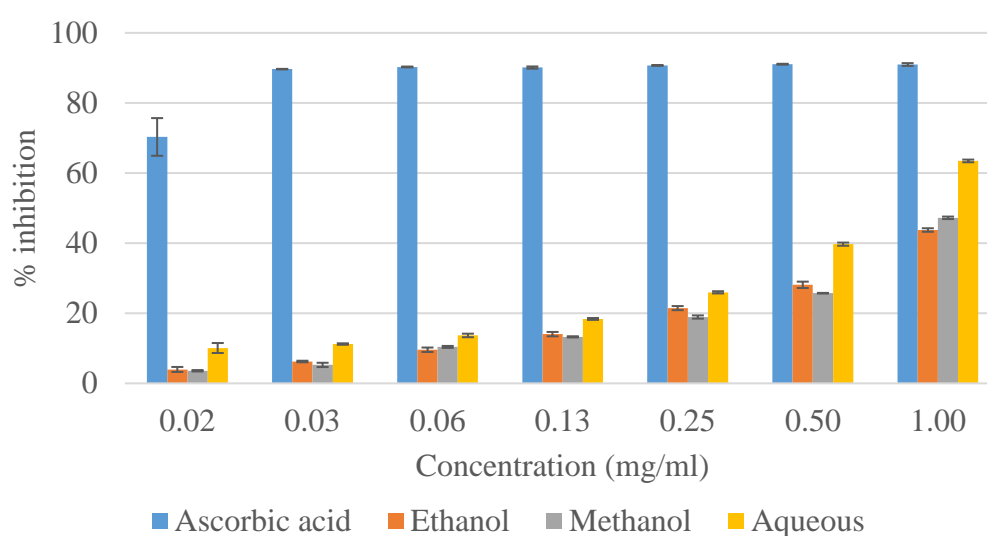


Figure 22. DPPH scavenging activity of different *H. gordonii* extracts compared to that of ascorbic acid. Values are express as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ plant samples.

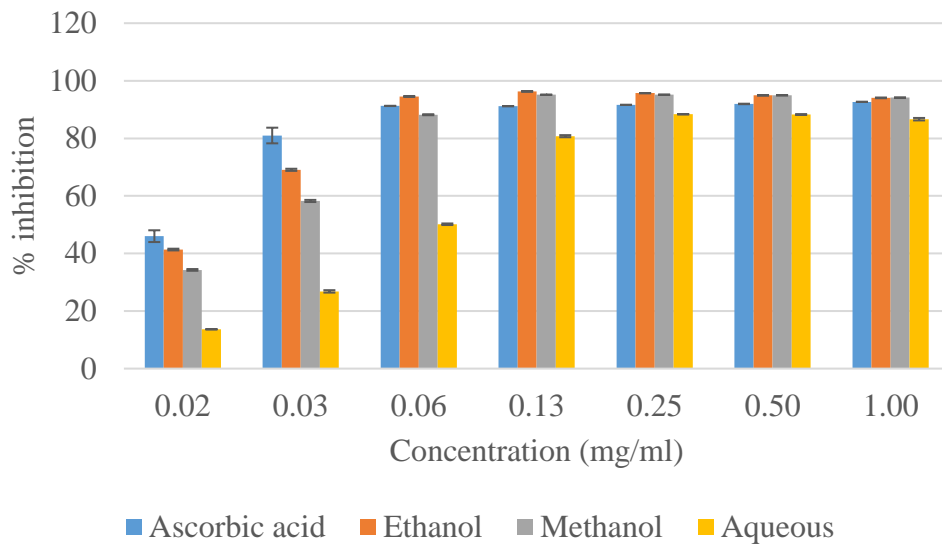


Figure 23. DPPH scavenging activity of different *C. tridens* extracts compared to that of ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ plant samples.

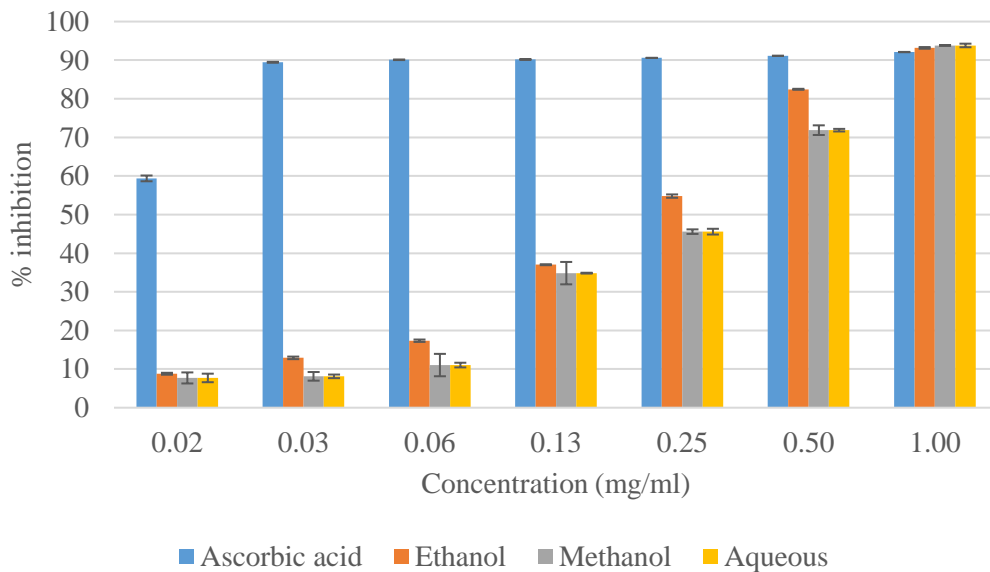


Figure 24. DPPH scavenging activity of different *Z. decumbens* extracts compared to that of ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ plant samples.

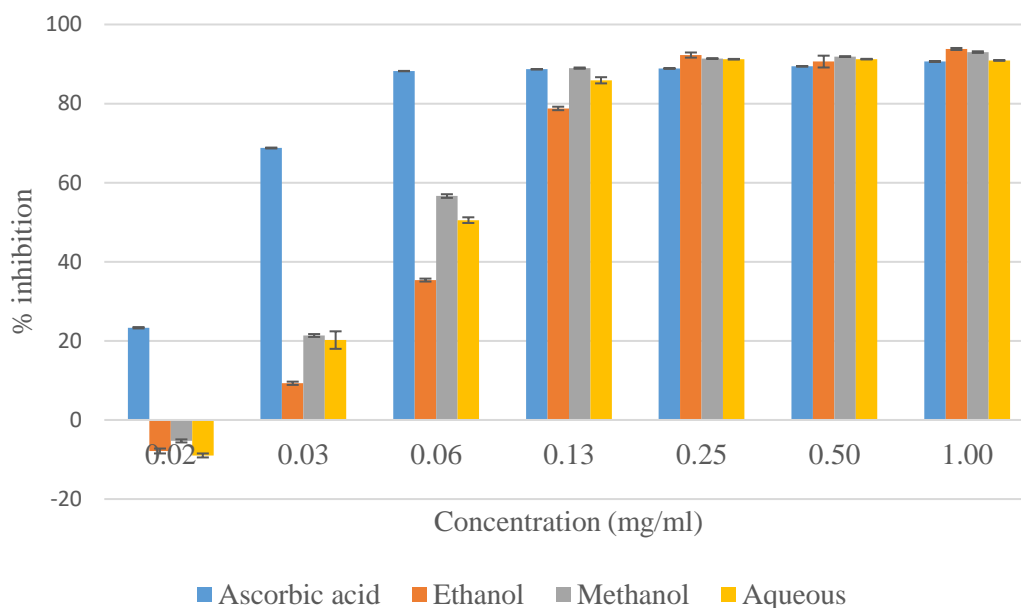


Figure 25. DPPH scavenging activity of different *S. salmoniflorum* extracts compared to that of ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ plant samples.

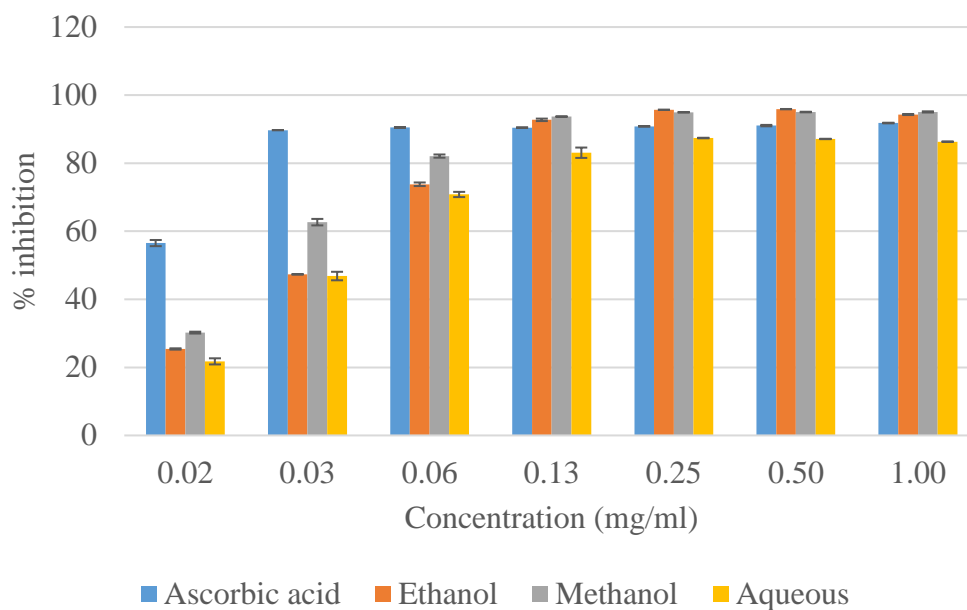


Figure 26. DPPH scavenging activity of different *H. fruticulosa* extracts compared to that of ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ plant samples.

Table 9. Half maximal inhibitory concentration IC₅₀ (mg/ml) for DPPH scavenging activity of the extracts of the plants under investigation.

Plants & Ascorbic acid	IC ₅₀ ±SD(DPPH)		
	Ethanol	Methanol	Water
Ascorbic acid	0.02794±17.09		
<i>C. tridens</i>	0.0312±21.05	0.03536±24.20	0.06106±31.77
<i>Z. decumbens</i>	0.2016±34.11	0.2535±33.81	0.4075±22.89
<i>H. fruticulosa</i>	0.04473±28.22	0.03386±24.64	0.04005±25.46
<i>S. salmoniflorum</i>	0.06588±43.10	0.04798±40.11	0.04926±40.84
<i>H. gordonii</i>	0.2837±14.14	0.3491±15.08	0.3801±19.46

Values are expressed as mean ± standard deviation (n=15), *p* < 0.05 concentration.

4.8.2 Reducing power assay

The extracts were further subjected to reducing power analysis, which assessed the ability of plant extracts to reduce ferric ion Fe³⁺ to Fe²⁺. The highest reducing power was observed in methanol extracts of *S. salmoniflorum* with an absorbance of 1.84 (Figure 28) followed by *C. tridens* 1.5 (Figure 27) when compared with the positive control, ascorbic acid which recorded an absorbance of 3.16. The lowest reducing power recorded was that of *H. fruticulosa* and *H. gordonii* with absorbance below 0.5. The plant extracts ability to reduce Fe³⁺ to Fe²⁺ is significantly different across the various concentrations (*p* < 0.05) and between aqueous and methanol extracts (*p* < 0.05). However, no statistically significant difference between aqueous-to-ethanol extracts (*p* > 0.05) and ethanol-to-methanol extracts (*p* > 0.05). The *C. tridens* (Figure 27) and *S. salmoniflorum* (Figure 28) reducing power capacity is consistent with that of the DPPH scavenging activity of *C. tridens* (Figure 27) and *S. salmoniflorum* (Figure 28) with DPPH scavenging capacity of above 90 % inhibition. A relationship was evident in the plant's ability to function as an antioxidant in that the extract scavenging activity was seen in the order of *C. tridens* > *S. salmoniflorum* > *Z. decumbens* > *H. fruticulosa* > *H. gordonii* while the reducing power was in the order

of *S. salmoniflorum* > *C.tridens* > *Z. decumbens* > *H.fruticulosa* > *H. gordonii*.

Interestingly the ability of the extracts to donate an electron and a hydrogen was seen to have increased with increasing concentrations.

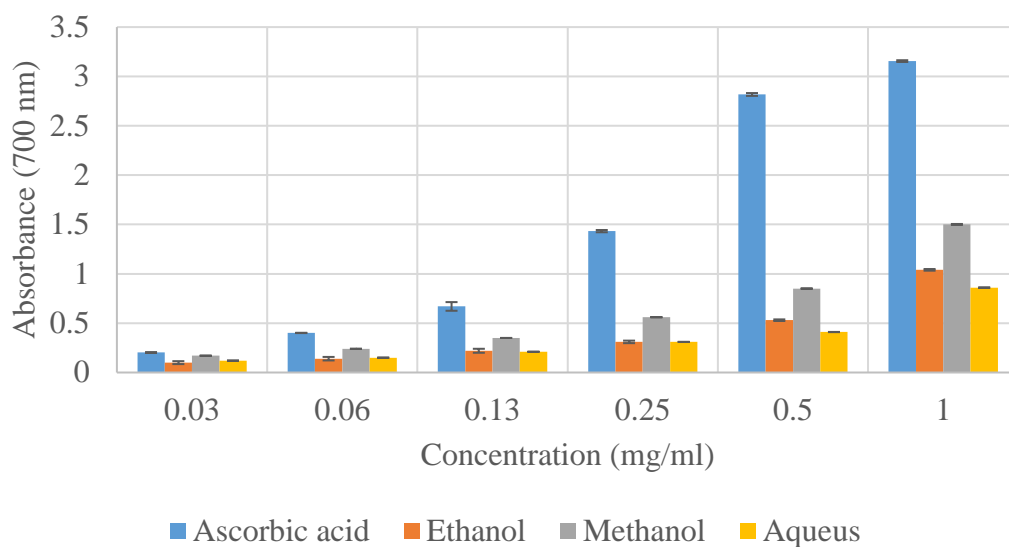


Figure 27. Reducing power of *C. tridens* extracts and ascorbic acid (standard). Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ different solvents.

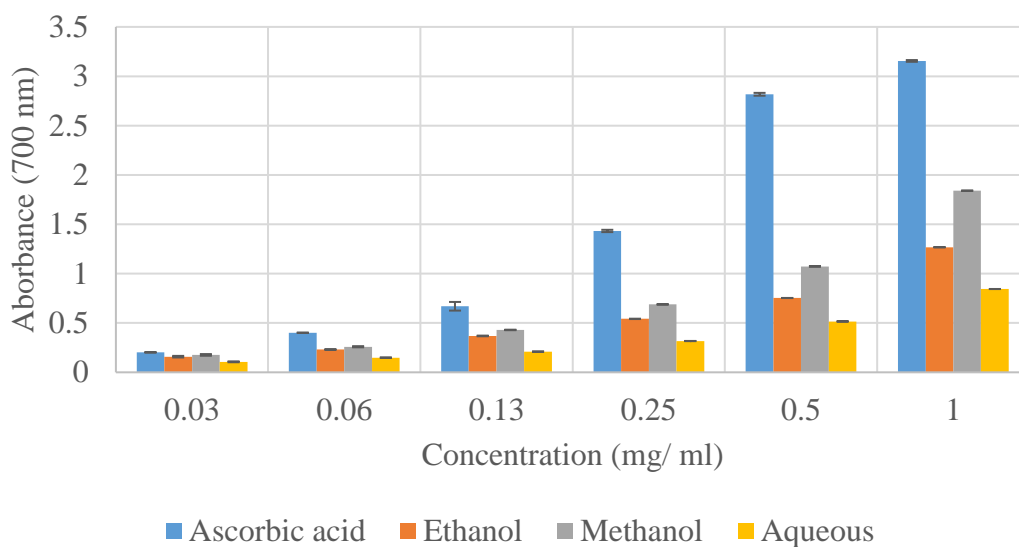


Figure 28. Reducing power of *S. salmoniflorum* extracts and ascorbic acid (standard). Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ different solvents.

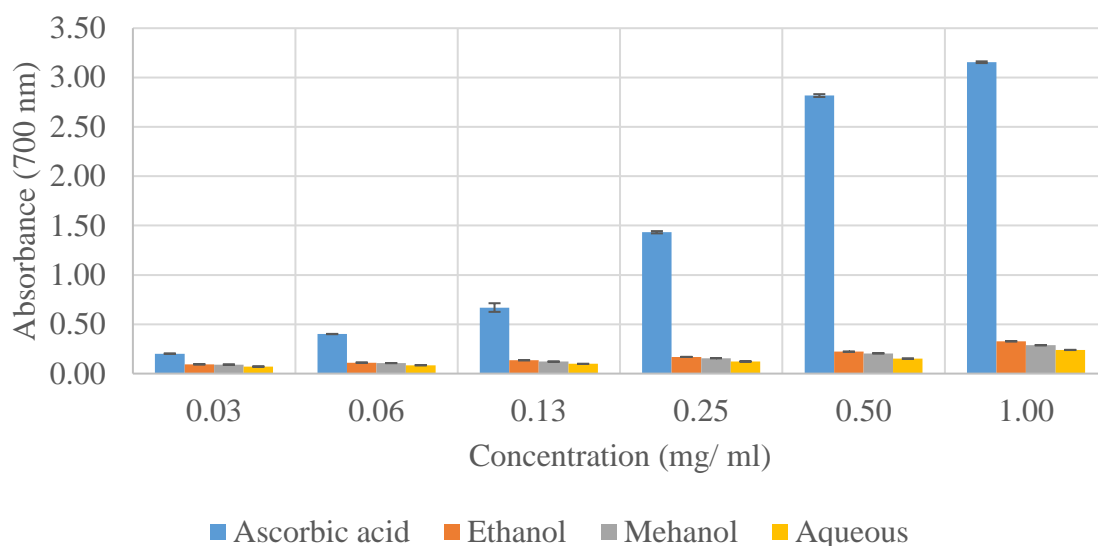


Figure 29. Reducing power of *H. fruticulosa* extracts with ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ different solvents.

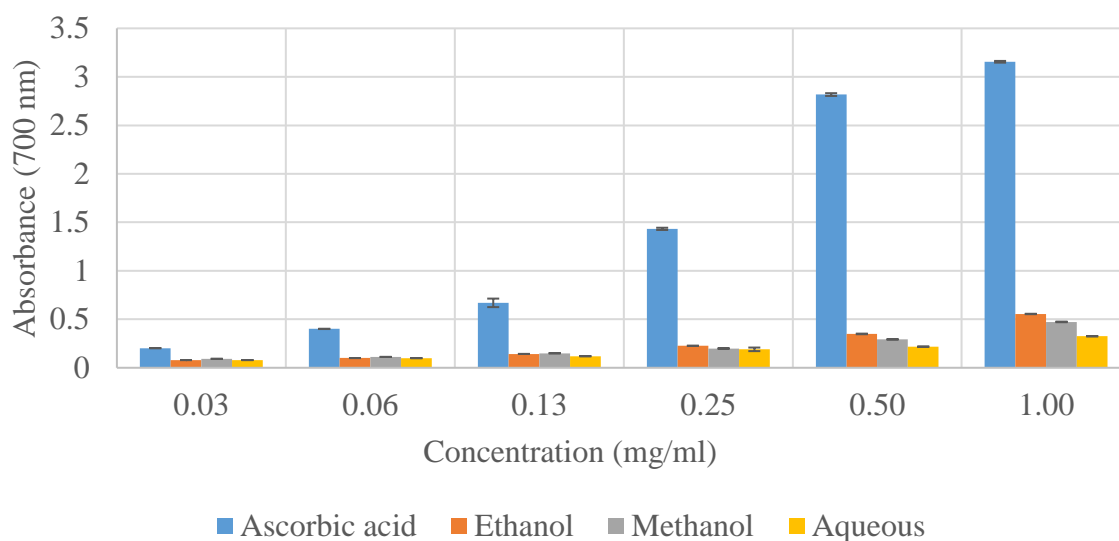


Figure 30. Reducing power of *Z. decumbens* extracts and ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ different solvents.

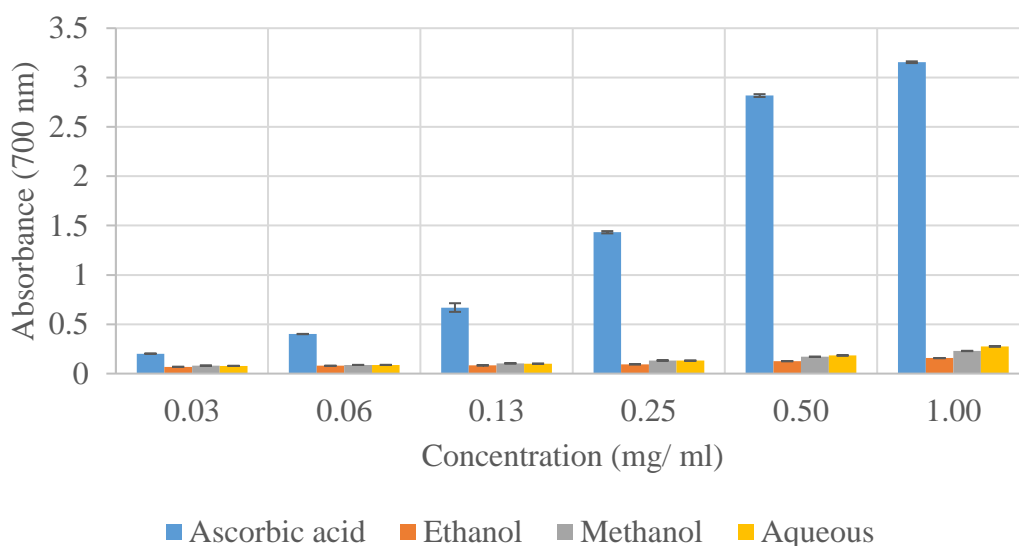


Figure 31. Reducing power of *H. gordonii* extracts and ascorbic acid. Values are expressed as mean \pm standard deviation ($n=15$), $p < 0.05$ concentration, $p > 0.05$ different solvents.

4.9 Antihyperglycemic activity

4.9.1 Alpha-amylase

The extracts of *H. gordonii*, *C. tridens*, *Z. decumbens*, *S. salmoniflorum*, and *H. fruticulosa* were evaluated for their possible alpha-amylase inhibitory activity alongside acarbose as a positive control. Activity data in the presence of varying concentrations of plant extracts were expressed as a percentage of inhibition. The alpha-amylase inhibitory activity is summarised in Figures 32 & 33 and IC_{50} values in Table 10, respectively. A concentration-dependent inhibition was observed with the extracts of the studied plants with an inhibition of 60 % and above. The highest inhibition observed was in ethanol extracts of *S. salmoniflorum* 92 %, *Z. decumbens* 90.1 %, and *H. gordonii* 83.5 %. The minimum inhibitory concentration of the extracts varied from 0.1667 to 0.5092 (IC_{50} mg/ mL). As expected, acarbose showed the lowest IC_{50} , establishing its relative potency as a glucosidase inhibitor. Moreover, aqueous

extracts of *H. gordonii* ($IC_{50} = 0.1667$) and *Z. decumbens* ($IC_{50} = 0.2382$) exhibited the lowest IC_{50} value amongst all extracts as the most potent extracts. The different concentrations did not significantly influence the inhibition activity ($p > 0.05$) but the difference in solvents used for extraction ($p < 0.05$).

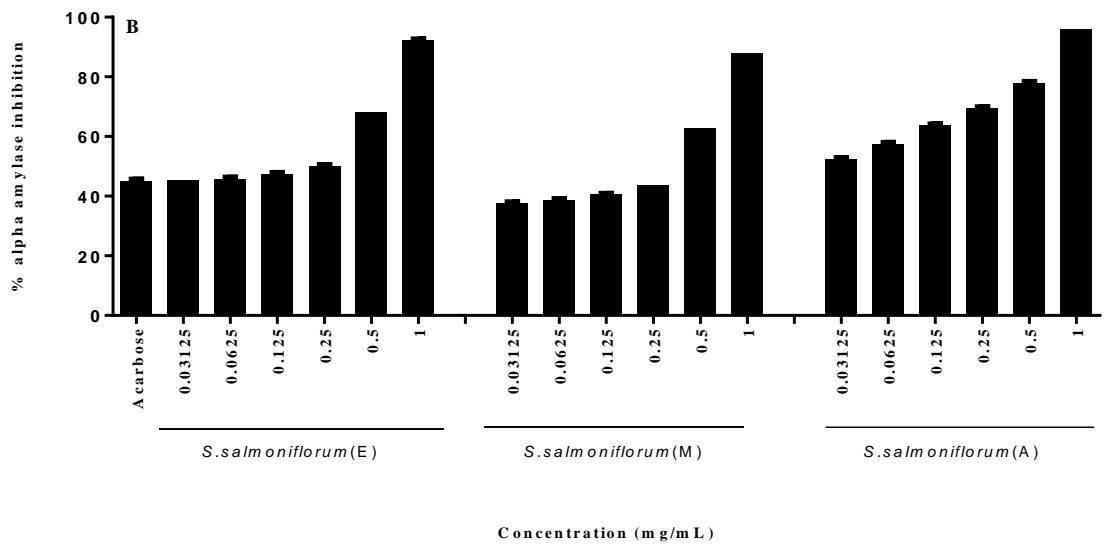
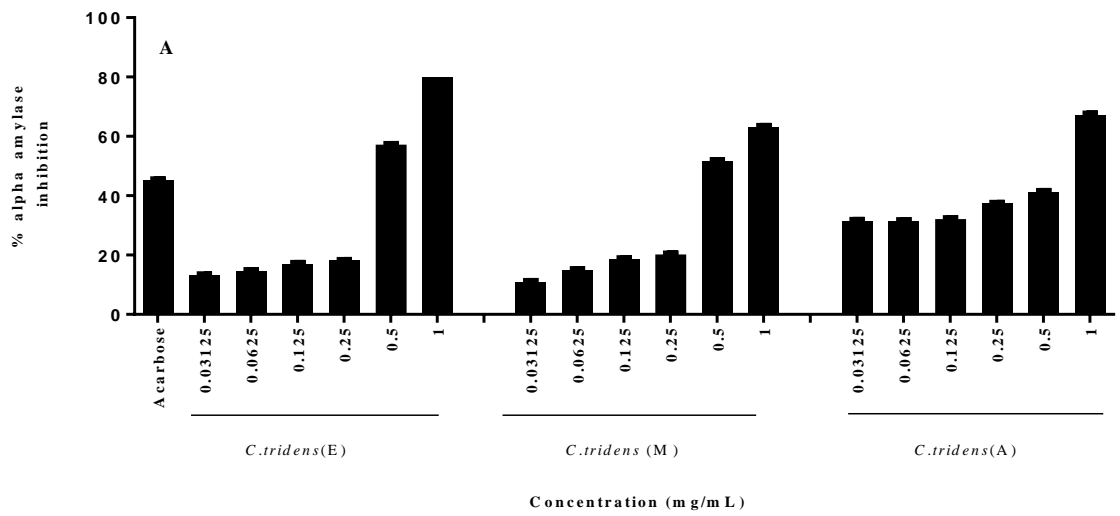


Figure 32. A-B. Inhibition effects of studied plant extracts used to treat diabetes in the Hardap region. Different superscript letters denote, E= ethanol, M=methanol & A= aqueous. Values are expressed as mean \pm standard deviation (n=15), $p > 0.05$ concentration difference, $p < 0.05$ different solvents.

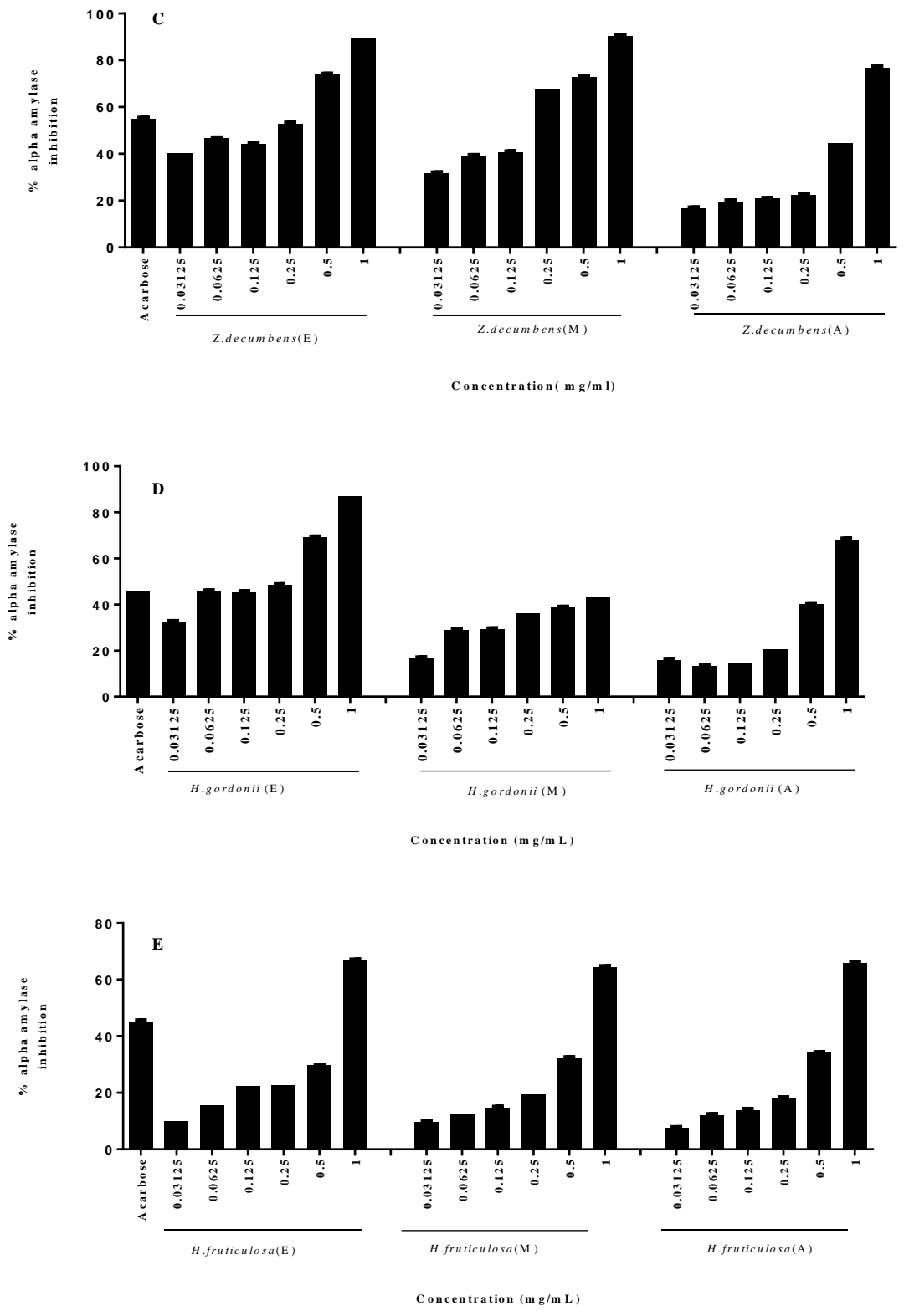


Figure 33. C-E. Inhibition effects of studied plant extracts used to treat diabetes in Hardap region. Different superscript letters denote, E= ethanol, M=methanol & A= aqueous. Values are expressed as mean \pm standard deviation (n=15), $p > 0.05$ concentration difference, $p < 0.05$ different solvents.

Table 10. Minimum inhibitory concentration IC_{50} (1 mg / ml) of the studied extracts against alpha amylase.

Plants & Ascorbic acid	Ethanol	Methanol	Water
Acarbose	0.08839±3.179		
<i>C. tridens</i>	0.4662±28.62	0.3772 ±23.15	0.4258±13.03
<i>Z. decumbens</i>	0.3648±19.47	0.509±23.52	0.2382±2334
<i>H. fruticulosa</i>	0.5092±20.20.25	0.4536±21.86	0.5164±20.7
<i>S. salmoniflorum</i>	0.4957±17.74	0.3069±15.77	0.3771±19.61
<i>H. gordonii</i>	0.3055±19.73	0.4822±26.38	0.1667±9.4

Values are presented as means ± standard deviation ($n=15$), $p > 0.05$ concentration difference, $p < 0.05$ different solvents.

4.9.2 Alpha-glucosidase inhibitory activity

The alpha-glucosidase inhibitory activity was observed when spots, where the samples were spotted, turned blue. All plants showed alpha glycosidase inhibitory activity with an increase in activity demonstrated by the intensity of dark blue spot by *H.fruticulosa* (Table 11 and Figures 34 & 35). The results of alpha-glucosidase are consistent with alpha-amylase activity, in that all extracts exhibited the ability to inhibit alpha-amylase with inhibition activity of 80 % and above, as well as inhibit alpha-glucosidase activity by staining blue the starch-containing agar.

Table 11. Alpha-glucosidase inhibition activity was quantified based on the stain formation of the spotted samples.

Plants	Ethanol	Methanol	Aqueous		
<i>Z. decumbens</i>	3	2	2	Positive (Acarbose) (+)	3
<i>C.tridens</i>	2	2	2		
<i>H.gordonii</i>	1	1	1	Negative (Ethanol) (-)	0
<i>H.fruticulosa</i>	3	3	2		
<i>S.salmoniflorum</i>	1	2	3	Methanol (*)	0

Key: 0-Dot did not stain, clear gel-no glucosidase inhibitor activity, 1-Dot stained to light blue traces of glucosidase inhibitor activity.2- Dot stained to blue- some glucosidase inhibitors activity, 3- Dot stained dark blue-glucosidase inhibitor activity.

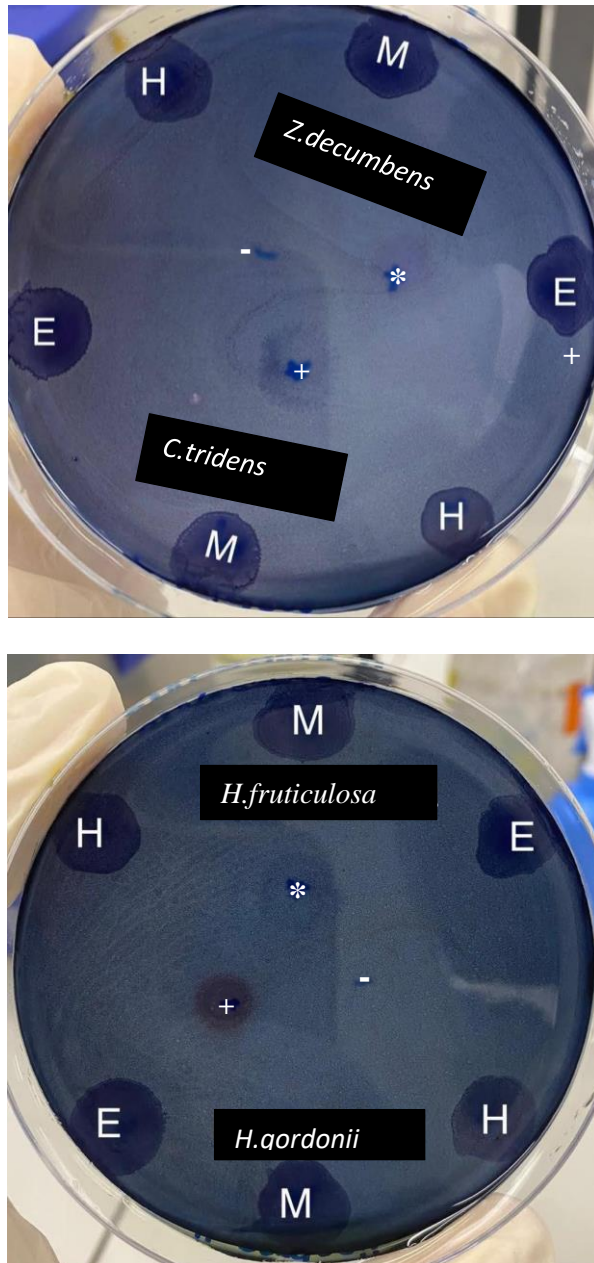


Figure 34. Alpha glucosidase activity of *C.tridens*, *Z. decumbens*, *H.fruticulosa* & *H. gordonii*. Different signs denote, (+) Acarbose, (-) Negative control (Ethanol), (*) Methanol.

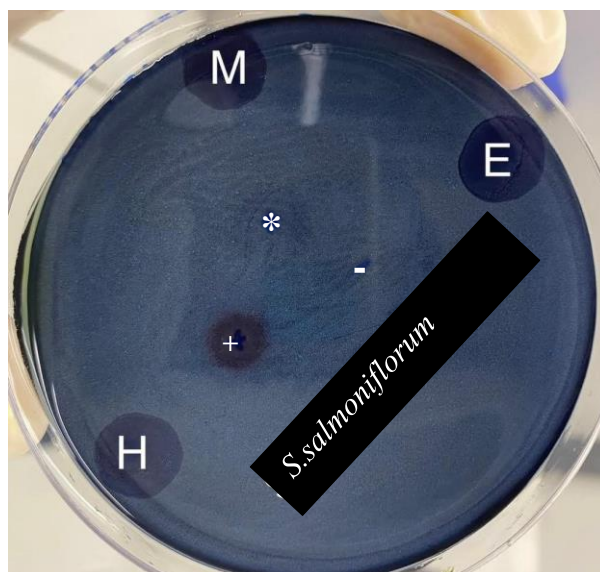


Figure 35. Alpha glucosidase activity of *S. salmoniflorum*. Different signs denote (+) Acarbose, (-) Negative control (Ethanol), and (*) Methanol.

4.10 Cytotoxicity effects of the extracts on the 3T3 cell line

The cytotoxicity analysis of ethanol, methanol, and aqueous extracts of *H. gordonii*, *C. tridens*, *Z. decumbens*, *S. salmoniflorum*, and *H. fruticulosa* on the 3T3 cell line, proliferated 90 % and more in the presence of the studied plant extracts together with triton X, as a positive control (Figure 36). Low cytotoxicity was revealed with methanolic extracts of *C. tridens* $IC_{50} = 0.2014 \pm 5.491 \mu\text{g}/\mu\text{l}$ (table12). The Kruskal-Wallis test revealed that the percentage of cell viability is significantly influenced by the concentrations $p < 0.05$, the different studied plants, $p < 0.05$, and the solvents used for extraction $p < 0.05$.

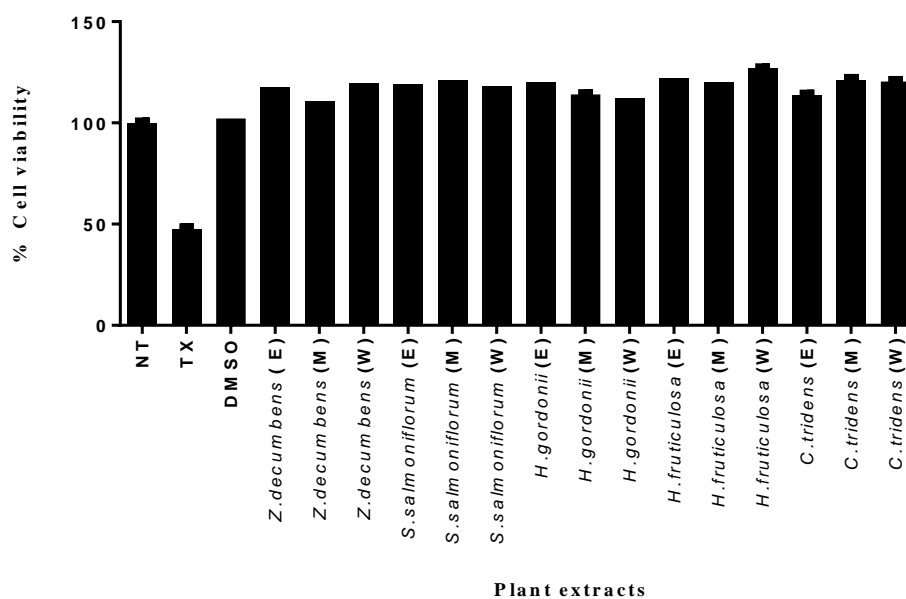


Figure 36. Cytotoxicity effects of the extracts on 3T3 cell line, Values are expressed as mean \pm standard deviation ($n=90$), $p < 0.05$ concentration, different plants and solvents.

Table 12. Minimum Inhibition Concentration IC 50 ($\mu\text{g}/\mu\text{l}$) of plant extracts against MTT.

Plants	Ethanol	Methanol	Water
Triton X	36.32 \pm 15.17		
<i>C. tridens</i>	20.46 \pm 4.037	0.2014 \pm 5.491	30.16 \pm 2.081
<i>Z. decumbens</i>	31.06 \pm 7.043	133931 \pm 4.009	58.99 \pm 6.263
<i>H. fruticulosa</i>	36.13 \pm 5.820	61.45 \pm 4.848	59.97 \pm 6.785
<i>S. salmoniflorum</i>	135 \pm 7.975	20.95 \pm 10.56	12.24 \pm 4.772
<i>H. gordonii</i>	76.49 \pm 9.622	024.28 \pm 7.985	480.3 \pm 7.586

Values are expressed as mean \pm standard deviation ($n=90$), $p < 0.05$ concentrations, plant species, and different solvents.

CHAPTER FIVE: DISCUSSION

The need for anti-hyperglycemic remedies is evident due to the increasing incidences of diabetes in Namibia as indicated by Dowson *et al.* (2022) and worldwide (World Health Organization, 2016). The use of plants to treat ailments has been prevalent since ancient times. Some of the currently used anti-hyperglycemic drugs are derived from plants. For example, metformin was derived from *Galega officinalis* (goat's rue), a herbaceous plant rich in guanidine, the active compound used in the production of metformin (Bailey, 2017).

Despite Namibia having a rich heritage of medicinal plants, some of which are used to treat diabetes, most of these plants are not documented and scientifically validated. The study identified fifteen such plants used in the management of diabetes, with most respondents being females (87%), owing to their roles as primary caregivers, learning about medicinal plants at a very young age when caring for their household and the elderly. This knowledge is shared with those around the elders, by word of mouth, with nothing documented (da Costa, Guimarães and Messias, 2021). The findings are in line with other studies that suggest women are more knowledgeable than men, regarding the use of medicinal plants (Cheikhoussef *et al.*, 2011; Weckmüller *et al.*, 2019; Kachmar *et al.*, 2021).

Moreover, the present study revealed that respondents over 40 years old were more knowledgeable about medicinal plants than their younger counterparts. This is similar to the observation by Mussarat *et al.* (2014) and Buwa-Komoreng *et al.* (2019), who reported that knowledge of medicinal plants is acquired over time and passed on from generation to generation. Weckmüller and others have identified several reasons that

may either limit or advantage a particular age group or gender. This includes the choice to keep the knowledge secret or consider the younger generation's lack of interest, among other factors (Weckmüller *et al.*, 2019). Therefore, emphasizing the importance of preserving ethnomedicinal knowledge through documentation before it disappears.

Among the different plant families identified in the study, the most prevalent plant family was Asphodelaceae (3 species) which include *Bulbine frutescens*, *Aloe zebrine*, and *Aloe hereroensis*. Members of this family are most common in arid areas, because of their ability to thrive in dry and hot conditions (Klopper, Van Wyk and Smith, 2010). Furthermore, the Asphodelaceae family is documented as an excellent source of anti-oxidants and anti-diabetic agents with the potential to mimic the activity of metformin (Odeyemi and Bradley, 2018; Oguntibeju, 2019). The second most prevalent family was Malvaceae 2 species (*Corchorus tridens* & *Hermannia fruticulosa*). Malvaceae are widespread and most prevalent in warmer regions similar to the Hardap region (Erarslan and Koçyiğit, 2019). Studies showed the use of Malvaceae by the traditional healer to treat diabetes (Oguntibeju, 2019).

Pedaliaceae and Lamiaceae families were among those unavailable at the point of collection. The family Pedaliaceae (*Harpogophytum procumbens* also known as Devil's claw) shares in the history of indigenous natural plant commercialization in Namibia, tea made from the tuber is used extensively for treating arthritis and rheumatoid arthritis according to Cole *et al.* (2014) and also used for blood purification, ailments related to gall bladder, pancreas, kidney (Von Koenen, 2007). Substantial studies have scientifically validated the use of *H.procumbens* in South Africa as an anti-inflammatory, anti-diabetic, and analgesic effect (Georgiev *et al.*, 2013; Brendler, 2021; Gxaba and Manganyi, 2022). The Lamiaceae family is popular

in southern African countries for their use in both folk medication and industries (Rattray and Van Wyk, 2021). A Lamiaceae (*Leonotis ocymifolia*) is documented for its potential as an alternative treatment for type 2 diabetes (Afolayan and O. Sunmonu, 2010; Etsassala *et al.*, 2020). However, the literature is silent on Namibian-based studies.

The participants interviewed for the current study indicated that they use the entire plant when preparing remedies, with leaves being the most frequently used plant parts. This suggests that harvesting is conducted sustainably with minimal impact on species exploitation, thus maintaining species' sustainability. This finding supports the observation by (Cheikhoussef *et al.*, 2011) and (Raj *et al.*, 2018) who studied the indigenous knowledge system of medicinal plants used by the Namibian and Indian communities respectively, and found leaves to be the most plant used. Likewise, Ofuegbe & Adedapo (2015) also reported that in Nigeria, leaves were the most frequently used plant parts and the most imperative constituent in herbal medicine preparation used to manage diabetes. The reason behind the frequent use of leaves can be attributed to the fact that they are easy to harvest and are rich in phytochemicals which are of therapeutic importance (Kachmar *et al.*, 2021).

In the current study, the most preferred method of remedy preparation was decoction at 47%, consistent with other studies (Baharvand-Ahmadi *et al.*, 2015; Hussain *et al.*, 2018). Furthermore, studies have shown a correlation between the preparation process and the effectiveness of traditional medicine (Yang and Ross, 2010). Highlighting the importance of optimizing conditions such as soak time, water-to-herb ratio, and decoction time during remedy preparation (Zhang *et al.*, 2017).

Hyperglycemia is concluded to be a risk in the progression of diabetes, due to insufficient insulin production or its effective use (De Vos *et al.*, 2021). Management of blood glucose levels through the inhibiting of two key enzymes; alpha-amylase and alpha-glucosidase is considered an effective approach (Kazeem, Adamson and Ogunwande, 2013). While many antihyperglycemic medicinal plants have been identified globally, this is not the case in Namibia (Sagbo and Hussein, 2022; Salehi *et al.*, 2019). To this end, the antihyperglycemic properties of extracts from *Hoodia gordonii*, *Chorchorus tridens*, *Hermannia fruticulosa*, *Sarcocaulon salmoniflorum*, and *Zygophyllum decumbens* were investigated. The qualitative phytochemical screening of ethanol, methanol, and water extracts of these plants revealed the presence of flavonoids, saponins, steroids, and tannins which might be responsible for the anti-hyperglycemic activity of the plant extracts. The ethanol and methanol extracts contained high levels of flavonoids, saponins, steroids, and terpenoids. This finding is consistent with Sonam, Singh, and Pooja's (2017) observation of high levels of these compounds when using these solvents due to their polarity.

Bioactive compounds such as steroids, flavonoids, alkaloids, and saponins are well-documented for their anti-hyperglycemic activity (Keller *et al.*, 2011; Aswathy and Jessykutty, 2017). According to a study by Bharti *et al.* (2018), flavonoids can restore pancreatic cells by triggering the secretion of insulin, decreasing glycosylated hemoglobin, and increasing glutathione peroxidase activity through oxidative damage reduction (Jere, Ezeala and Prashar, 2021). The current study also demonstrated the presence of alkaloids in the methanol extracts of *H. fruticulosa* and ethanol extracts of *C. tridens*, *H. fruticulosa*, and *H. gordonii* respectively. The presence of alkaloids is important because of their ability to inhibit both alpha-glucosidase and alpha-amylase enzymes, as well as ameliorate diabetic-induced oxidative stress (Kumar *et al.*, 2019).

Anti-oxidant potential in plant extracts affords them the ability to neutralize free radicals, quench singlet and triplet oxygen, and decompose peroxides (Rodríguez-García *et al.*, 2019). This is significant in many ailments especially in diabetes, as it affects the progression and complications of diabetes (Pieme *et al.*, 2017). A higher DPPH radical scavenging activity is associated with lower IC₅₀ values (Owolabi *et al.*, 2018). The high DPPH scavenging activity of *C. tridens* and *H. fruticulosa* in this study was consistent with a study by Fernandes de Oliveira *et al.* (2012) who demonstrated excellent scavenging activity by *C. tridens* and *H. fruticulosa*. Even though *H. gordonii* was not among those who exhibited high radical scavenging activity, it is worth noting that the *H. gordonii* ethanol extracts IC₅₀ for radical scavenging is in line with Kapewangolo *et al.* (2016) who reported an IC₅₀ value of 0.1246 ± 11.3 mg/ml compared to the current study of 0.2837±14.14 mg/ml.

Compounds or molecules with reducing power are potential electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, and thus function as primary and secondary antioxidants (Benslama and Harrar, 2016). The anti-oxidants and reducing power of the extracts can be attributed to the presence of phenolic and alkaloids, due to the hydrogen-donating capacity of their hydroxyl groups (Aluko, 2017; Gan *et al.*, 2017).

The studied plants showed alpha-amylase and alpha-glucosidase activity. These enzymes are vital in the hydrolysis of carbohydrates and postprandial glucose blood concentration in diabetic patients (Poovitha and Parani, 2016). Thus, inhibiting these enzymes can aid in modulating postprandial hyperglycemia, as well as delay or prevent the progression of type II diabetes (Thovhogi, 2009; Telegari & Hullatti, 2015; Poovitha & Parani, 2016). This was evident in a study by Sharma *et al.* (2018) that

showed human pancreatic alpha-amylase activity correlating with increased postprandial glucose levels.

The results of this study confirmed *Hoodia gordonii*, *Corchorus tridens*, *Hermannia fruticulosa*, *Sarcocaulon salmoniflorum*, *Zygothymum decumbens* ability to inhibit alpha-amylase and alpha-glucosidase. All the studied plant extracts exhibited excellent antihyperglycemic activity, with the highest alpha-amylase inhibition exhibited by methanol extracts of *S.salmoniflorum* at 96 %, followed by *S.salmoniflorum* ethanol extracts at 92% and lastly, *Z.decumbens* methanol extracts with 90 %. *S.salmoniflorum* showed increasing results that were dependent on the dose for all three extracts. The activity demonstrated by *S. salmoniflorum* could be attributed to the presence of flavonoids, tannins, and terpenoids which are known to bind to carbohydrates eliciting the inhibitory effect (Chelladurai and Chinnachamy, 2018). The starch agar gel diffusion test showed traces of alpha-glucosidase inhibitor activity for all the tested extracts (Figures 34 &35) with spots of extracts turning from clear to dark blue. A darker stained spot was reported in extracts of *H. fruticulosa* and *Z. decumbens*. The fact that the studied plants demonstrated inhibition for alpha-amylase and alpha-glucosidase could justify their use in the traditional setting.

The positive control, acarbose is a competitive and reversible inhibitor of alpha-amylase and alpha-glucosidase. It delays the hydrolysis of carbohydrates, therefore slowing down the absorption of glucose and subsequently reducing postprandial blood glucose concentration (McIver, Preuss & Tripp 2022). In the present study, acarbose revealed rather low inhibitory activity of 55% when compared with all the tested plants at 1mg/ml. This is parallel with the observation made by Poovitha and Parani (2016) who reported on a study that revealed acarbose inhibition activity to range from 55 %

and 82 % depending on experimental conditions and with their study revealing acarbose inhibitory activity of 68 % at 10 mg / ml. However, a study by Granados-Guzmán *et al.* (2018) demonstrated how increasing concentration, incubation time, and incubation temperature affect the activity of the tested compounds and acarbose activity. Consequently, in the same study, acarbose demonstrated 70% inhibition activity at 310.2 µg/ ml and incubated at 37 ° C for 17.5 minutes.

The use of herbal medicine, as one element of complementary and alternative medicine (CAM) continues to grow globally and many new products are introduced into the market (Welz, Emberger-Klein and Menrad, 2018). While some of these herbal medicines have promising potential and are widely used, Mensah *et al.* (2019) report that their chemical constituents make them intrinsically toxic and therefore recognize concerns about their safety. Likewise, a study on the aqueous extracts of the African *C. sanguinolenta* (Apocynaceae) revealed their use for centuries in the treatment of malaria, bacterial infections, hepatitis, and rheumatism among many other ailments (Osafo, Mensah and Yeboah, 2017). However, when *C. sanguinolenta* was assessed for safety, an aqueous extract of the root suggested general safety at oral dosages below 500 mg/kg in Sprague Dawley rats. Yet, the ethanol extract of the stem demonstrated localized systemic acute and sub-chronic toxicity in albino rats (Mensah *et al.*, 2019).

In a genetic toxicity study, aqueous root extracts of *C. sanguinolenta* also demonstrated genotoxicity at 50µg/ml causing DNA damage (Ansah, Khan and Gooderham, 2005). In some cases, the toxicity of plants can be specific or has multiple targets, for example, the hepatotoxic of *Aphania senegalensis* reported in rats (Fall *et al.*, 2011) and *Herniaria cinerea* gastrointestinal tract toxicity (Sokar *et al.*, 2003). However, the toxic substances in African herbal medicines, along with their toxicology

and pathogenesis, are still largely unknown, as mentioned by Anywar *et al.* (2021). MTT assay was thus used to evaluate the toxicity of the plant extract's toxicity. MTT is a rapid, sensitive, and highly reliable colorimetric assay that detects the conversion of a water-soluble substrate 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) to a water-insoluble formazan product from functioning cellular mitochondrial dehydrogenase enzyme. The amount of formazan produced reflects and is directly proportional to the number of viable cells (Ogbole *et al.*, 2017; Putthawan *et al.*, 2018; Uğur *et al.*, 2017). In the presence of MTT, all the studied extracts revealed general safety at all the tested concentrations with the highest concentration of 100 µg/ml. According to Adegbaaju *et al.* (2020), any crude plant extract at a concentration of 20 µg/ml and below that produces 50% of cell death within 72 hours in vitro is cytotoxic. Worth noting is that the cytotoxicity response of the 3T3-cell to all extracts was above 50 % at all concentrations. This thus implies that the tested extracts are not toxic. The significantly increased cellular proliferation observed would mean extracts support proliferation translating into properties of wound healing, tissue repair, and aging (Boyette and Tuan, 2014).

The findings of the present study suggest that the limited number of plants collected and the assay used for alpha-glucosidase may have directly or indirectly influenced the study's outcome. Due to the limited number of plants, the comparison of samples across different data points was not comprehensive. The alpha-glucosidase inhibitory effect was qualitatively analyzed, while the alpha-amylase effect was quantitatively analyzed, making it less likely to statistically compare the two data sets. Despite these limitations, one of the main objectives was to document and validate the knowledge of medicinal plants in Namibia, and based on the results, this objective was accomplished.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

The current study revealed the first pharmacological insight into the antioxidant, antihyperglycemic, and cytotoxic activity of *C.tridens*, *S.salmoniflorum*, *H.fruticulosa*, *H.gordonii* and *Z.decumbens* plants used to manage diabetes in the Hardap region of Namibia in an attempt to validate their ethnomedicinal uses.

The plant extracts of *C.tridens*, *S.salmoniflorum*, *H.fruticulosa*, *H.gordonii*, and *Z.decumbens* revealed the presence of phytochemical compounds such as alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. These compounds are known for their anti-oxidant activity and antihyperglycemic activity, as they inhibit the catabolic effect of alpha-amylase and alpha-glucosidase enzymes. In vitro, anti-hyperglycemic activity showed that aqueous extracts of *H.gordonii* and *Z.decumbens* exhibited moderate anti-hyperglycemic activity with *H.gordonii* being the most potent with the lowest IC₅₀ value. Cytotoxicity screening of all plants against the 3T3 cell line revealed cell proliferation and low cytotoxicity potency observed in *C.tridens*, with the lowest IC₅₀.

In summary, the study findings provide moderate support for the use of *C.tridens*, *S.salmoniflorum*, *H.fruticulosa*, *H.gordonii*, and *Z.decumbens* in the management of diabetes mellitus in the Hardap region community. However, the in-vivo anti-hyperglycemic activity and in-vivo toxicity using animal models should be investigated to ascertain their potency, and safety and thus advise on dosage regime based on how the liver metabolizes the extracts. The identification and quantification of phytochemical compounds using advanced analytical techniques such as HPLC should also be explored. As well as characterize the mode of action of the principal compounds responsible for the inhibitory action of alpha-amylase and alpha-glucosidase and also the above-mentioned anti-oxidant activity. This will be useful in

their integration into mainstream anti-hyperglycemic management and consequently contribute to the development of alternative anti-hyperglycemic agents from plants.

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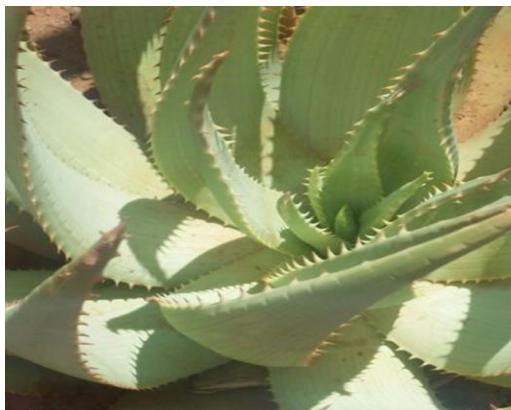
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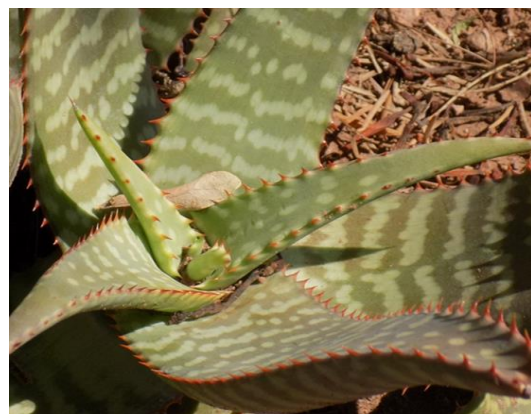
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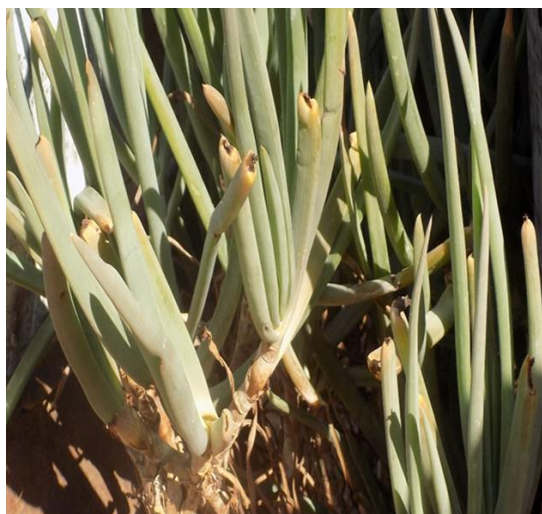
APPENDIX A- Plants used in the study voucher specimen



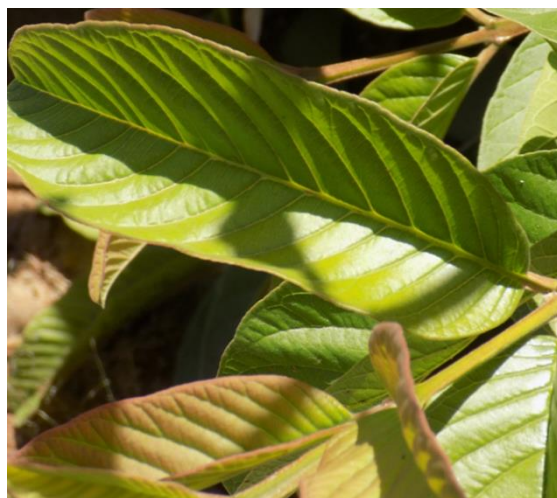
Aloe hereroensis (BRL21)



Aloe zebrine (BRL25)



Bulbine frutescens (BRL27)



Chorchorus tridens (BRL27)



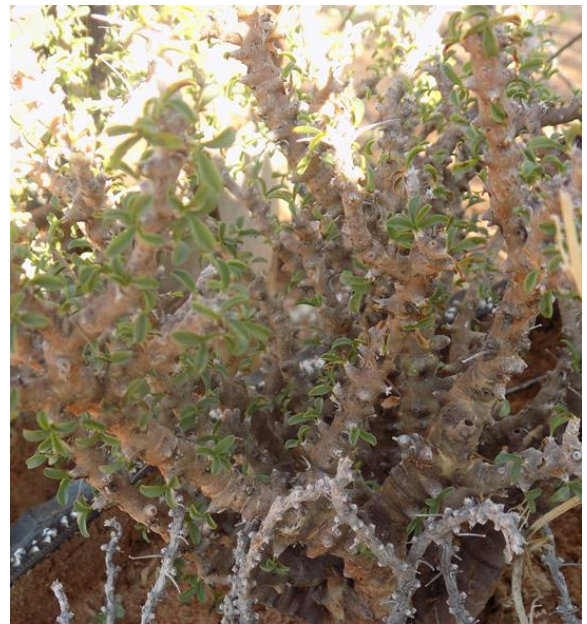
Harpogophytum procumbens (BRL24)



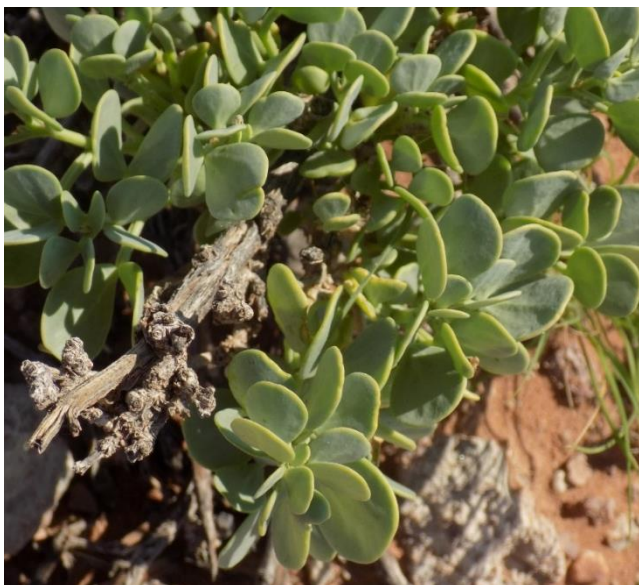
Hoodia gordonii (BRL20)



Leonotis ocymifolia (BRL29)



Sarcocaulon salmoniflorum (BRL28)



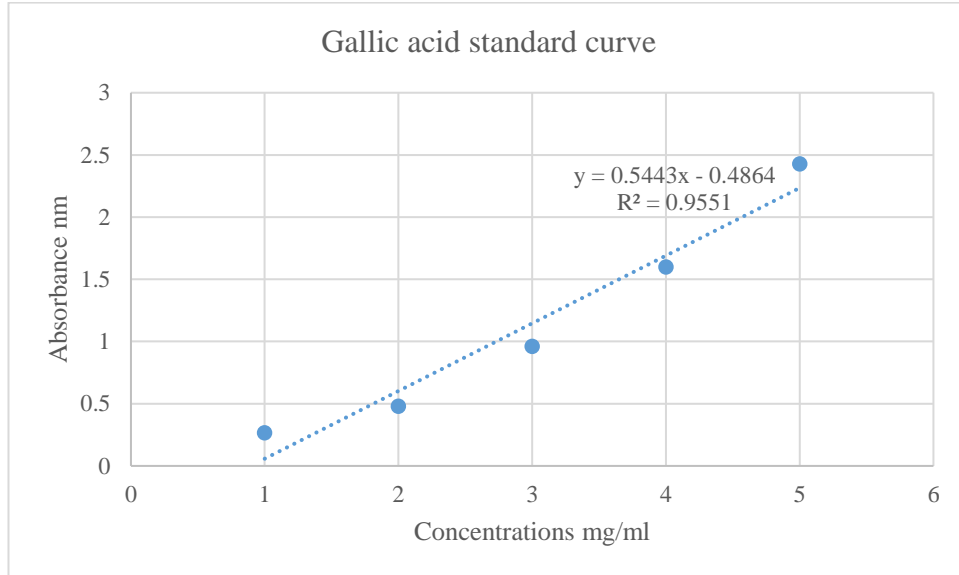
Zygophyllum decumbens (BRL23)



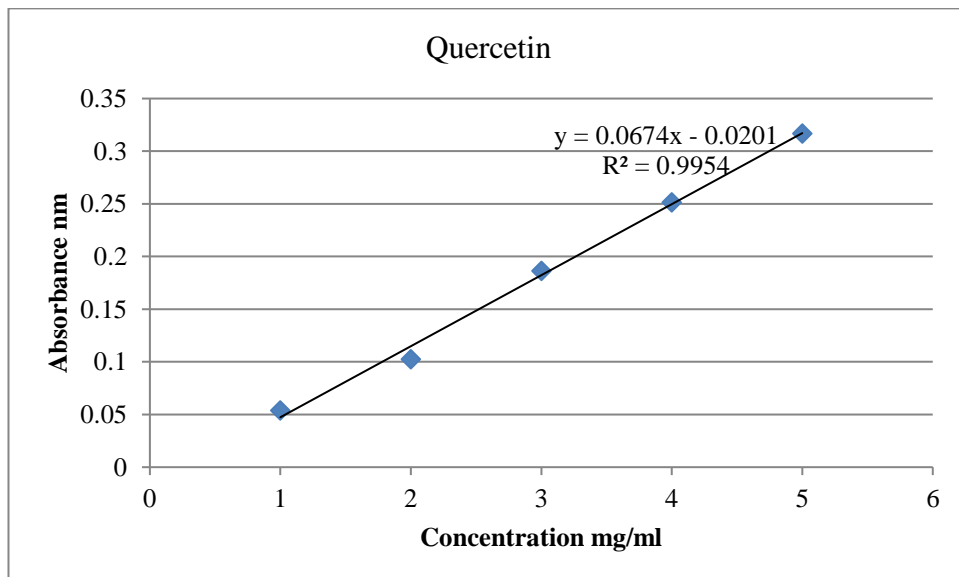
Hermannia fruticulosa (BRL26)

APPENDIX B- Standard curves

Estimation of the total phenolics calibration curve

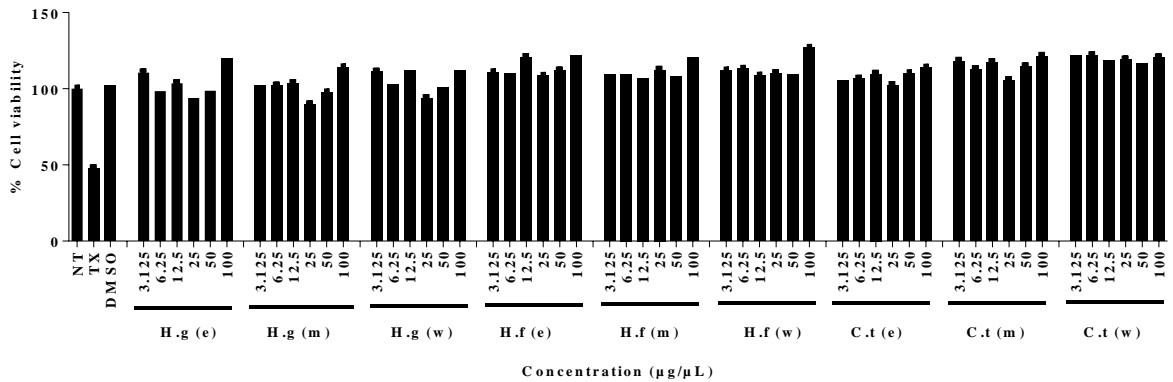
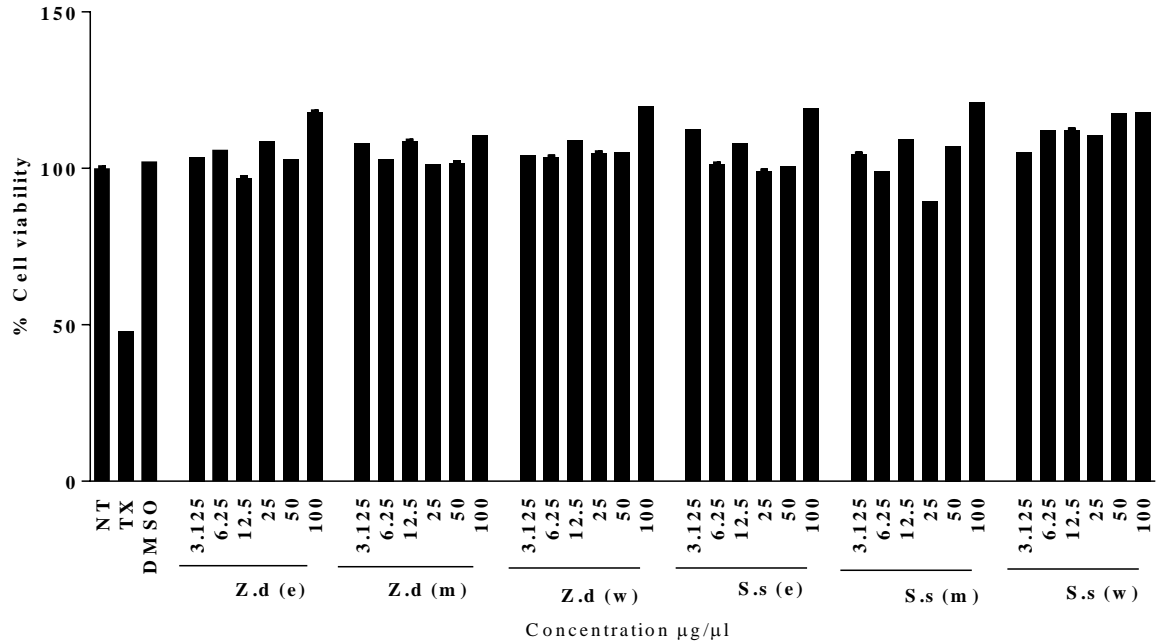


Estimation of the total flavonoids calibration curve



APPENDIX C- Graphs for the cytotoxicity effect of the plant extracts

Cytotoxicity activity of extracts at different concentrations



Cytotoxic effects of plants extracts 3T3 cell line (C. t=C.tridens, Zd=Z.decumbens, S. s= S.salmoniflorum, H.g= H.gordonii, H.f= H.fruticulosa)

APPENDIX D- Research Permit



MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 2221/2017

Valid from 9 November 2016 to 31 October 2017

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: Ms C.I. Du Preez
Address: Multidisciplinary Research Centre
University of Namibia
Private Bag 13301
Windhoek
Namibia

Coworkers: Dr. D. Mumbengegwi, Ms. K. Kaitjizemine, Dr. H. Winschiers, Mr. G. Katjipure, Mr. C. Stanley, Mr. M. Chamunorwa, Mr. M. Shirungu, Mr. W. Embashu and Dr. A. Cheikhousseff

National survey on documentation of Indigenous Knowledge System (specifically on medicine, food and beverage) in Hardap, Omusati, Erongo and Omaheke Regions, subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.





Authorising Officer

IMPORTANT

This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Conservation Scientist, email illa.matheus@met.gov.na
Private Bag 13306, Windhoek, Namibia

APPENDIX E- NBRI report



Ministry of Agriculture, Water and Forestry

National Herbarium of Namibia (WIND)

Identification Report

Report No.: 2017/390

25 July 2017

Collector/s: Du Preez, I

Address: University of Namibia
P/Bag 13301
Pionierspark
Windhoek

Number	ID cat.	Identification
s.n	1	<i>Marsdenia macrantha</i> (Klotzsch) Schltr.
s.n	4	
BRL 20	1	<i>Hoodia gordonii</i> (Masson) Sweet ex Decne.
T 02-05	3	<i>Hoodia</i> sp.
BRL 40	1	<i>Terminalia sericea</i> Burch. ex DC.
BRL 43	1	<i>Adenium boehmianum</i> Schinz
BRL 51	1	<i>Terminalia sericea</i> Burch. ex DC.
BRL 30	1	<i>Chorchorus tridens</i> L.
BRL 33	1	<i>Grewia tenax</i> (Forsk.) Fiori
BRL 23	1	<i>Zygophyllum decumbens</i> Delile var. <i>decumbens</i>
BRL 35	1	<i>Terminalia prunioides</i> M.A.Lawson
BRL 36	1	<i>Drimys sanguinea</i> (Schinz) Jessop
T 03-04	2	<i>Tulbaghia violacea</i>
T 01-05	3	<i>Carpobrotus</i> sp.
BRL 38	1	<i>Asparagus exuvialis</i> Burch.
T 02-04	3	<i>Aloe</i> sp. Baker
BRL 21	3	<i>Aloe hereroensis</i> Engl.
BRL 25	1	<i>Aloe zebrina</i> Baker
T 03-03	1	<i>Bulbine frutescens</i> (L.) Willd.
BRL 27	1	<i>Bulbine frutescens</i> (L.) Willd.
BRL 26	1	<i>Hermannia fruticulosa</i> K.Schum.
BRL 28	1	<i>Sarcocaulon salmoniflorum</i> Moffett
BRL 30	3	<i>Psidium</i> sp.
BRL 36	2	<i>Moringa ovalifolia</i> Dinter & A.Berger
BRL 37	1	<i>Ziziphus mucronata</i> Willd.
BRL 42	1	<i>Pollichia campestris</i> Aiton
T 01-06	1	<i>Punica granatum</i> L.
T 01-07	2	<i>Petroselinum crispum</i> (Mill.) Fuss
T 01-09	2	<i>Petroselinum crispum</i> (Mill.) Fuss
T 01-10	2	<i>Petroselinum crispum</i> (Mill.) Fuss
T 01-12	2	<i>Myrothamnus flabellifolius</i> Welw.
T 02-03 (a)	1	<i>Blepharis obmitrata</i> C.B.Clarke

Identification categories: 1. Certain identification 2. Closest to 3. Certain to genus only 4. Unable to identify

Private Bag 13184, Windhoek Tel: +264 - 61 - 202 - 2021 Fax: +264 - 61 - 259 - 153 e-mail: Frances.Chase@mawf.gov.na

1

Number	ID cat.	Identification
T 02-03 (b)	1	<i>Dicoma schinzii</i> O.Hoffm.
T 02-06	1	<i>Thamnosma africana</i> Engl.
T 02-09	1	<i>Ziziphus mucronata</i> Willd.
T 03-01	2	<i>Nymania capensis</i> (Thunb.) Lindb.
T 01-08	3	<i>Amaranthus</i> sp. Thell.
T 01-07	1	<i>Senna italica</i> Mill.
BRL 41	1	<i>Ximenia americana</i> L. var. <i>americana</i>
BRL 34	1	<i>Albizia anthelmintica</i> (A. Rich.) Brongn.
BRL 52	1	<i>Acacia erioloba</i> E.Mey.
T 02-01	1	<i>Catophractes alexandri</i> D.Don
BRL 44	1	<i>Colophospermum mopane</i> (J.Kirk ex Benth.) J.Kirk ex J.Léonard
BRL 39	1	<i>Colophospermum mopane</i> (J.Kirk ex Benth.) J.Kirk ex J.Léonard
BRL 24	1	<i>Harpagophytum procumbens</i> (Burch.) DC. ex Meisn. var. <i>procumbens</i>
BRL 20	1	<i>Acacia erioloba</i> E.Mey.
T 02-08	4	
T 03-02	1	<i>Kleinia longiflora</i> DC.
BRL 29	1	<i>Leonotis ocyimifolia</i> (Burm.f.) Iwarsson var. <i>schinzii</i>
T 1-13	4	
T 01-11	1	<i>Rosmarinus officinalis</i>
T 01-03	3	<i>Mentha</i> sp.
T 01-04	1	<i>Ocimum americanum</i> L.
T 01-02	2	<i>Ocimum filamentosum</i> Forssk.

Comment:

Curator
National Herbarium of Namibia (WIND)

APPENDIX F- Consent form

MULTIDISCIPLINARY RESEARCH CENTRE (MRC)

Science, Technology and Innovation Division

University of Namibia, Private Bag 13301, Windhoek, Namibia

340 Mandume Ndemufayo Avenue, Pioneers Park

☎ +264 61 206 3051/2; Fax: 061-206 3684/3050; URL: <http://www.unam.edu.na/mrc/>



Consent to participate in study: Screening of anti-hyperglycemic activity of selected medicinal plants in the Hardap region of Namibia

My name is Kaveire Kaitjizemine. I am a student at the University of Namibia (UNAM), doing my Master's degree on a project that is a collaboration between the Multidisciplinary Research Centre and the Faculty of Science (Department of Biological Sciences).

We are conducting a follow-up study to the Medicinal IKS survey that was conducted in November 2016 where you indicated you had knowledge on plants used to manage diabetes. We would like to ask you questions relating to this knowledge and to identify and collect the plants in order to analyse them in the lab for antidiabetic properties. The findings of such studies will be shared with you, and you will be consulted before any further studies are conducted. The results of the study may be published.

The study aims at validating plants used to manage diabetes Mellitus (DM), and we would like your consent to participate in this study. Please indicate by signing this form that you are willing to participate in this study. Your sharing of this information is voluntary. It will be treated as confidential and used for research purposes only.

I confirm that this study and its objectives has been explained to me. I fully understand the nature and purpose of this study and I voluntarily consent to take part in this study.

Signature:..... Date:.....

Name and signature of witness:.....

SECTION A. Participant's information

Full names	
Date of birth	
Place of information transfer	
Contact details	

SECTION B. Level of knowledge on Diabetes Mellitus

Question	Response
What do you know about DM?	
What do you think are the causes of DM?	
What are the signs and symptoms of DM?	
Do you treat people with DM or have you shared this information with DM?	
Why do you think they use medicinal plants instead of Western medicine?	
Do you/they think medicinal plants works?	

SECTION C. Plant(s) used to manage DM

Question	Response
What plant(s) do you use to manage/treat DM? [Add voucher specimen no. to plant name]	
Is it used in combination with any other plant?	
Which part(s) of the plant is/are used?	
How is it prepared?	
How is it administered?	
How long does it take for the treatment to work?	
When harvested, are there ways to ensure the plant does not die?	

Question	Response
What plant(s) do you use to manage/treat DM? [Add voucher specimen no. to plant name]	
Is it used in combination with any other plant?	
Which part(s) of the plant is/are used?	
How is it prepared?	
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How long does it take for the treatment to work?	
When harvested, are there ways to ensure the plant does not die?	

SECTION C. Plant(s) used to manage DM

Question	Response
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