ETHNOBOTANICAL SURVEY AND PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANTS USED IN THE MANAGEMENT OF DIABETES AND HYPERTENSION DISEASES IN MPIGI DISTRICT, UGANDA

BY

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DECLARATION

This dissertation is my original work and has never been presented for a degree in any other University.

…….…. …… ……

Signature Date

APPROVAL

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

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DEDICATION

I dedicate this dissertation to my family, whose unwavering support and encouragement have been my constant source of strength and inspiration. To my mentors and educators, whose guidance and wisdom have shaped my academic journey, I express my deepest gratitude.

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LIST OF ABBREVIATIONS

AAPH	2,2′ -azobis(2- amidinopropane) dihydrochloride
ABTS	2,2′-azinobis-3-ethylbenzothiazolin-6-sulfonic acid
ALB	Albumin
AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline phosphatase
ALT/GPT	Alanine aminotransferase
AST/GOT	Aspartate aminotransferase
BASO	Basophils
BILD	Direct Bilirubin
BILT	Total Bilirubin
CAT	Catalase
CRE	Creatinine
DM	Diabetes Mellitus
DPPH	1,1-diphenyl-2-picryhydrazyl
EO	Eosinophils
GC-MS	Gas Chromatography-Mass Spectrometry
GGT	Gamma-Glutamyl Transferase
GPx	Glutathione peroxidase
HCT	Haematocrit
HGB	Haemoglobin
HIV	Human Immunodeficiency Virus
IG	Immunoglobulin
LYMPH	Lymphocytes
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration

MCV	Mean Corpuscular Volume
MDA	Malondialdehyde
MONO	Monocytes
MPV	Mean Platelet Volume
NCDs	Non-Communicable Diseases
OECD	Organization for Economic Cooperation and Development
PCT	Procalcitonin
PCV	Packed Cell Volume
PDW	Platelet Distribution Width
P-LCR	Platelet Larger Cell Ratio
PLT	Platelets
RBC	Red Blood Cells
RDW-CV	Coefficient of Variation in Red Cell Distribution Width
RDW-SD	Standard Deviation in Red Cell Distribution Width
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SSA	Sub-Saharan Africa
TH	Traditional Healers
TMP	Traditional Medical Practitioners
TP	Total Protein
UBOS	Uganda Bureau of Statistics
UV	Ultraviolet
WBC	White Blood Cells
WHO	World Health Organization

WMP	Western Medical Practitioners
WTD	Western Trained Medical Doctors

ABSTRACT

Diabetes and hypertension have emerged as significant global health challenges due to their increasing prevalence within populations and status as major contributors to mortality worldwide, with projections indicating a further rise in deaths, particularly in less developed regions. Various allopathic medicines have been produced but with low curative efficiency and side effects. On the other hand, medicinal plants have emerged as a significant alternative for treating various human conditions, such as diabetes and hypertension. Despite their importance, and high usage globally, majority of them have not been studied and documented. This study was conducted to assess the use of medicinal plants in the management of diabetes and hypertension in Mpigi district, Uganda. Individual interviews with traditional herbalists were carried out in a semi-structured format followed by field visits for taxonomic classification of the plants mentioned in the local language. Furthermore, three highly used plants were selected for phytochemical analysis qualitatively, quantitatively, and using the GCMS method. These plants were also analysed for their safety profile using rats as model species. Fifty-four (54) herbalists were interviewed, and 64% of them had an experience of more than 6 years in treating diabetes and hypertension. Herbalists reported the utilization of one hundred and twenty (120) plant species for the treatment of diabetes and hypertension. In the phytochemical analysis, the results revealed the presence of a majority of the analysed phytochemicals, encompassing alkaloids, steroids, phenols, tannins, flavonoids, coumarins, and terpenoids. The FSM extract exhibited high levels of total phenols (217±25.05mg/g) and total tannins (179.75±3.44mg/g). The CRM extract contained substantial amounts of total flavonoids (28.75±0.98mg/g), total saponins (225.07±4.11mg/g), and total alkaloids (116.15±3.73mg/g). Additionally, FSM extract contained 14 detected compounds, while both CRM and MPM extracts contained 30 compounds each. Several of the identified compounds exhibit pharmacological activities that are pertinent to the treatment of diabetes and hypertension. The LD_{50} value, surpassing 5000mg/kg in toxicity assessment, confirms the safety of these plants within the context of traditional use. However, the administration of the three extracts significantly altered certain haematological (White Blood Cells) and biochemical parameters (liver enzymes), indicating potential toxicity. Traditional healing knowledge remains crucial for addressing human ailments, and the study underscores the significance of the identified plants in managing diabetes and hypertension, albeit with a need for cautious use to prevent adverse health effects. The three analysed plants possess compounds with antidiabetic and antihypertensive abilities signalling the potential for drug development.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Several indigenous plants and/or their products have been used in traditional systems to cure several medical problems since ancient times (Nakavuma *et al*., 2016). This dates back to when human beings required a means in their environment to improve their health and recover from diseases, and the only choice that they had was to use plants. There is evidence suggesting that the cultivation of plants for medicinal use has a history dating back around 60,000 years (Fabricant & Farnsworth, 2001; Jamshidi-Kia *et al*., 2018).

Over the centuries, the demand, use, and acceptance of medicinal plants have been globally increasing (Tugume *et al*., 2016; Ssenku *et al*., 2021). The World Health Organisation reports show that approximately eighty percent of the global population relies on plants and their derivatives to meet their basic healthcare needs, particularly for millions of persons residing in extensive rural regions of low-income nations (Kamatenesi-Mugisha & Oryem-Origa, 2007: Tugume *et al.*, 2016). Similarly, the demand for and utilization of herbal plants is not the case in only developing nations but also in developed countries like the USA where good and up-to-date health facilities are in place (Ssenku *et al*., 2021).

In Africa, the situation is not different, with more than eighty percent of the population totally relying on plants as their major solution to different ailments and this has been mainly due to very poor access to healthcare facilities (Ofori *et al*., 2012). In Ghana, the ratio of medical doctors to patients exceeds one to ten thousand, illustrating a scarcity of conventional healthcare resources. Consequently, a significant portion of the population depends on traditional healthcare delivery (Ofori *et al*., 2012). In South Africa, over 60% rely on medicinal plants for treatment, with one traditional medical practitioner (TMP) for 700-1200 patients, a ratio significantly higher than one western medical practitioner (WMP) for 17-400 patients

(Galabuzi, 2015). In recent years, there have been notable advancements in health facilities and increased accessibility to Western industrial medicines in Africa. However, despite these improvements, medicinal plants continue to serve as a fundamental and prevalent form of treatment in the region (Delbanco *et al*., 2017).

In the Eastern Africa countries such as Tanzania, Uganda, and Burundi, medicinal plants play a vital role in healthcare, leading to over 80% of the population depending on herbal medicine, mostly in rural areas (Tugume *et al.*, 2016). For instance in Uganda, there is at least 1 TMP who treats 290 patients, when compared to one WMP for ten thousand patients in urban and one for fifty thousand patients in rural areas (Kamatenesi-Mugisha & Oryem-Origa, 2006). Every single village in Uganda has at least one Traditional Healer (TH) and among every five people, four mainly seek out medical help from TH (Abbo, 2011). Uganda has also more THs than Western-trained medical doctors (WTD) with a TH-to-population ratio of 1:200 when compared to 1:20,000 for WTDs. This outcome has led to the number of THs being 100 times greater than that of Western-trained physicians (Schultz *et al*., 2020). WHO recommends at least a ratio of 1:1000 for physicians to patients (Schultz *et al*., 2020). This makes the recommended WTD far below the WHO recommendations.

Traditional therapeutic plants continue to be the most utilized form of the healthcare system in managing and treating several human ailments (Nakavuma *et al*., 2016; Kakudidi *et al*., 2017). This has been due to cultural preferences, easy accessibility, and availability of plants among other factors (Kamatenesi-Mugisha & Oryem-Origa, 2006; Ssegawa & Kasenene, 2007; Tugume *et al*., 2016; Ssenku *et al*., 2021). For example, a study conducted in the Katikekile sub-county, Uganda documented that, challenges in accessing modern treatments such as long distance to health facilities, inadequate medicines, and unaffordable medical fees are among the socio-economic factors that facilitate their use (Logiel *et al.*, 2021). The day-to-day usage

and needs of medicinal plants in rural populations of Uganda is nearly ninety percent and form a core part of treatment.

Non-Communicable Diseases (NCDs) pose a global health crisis, surpassing all other causes of death combined (Hughes *et al*., 2015). Predictions indicate a rise from 38 million in 2012 to 52 million deaths by 2030, and among these, 82% are attributed to major NCDs like diabetes, cancers, heart diseases, and respiratory diseases, with 18% stemming from other NCDs (Hughes *et al.*, 2015). The majority of these fatalities, about 75 %, take place in countries with low and middle incomes (Hughes *et al*., 2015). Hypertension ranks among the most widespread non-communicable diseases globally, impacting approximately 20% of the global adult population, with Uganda consistently identifying it as the most prevalent NCD based on multiple studies (Kakudidi *et al.*, 2017). Similarly, diabetes poses a rising concern in Uganda, affecting four percent of the total population, with the number of patients increasing from 560,000 in 2006 to 693,200 in 2014, and an estimated additional 560,000 people living with diabetes unaware in 2006 (Kakudidi *et al*., 2017). Globally, both in developed and developing nations, the utilization of herbal medicine continues to be a prevalent and increasing approach to addressing NCD (Kakudidi *et al*., 2017). This has been triggered by various factors among them include efficiency of plants and pharmaceutical drug resistance (Nguanchoo *et al.*, 2023).

Given that NCDs are the major global health concerns currently, with diabetes and hypertension as the most prevalent, proper understanding and documentation of herbal plants employed in the treatment of diabetes and hypertension are necessary to be undertaken. This is of key importance to inform the public on the plants that are used which can prevent this traditional knowledge from being lost. This information is also important and necessary as a foundation/basis for further scientific studies on developing drugs to manage these two diseases, to prove the claims of used plants in treatment, and also for the scientific evaluation

of the documented medicinal plants. This study was undertaken to assess plants that are used to alleviate diabetes and hypertension diseases in the Mpigi district in Uganda.

1.2 Problem statement

The escalating prevalence of diabetes and hypertension worldwide has resulted in a burgeoning global health crisis, leading to increased mortality rates and posing significant challenges to public health, particularly in less developed countries (Hughes *et al.*, 2015). This crisis is also persistent in Uganda, where hypertension stands out as the most prevalent NCD, impacting a substantial portion of the population, while diabetes, currently affects four percent of the total populace and is anticipated to massively increase in current years (Kakudidi *et al*., 2017). These diseases, apart from their evident impact on public health, have presented formidable challenges to the attainment of Sustainable Development Goals (SDGs) and the objectives outlined in the Third National Development Plan (NDP III). This is exemplified in their adverse effects on the promotion of overall social well-being, the targeted reduction of premature mortality, and the alleviation of poverty.

In the face of challenges such as low curative efficacy and adverse side effects associated with developed allopathic drugs, medicinal plants have emerged as pivotal options, presenting a noteworthy alternative for the treatment of these diseases. Nevertheless, a substantial portion of utilized medicinal plants and their applications remain unexplored and undocumented, presenting a dual challenge of potential loss of traditional knowledge and the absence of scientific validation regarding the quality, potential efficacy, and safety of the employed plants for users. Therefore, given this context, the aim of this study was to investigate and document the use of medicinal plants by herbalists in the Mpigi district for alleviating diabetes and hypertension. In addition, phytochemical constituents as well as toxicity profiles of the selected documented medicinal plants were investigated.

1.3 Objectives

1.3.1 General objective

This was to investigate the use of medicinal plants in the treatment of diabetes and hypertension in the Mpigi district.

1.3.2 Specific Objectives

This study was structured around three specific objectives which were to;

I. Document medicinal plants used by traditional herbalists for the management of diabetes and hypertension in the Mpigi district.

II. Analyse phytochemical constituents of the selected medicinal plants used by traditional herbalists to manage the two targeted diseases in the Mpigi district.

III. Determine the toxic and neutralizing dose levels of the selected plants used to manage the two targeted diseases in the Mpigi district.

1.4 Research questions

This study aimed to address three research questions, which are,

I. What are the plants used by traditional herbalists to manage diabetes and hypertension in Mpigi district?

II. What are the phytochemical constituents within the documented medicinal plants used to manage diabetes and hypertension diseases?

III. What are the toxic and neutralizing dose effects of medicinal plants used by THs to manage diabetes and hypertension?

1.5 Significance of the study

The main goal of this study was to document the plants employed by herbalists in Mpigi for treating diabetes and hypertension. This contributes to enhancing our comprehension of plant-based therapies for human diseases, focusing on chronic conditions (NCDs) like diabetes and hypertension, which are significant global health concerns. Additionally, documenting the use of therapeutic plants supports the conservation of biological resources, particularly medicinal plants, and preserving indigenous knowledge crucial for future generations.

In addition to documentation, this study conducted a comprehensive assessment of the phytochemical composition of the pivotal and extensively utilized plants. This analysis holds paramount significance as it offers insights into the quality and purity of the utilized plants, thereby gauging their effectiveness in treating the targeted diseases. The acquired data furnishes scientific information that proves particularly valuable for traditional herbalists (THs), patients, and local communities relying on these plants for addressing specified health conditions. This information is also crucial and serves as a foundational basis for further scientific studies aimed at developing drugs to manage these two diseases.

Furthermore, this study evaluated the toxicity and dose effects of the predominantly utilized plants. The aim is to provide crucial information regarding the safety of employing these specific plant species for medicinal purposes. This assessment caters to the informational needs of medicinal users, traditional healers, and the broader public, shedding light on potential health risks associated with the usage of these targeted plants. By discerning the toxicity levels and appropriate dosages, this research contributes to ensuring the safe and responsible utilization of these plants, ultimately enhancing the overall well-being and health outcomes of individuals relying on them for therapeutic purposes.

1.6 Scope of the study

1.6.1 Content scope

This study aimed to evaluate traditional healing practices for treating diabetes and hypertension in the Mpigi district, while also examining the phytochemical composition and toxicity of the identified medicinal plants.

1.6.2 Geographical scope

This study was conducted in Buyijja, Buwama Sub County in the Mpigi district in Central Uganda. The district is situated along the shores of Lake Victoria at the latitude of 0° 13′ 38.4708″ N and longitude 32° 19′ 29.7264″ E. The total area of the district is 1,541.13 km^2 of which 1,397.19 km^2 is the total land area. PROMETRA is located at the latitude of 00° 06.979′ N and longitude of 032° 07.710′, at an altitude of 1161m.

1.6.3 Time scope

This study was conducted between April 2023 to December 2023. Interviews were conducted for two months, April and May. After interviews, field visits were made for three weeks to collect and identify all the plants mentioned. Laboratory work for phytochemical analysis was conducted for one month (August), after drying the plants in July. Toxicity assessment was done from September to December 2023.

CHAPTER TWO

LITERATURE REVIEW

2.1 Non-Communicable diseases

Non-communicable diseases, synonymous with chronic diseases, are primarily lifestyle-induced and not transmissible between individuals. The primary categories of NCDs comprise diabetes, cancers, cardiovascular diseases, and chronic respiratory diseases (Kakudidi *et al*., 2017). The diseases are triggered by both external and internal causes, which are divided into genetic, physiological, environmental, and behavioural factors (Hachlafi *et al*., 2020). Behavioural risk factors encompass elements such as unhealthy diets, physical inactivity, stress, alcohol consumption, and smoking. These factors contribute to the accumulation of high levels of calories, salt, and fat, coupled with a low intake of fibre (Kakudidi *et al*., 2017).

NCDs have become a serious and threatening diseases all over the world with higher effects and rates of growth observed in developing countries (Terzic & Waldman, 2020). These diseases are projected to be a global problem in recent years by being a cause of seven of every ten deaths and many of these deaths are premature. In 2008, non-communicable diseases accounted for 36 million fatalities worldwide, constituting 63 percent of the total deaths (Bhandari *et al*., 2014). Overall data showed an increase in deaths from eight million in 1990 to 52.8 million in 2010 (Nojilana *et al.,* 2016). Furthermore, projections suggest that by the year 2030, the four primary non-communicable diseases are expected to account for 75% of global mortality (Terzic & Waldman, 2020). Moreover, reports indicate that over 80% of non-communicable disease-related deaths have occurred in developing nations (Bhandari *et al*., 2014). NCDs are also a major threat to economic development by being a cause of poverty that hinders the attainment of Millenium and Development Goals (Beaglehole *et al*., 2011) such as the promotion of general wellbeing, target to reduce premature death and poverty (Islam *et al*., 2014). Beyond economic repercussions and poverty, non-communicable diseases pose a dual

burden on health facilities in both developing and developed nations, with 50 to 80 percent of the health sector's budget allocated to managing NCDs (Bhandari *et al*., 2014).

In Sub-Saharan Africa (SSA), infectious diseases like HIV/AIDS and malaria have historically been the predominant sources of disease burden, though, in recent years, there has been a noted shift in epidemiology, transitioning from communicable diseases to NCDs (Gouda *et al*., 2017), a transformation attributed to various factors but the main being changes in lifestyle, economic development, and urbanization (Lange-jacobs *et al*., 2020). It is projected by WHO that by 2030 these chronic diseases will be the main cause of death in Africa (Nojilana *et al*., 2016), accounting for fivefold as many deaths as transmissible diseases (Hofman, 2015). In 2011 more than 14.7 million individuals were affected with DM in SSA and the majority were affected with type 2 diabetes (Marquez & Farrington, 2013). The number of people with diabetes is anticipated to increase to over 28 million by the year 2030. Not only diabetes but the incidence rate of hypertension in SSA was between 25-35 percent and mainly in the adult population (Marquez & Farrington, 2013). Some other countries in SSA have experienced a higher prevalence of hypertension of up to 48% in the adult population. In Southern Africa, by 2010, NCDs were responsible for 39% of deaths which was higher than the deaths from Tuberculosis and HIV/AIDs combined and the majority of deaths were below 60 years (36%) (Nojilana *et al*., 2016). Not only this but these massive deaths are anticipated to increase much in developing countries (Samoisy & Mahomoodally, 2015).

2.1.1 Diabetes mellitus

Diabetes mellitus is a metabolic condition marked by elevated blood glucose levels resulting from issues in insulin production or abnormality in insulin action (Karou *et al*., 2011). It is a major NCD affecting millions of people all over the world. Diabetes is one of the most common and most predominant chronic and endocrine gland diseases with more than 422 million patients recorded in 2014, a rise from 180 million patients in 1980 (Hoda *et al*., 2019). The

global incidence of diabetes is projected to rise to 590 million patients by 2025 (Ogurtsova *et al*., 2017), and 642 million by the year 2040, according to the WHO (Zhao *et al*., 2019). Every year more than 1.5 million deaths occur as the result of only high blood glucose and more than 2 million deaths as the result of increased risk of diabetes implications which include cancer, TB, heart diseases, and kidney failure (World Health Organization, 2016). More diabetes cases remain unidentified due to a lack of healthcare facilities and infrastructure. Diabetes Mellitus was a very rare disease up to the 20th Century but significant changes and a higher prone of the population were observed during the beginning of the industrial revolution. The onset of Diabetes Mellitus can be prompted by external factors like diet, physical activity, occupational risks, and environmental influences. Nevertheless, internal factors, including genetic changes, and the dysfunction of various receptors and proteins, significantly contribute to its development (Hoda *et al.*, 2019).

Diabetes has various types and forms which are divided into primary and secondary. The categories comprise various types, include type 1 DM, Type 2 DM, Gestational DM, and Monogenic diabetes. The first two mentioned are the most common, while type 2 is the most prevalent and severe, accounting for 90% of all diabetes cases (Hoda *et al*., 2019). Type 1 is mainly triggered by insulin production problems and type 2 by advanced/progressive insulin resistance and deficiency (Mathers & Loncar, 2015).

Diabetes mellitus not only heightens the susceptibility (by 8 times) to infections from other diseases but also exerts adverse effects on vital organs including the heart, liver, brain, and kidneys (Zhao *et al*., 2019). Diabetes is also responsible for microvascular and macrovascular clinical complications. Microvascular consists of 3 main clinical situations, retinopathy (effects on the retina), nephropathy (kidney/renal failure), and neuropathy (effects on the nervous system). The second type which is macrovascular is responsible for implications such as increased risks of cardiovascular (CVD), cerebrovascular, and atherosclerosis diseases

(Fowler, 2011). Other risks include damage to the brain, sleep disorders, lowered male fertility, tuberculosis, gum diseases, and delayed wound healing among few to mention (Fowler, 2011; Wojciechowska *et al*., 2016).

Worldwide many pharmaceutical remedies have been discovered and developed for the management and treatment of this disease but no even a single drug has proved to be effective in alleviating the disease origin (Nazarian-Samani *et al*., 2018), and most of the drugs cause several adverse effects (Bahmani *et al.*, 2014). Due to this failure of allopathic drugs, there has been an urgent need for alternative forms of treatment for managing the disease and the common method that has been adopted is the use of medicinal plants (Bahmani *et al*., 2014). These plants have various mechanisms to control and treat diabetes, with more than 200 chemical elements with antidiabetic pharmacological activities (Singh, 2011). Some of these include the capability of restoring the pancreatic tissue function by rising the insulin output or avoiding the absorption of glucose from the intestine, etc. (Malviya *et al*., 2010).

To show the importance of these herbal medicines, the WHO recommended the utilization of this alternative to cure and manage the complications that arise from DM. Various researches have documented medicinal plants that have anti-diabetic potentials, not only this but plants have proven to have important properties such as phytochemicals and biological activities that are essential in treating and managing DM complications (Nazarian-Samani *et al*., 2018).

2.1.2. Documentation of anti-diabetic medicinal plants

Across the globe, several researchers have compiled information on therapeutic plants with anti-diabetic properties. For example, in Asia, multiple studies have documented traditional herbal remedies employed for the management of this disease. A study in Urmia, Northwestern Iran, by Bahmani *et al*. (2014) reported 30 plants used by THs to manage DM. *Citrullus colocynthis* was the most common and highly utilized plant species by THs. In Shiraz, southwest of Iran, (Baharvand-Ahmadi *et al*., 2016) documented 24 plants that are used to treat

DM. The most frequently used plants were *Trigonella monspeliaca, Amygdalus scoparia, Juglans regia,* and *Urtica dioica.* In the two above-mentioned studies conducted in Iran, four plant species were common and mentioned in both studies. The plants were *Juglans regia, Cinnamomum verum, Arctium lappa,* and *Urtica dioica.* In Bangladesh, the study by Rahmatullah *et al*. (2012) in the Mymensingh district using tribal medicinal practitioners documented the use of twelve remedial plants. In Thailand, the research conducted by Neamsuvan *et al*. (2015) in Chana and Nathawee districts, recorded the use of 38 species to manage DM by THs. The most used herbal plants were *Lagerstraemia speciose*, *Padanus amaryllifolius,* and *Tiliacora triandra*.

In the African continent, studies have been also conducted. In Limpopo Province, South Africa 24 plants were reported to be utilized by THs to manage DM. The highly used species were *Aloe marlothii, Plumeria obtusa, Mimusops zeyheri, Helichrysum caespititium, Hypoxis iridifolia,* and *Moringa oleifera* (Semenya *et al*., 2012). In Eastern Africa, studies have also been conducted, For instance, in Kenya, the study by Keter and Mutiso, (2012) in the Lower Eastern province documented thirty-nine species. The frequently cited plants included *Azadirachta indica Momordica foetida, Cassia abbreviata, Urtica massaica,* and *Zanthoxylum chalybeum*. In Tanzania, a study conducted by Moshi and Mbwambo, (2008) revealed 54 plants to be used by THs to treat DM. In Uganda, there has been limited research focused on documenting medicinal plants used for treating DM. A study conducted in the vicinity of the Mabira Forest Reserve identified five plant species used in the management of diabetes. These species include *Canarium schweinfurthii, Garcinia buchananii* Baker, *Acacia constricta* Benth., *Sesbania sesban* (L.) Merr., and *Oxalis corniculata* L. (Tugume *et al*., 2016). A study by Ssenyange and colleages, (2015) documented 18 plants for type II diabetes in some selected districts in central Uganda. Key among them included *Anona muricata, Aloe vera* var. *Solanum indicum, Cucurbita maxima, Vernonia amygydalina,* and *Carissa macrocarpa*.

2.1.3 Hypertension

Hypertension (HTN) is a persistent medical condition identified by elevated systemic arterial blood pressure (Karou *et al*., 2011), when it is consistently higher than 140 mmHg systolic over 90 mmHg diastolic (Lange-jacobs *et al.*, 2020; Kamyab *et al*., 2021). HTN is one of the problematic chronic diseases in both developed and low-income countries. The worldwide prevalence of hypertension is anticipated to increase from 26.4% in the year 2000 to encompass 60% of the global population by 2025, with a corresponding epidemiological study across multiple countries indicating an annual mortality rate of 7 million deaths worldwide attributed to hypertension (Lange-Jacobs *et al*., 2020). As per the African Regional Health Report, Sub-Saharan Africa had 80 million individuals with hypertension in 2000, and it is anticipated that this number will increase to 150 million by the year 2025 (Lange-Jacobs *et al*., 2020). As with other NCDs such as diabetes, hypertension has been also difficult to control and treat. Furthermore, hypertension poses life-threatening risks by subjecting patients to complications such as brain and retinal artery damage, renal dysfunction, disabilities, and increased susceptibility to diseases like diabetes and cardiovascular conditions, ultimately leading to mortality. Hypertension and diabetes disease are interlinked. Hypertension affects about 70% of people with type 2 DM, and more than thirty percent of people with type 1 DM (Tsabang *et al*., 2015). Owing to the ineffectiveness of contemporary drugs and unaffordability, fewer health facilities and drugs effects to the patients such as erectile dysfunction, constipation, coughing and loss of appetite, etc. (Lange-Jacobs *et al.*, 2020), herbal medicines have been playing significant roles in treating this disease, therefore, documentation of the plants used is of key importance (Pourjabali *et al*., 2017). Most of the documented plants display effectiveness due to the secondary metabolites within them.

2.1.4 Documentation of anti-hypertension medicinal plants

Research has highlighted the use of herbal remedies for managing hypertension in different regions across the globe. In Cameroon, for instance, over thirty-three plant species have been identified as being used to treat both diabetes and hypertension (Tsabang *et al*., 2015). In Togo, ethnobotanical research by Gbekley *et al*. (2018) in the Maritime region documented 116 plants from 46 families to be used by THs to cure hypertension. The mostly used and important plant species included *Boerhavia diffusa, Xylopia aethiopica, Byrsocarpus coccineus, Crateva religiosa*, and *Mangnifera indica*. Traditional healers in the central region of Togo identified 64 plants for the management of hypertension and diabetes, with *Khaya senegalensis*, *Psidium guajava*, and *Securidaca longepedunculata* being predominantly used for diabetes, and *Persea americana, Allium sativum, Gardenia ternifolia*, and *Parkia biglobosa*, being the primary choices for hypertension (Karou *et al.*, 2011). In South-eastern Morocco, 64 plants were documented to alleviate diabetes and hypertension. Of the total 64 species, 45 were used only for diabetes, 36 for hypertension, and 18 for both hypertension and diabetes (Tahraoui *et al*., 2006). Research conducted in Shiraz, Iran, identified 27 plants employed by THs for treating hypertension, with *Borago officinalis* and *Berberis vulgaris* being the highest used species (Baharvand-ahmadi *et al*., 2016). In the Dir Lower district of Pakistan, herbalists and local communities were found to use 46 plant species for the treatment and control of hypertension, with *Sarcococca saligna, Paeonia emodi, Fumaria indica, and Teucrium stocksianum* being the most frequently used species (Ahmad *et al.*, 2015). In Uganda, there are few reports documenting medicinal plants used to treat hypertension. For instance, a review paper by Kakudidi and colleages (2017) identified medicinal plants used for NCDs as reported in other studies. This compilation reported 19 plants for hypertension. Key among them included *Amaranthus spinosus, Citrus limon, Chamaecrista nigricans, Gomphocarpus physocarpus, Hibiscus sabdariffa, and Hoslundia opposite*. A comprehensive study conducted in the Mabira

Forest Reserve documented nine plant species used in the treatment of hypertension. These species include *Canarium schweinfurthii*, *Sesbania sesban* (L.) Merr., *Oxalis corniculata* L., *Kigelia africana* (Lam.), *Tragia benthamii* Baker, *Ficus cyathistipula* Warb., *Vangueria apiculata* K., *Citrus limon* (L.) Osbeck., and *Solanum anguivi* Hook (Tugume *et al*., 2016).

2.2 Diabetes, hypertension, and oxidative stress

Oxidative stress is a state marked by an imbalance in reactive molecule generation and elimination, involving radicals like Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Vijay & Vimukta, 2014). These radicals have unpaired electron(s) that can swiftly react with molecules such as proteins and lipids, causing energy release (Golbidi *et al*., 2011; Rajendiran *et al*., 2018). Such radicals contribute to tissue injury, leading to health disorders like diabetes and high blood pressure. In diabetes, oxidative stress causes complications, including beta cell reduction, endothelial damage, inflammation, and reduced glucose uptake (Vijay & Vimukta, 2014). In general, diabetes is influenced by oxidative stress, which exacerbates insulin resistance or hampers insulin secretion (Bajaj & Khan, 2012).

Under normal conditions, an equilibrium exists between the generation of free radicals and their elimination. The connection between oxidative stress and diabetes mellitus lies in the escalation of free radical production and a reduction in the ability of antioxidant defence (Khan *et al*., 2015). Evidence suggests a decrease in antioxidant defences in diabetes, attributable to various factors. These factors include diminished levels of specific antioxidants like vitamin E, a reduction in the overall antioxidant capacity or free radical scavenging activity in plasma/serum, and an increase in plasma oxidizability. Additionally, studies have demonstrated a decline in the activities of antioxidant enzymes (Bajaj & Khan, 2012).

On the other hand, antioxidants are the key chemical and biological compounds that protect cells and tissues against possible injuries instigated by reactive radicals. These substances can

effectively impede or avert the oxidation of other small molecules involved in oxidative stress. They play a vital role in neutralizing reactive species, thereby shielding cells from their harmful effects. Hence, these compounds are highly significant for individuals with diabetes, serving both as alternatives and aiding in the neutralization of ROS. Ultimately, they contribute to lowering the risk of developing complications associated with diabetes (Vijay & Vimukta, 2014). Antioxidants operate through three mechanisms: enzymatic degradation of free radicals, proteins binding metals that kindle free radical production, and specific antioxidants acting as scavengers for free radicals (Rahimi *et al.*, 2005). In diabetes mellitus, antioxidants play crucial roles, including safeguarding beta cells from oxidative stress to maintain their function, diminishing diabetic complications, and recuperating insulin sensitivity recovery (Rajendiran *et al.*, 2018).

2.3 Phytochemicals

Phytochemicals, also known as phytobiotics, secondary metabolites, or phytogenics are naturally occurring chemical compounds in plants that are biologically non-nutritive but active (Nyamai *et al*., 2016). These chemical compounds provide plants with colour, flavour, aroma, and texture (Barbieri *et al*., 2017). Phytochemicals are systematically synthesized by specific plant tissues and organs in response to changes in natural conditions, with the purpose of fulfilling specific roles (Ahad *et al*., 2021) which range from cellular to organismic levels (Lila & Raskin, 2005). Some of these roles include defence from the impacts of free radicals, bacteria, and viruses (Barbieri *et al*., 2017), threats such as drought, UV light, pollution, and attracting insects for pollination (Nyamai *et al*., 2016). They also help plants fight against predators, allopathic agents and recover from damage (Naikoo *et al.*, 2019; Ahad *et al*., 2021). Phytochemicals mainly occur in several parts such as in the leaves, seeds, roots, stems, fruits, and flowers (Saxena *et al.*, 2013).

Phytochemicals not only play important roles in plants but recent studies have shown that they are utilized by human beings and play key roles and benefits in human well-being (Nyamai *et al*., 2016; Kytidou *et al.*, 2020; Ahad *et al*., 2021). They are unessential nutrients and not required in human growth but are essential in the treatment of numerous ailments by causing a certain physiological response in the human body (Edeoga *et al*., 2006). They exhibit therapeutic effects, encompassing the prevention and treatment of diseases, alleviation of symptoms, and positive regulation of both physical and mental well-being (Ahad *et al*., 2021). These bioactives have pharmacological values for instance anti-inflammatory and antibacterial (Okwu & Ekeke, 2003). Currently, more than 4000 phytochemicals have been classified and among them, 150 have been critically studied although there are still many unknown (Almodaifer *et al.*, 2017). These chemical compounds are recognized for their functions in reducing the risk of NCDs (Liu, 2004).

Therapeutic plants produce and store a diverse array of these chemicals namely alkaloids, phenolic, flavonoids, tannins, terpenes, etc. which are important due to their therapeutic purposes (Sofowora, 1993; Edeoga *et al.*, 2005). Phytobiotics in medicinal plants are most spread and rich in stems and leaves (Sofowora, 1993). Furthermore, plant phytochemicals not only differ from plant to plant, or due to geographical location, or depending on the season but even day and night patterns influence the variation of plant chemical content (Verpoorte, 1989).

Generally, phytochemicals play major roles in fighting, preventing, and managing NCDs like diabetes and hypertension (Liu, 2004) through various mechanisms. For example, phenolic compounds; such as phenolics acid possess anti-diabetic potentials such as inducing glucose uptake through facilitating GLUT4 translocation (Zhao *et al*., 2019), flavonoids control hyperglycaemia by rising blood insulin levels, facilitating insulin production, and impeding aldolase reductase (Bahmani *et al.*, 2014; Zhao *et al*., 2019). Tannins are also important in managing and treating NCDs (Saxena *et al.*, 2013). Terpenoids have various mechanisms in

managing diabetes such as reducing serum glucose levels, increasing glycogen synthesis, lessening the conversion of glycogen into glucose, and obstructing the aldose reductase enzyme (Zhao *et al.*, 2019). Steroidal alkaloids are also important in NCD treatment because they contain antihypertensive properties (Li *et al.*, 2006; Gunaherath & Gunatilaka, 2014). Furthermore, some phytochemicals identified in medicinal plants possess antioxidant potential. Recent research underscores the value of plant-based antioxidants, offering potential benefits for disorders linked to oxidative stress like diabetes and hypertension (Rahimi *et al*., 2005). Given their significance, examining the chemical compounds in medicinal plants is crucial to understanding and confirming their pharmacological potential.

2.3.1 Phytochemical profiling of antidiabetic and antihypertensive medicinal plants

Research has investigated the chemical components of plants traditionally used to treat diabetes and hypertension across various regions around the world. In Kenya, a study examining the phytochemical makeup of commonly used plants for diabetes management found that flavonoids and terpenoids were the primary chemical classes identified. These compounds are associated with potent biological activity against diabetes (Muema *et al*., 2023). Bouyahya *et al.* (2021) conducted a comprehensive review of Moroccan plants traditionally used for diabetes treatment, focusing on their phytochemical composition. The study identified 148 secondary metabolites across various chemical groups, including alkaloids, terpenoids, flavonoids, phenolic acids, and fatty acids. Of these, 95 compounds were tested for their antidiabetic properties with positive results. A study reviewing the phytochemical makeup of 20 antidiabetic medicinal plants from Jordan found that flavonoids were the predominant secondary metabolites in most of the plants analysed (Afifi & Kasabri, 2013). Sunmonu and Lewu, (2019) conducted a research examining the chemical composition of 21 Nigerian plants used for diabetes treatment. The research identified seven plants—*Anacardium occidentale*, *Carica papaya*, *Ficus asperifolia*, *Hibiscus sabdariffa*, *Khaya senegalensis*, *Ocimum*

gratissimum, and *Parkia biglobosa*—as having notably higher concentrations of total flavonoids and polyphenols compared to the other plants studied.

Nigella sativa, a significant antidiabetic medicinal plant in India, has been extensively studied for its phytochemical properties. Key active compounds identified in its seeds included thymoquinone, carvacrol, dithymoquinone, thymohydroquinone, p-cymene, 4-terpineol, t-anethole, sesquiterpene longifolene, α-pinene, and thymol (Desai *et al*., 2015) . The seeds of *Phaseolus vulgaris*, a key antidiabetic plant in Côte d'Ivoire, were analyzed for their chemical composition. The study identified the presence of saponins, alkaloids, tannins, flavonoids, fiber, proteins, terpenoids, quercetin, catechin and anthocyanin in the plant (Atchibri *et al*., 2010).

A phytochemical analysis of four major antidiabetic plants from Sri Lanka—*Syzygium cumini*, *Trigonella foenum-graecum*, *Brassica alba*, and *Nigella sativa*—revealed the presence of alkaloids, flavonoids, tannins, phenols, and saponins in all the plant extracts. *Syzygium cumini* was found to contain the highest concentrations of flavonoids, phenolics, alkaloids, and tannins among the plants studied (Rajkumar *et al.*, 2021). Chemical compositions of *Azadirachta indica, Gongronema latifolium* and *Vernonia amygdalina* important medicinal plants was also analysed. The results indicated that flavonoids, polyphenols and saponins were significantly higher in *V. amygdalina*, while alkaloids were highest in *A. indica* and tannins in *G. latifolium* (Atangwho *et al*., 2009).

A review paper gathered information on plants utilised in Nigeria for managing hypertension, along with their phytochemical compositions. The study identified 136 medicinal plants, with 89.71% containing phenolic compounds, while 64.71% and 39.71% were found to contain alkaloids and terpenes, respectively (Obode *et al*., 2020). *Lippia multiflora*, a widely used plant for hypertension management in Benin, was analysed for its secondary metabolites composition. The study revealed the presence of gallic tannins, terpenes, and steroids

(Gandonou *et al*., 2017). Mangifera indica, a plant commonly used to treat hypertension in Brazil, has been studied for its phytochemical composition. The analysis identified the presence of gallic acid, caffeic acid, ferulic acid, apigenin, and quercetin (Ronchi *et al*., 2015). In conclusion, a portion of documented curative plants used to treat diabetes and hypertension have been scientifically studied for their chemical compositions. These studies have confirmed that most of these plants contain phytochemicals with significant biological activities, reinforcing their effectiveness in supporting traditional medicinal practices.

2.4 Toxicity of medicinal plants

Toxicity is the potential of substance from plant extracts to exert a detrimental effect on an animal or human which can be on only a certain organ, tissue, or the whole human body (Kharchoufa *et al.*, 2018). Plants produce secondary metabolites that form complex chemical compounds that offer both advantages for human health while also posing potential risks (Kpemissi *et al.*, 2020). Initially, there was a belief that herbal medicines had no side effects; however, research has revealed that many plants traditionally used for medicinal purposes exhibit side effects at both low and high doses (Gupta & Raina, 1998).

Setting aside the impacts of xenobiotics, the absence of established procedures and protocols for cultivating, harvesting, and post-harvesting (preparation and processing) of medicinal plants may introduce impurities, potentially leading to adverse drug reactions and impacting human health (Ahad *et al*., 2021). Not only this but there is an absence of evidence-based strategies, for instance, the legal and governing framework, pharmacovigilance, and insufficiency of toxicological assessments of medicinal plant preparations (Kpemissi *et al*., 2020).

Among the investigated plants that are used as medicines, some of them have proved to possess toxic elements and are mainly from plant secondary metabolites (Mounanga *et al*., 2015). The

toxicity of plants is determined by multiple factors, including the plant's inherent properties, the human body, and the methods of preparation or dosage administration. These factors encompass the specific plant parts used (for instance roots, leaves, stem bark, and seeds), the amount of the plant ingested, environmental conditions, soil quality, potency of secondary metabolites, duration of using the plant, individual body chemistry, and the genetic makeup of the plant species (Mounanga *et al*., 2015).

The plant toxic substances mainly affect the physiology of different organs (Kpemissi *et al*., 2020). The organs most vulnerable to the toxicity of medicinal plants are the liver and kidneys, as they are directly engaged in crucial functions related to the breakdown and elimination of ingested chemical compounds (Oliveira *et al*., 2010). For example, the use of herbal medicine by DM patients puts them at risk of renal damage and failure (Oliveira *et al*., 2010).

Toxicity from plants can pose significant health risks, including adverse effects on haematology, liver, nervous system, kidneys, and genetics, as well as injuries to the lungs, heart, testes, spleen, and gastrointestinal tract, to name a few (Mounanga *et al*., 2015). The risk is heightened due to the fact that a single plant can encompass over 400 chemical compounds, making it challenging to trace the specific chemical responsible for causing health problems and complicating the monitoring process (George, 2011). Despite the wide spread use of plants to cure human ailments, there remains a shortage of scientific and experimental proof regarding their possible toxicity (Alelign *et al*., 2020; Kpemissi *et al*., 2020). The majority of research efforts have been focused on investigating the pharmacological potentials of these plants resulting in just a few reports of their detrimental effects (Oliveira *et al*., 2010). With these alerts and for the safety of medicinal plant consumers, toxicity evaluation of medicinal plants is critical to be undertaken to establish a safety profile of used plants. This usually involves conducting toxicity studies using suitable animal models, to assess the likelihood of

encountering the specific risk at a particular dose level or concentration (I. Bello et al., 2016) on important systems such as haematopoietic and serum biochemicals.

The hematopoietic is one of the most sensitive systems and has been used to study the effects of medicinal plant toxicity and drug safety (Tchoumtchoua *et al*., 2014; Sureshkumar *et al*., 2018). Its evaluation provides vital information on bone marrow activity (Tchoumtchoua *et al*., 2014), and it extracts crucial details regarding the body's response to stress or injury, encapsulated within the blood profile (Prasanth *et al*., 2015).

An assessment of the toxicity of medicinal plants remains incomplete without the examination of hepatic and renal functions. These organs play a pivotal role due to their direct involvement in metabolizing and excreting xenobiotics in the body. Assessing their status is crucial, and biochemical evaluation is employed for this purpose (Kpemissi *et al*., 2020). For instance, the examination of Liver Function Tests offers valuable insights into evaluating plant safety, as many plant toxins tend to accumulate in the liver for detoxification (Adeneye *et al*., 2006). Liver serum biomarker enzymes like AST, ALT, and ALP are commonly analysed to evaluate the potential hepatotoxic effects of herbal medicines on the livers of treated animals (Prasanth *et al*., 2015).

2.4.1 Evaluation of toxicity in antidiabetic and antihypertensive medicinal plants

Numerous studies have evaluated the toxicity of medicinal plants using various model organisms. One particular study examined the toxicity of six Sudanese medicinal plants: *Ambrosia maritima*, *Ammi visnaga*, *Foeniculum vulgare*, *Sesamum indicum*, *Acacia senegal*, and *Nigella sativa* (Hilmi *et al*., 2014). The findings revealed the significant toxicity effects of all aqueous extracts and ethanol extracts (except *Nigella sativa*) in model species. A toxicity study on *Spondias mombin*, *Carica papaya, Xylopia aethiopica,* and *Senecio biafrae*, using rats as the model species, found no noticeable changes in the animals' behaviour, even at high doses.

The lethal dose (LD_{50}) for all the plants tested exceeded 5000 mg/kg, indicating a relatively low toxicity. Although the haematological analysis showed variations in white blood cell counts, no significant changes were observed in other blood parameters (Bello *et al*., 2022).

The toxicity evaluation of *Lycium shawii* in mice resulted in noticeable alterations in body weight, as well as significant changes in both biochemical and hematological parameters. Additionally, the study revealed that the plant possesses considerable potential for causing spermatotoxic effects (Sher & Alyemeni, 2011). A study of *Heracleum persicum* in rats revealed its impact on liver health. The results showed that the plant extract caused elevated levels of serum enzymes associated with liver damage, indicating significant hepatocyte injury (Alkan & Celik, 2018). A study on *Phragmites karka* found no mortality in the preliminary test. Nevertheless, signs of cellular toxicity were witnessed, and histopathological analysis of key organs revealed necrosis in both the liver and kidneys, indicating potential organ damage despite the absence of immediate fatal effects (Mazumder *et al*., 2021). The toxicity study on *Chenopodium album* demonstrated the extract's potential to significantly affect most of the haematological and biochemical parameters examined. However, no observable effects were noted in the histopathological analysis (Choudhary *et al*., 2021). Similarly, the study on *Physalis peruviana* revealed significant changes in several blood, kidney, and liver markers, along with observed mortality, with the LD_{50} estimated at approximately 1,280 mg/kg. Additionally, vital organs showed signs of hemorrhaging and swelling, indicating severe damage as a result of the plant extract (Kasali *et al*., 2013).

A toxicity study on the alcoholic extract of *Cassia kleinii* revealed no significant signs of acute or short-term general toxicity (Babu *et al*., 2003). Similarly, a study by Goodies *et al*. (2015) assessed the toxicity of *Spondias mombin* and *Costus afer* in Wistar rats. The analysis revealed no significant changes in the haematological and biochemical parameters analysed, indicating that the plant extracts did not cause any notable adverse effects under the conditions tested.

2.5 Summary of literature and research gaps

Non-communicable diseases present a growing health challenge globally, including in Africa, largely due to lifestyle changes linked to industrialization and increased exposure to risk factors. In developing countries, limited healthcare facilities and inadequate monitoring result in a lack of accurate data on NCD cases. This hampers governments and health organizations in effectively managing and preventing these diseases.

Medicinal plants have long been used to treat NCDs and various ailments, particularly in rural communities. However, the lack of documentation for this traditional knowledge, which is mainly passed down orally, poses a risk of its loss. As a result, valuable healing practices may disappear when individuals pass away without sharing their expertise.

Eighty percent of the world's populace depend on plants for disease treatment, yet there is a lack of standardized information on the quality, purity, and chemical composition of many medicinal plants. Many herbal medicines have not been thoroughly studied for their phytochemical constituents, raising concerns about their efficacy. Studies in Uganda, for example, have often omitted such analyses, highlighting the need for more research into the chemical compounds of medicinal plants.

The safety of medicinal plant users is a global concern due to health risks associated with their use. While once considered entirely safe, recent studies have shown that certain chemical components in these plants can have adverse effects, particularly on key organs like the liver and kidneys. Despite these risks, there is limited information on the toxicity profiles of many therapeutic plants (Alelign *et al.*, 2020; Kpemissi *et al*., 2020), highlighting the urgent need for comprehensive toxicity assessments.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the materials and methods employed in this study, organized into several sections. The first section provides a description of the study area, followed by the second section, which details the research design and sampling techniques. The third section delineates the materials, methods, and steps undertaken to achieve the objectives. Ethical clearance is covered in the fourth section, while the fifth section addresses data analysis techniques.

3.2 The Study area

3.2.1 Location

This study was conducted in Buwama Sub County, Mpigi district, Central Uganda. The Sub County has 10 parishes and 62 villages. Buwama lies at the latitude of 0° 03′ 39.0″ N and a longitude of 32° 05′ 47.0″ E. The sub-county is located approximately 32 kilometres, south-west of district headquarters and approximately 71 kilometres south-west of Kampala, Uganda's capital city. Generally, the district lies between latitudes 0° 13′ 38.4708″ N and longitude 32° 19′ 29.7264″ E, with an altitude that ranges from 1100-1400m asl. The district boarders five other districts, Wakiso, Mityana, Butambala, Kalangala, and Kalungu districts. Mpigi comprises eight sub-counties, fifty-nine parishes, and four hundred sixty-one villages. The district lies on the shores of Lake Victoria, the largest fresh water lake in African. The district covers 1,541.13 km^2 (0.07 percent of Uganda's size). Approximately 91% of the total district area is land while the remaining 9% is covered by water. This district was chosen due to the lack of previous studies documenting plants used for managing diabetes and hypertension. Additionally, the district is renowned for its rich and diverse flora. Its ecosystems, including forests, wetlands, and grasslands, host a wide variety of medicinal

plants. This study concentrated on the Buyijja parish in the Buwama subcounty. Buyijja parish is where the PROMETRA traditional medicine institution headquarters is located.

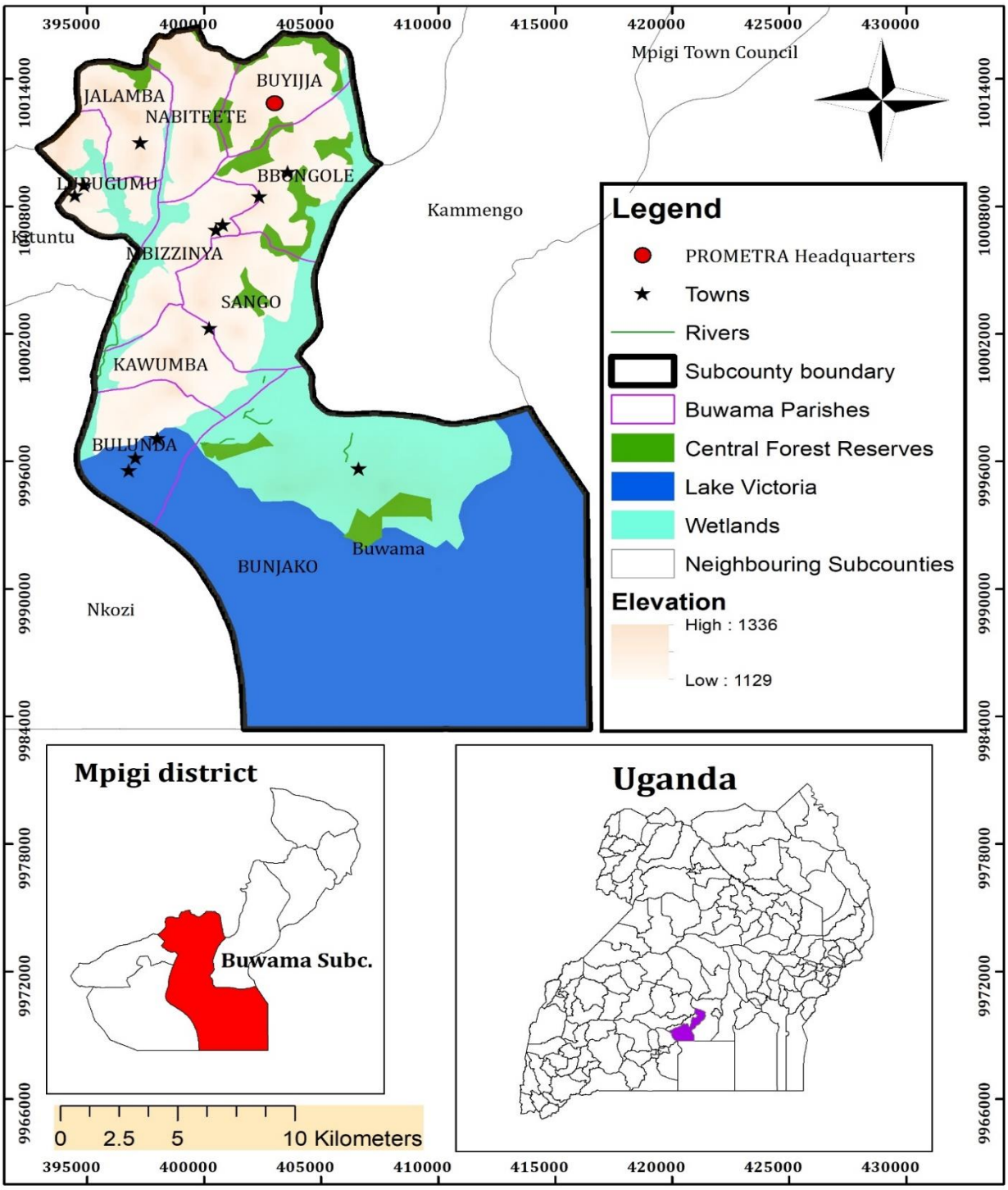

Figure 3.1 A map showing the study area (Buwama subcounty) in Mpigi district.

3.2.2 Climate

3.2.2.1 Rainfall and humidity

The district experiences two distinct rainfall seasons, with the primary season typically spanning from February to May, followed by a secondary season occurring between September and November. The remaining months are generally considered dry seasons, occasionally receiving brief spells of rain. While the district's average rainfall is 1320 mm, certain areas around the lake experience amounts ranging between 1750 mm and 2000 mm. The monthly average days with rainfall amount to eleven. Humidity levels average between eighty and ninety-five percent, particularly in forested areas. The rainfall and humidity in Mpigi district create ideal conditions for the diverse growth of medicinal plants. The impact of seasonal weather patterns on NCDs like diabetes has been explored in studies. For example, hospitals tend to admit more diabetic patients during the rainy season than in dry periods. This could be attributed to changes in nutrition, as physical activity often decreases due to frequent rainfall, and people may consume more high-fat, high-calorie foods while waiting for the harvest, potentially leading to higher blood sugar levels (Lontchi-Yimagou *et al.*, 2016).

3.2.2.2 Temperature

Mpigi district maintains a relatively constant temperature throughout the year, with an average range between 22.5°C and 27°C. The district experiences minimal temperature variation, and the recorded extremes include a minimum of 11°C and a maximum of 33.3°C. Temperature patterns also has an influence on medicinal plants growth (normal behaviours and physiology) and on phytochemical constituents. For instance, plant growth improves as temperatures approach the optimal level for a given species, while colder temperatures restrict growth, leaf development, and photosynthesis (Pant *et al*., 2021). There is no clear link between typical temperature fluctuations and NCDs like hypertension and diabetes. However, the effects become evident during extreme weather conditions, such as intense heat or cold. For instance,

prolonged exposure to cold can cause hypothermia, which may result in a decrease in blood pressure. Likewise, hypertension tends to occur more frequently in winter compared to the summer months (Schreier, 2014).

3.2.2.3 Vegetation

Mpigi is distinguished by its evergreen vegetation, featuring numerous intermittent wetlands and some areas with savanna vegetation. The overall vegetation makeup consists of 5% dense moist natural forests, 90% savanna woodland, and the remaining portion in swampy areas. The rich vegetation diversity in Mpigi makes it one of the most herbally diverse districts in the country. In addition to its herbal benefits, this vegetation cover offers the important advantage of potentially reducing the risk of non-communicable diseases (NCDs), especially among the elderly (Sogno *et al*., 2020). The primary economic activity in Mpigi is agriculture, focusing on the cultivation of key crops like sweet potatoes, cassava, maize, bananas, and avocados, alongside cash crops such as coffee and cotton.

3.2.3 Soil

The soil in the Mpigi district is predominantly ferralitic, characterized by red or yellow sandy/clay loams (latosols) with a pH ranging from 5.5 to 6. Although the soil is moderately leached, it is generally well-drained. Valleys in Mpigi exhibit colluvial grey sandy loams, while swamp bottoms consist of grey clays and silt with a pH between 4.5 and 5.2. In comparison to soils in other Ugandan districts, the soils in Mpigi are generally considered less fertile.

3.2.4 Human population

In 1991, the estimated population of the district stood at 157,400. By the 2002 census, the population had increased to approximately 187,800, reflecting an annual growth rate of 1.4%. In 2012, the district's population was estimated to be around 215,500. Notably, only 8.4% of the population resides in urban areas, indicating that Mpigi is predominantly a rural region. As

of the most recent data from the 2024 census by the Uganda Bureau of Statistics (UBoS), Mpigi's current population stands at approximately 306,003, reflecting a growth rate of 2.1% compared to the 2014 census results (UBOS, 2024).

3.3 Research design

This study employed a mixed-methods research design, which involved the collection and analysis of data, synthesizing results, and making conclusions through both qualitative and quantitative techniques (Doyle *et al*., 2009; Caruth, 2013). This involved a social survey that was carried out for the identification and documentation of medicinal plants used to manage diabetes and hypertension (a qualitative and quantitative approach), and experimental procedures that were used to analyse the phytochemicals and toxicity profile of the selected medicinal plants (a quantitative approach).

3.4 Target population, sampling techniques, and size

The sampling framework for this study comprised of traditional herbalists, field observations, and laboratory experiments. The primary focus for understanding ethnomedicine practices was on traditional herbalists possessing the requisite knowledge. The study employed a purposive sampling technique to select the target population. This involved identifying and choosing traditional herbalists with significant knowledge and experience in using plants to treat the specified diseases as key respondents. The respondents were selected based on their prior expertise and experience in the field. Their expertise was determined by the length of time they had been practicing traditional medicine, having treated at least one patient with diabetes or hypertension (indicating a high level of knowledge), and their ability to accurately describe the key symptoms and causes of diabetes and hypertension. All respondents were selected by their institutional leaders, considering the aforementioned criteria. Given the focus on experienced herbalists, a total of 54 herbalists were interviewed. The determination of the sample size was

dependent on the availability of knowledgeable herbalists with expertise in the specific topic under investigation.

All the herbalists who were interviewed are affiliated with Dr. Sekagya Institute of Traditional Medicine, also known as PROMETRA UGANDA. The institute is under the leadership of Dr. Sekagya Yahaya Hills, a renowned traditional medicine practitioner in Uganda. The headquarters of the institution is situated in Buyijja parish, Buwama. This institute provides formal training, conducted in Luganda, to traditional healers, evaluating them through examinations to qualify as certified traditional healers. Following the completion of these formal training sessions, herbalists gather at the institution weekly to exchange any newly acquired traditional knowledge.

3.5 Data collection instruments

In this study only primary data were collected, both qualitative and quantitative.

3.5.1 Ethnobotanical data and documentation of medicinal plants

3.5.1.1 Semi-structured individual interviews

In ethnobotanical research, individual interviews stand out as the predominant method of data collection (Jeffery, 2018). The interviews were structured with the guidance of pre-prepared (semi-structured) questions. Depending on the language proficiency of the respondents, interviews were carried out in both English and Luganda. To facilitate communication, two research assistants proficient in the local language of the study area were enlisted. All responses were documented in English, with the exception of the vernacular names of plants. A total of fifty-four respondents were interviewed, with the number of participants purposively determined based on the availability of knowledgeable individuals on the specific topic being investigated. The information gathered through this method encompassed traditional knowledge held by herbalists, details about the plants used, specific plant parts employed,

dosage administration practices, identified threats to medicinal plants, conservation strategies, and other pertinent information related to the treatment of the two targeted diseases using herbal medicines.

3.5.1.2 Botanical survey/Field visits

Following comprehensive interviews with the respondents, the subsequent step involved the collection and scientific identification of plants cited for treating diabetes and hypertension. The scientific identification of some plants was carried out in the field utilizing field manuals. Plants/ voucher specimens were collected and subjected to further evaluation and identification at the Makerere University herbarium. The accuracy of scientific names was also verified using available databases and software during the process.

The parts of the selected plants used to treat the two targeted diseases were gathered for laboratory evaluation in the subsequent phases of this study. This process entailed acquiring fresh plant parts, including leaves, stems, and bark, using a knife, panga, and scissors. The collected plant material was promptly packed into labelled sterile polyethylene sampling bags, utilizing marker pens for identification, and then transported to the laboratory. Special attention was given to collecting parts that were devoid of extraneous substances, diseases, or signs of stress.

3.5.2 Phytochemical analysis of selected medicinal plants

Under this section, the three selected anti-diabetic and anti-hypertension medicinal plants that were identified by interview method were screened both qualitatively and quantitatively for phytochemicals detection. This involved applying standard protocols for phytochemicals detection (Hashmi *et al*., 2021). Three plant species (*Ficus saussureana*, *Clerodendrum rurundifolium*, and *Microglossa pyrifolia*) were selected for this analysis. The selection was based on the common plants that were highly used by traditional herbalists and that are not yet

studied. All phytochemicals were analysed at the Department of Government Analytical Laboratory (DGAL), and at the Natural Chemotherapeutics Research Institute (NCRI), both under the Ministry of Health, Uganda.

3.5.2.1 Plant samples collection, cleaning, and drying

The plants selected for phytochemical analysis were gathered from a study area in June 2023. For each plant, 5 kg of the selected part was collected. Post-collection, the plant samples underwent a thorough washing with water to prevent the degradation of phytochemicals. Immediate drying was imperative to eliminate water content and avert spoilage (Banu & Cathrine, 2015). The drying process was done under the shade for one month. Subsequently, the dried samples were meticulously diced, crushed, and pulverized using an electrical grinder. The resulting finely powdered samples were then stored in polyethylene bottles, poised for the subsequent stages of the analytical procedures.

3.5.2.2 Samples extraction

For phytochemical analysis, two solvents (methanol and aqueous) were used. These two solvents were chosen for their exceptional effectiveness, as they not only yield the highest amounts but also maximize the concentration of phytochemical constituents (Truong *et al*., 2019). Additionally, methanol in different concentrations has proven to be an effective solvent for reliably extracting active compounds, as many of these components are readily soluble in methanol (Tejavathi & Sujatha, 2019). Two extraction methods were used to obtain the crude extracts from the plants. The methods included maceration and decoction. The maceration method was mainly used for fluid extracts. The obtained powdered plants were soaked in methanol (80 %) for 48 hours in a stoppered jar, the mixture was then incubated in a dark place and at normal room temperature with occasional agitation. After 48 hours of maceration

process and occasional shaking, the extracts were filtered separately using filter paper and funnel. The obtained extract was then kept for the next procedures. The second method used was decoction, mainly to obtain aqueous extracts. The powdered plant extracts were boiled in distilled water for 20 minutes. The obtained mixture was then cooled and finally filtered using filter paper and funnel. The obtained liquid extract was then kept for the following experimental procedures.

3.5.2.3 Qualitative phytochemical screening

To conduct a qualitative phytochemical analysis, the presence and abundance of the compound were determined by the change and intensity of the colour after the addition of reagents to the sample extracts (Biapa *et al*., 2007). Procedures, reagents, and various tests that were used per each type of phytochemical are explained below:

3.5.2.2.1 Detection of alkaloids

1 ml of extracts was mixed with 1 ml of 1% Hydrochloric acid. The mixture was then warmed and filtered. Subsequently, three drops of Mayer reagent were added. A positive alkaloids test was shown by the development of a yellowish-white precipitate (Morsy, 2014).

3.5.2.2.2 Detection of saponins

Foam test: 5 ml of plant extract were combined with an equal amount of distilled water and shaken thoroughly, then allowed to stand for 10 minutes. Positive saponin was shown by the formation of stable froth (Gracelin *et al*., 2013; Morsy, 2014; Longbap *et al.*, 2018; Alqethami & Aldhebiani, 2021).

3.5.2.2.3 Detection of steroids

5 ml of plant extract were blended with 2 ml of chloroform, then with 2 ml of concentrated sulphuric acid. Positive steroid test was indicated by the appearance of red colour (Gracelin *et al*., 2013; Morsy, 2014).

3.5.2.2.4 Detection of phenols

Ferric chloride test: 10 ml of plant extract was mixed with 3-5 drops of ferric chloride solution. A positive phenols test was shown by a bluish-black colour (Tyagi & Agarwal, 2017; Longbap *et al.*, 2018; Nortjie *et al.*, 2022).

3.5.2.2.5 Detection of tannin

Braymer's test: 5 millilitres of extracts were mixed with a few drops of 10% $FeCl_3$. A positive tannin test was shown by a dark blue/greenish-grey colour (Gracelin *et al*., 2013; Morsy, 2014; Tyagi & Agarwal, 2017; Longbap *et al.*, 2018; Alqethami & Aldhebiani, 2021).

3.5.2.2.6 Detection of flavonoids

Shinoda test: 5 ml of extracts were mixed with a piece of magnesium ribbon followed by 2 ml of concentrated HCl. A positive flavonoids test was shown by a red or pink-red colour (Morsy, 2014; Alqethami & Aldhebiani, 2021).

3.5.2.2.7 Detection of resins

Precipitate test: 10 ml of plant extract were blended with 15 millilitres of distilled water. A positive resins test was shown by the formation of a precipitate (Alqethami & Aldhebiani, 2021).

3.5.2.2.8 Detection of glycosides

Salkowski's test: 5 ml of extract was treated with 2 ml of concentrated H_2SO_4, then carefully shaken and left to stand for two minutes. A positive glycoside test was shown by a red colour precipitate (Gracelin *et al.*, 2013)**.**

3.5.2.2.9 Detection of coumarins

Five ml of extracts were mixed with 1 N NaOH solution and placed in boiling water with the mouth of the test tube covered. After boiling the test tube cover was removed and inspected

under UV light for the occurrence of yellow fluorescence which indicated a positive coumarins test.

3.5.2.2.10 Detection of terpenoids

Salkowski test: 5 millilitres of plant extract were mixed with 2 millilitres of chloroform, then 3 millilitres of concentrated H_2SO_4 which was added carefully create a distinct layer. A positive terpenoids test was shown by a layer of reddish-brown colour (Morsy, 2014; Tyagi & Agarwal, 2017; Longbap *et al.*, 2018; Nortjie *et al.*, 2022).

3.5.2.4 Quantitative phytochemical analysis

After preliminary screening, selected groups among the detected phytochemicals were analysed quantitatively using standard procedures. The same extracts and solvents were used for quantification. For this section, five phytochemical groups were selected.

3.5.2.4.1 Quantitative estimation of total alkaloids

Harborne method: 5 grams of the sample were measured and placed in a 250 ml beaker. Subsequently, 200 ml of 10% acetic acid in ethanol was introduced into the beaker, and the mixture was covered, and left undisturbed for a duration of 4 hours. Following this, the extract underwent filtration and concentration in a water bath until it reached one-quarter of the original volume. Concentrated ammonium hydroxide was then gradually added drop by drop to the extract until precipitation was complete. The entire solution was allowed to settle, and the precipitate was collected, washed with dilute ammonium hydroxide, and subsequently sieved. The resulting residue, identified as the alkaloid, was dried and weighed once complete dryness was attained. The determination of total alkaloids was carried out in triplicate (Khan

et al., 2011; Morsy, 2014; Longbap *et al*., 2018; Alqethami & Aldhebiani, 2021; Nortjie *et al*., 2022).

3.5.2.4.2 Quantitative estimation of total flavonoids

The method described by Ordoñez *et al*. (2006) was employed for the estimation of total flavonoids. One gram of the sample was extracted in 10 ml of 80% methanol. A 0.1 ml aliquot of the sample was then mixed with 0.5 ml of a 2% $AlCl_3$ ethanol solution. After one hour at room temperature, the absorbance was measured at 420 nm using a UV-visible spectrophotometer (U-2602, LaboMed-Inc. USA). The presence of flavonoids was signalled by the appearance of a yellow colour. Reference standard solutions of rutin (0, 10, 20, 40, 80 µg/mL) were prepared. Total flavonoid content, expressed as quercetin (mg/g), was calculated using the equation derived from the calibration curve. The estimation of total flavonoid content was conducted in triplicate, and the results for extract samples were expressed as quercetin equivalent (QE) milligrams per gram of the extract.

3.5.2.4.3 Quantitative estimation of saponin

The saponin content of the samples was assessed using the double extraction gravimetric method outlined by Hashmi *et al*. (2021) with some modifications. Five grams of the powdered sample were combined with 50 ml of a 20% aqueous ethanol solution in a flask. The mixture was heated in a water bath at 55ºC for 90 minutes with occasional stirring, and then filtered using Whatman filter paper (No. 42). The residue was subjected to a second extraction with 50 millilitres of 20% ethanol, and both extracts were combined. The resulting extract was reduced to approximately 40 ml at 90ºC and transferred to a separating funnel, where 40 ml of diethyl ether was added and vigorously shaken. Re-extraction through partitioning was repeated until

the aqueous layer became clear. Saponins were then extracted using 60 ml of normal butanol. The combined extracts were washed with a 5% aqueous sodium chloride (NaCl) solution, evaporated to dryness in a pre-weighed evaporation dish, and dried at 60ºC in the oven. The dish was reweighed after cooling in a desiccator, and this process was repeated twice more to obtain an average. The saponin content was determined by the difference and expressed as a percentage of the original sample.

3.5.2.4.4 Quantitative estimation of total tannin

The total tannins were measured using the Folin-Ciocalteu method with slight modifications. A sample weighing 0.1g was extracted in 10 millilitres of distilled water. For the analysis, 0.1 mL of the sample extract was added to a 10 mL volumetric flask containing 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent, and 1 mL of 35% sodium carbonate solution. The mixture was then diluted to 10 mL with distilled water, thoroughly shaken, and left at room temperature for 30 minutes. Reference standard solutions of tannic acid (0, 10, 20, 40, 50 µg/mL) were prepared. Absorbance for both the test and standard solutions was measured using a UV/Visible spectrophotometer (U-2602, Labomed Inc, USA) at 725 nm against the blank (distilled water). The determination of total tannin content (TTC) was conducted in triplicate, and the tannin content was expressed in terms of mg/g of gallic acid in the sample.

3.5.2.4.5 Quantitative estimation of total polyphenols

The spectrophotometric determination of total phenolic contents in the extracts was conducted using the Folin–Ciocalteau method as outlined by Singleton *et al.* (1999). In this process, 0.1g of the sample was extracted in 10 ml of distilled water. To the extract solution (0.1 ml), 0.5 ml of Folin–Ciocalteau reagent was added, and the total volume was adjusted to 8.5 ml with distilled water. After maintaining the tubes at room temperature for 10 minutes, 1.5 ml of sodium carbonate (20%) was introduced. The tubes were then incubated in a water bath at 40°C

for 20 minutes, and the intensity of the developed blue colour was measured by recording the absorbance at 755 nm using a UV–visible spectrophotometer (U-2602, LaboMed-Inc. USA). A reagent blank was prepared using distilled water. For quantifying the total phenolic content in the extract, a standard calibration curve was established using gallic acid. Reference standard solutions of gallic acid (0, 10, 20, 40, 80 µg/mL) were prepared. Absorbance for both the test and standard solutions was measured with a UV/Visible spectrophotometer (U-2602, Labomed Inc, USA) against the blank (distilled water) at 755 nm. The determination of total polyphenol content was conducted in triplicate, and the results for extract samples were expressed as gallic acid equivalent (GAE) milligrams per gram of the extract.

3.5.2.5 Gas chromatography-mass spectrometry analysis

For the general identification of the phytochemical compounds within the plants, the methanolic extract of individual plants was analysed by GC-MS of Agilent Technologies (USA) model (Intuvo 9000 GC connected to 19091S-433UI-INT MS) having HP-5MS UI column with 30 m length, 250 µm dimensions, and 0.25 µm film thickness. Helium served as the carrier gas and the flow rate was set at 3 mL/min. The sample injection was done using a split-less mode. The sample volume used was 5 µL. The temperature at the injector was set at 280° C. The oven temperature was programmed as follows: 70° C for 2 minutes and increased at a rate of 25°C/min to 150° C and held for 2 minutes, then it was increased at a rate of 3°C/min to 200°C and held for 2 minutes, and finally 8°C/min to 280° C and held for 10 minutes. The ionization voltage of MS-analysis was controlled by the EI procedure with the ion source heat of 280° C. The total GC-MS running time was 45.867 min. The relative propotional percentage of each component was calculated by comparing its average peak area to the total area. The analysis of the mass spectrum of the GC-MS utilized the National Institute of Standard and Technology (NIST) database, which contains over 62,000 patterns. The spectrum of the unidentified components was matched against the spectrum of known components stored in

the NIST library. This process determined the names, molecular weights, and molecular formulas of the components present in the extracts. To ensure the results accurate the instrument was tuned using perfluorotributylamine (PFTBA) to ensure that calibration has not shifted. This was performed before injecting the sample. Additionally, the blank of methanol solvent used for extraction was also injected to the machine.

3.5.3 Toxicity and neutralizing dose levels determination

3.5.3.1 Plants preparation and extraction

The samples of the selected three medicinal plants that were used to manage diabetes and hypertension were collected, pilled, dried, and then powdered. For this objective, plant samples were extracted using ethanol. Ethanol was chosen as the solvent for extraction in the toxicity study due to its effectiveness in extraction and its safety for human consumption and therefore to the experimental animals (Tejavathi & Sujatha, 2019). This involved soaking 300g of each plant powder in 3000 ml of ethanol (95%). The mixture was then incubated in a dark place and at normal room temperature for 72 hours with occasional agitation at least twice a day. After 72 hours of the maceration process and occasional shaking, the extracts were filtered separately. Extreme care was taken to avoid any contamination during the filtration process. After filtration, the obtained liquid extract was concentrated to dryness using an air oven at a maximum temperature of 45° C. The yields were 14.5% for FS, 13.6% for CR, and 8.87% for MP. The obtained solid/semisolid crude extract was then put in storage containers, and refrigerated at -20° C until use.

3.5.3.2 Model animals for toxicity assessment

For toxicity assessment of the three selected medicinal plants Wistar albino rats were used as a model species. Nulliparous female (non-pregnant) albino rats, having an age of 8-10 weeks and weighing 80-106g were obtained from animal houses under the College of Veterinary

Medicine, Animal Resources and Biosecurity (COVAB), Makerere University. The obtained rats were kept in polyvinyl chloride (PVC) cages and stored in a well-ventilated room, with good access to tap water and rat pellets. The rats were exposed to a circle of twelve hours of natural light (day) and twelve hours of darkness (night). All rats were housed at the Pharmacology and Toxicology Laboratory, COVAB. Before the experiment, the rats were acclimatized in the experiment room for two weeks. Before any dosing and on each instance, rats underwent an overnight fast (only pellets but all had access to tap water *ad libitum*), and after dosing food was withheld for 4 hours. Toxicity assessment was done following the standard operating procedures for using animals in scientific research set forth by the Organization for Economic Cooperation and Development (OECD, 2001; OECD, 2016). For Acute and Subacute toxicity, this study adopted OECD Guideline No. 425.

3.5.3.3 Experimental design for Acute toxicity assessment (14 days)

A total of thirty-five rats were used for acute toxicity assessment. Rats were divided into seven groups with five rats each, corresponding to each plant extract (3 plants) and Control. Each group of experimental rats was given a single dose administered orally (using oral gavage feeding tubes) once and observed for 14 days. Group one included CONTROL rats that received only distilled water administered orally until the end of the experiment. For groups II, III and IV, rats were orally administered with 2000mg/kg body weight of *Ficus saussureana (FS), Clerodendrum rutundifolium (CR),* and *Microglossa pyrifolia (MP)* extracts respectively. Groups V, VI, and VII were administered a dose of 5000mg/kg body weight of *FS*, *CR*, and *MP* respectively. For standardization, the CONTROL group was given 2 ml of distilled water using the same administration method as the experimental groups. After dose administration, each rat was closely monitored for the first five minutes to identify any sign of regurgitation.

To determine the short-term effects of the doses, rats were monitored every 15 minutes for the first four hours, then 30 minutes for the successive 6 hours. To determine the long-term effects

of the dose for the remaining 13 days, rats were observed daily to determine the acute oral median lethal dose (LD_{50}) if any death occurred (Adeniyi *et al*., 2007). To assess acute toxicity on rats' behaviour, each behavioural change was systematically recorded. Indicators observed included reduced movement or hyperactivity, signs of imbalance, tremors, abnormal posture, laboured or irregular breathing, changes in social behaviour such as increased aggression or lethargy, and increased or abnormal vocalization. Other clinical observations were also done such as moribund, bad health, or treatment-related side effects such as salivation, tremors, changes to the skin or fur, mucus membranes, diarrhoea, discharge, and coma.

3.5.3.4 Experimental design for subacute toxicity (28 days)

To evaluate subacute toxicity, rats were orally administered with plant extracts every day for a duration of 28 days. A sum of 35 rats was once more subdivided into seven groups, each consisting of five rats. Prior to the commencement of the experiment, the weight of all rats was measured and documented, then weights were measured at an interval of 7 days. Group one was CONTROL rats administered exclusively with distilled water (2ml). For experimental groups, two doses were selected with the aim of determining the effect of repetitive doses. The selection was based on the criteria of the doses that did not cause death or severe acute toxicity from the step one experiment (acute setup) (OECD guidelines). Rats in groups II (*FS),* III(*CR),* and IV (*MP)* were administered a dose of 500mg/kg daily for 28 days. Rats in groups V (*FS),* VI (*CR),* and VII (*MP)* received 1000mg/kg dose daily for 28 days.

3.5.3.4.2 Determination of haematological and biochemical parameters

At the end of the experiment period, for both Acute (14 days) and Subacute (28 days), all rats were humanely sacrificed. Euthanasia was administered using chloroform LR ($CHCl_3$) inhalant in a sealed glass chamber which resulted in a peaceful sleep. The blood sample of each rat was collected by a cardiac puncture once unconsciousness had been achieved. Blood samples for haematological and biochemical analysis were collected using both plain vacutainers (red top)

and vacutainers containing dipotassium ethylenediamine tetra-acetic acid (K_2EDTA) (purple top). The determined clinical biochemistry parameters involved aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), Total protein (TP), Gamma-Glutamyl Transferase (GGT), Total Bilirubin (BILT), Direct Bilirubin (BILD), Creatinine (CRE), Albumin (ALB), Urea and electrolytes (Na+, K+, Cl-). These parameters were assessed as indicators of liver and renal toxicity. Determination of all these parameters was done using Cobas Clinical Chemistry Analyser, (Cobas 6000 model). Haematological parameters analysed included White Blood Cells count (WBC), Lymphocytes (LYMPH), Monocytes (MONO), Eosinophils (EO), Basophils (BASO), Immunoglobulin (IG), Red Blood Cells count (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Standard Deviation in Red Cell Distribution Width (RDW-SD), Coefficient of Variation in Red Cell Distribution Width (RDW-CV), Platelet (PLT), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Larger Cell Ratio (P-LCR), and Procalcitonin (PCT). Haematological examination was done using Sysmex Haematology Analyser, XNL-550, SN: 14275.

3.6 Ethical consideration and clearance

In adherence to ethical guidelines, research approval and clearance were obtained from the Uganda Christian University Research Ethics Committee (REC) before the commencement of the study (Registration number; **UCUREC-2023-504**). Additionally, an introductory letter was secured from the Directorate of Research and Graduate Training of Kyambogo University. Moreover, respondent participation was entirely voluntary, with full autonomy to withdraw at any stage. Informed consent was paramount; participants received prior information about the study's aim and implications, and each participant signed a written informed consent form, ensuring voluntary and informed participation.

3.7 Data analysis

Data from interviews were statistically analysed using Statistical Package for Social Sciences (SPSS Version 20). Because most of the questions had multiple responses, Multiple Response Analysis (MRA) was employed. This involved data coding which is a technique of transforming responses into numbers based on each category of choice. After coding and data entry, outputs were made into frequencies and percentages and presented in tables and figures. Data on treatment perspectives such as plants identified, preparation methods, parts of the plants utilized, etc. were entered and organized in Excel spreadsheets (Microsoft Excel 2016). Further analysis was done to estimate the Frequency Index (FI) as a quantitative measure to determine the percentage frequency of citation for each plant species. The frequency is high for plant species mentioned by many respondents and low when there are few reports from respondents. These data were also presented in tables.

Preliminary phytochemicals and GCMS results were summarized in excel and presented in tables. Quantitative phytochemical data were expressed and presented in means ± SEM. Toxicity data were analysed using GraphPad Prism version 8 Software (Graph Pad Software, San Diego, CA, USA). All data were expressed as mean ± SEM. One-way Analysis of Variance (ANOVA) was employed to assess the statistical significance of the mean among treatment groups. Dunnett's comparison test served as a post-hoc analysis to identify the statistical significance between the control group and each treatment group. A P-value < 0.05 was considered indicative of significance for both tests.

CHAPTER FOUR

RESULTS

4. Introduction

Results of the study are presented in this chapter and they are presented in three sections as per objectives. Part-one includes general indigenous knowledge perspectives, part two reports the phytochemical components of the three selected medicinal plants, and part three presents the toxicity profiles of these selected plants.

4.1 ETHNOBOTANICAL INDIGENOUS KNOWLEDGE

Indigenous knowledge included the biodata of respondents, traditional knowledge perspectives, herbalist understandings of the two diseases, medicinal plants used, threats, and conservation strategies.

4.1.1 Socio demographics of herbalists (respondents)

Fifty-four herbalists were interviewed, belonging to PROMETRA-UGANDA, an institution of traditional medicine found in Mpigi Uganda. Out of 54 herbalists, 68.5 % were females while 31.5 % were males. The majority (87 %) had an age of more than forty years where 31.5 % had an age of between 51-60 years and 24.1 % had an age of between 41 – 50 years. Seventy percent (70.4 %) of herbalists had only attained primary education and only 1.9 % had university education. A hundred percent of respondents were herbal healers who also practice other economic activities such as agriculture (77.8 %) (Table 4.1). Due to the fact that this study was conducted in Central Uganda, 92.6 % of respondents were Baganda who belonged to the Buganda Kingdom, and the majority of them (37 %) had stayed in their current residence

for more than 30 years. Out of a hundred percent of interviewed respondents, 55.6 % were married. Generally, herbal healing was dominated by less educated (primary education) people and more aged (87.1 % had an age of > 41 years) (Table 4.1).

Table 4.1: Biodata of interviewed Traditional Herbalists (respondents) (N = 54)

Variables	**Frequency response**	**Percentage (%)**
Gender of respondents		
Male	17	31.5
Female	37	68.5
Age of respondents (Years)		
18-30	3	5.6
31-40	4	7.4
41-50	13	24.1
51-60	17	31.5
61-70	7	13.0
More than 70	10	18.5
Education level attained		
No formal education attained	3	5.6
Primary education	38	70.4
Secondary education	10	18.5
Technical/College Education	2	3.7
University education	1	1.9
Are you a traditional herbal healer?		
Yes	54	100.0
Employment status		
No formal	1	1.9
Farmers	42	77.8
Employed	4	7.4
Business person	3	5.6
Community Health Promoter (CHP) /Village Health Team (VHT)	2	3.7
Midwife	1	1.9
Local Government Officer (LC)	1	1.9
Residence duration (Years)		
1-10	9	16.7
11-20	14	25.9
21-30	11	20.4

More than 30	20	37.0
Tribe		
Mufumbira	1	1.9
Baganda	50	92.6
Mukiga	1	1.9
Munyankole	1	1.9
Munyarwanda	1	1.9
Marital status		
Single	14	25.9
Married	30	55.6
Widowed	8	14.8
Divorced	2	3.7

4.1.2 Sources of traditional healing knowledge and training

Interviewed herbalists attained their traditional knowledge through various means. This included self-experience such as dreaming about certain plants or experiencing sickness (themselves/relatives) from some diseases that made them find treatment alternatives and ending up exploring some plants and then starting treating others. Example, one of the herbalists said,

> "*I previously faced challenges with Malaria and was concerned about the side effects associated with Western medicine, such as Chloroquine and Quinine. In my search for alternatives, I discovered Bro Kiganda, a traditional institute. Following training, I successfully used their medicine to overcome Malaria and then began treating others. Additionally, I learned about the Sekagya Institute in 2003 and have continuously expanded my knowledge since then. Over the years, I have not only gained knowledge but also generated income by providing treatment to patients.*"

Others learned from parents or grandparents, this included scenarios where they were sent to collect certain plants and when they grew up ended up collecting the plants by themselves for personal treatment and eventually extended their services to others. Some herbalists also had specific pieces of training from parents. Another herbalist said,

> "*My parents were traditional herbalists, and they relied on herbs to treat us, their children. As a child, I was sent to collect these herbs for our family's medicinal needs.*

As I matured, I continued the practice of gathering herbs and utilizing them for self-treatment and the well-being of others."

This guidance and teachings from their elders serve as a valuable foundation for their herbal practice. Others learned from fellow herbalists as well as through formal training where all herbalists met at the institution headquarters every Wednesday to learn, share, and do some practicals together. This apprenticeship model allows for a rich exchange of information. Overall, the journey to becoming an herbalist involved a diverse range of learning experiences as explained above, these varied paths contributed to the rich tapestry of traditional herbal knowledge and ensured its preservation for future generations.

Respondents when asked about sharing the knowledge, one hundred percent replied positive, although some of them pointed out that sharing at their home places with some villagers was a challenge because some village members considered them as Witches. For example, one herbalist said,

"*I don't share my knowledge with other people because where I live, they call me a witch.*"

Another one said,

"*I only share this knowledge with my children since other people call us witches.*"

Apart from sharing with fellow herbalists at the institution, the majority of them (57.4 %) shared their knowledge with family members and relatives. Only 1.9 % shared with workmates or the general public (Figure 4.1). When asked about age groups they shared this knowledge with, 53.7 % shared with only people aged above eighteen years, and the herbalists reiterating the methods they used to share this knowledge, the majority pointed out that this was through formal training at the institution (100 %) and through practicals (hands-on) (33.3 %) (Figure 4.1).

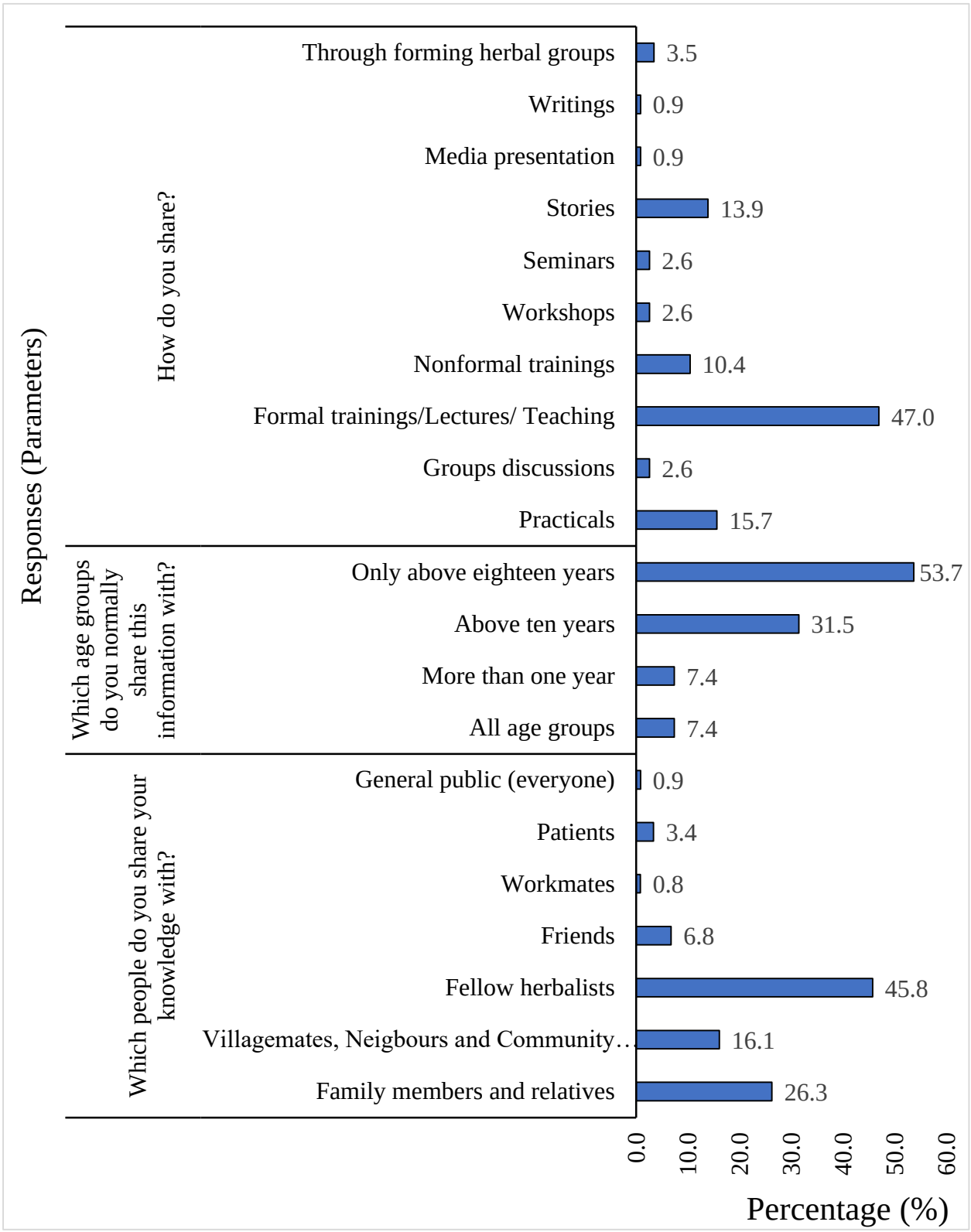

Figure 4.1: Traditional knowledge sharing from respondents

4.1.3 Causes of diabetes and hypertension

When the respondents were asked about what they believe caused diabetes and hypertension, their responses were as presented in Figure 4.2. Out of all interviewed herbalists, 83.3 % believe that hypertension and diabetes were caused by poor feeding habits such as high consumption of highly processed foods (fried foods) (7.4 %), high consumption of foods with high sugar contents (35.2 %), foods with high-fat contents (22.2 %), eating raw salts (Hypertension) (5.6 %) as well as poor diet (51.9 %). Apart from feeding habits, 50 % of herbalists believed that the two diseases were caused by stress or anxiety while only 5.6 % mentioned smoking (Figure 4.2).

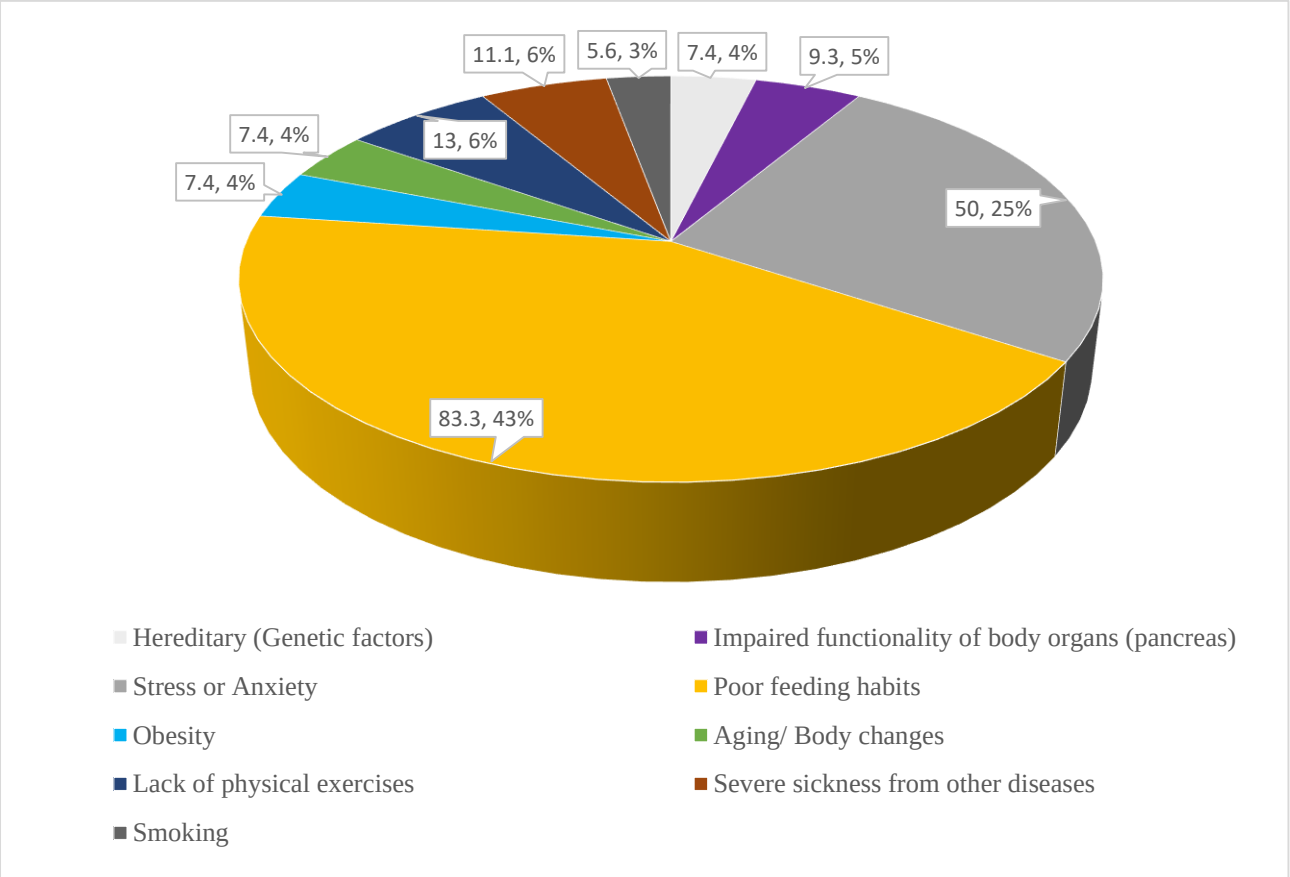

Figure 4.2: Presumed causes of diabetes and hypertension from study respondents

4.1.4 Symptoms of diabetes and hypertension as mentioned by herbalists

To capture further understanding of respondents on the two targeted diseases, herbalists were asked to mention some of the symptoms that either they treat, they know or they use to confirm diabetes or hypertension sickness in patients. The responses are recorded in Tables 4.2 and 4.3. Diabetes is locally known as *Sukali* and Hypertension is *Entununsi* in Luganda. Among all mentioned symptoms of diabetes, Body weakness or loss of energy was mentioned by 40.7 % of respondents, while frequent urination was mentioned by 29.6 % of all respondents. Other highly mentioned symptoms included excessive sweating (27.8 %), swollen legs (25.9 %), and slow-healing wounds (16.7 %).

Table 4.2: Reported Diabetes symptoms by herbalists

Symptoms	Frequency	Percentage (%)	Relative %
Excessive sweating	15	27.8	10.5
Swollen legs	14	25.9	9.8
Joints pain	4	7.4	2.8
Body weakness/ losing energy	22	40.7	15.4
Excessive thirst	8	14.8	5.6
Frequent urination	16	29.6	11.2
Passing out smelling urine	3	5.6	2.1
Weight loss	4	7.4	2.8
Fatigue	1	1.9	0.7
Loss of appetite	4	7.4	2.8
Fainting/ loss of consciousness	5	9.3	3.5
Severe headache	6	11.1	4.2
Slow healing wounds	9	16.7	6.3
Body pain	5	9.3	3.5
Blurred vision	7	13.0	4.9
Frequent hunger	1	1.9	0.7
Body spots	1	1.9	0.7
Fever	2	3.7	1.4
Body itching	3	5.6	2.1
Passing sweet urine that attracts insects	6	11.1	4.2
Skin infections and colour change	7	13.0	4.9

Respondents mentioned fourteen hypertension symptoms. Highly mentioned among them included, body weakness, which was mentioned by thirty-five percent (35.2 %) of respondents,

excessive sweating (29.6 %), abnormal heartbeat (18.5 %), severe headache (14.8 %), and difficulty in breathing (11.1 %) (Table 4.3).

Table 4.3: Reported hypertension symptoms by herbalists

Symptoms	Frequency	Percentage (%)	Relative %
Stroke	3	5.6	3.3
Paralysis	4	7.4	4.4
Abnormal heartbeat	10	18.5	11.1
Excessive sweating	16	29.6	17.8
Anxiety	3	5.6	3.3
Loss of sleep	1	1.9	1.1
Body weakness	19	35.2	21.1
Poor vision	7	13	7.8
Dizziness	3	5.6	3.3
Difficulty in breathing	6	11.1	6.7
Severe headache	8	14.8	8.9
Inability to walk long distances/ lifting heavy things	2	3.7	2.2
Easily scared	2	3.7	2.2
Gaining weight	6	11.1	6.7

4.1.5 Herbalists' experience and diagnosis methods

Figure 4.3 below illustrates the insights gained from herbalists regarding their experiences in treating diabetes and hypertension, along with the diagnostic methods employed to confirm these diseases prior to initiating treatment. The results revealed that the majority of herbalists (35.2 %) had an experience of 1 – 5 years followed by 6 – 10 years (33.3 %). Each herbalist had treated at least one diabetes or hypertensive patient. On diagnosis methods, 79.6 % of herbalists rely on laboratory results (patients who have been diagnosed already in hospitals). Even if the patient went to see these healers without laboratory results most healers request the patients to go for a checkup first to confirm if the symptoms are for diabetes, hypertension, or other disease. For example, one herbalist said,

"*I initially direct individuals to seek medical attention at hospitals for proper diagnosis. Then, once they have received a diagnosis from the hospital, I advise them to return to me for herbal treatment*."

Few TH (11.1 %) diagnosed the patients by themselves. This self-diagnosis (using the traditional method) was only used for Diabetes, where herbalists told the patients to urinate on the soil and then observe the presence of insects (bees and ants) surrounding the urine spot as an indicator of sugar in the urine (Diabetes). One herbalist said,

"*If a patient has not yet visited the hospital, I instruct them to urinate on the soil. After a few minutes, the presence of insects such as ants and bees surrounding the urine serves as an indicator of Sukali."*

When asked about gender which forms the majority of diabetes and hypertension patients as well as the most frequent age groups of diabetes and hypertension, fifty-five percent (55.6 %) of herbalists said the majority of their patients were females, 38.9 % said were from both genders and 5.6 % said were males. On the most frequent age groups of patients, 38.9 % of herbalists said the patients were 40 years and above, 33.3 % said were 30 years and above, 20.4 % said they were 50 years and above, 5.6% said 60 years and above while 1.9 % said the patients were below 30 years old. This indicated that the majority of DM and HT patients were elderly (> 40 years) and mostly females.

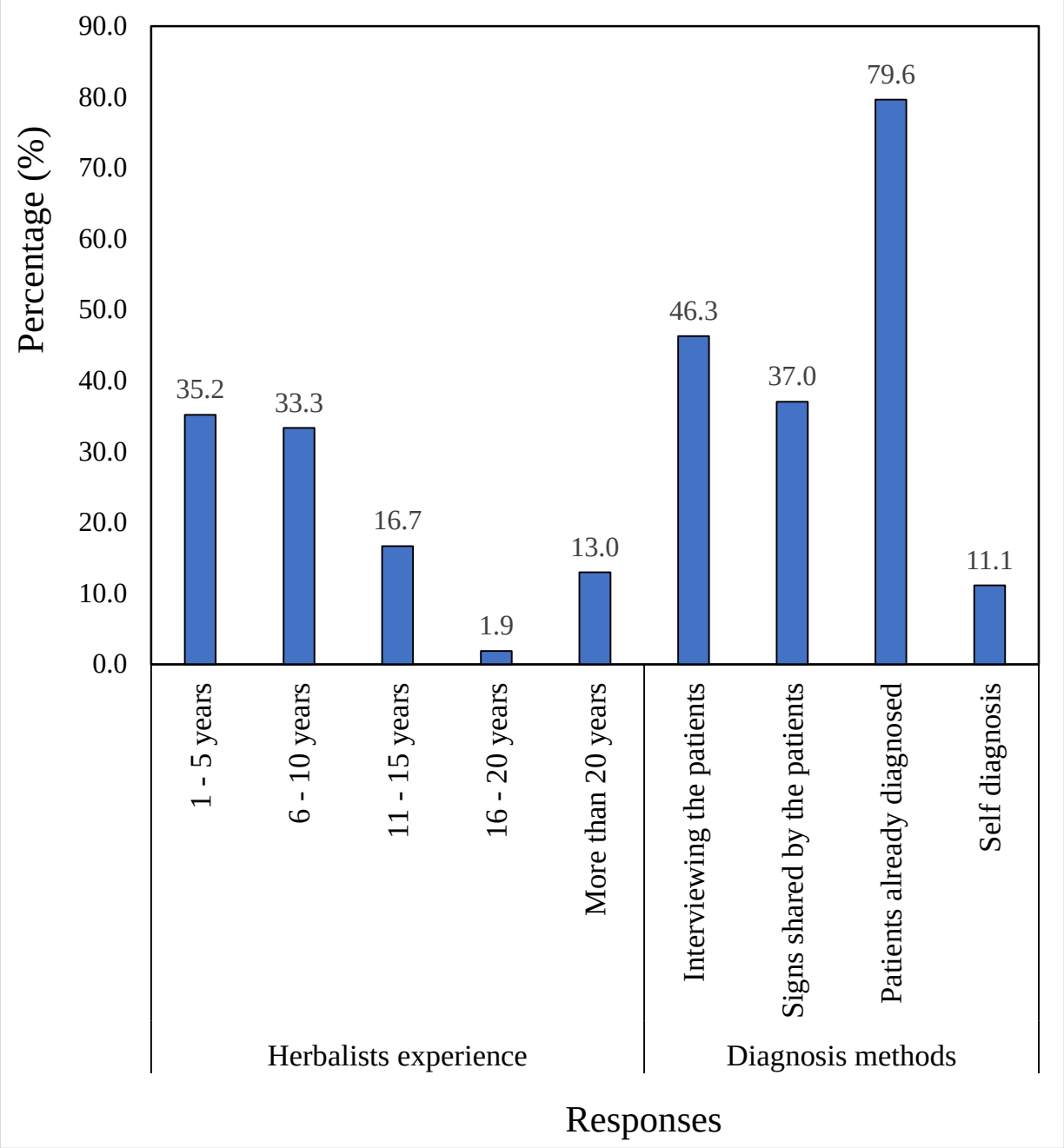

Figure 4.3: Respondents experiences and diagnosis methods for diabetes and hypertension

4.1.6 Anti-diabetic and anti-hypertension medicinal plants

One hundred and twenty plant species were reported to be used to treat Diabetes and Hypertension by traditional herbalists in the study area (Table 4.4). These plants are distributed into 52 different families. Families with the majority of plants include Asteraceae, Solanaceae, and Eurphobiaceae with 12, 8, and 7 plants respectively (Figure 4.6). Among the total plant species, 56 treat diabetes, 29 treat hypertension and 35 species treat both diabetes and

hypertension. The highly mentioned plants include *Tithonia diversifolius*, *Aloe vera*, *Catharanthus roseus, Kigelia africana, Prunus africana,* and *Momordica foetida*. Traditionally a single species with minor differences would be used separately to treat different symptoms of a single disease or two different diseases. For example, during interviews, some herbalists mentioned using *Catharanthus roseus* with white flowers only to treat high blood sugar and red flowers to treat low blood sugar although others use this species without considering the difference in flower colour.

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents

Scientific name	Local Name (Luganda)	Family	Growth form	Part Used	Disease treated	Preparation	Frequency Index (FI)
Tithonia diversifolius (Hemsl.) Gray	Ekimyula	Asteraceae	S	S, B, L	D	Decoction of fresh parts or powder	40.7
Aloe vera (L.) Burm.f.	Ekigajji	Asparagaceae	H	L	D	Blended, or boiled fresh or powder	33.3
Catharanthus roseus (L.) G. Don	Ssekajja/ Akakukulu	Apocynaceae	H	Wh	D & H	Boiled fresh or powder	31.5
Kigelia africana Benth.	Omussa/Ebeere	Bignoniaceae	T	AP	D & H	Decoction in fresh or in powder form	27.8
Prunus africana (Hook.f.) Kalkman	Entaseesa	Rosaceae	T	Wh	D & H	Boiled in powder form	22.2
Momordica foetida Schumach.	Ebbombo	Cucurbitaceae	Sr	Wh	D	Squeezed to make juice or boiled fresh or powder	22.2
Centella asiatica (L.) Urb.	Mbutamu	Apiaceae	Cr	Wh	D & H	Decocted fresh or powder	20.4
Piptadeniastrum africanum (Hook.f.) Brenan	Empewere	Mimosoideae	T	B, L	D & H	Decoction in powder form	18.5
Bidens pilosa L.	Seere	Asteraceae	H	Wh	D & H	Boiled fresh or in powder form	16.7
Aspilia africana (Pers.) C. D. Adams	Makayi	Asteraceae	H	L	D & H	Leaves are boiled	16.7
Solanum anguivii Lam.	Katunkuma/ Obutura	Solanaceae	H	Fr, S	D & H	The powder is boiled or steamed in water	14.8
Syzygium cuminii (L.) Skeels	Jambula	Myrtaceae	T	B, S	D & H	Decoction in powder form	13.0
Albizia coriaria Oliv.	Omugavu	Mimosoideae	T	B	D & H	Boiled in powder form	13.0
Vernonia amygdalina Del.	Omululuza	Asteraceae	T	L, R	D & H	Boiled either fresh or powder	13.0

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued*)

Eucalyptus grandis Maiden	Kalitunsi	Myrtaceae	T	L	D & H	Leaves are boiled fresh	9.3
Cleome gynandra (L.) Briq.	Ejjobyo	Capparaceae (Cleomaceae)	H	L	D & H	Boiled	9.3
Clerodendrum rotundifolium Oiv.	Ekisekeseke	Verbenaceae	H	L	D & H	Fresh leaves are decocted	9.3
Ficus saussureana DC.	Omuwo	Moraceae	T	B	D	Fresh stem bark or powder is boiled	7.4
Senna didymobotrya Fres.	Omucuura	Caesalpinioideae	S	L	D	Fresh leaves are boiled	7.4
Aristolocchia elegans Mast.	Serumbete/ Kaseero	Aristolochiaceae	Sr	L, S	D	Decocted fresh or in powder form	7.4
Hoslundia opposita Vahl	Kamunye	Lamiaceae	S	L, Fl	D & H	Boiled fresh or in powder form	7.4
Canarium schweinfurthii Engl.	Omuwafu	Berseraceae	T	B	D & H	Boiled in hot water	7.4
Persea americana Mill.	Avocado	Lauraceae	T	S	D & H	Decocted in powder form	7.4
Punica granatum L.	Enkoma-mawanga	Punicaceae	T	L, Fr, S, R	D & H	Fruits are eaten in raw form, other parts are boiled in powder form	7.4
Stachytarpheta urticifolia Sims	Nayire	Verbenaceae	H	B, L	D & H	The powder is boiled in hot water or taken in tea	7.4
Microglossa pyrifolia (Lam.) O. Ktze.	Kafugankande	Asteraceae	S	L, B, R	H	Parts are boiled fresh or in powder form	5.6
Warburgia ugandensis Sprague subsp. ugandensis	Abasi	Canelaceae	T	L	D	The powder is decocted in hot water	5.6
Plectranthus barbatus Andr.	Ekibwankulata	Lamiaceae	S	L	D & H	Boiled fresh	5.6
Annona muricata L.	Ekitafeeri	Annonaceae	T	L, Fr, S, R	D & H	The powder is either boiled or added to hot water	5.6

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued)*

Bridelia micrantha (Hochst.) Baill.	Katazamiti	Euphorbiaceae	T	B	D & H	The stem bark is boiled in hot water	5.6
Markhamia lutea (Benth.) K. Schum.	Omusambya	Bignoniaceae	T	L, Fl	H	Decocted either fresh or powder	5.6
Tamarindus indica L.	Enkoge	Caesalpinioideae	T	S, B, L	H	Parts are boiled in powder form	5.6
Digitaria abyssinica (A. Rich.) Stpf	Olumbugu	Poaceae	G	Wh	D	Fresh parts are boiled	5.6
Callistemon citrinus (Curt.) Stapf	Mwambala butonya	Myrtaceae	T	L	D	Fresh leaves are boiled	5.6
Maesa lanceoata Forssk.	Ekiwondowondo	Myrsinaceae	T	L	D & H	Decocted in powder form	5.6
Zanthoxylum leprieurii Guill. & Perr.	Omunyenye	Rutaceae	T	B, L	D	Boiled in powder form	5.6
Leonotis nepetifolia (L.) Ait.f.	Ekifumufumu	Lamiaceae	H	L	D	Leaves are boiled fresh	5.6
Siegesbeckia orientalis L.	Seziwundu	Asteraceae	H	L	D & H	Decocted fresh	5.6
Phoenix reclinata Jacq.	Empirivuma	Arecaceae	P	S	D & H	Boiled in powder form	5.6
Physalis peruviana L.	Entuntunu	Solanaceae	H	L	D	Leaves are boiled fresh	3.7
Spathodea campanulata P. Beauv.	Ekifabakazi	Bignoniaceae	T	Fl, B	D	Flowers are boiled fresh. The bark is decocted in powder form	3.7
Dracaena steudneri Engl.	Ekajjolye njjovu	Dracaenaceae	T	B	D	The bark is ground into powder and then boiled	3.7
Rhus vulgaris Meikle	Kakwansokwanso/ Tebbuda	Anacardiaceae	S	R	D	Roots are ground into powder and boiled	3.7
Rumex usambarensis (Dammer) Dammer	Kaseke kambajwe	Polygonaceae	H	Wh	D	Decocted fresh or in powder form	3.7

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued)*

Justicia betonica L.	Nalongo	Acanthaceae	H	L	D & H	Leaves are either squeezed to make juice or boiled	3.7
Agelanghus entebbensis (Sprague) Polh. & Wiens	Enzirugaze	Loranthaceae	Pr	L	D	Leaves are boiled fresh or in powder form	3.7
Alchornea cordifolia (Schumach. Thonn.) Muell. Arg.	Oluzibaziba	Euphorbiaceae	T	L	D & H	Leaves are boiled.	3.7
Ficus asperifolia Miq.	Ekitonto	Moraceae	T	Wh	H	Squeezed to make a juice or decocted in powder form	3.7
Citrus limon (L.) Burm.f.	Nnimu/ Eniimu	Rutaceae	T	Fr	D & H	Squeezed to make a juice	3.7
Azadirachta indica A Juss.	Neem	Meliaceae	T	L, B	D	Decocted fresh or in powder form	3.7
Mangifera indica L.	Omuyembe	Anacardiaceae	T	L	D & H	Leaves are boiled fresh.	3.7
Lillium cepa L.	Akatunguru	Alliaceae	T	Fr	D & H	Fruits (bulb) are ground and either smelt or smeared under the foot, also boiled	3.7
Aframomum angustifolium	Ettunguru	Zingiberaceae	H	L	D & H	Leaves are boiled fresh	3.7
Oxalis cornicula L. var. corniculata	Kajampuni	Oxalidaceae	H	Wh	D & H	Decocted fresh or in powder form	3.7
Artocarpus heterophylla Lam.	Feene	Moraceae	T	S	D	Boiled in powder form	3.7
Amaranthus dubius Thell.	Dodo	Amaranthaceae	H	L, B	D	Boiled	3.7
Cassia mimosoides L.	Akoloola akomudo/ Kasere akatono	Caesalpinioideae	H	L	H	Boiled in powder form	3.7
Portulaca quadrifida L.	Bwanda	Portulacaceae	Cr	L	D	Leaves are boiled	3.7
Tetradenia riparia (Hochst.) Codd.	Ekiwamala	Lamiaceae	S	L	D	Boiled	3.7
Toddolia asiatica (L.) Lam.	Kawule	Rutaceae	S	R	D	Boiled in powder form	3.7

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued)*

Fleroya rubrostipulata (K. Schum.) Y. F. Deng.	Enzigu	Rutaceae	T	B	H	Decocted in powder form	3.7
Aerva lanata (L.) Schultes	Olweza	Amaranthaceae	H	L	D	Fresh leaves are boiled	3.7
Vernonia cinerea (L.) Less.	Kayayana	Asteraceae	H	L	D	Fresh leaves are boiled in hot water	3.7
Erythrina abyssinica DC.	Ejirikiti	Papilionaceae	T	B	H	Boiled in powder form	3.7
Abutilon mauritianum (Jacq.) Medic.	Ekifuula	Malvaceae	H	L	H	Fresh leaves are boiled	3.7
Kalanchoe glaucescens Britten	Kiyondo ekyeru	Crassulaceae	H	L	D	Fresh leaves are boiled	3.7
Cyanotis arachnoidea C. B. Clarke	Nsenekerezi	Commelinaceae	H	Wh	H	Fresh parts are boiled in hot water	3.7
Justicia engleriana Lindau	Muwanga	Acanthaceae	S	L	H	Fresh leaves are squeezed to make a juice or chewed	3.7
Acalypha vallicaulis A. Rich.	Magunda	Euphorbiaceae	H	Wh	D	Fresh parts are boiled in hot water	3.7
Tragia brevipes Pax	Kamyu	Euphorbiaceae	C	Wh	H	Squeezed to make juice or boiled in hot water	3.7
Solanum mauritianum Scop.	Omunyera nyonyi/ Setaaba	Solanaceae	S	L	D	Fresh leaves are boiled in hot water	3.7
Siegesbeckia orientalis L.	Sekoteka	Asteraceae	H	L	H	Fresh leaves are boiled	3.7
Eriobotrya japonica (Thunb.) Lindl.	Omusaali	Rosaceae	S	L, B	H	Leaves and stem powder are boiled or taken in hot water	3.7
Lantana camara L.	Kapanga	Verbenaceae	S	L	D	Fresh leaves are boiled	3.7
Moringa oleifera Lam.	Molinga	Moringaceae	T	L	H	Boiled fresh	3.7
Acanthus polystachius Delile	Amatovu	Acanthaceae	S	R	H	Decocted fresh or powder	3.7
Aleurites maluccana Willd.	Kabaka enjjagala	Euphorbiaceae	T	B	D	Boiled in powder form	3.7
Shiraklopsis elliptica (Hochst.) Esser	Omusasa	Euphorbiaceae	T	B	D	Decocted in powder form	3.7

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued)*

Solanum macrocarpon L.	Ntengotengo	Solanaceae	H	R	H	Boiled fresh or in powder	1.9
Saccharum officinarum L.	Ekikajjo	Poaceae	G	B	D	Boiled after ground into powder	1.9
Abrus precatorius L.	Olusitii	Papilionaceae	Cl	L	D	Decocted after ground into powder	1.9
Citrus sinensis (L.) Osbeck	Ebibbala (Muchungwa)	Rutaceae	T	B	H	Boiled in powder form	1.9
Myrianthus holstii Engl.	Ekibbala nantooke	Moraceae	T	B	H	Blended to make a juice	1.9
Tristemma mauritianum J. F. Gmel.	Nantooke	Melastomataceae	H	L	H	Leaves are decocted fresh	1.9
Craterispermum schweinfurthii Hiern	Empomerezi	Rubiaceae	S	B	D	Boiled after drying and ground into powder	1.9
Phyllanthus amarus Schumach. & Thonn.	Kabalira kumngongo	Euphorbiaceae	H	L	H	Decocted fresh.	1.9
Ananas comosus (L.) Merr.	Akananasi ekiganda	Bromeliaceae	H	L	D	The inner part of the fruit (tongue) is removed then mixed with leaves and boiled while covered then kept for three days	1.9
Termitomyces microcarpus	Obutiiko	Tricholomataceae	M	Wh	H	Boiled fresh	1.9
Curcuma longa L.	Ekizaali ekiganda	Zingiberaceae	H	Fr	D	Pound when it is fresh and put in a tea	1.9
Dioscorea odoratissima Pax	Kaama	Dioscoreaceae	Sr	Fr, R	D	Parts are dried, ground, and boiled in warm water	1.9
Colocasia esculenta (L.) Schott	Obukopa	Araceae	H	L	D	Leaves are dried and ground then boiled or taken in hot water	1.9
Mukia maderaspatana (L.) M. J, Roem.	Akasunsa mukira	Cucurbitaceae	Sr	Wh	H	Decocted fresh	1.9
Cyphomandra betacea (Cav.) Standtner	Ekinyanya	Solanaceae	T	Fr	D	Fruits are eaten in raw form	1.9

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued)*

Bothriocline longipes (Oliv. & Hiern) N. E. Br.	Etwatwa	Asteraceae	S	L	D	Leaves are boiled fresh	1.9
Rubus pinnatus Willd. var. afrotropicus (Engl.) C. E. Gust	Olukenene	Rosaceae	Sr	R	D	Roots are dried, ground then boiled	1.9
Voacanga thouarsii Roem. & Schult	Kinywa mazzi	Apocynaceae	T	L	D	Decocted fresh or in powder form	1.9
Vernonia lasiopus O. Hoffm.	Akaluluza ekasajja	Asteraceae	S	S	D & H	Decocted and drunk after a meal	1.9
Commelina africana L.	Enanda	Commelinaceae	H	Wh	D	Decocted in powder form	1.9
Conyza steudelii A. Rich.	Omuzikiza	Asteraceae	H	L	H	Boiled	1.9
Oxygonum sinuatum (Meisn.) Dammer	Kafumita bagenda	Polygonaceae	H	L	D	Leaves are either boiled fresh or in powder form	1.9
Cymbopogon nardus (L.) Rendle	Ekiteete	Poaceae	G	Wh	D	Decocted	1.9
Cupressus lusitanica Mill.	Akakomera	Cupressaceae	T	L	H	Fresh leaves are boiled	1.9
Blighia unijugata Bak.	Nku za nyana	Sapindaceae	T	B	D	Decocted fresh or in powder form	1.9
Panicum trichocladum K.Schum.	Kalandaluggo	Poaceae	G	Wh	D	Fresh parts are boiled	1.9
Mimosa pudica L.	Muke wewumbeko	Mimosoideae	H	Wh	H	Decocted fresh	1.9
Curcuma maxima Lam.	Ensujju ento	Cucurbitaceae	H	Fr	D	Decoction either fresh or powder	1.9
Capsicum frutescens L.	Kamulari	Solanaceae	H	Fr	H	Added to other plants as a catalyst	1.9
Vigna unguiculata (L.) Walp.	Egobe lyempindi	Papilionaceae	H	L	D	Leaves are boiled fresh	1.9
Allium sativum L.	Katuguluccumu	Alliaceae	H	Bulb	D	Boiled	1.9
Maesopsis eminii Engl.	Omusizi	Rhamnaceae	T	B	D	Decocted in powder form	1.9

Table 4.4: Anti-diabetes and anti-hypertension medicinal plants mentioned by respondents (*Continued)*

Artemisia annua L.	Pasile	Asteraceae	H	L	D	Fresh leaves are boiled	1.9
Cinnamomun verum Ptesl.	Mudalasini	Lauraceae	T	L	D	Boiled in powder form	1.9
Hydnora abyssinica A. Braun	Omutima gwe'taka	Hydnoraceae	Pr	Wh	H	Dried and ground into powder, then taken in a tea	1.9
Biophytum abyssinica A. Rich.	Mutigumu	Oxalidaceae	H	Wh	H	Decocted fresh or in powder form	1.9
Solanum nigrum L.	Nakati (Nsuuga)	Solanaceae	H	Wh	D	Boiled fresh	1.9
Caralluma distincta E. A. Bruce	Akawulira	Apocynaceae	H	Wh	D	Fresh parts are boiled	1.9
Not identified	Ekimeere kyenkoko			L, B	D & H	Parts are boiled or steamed	7.4
Not identified	Ngaboya kabaka			B, L	D & H	Decocted fresh	5.6
Unknown	Kabajja nsaayi			B	D	Decocted fresh	1.9
Not identified	Akayiri kiakasaja			R	H	Roots are boiled fresh or in powder form	1.9

Plants life forms: S-shrub, **H**-herb, **T**-tree, **Cr**-creeper, **P**-palm, **Pr**-parasitic, **G**-grass, **M**-mushroom, **Sc**-Scandet, **Sr**-scrambler. Parts used: **S**-seeds, **L**-leaves, **Fr**-fruits, **Wh**-whole, **B**-bark, **AP**-aerial parts, **Fl**-flowers, **R**-roots. **Diseases treated**: **D**-diabetes, **H**-hypertension

4.1.7 Herbs preparation and dosage administration

Herbalists prepared the majority of anti-diabetic and anti-hypertension herbs by either boiling them individually or in combination with other species, except some fruits that were consumed in their raw form. Some TH simply provided patients with a powdered form of the medicinal herbs, instructing them to swallow it directly with water. Some of the other medicines were important vegetables and fruits that were consumed in conjunction with meals. Following the boiling process, the doses administered to patients did not exhibit significant variations among different traditional healers, although minor differences in dosage length were observed even for the same herb species. All doses were measured in either a cup (locally known as Nice (a manufacturer brand name)) or a glass. Most doses were given in half a cup (72.2 % of herbalists) and three times a day (88.9 %). A minimum single dosage length recorded was five days and the maximum dose was three months. Notably, 22.7% of herbalists opted for a one-month dosage regimen. In certain instances, the dosage was individualized for each patient, and, in some cases, patients were instructed to continue taking the medicines until complete recovery (Table 4.5). Dosage lengths were not uniform across age groups, as they were tailored to factors such as age, gender, and the overall health condition of each patient. Additionally, doses were occasionally determined based on the specific plants used; if the herbalist perceived a particular plant as potent due to its pronounced sourness or bitterness, a minimum dosage was prescribed.

Table 4.5: Anti-diabetes and anti-hypertension dosages administration by herbalists

Categories	Percentage (%)	Relative %
Single dosage administration		
Quarter a cup	44.4	34.8
Half a cup	72.2	56.5
A full cup	11.1	8.7
Frequency of dosage per day		
Once a day	9.3	6.8
Twice a day	31.5	23.3
Thrice a day	88.9	65.8
Fourfold	5.6	4.1
Single dosage duration		
Less than one week	13.0	7.9
One week	22.2	13.5
Two weeks	20.4	12.4
Three weeks	9.3	5.6
Four weeks (one month)	27.8	16.9
Two months	22.2	13.5
Three months	7.4	4.5
Up to recovery	16.7	10.1
Determined per each patient	25.9	15.7

4.1.8 Plant parts used and life forms

Leaves were the most used parts with 38.7 % usage, followed by bark at 20.6 % and whole plant at 14.2 % (Figure 4.4). It's noteworthy that in some instances, multiple parts of a single plant were combined in treatment formulations. For instance, a mixture of the stem and leaves of *Microglossa pyrifolia* was utilized, along with combinations like *Spathodea campanulata* flowers and bark, *Zanthoxylum leprieurii* bark and leaves, among others. In terms of plant life forms, the predominant categories of plants utilized by herbalists for treating the two diseases were herbs at 36.8%, followed by trees at 34.2%, and shrubs at 13.7% (Figure 4.5).

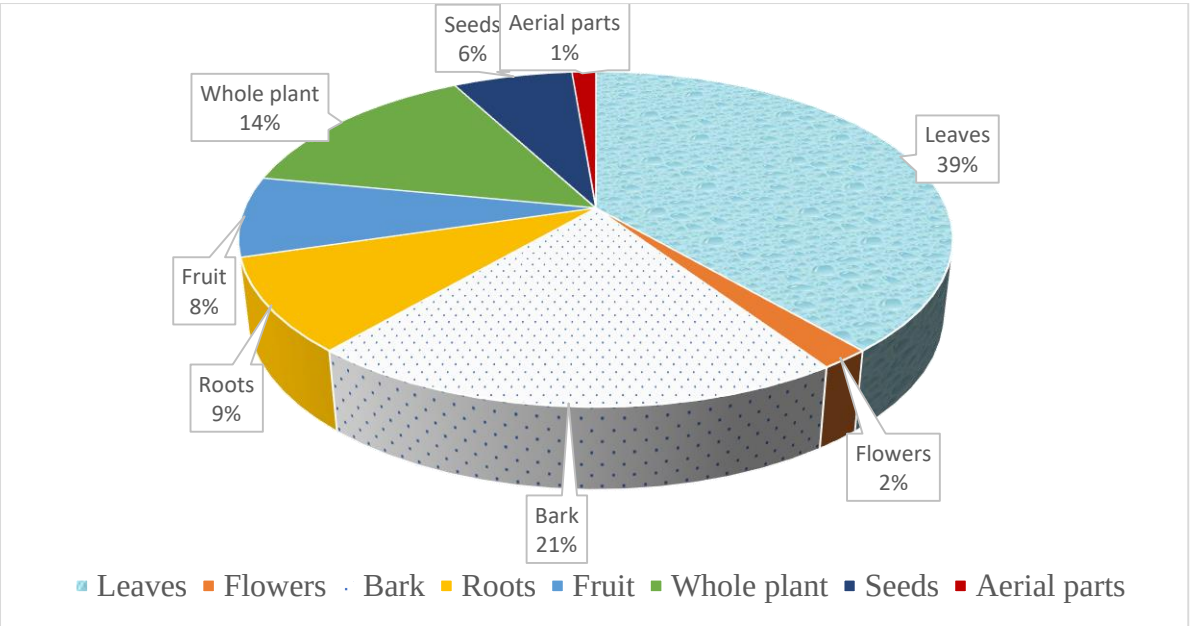

Figure 4.4: Plant parts used for diabetes and hypertension treatment

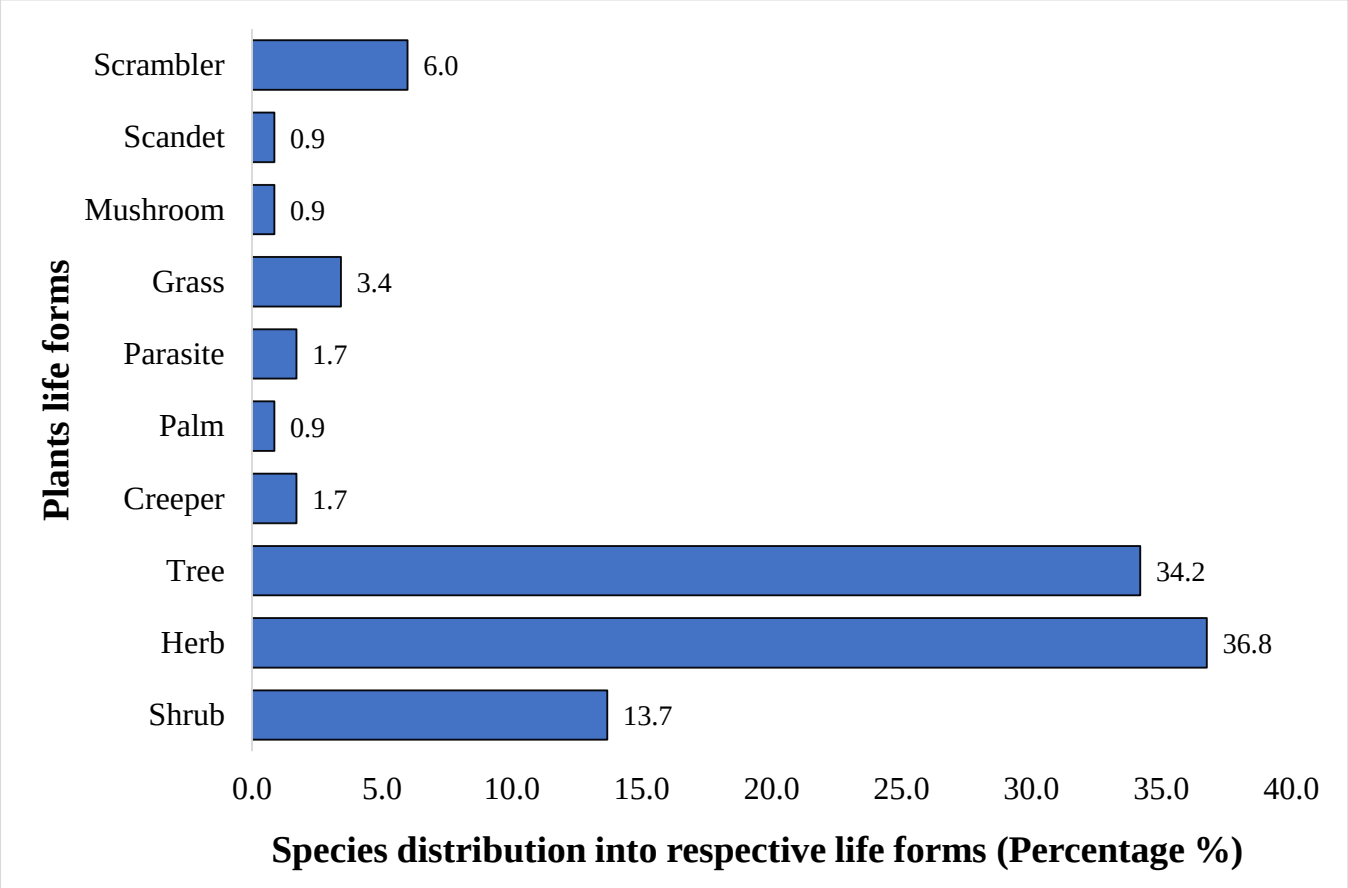

Figure 4.5: Diabetes and hypertension medicinal plants life forms

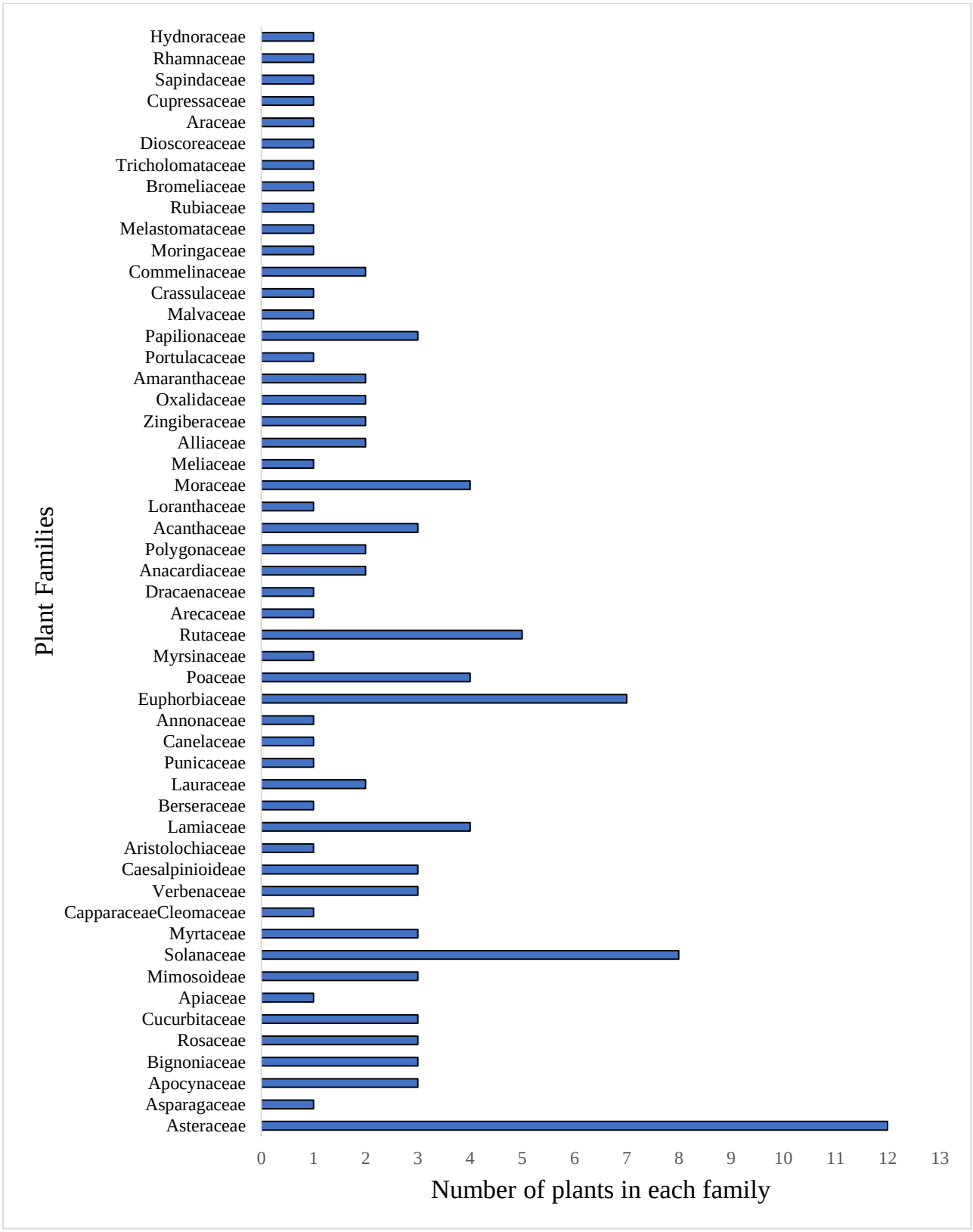

Figure 4.6: Distribution of anti-diabetes and anti-hypertension medicinal plants into different families

4.1.9 Sources and practices of harvesting medicinal plants

Some of the practices of harvesting antidiabetic and antihypertensive medicinal plants are recorded in Figure 4.7 below. First respondents were asked where they harvest the mentioned medicinal plants from, 85.2 % harvest them from home gardens, 66.7 % from wild areas or forests, and 14.8 % buy some of the plants in markets. On the time of harvesting, 94.4 % said they harvest during midmorning from around 10 am to 12 pm. This time was favourite to most herbalists because traditionally they believe all plants sleep during the night and wake up in the morning around 10 am. This is to say plants are more active around 10 am and therefore healing efficiency when harvested at this time.

In terms of preservation practices, the majority of herbalists (63 %) ground the harvested plants and stored the obtained powder in non-transparent tins (Figure 4.7). Twenty-five percent of herbalists revealed using natural preservatives such as bee honey and ash. Additionally, other plants were added as preservatives on boiled plants and these include *Bidens pilosa* and *Eucalyptus grandis*. Fifty-one percent (51.9 %) practiced proper drying without grounding into powder and 44.4 % of herbalists boil the plants and store them in jerrycans. These diverse methods highlight the range of approaches herbalists employ to maintain the efficacy and longevity of medicinal plants in their possession.

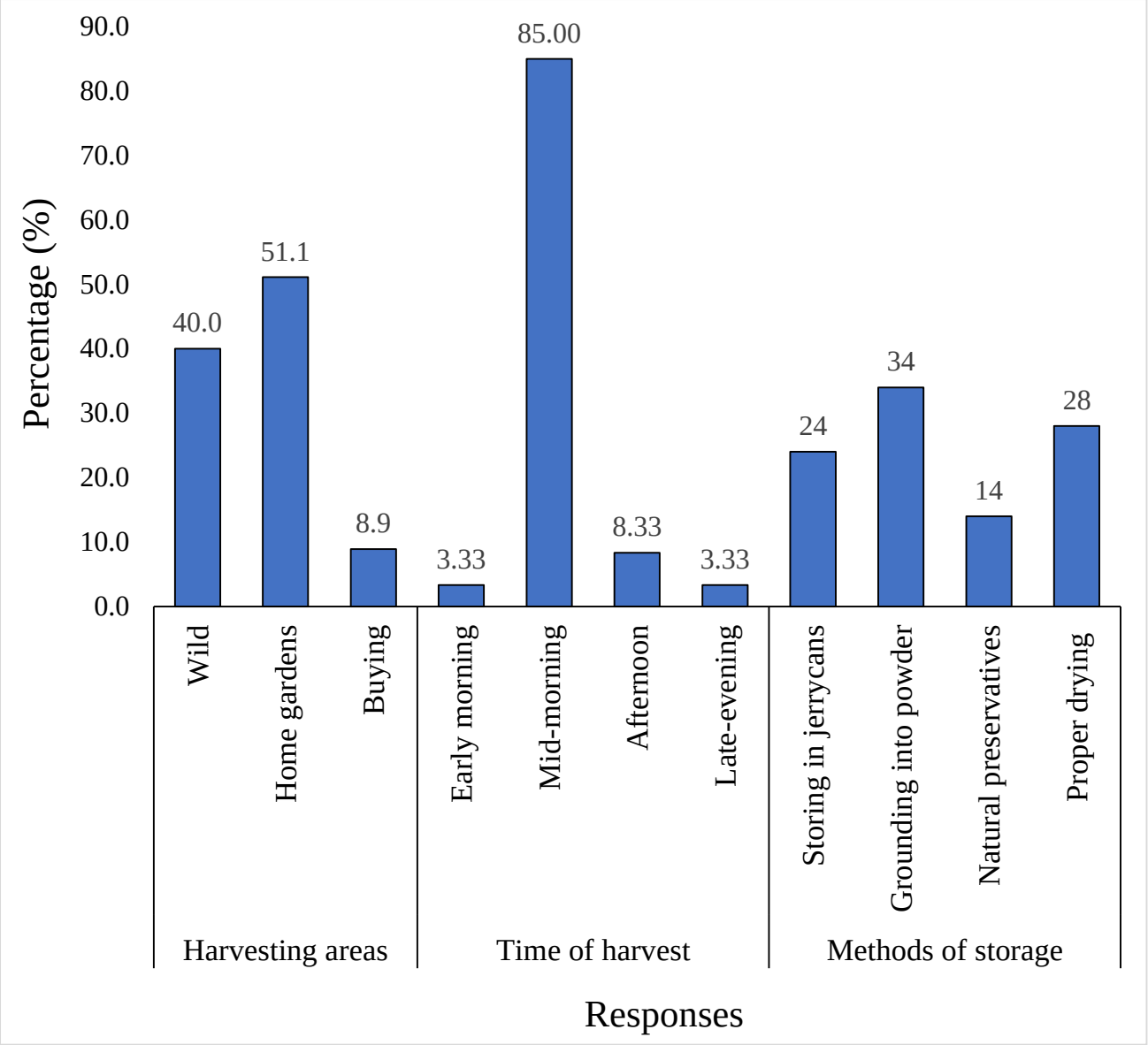

Figure 4.7: Some harvesting practices of diabetes and hypertension medicinal plants

4.1.10 Patients' preference for medicinal plants

The respondents believed that there was an increasing preference for using medicinal plants by patients to treat the two targeted diseases and other diseases. Some factors contributing to this shift in paradigm as by the respondent's responses are summarized in Table 4.6. These include among others, affordability of plants because they are either cheap or free of charge (51.9 %), easy availability and accessibility of plants (38.9 %), healing efficiency (31.5 %), and less

toxicity or side effects in plants compared to industrial drugs (29.6 %). One of the herbalists said,

> "*I believe patients lean towards medicinal plants because the prolonged and consistent use of industrial drugs tends to result in the accumulation of chemicals in the body, potentially leading to adverse effects.”*

Another herbalist said,

> "*Patients who seek medical care in hospitals often report unsatisfactory results. Additionally, prolonged and excessive use of prescribed drugs from hospitals cause effects on their liver*."

Also, another herbalist said,

> *"Hospitals are often unable to achieve complete healing, whereas patients utilizing herbal medicines experience lasting and permanent recovery.”*

Few respondents (1.9 %) believed patients opt for herbs because they fear using pharmaceutical drugs and tabs. Eleven percent of herbalists believed patients prefer plants because every single plant has a multi-curative potential in it (Table 4.6). For example, some herbalists said *Centella asiatica* is given to the patient for diabetes and hypertension treatment but also the plant provides other healing benefits such as improving body immunity, detoxification, as well as treating hepatitis.

Table 4.6: Factors for the high usage of medicinal plants as identified by respondents

Responses	Percentage (%)	Relative %
Easy availability and accessibility of plants	38.9	17.2
Low toxicity/ side effects	29.6	13.1
Affordability (Cheap/Free)	51.9	23.0
Multi-nutrient contents in plants	13.0	5.7
Phytochemicals variety in plants	3.7	1.6
Multi-curative potentials of a single plant	11.1	4.9
Healing efficiency	31.5	13.9
Unaffordability of pharmaceutical drugs (Poverty)	5.6	2.5
Abundant and easy accessibility of herbalists	3.7	1.6
No overdose	3.7	1.6
Less bureaucracy and prescription in dosage administration	5.6	2.5
Less efficiency of pharmaceutical drugs	16.7	7.4
Plants are fresh, natural, and contain no additional substitutes	9.3	4.1
Pharmacophobia	1.9	0.8

4.1.11 Threats facing medicinal plants and conservation strategies

In the final part of the survey, respondents were asked if there are any threats facing documented medicinal plants. All of the herbalists interviewed acknowledged that certain medicinal plants are currently under threat. Among the threats highly cited by herbalists included chemical spraying specifically to kill weeds (herbicides) and pesticides in farms and around homes (53.7 %). Forty percent (40.7 %) said these plants are affected by pests mostly caterpillars, some of the highly affected plants include *Bridelia micrantha* and *Senna didymobotrya*. Deforestation of some of the important forests where these plants were found

was also mentioned as a key threat by 27.8 % of herbalists. The least mentioned threat included the effects of domesticated animals on plants cultivated in home gardens (5.6 %) (Table 4.7).

Respondents suggested some of the conservation strategies that can be adopted to conserve and ensure the long-term survival of these medicinal plants. Some of the strategies proposed included the cultivation of medicinal plants at home gardens (74.1 %), proper harvesting (51.9 %), and controlled spraying (31.5 %) (Table 4.7).

Table 4.7: Threats facing medicinal plants and suggested conservation strategies by respondents

Threats/ Conservation strategies	**Percentage (%) response**	**Relative percentage**
Are there any threats facing these medicinal plants?		
Yes	100.0	
What threats?		
Spraying	53.7	27.6
Over-harvesting	25.9	13.3
Improper harvesting strategies	16.7	8.6
Pests/diseases	40.7	21.0
Charcoal burning	5.6	2.9
Bush burning (fire)	7.4	3.8
Deforestation	27.8	14.3
Forest encroachment/ land grabbing	11.1	5.7
Reared/ Domesticated animals in home gardens	5.6	2.9
What strategies do you think can be adopted to conserve these plants?		
Proper-harvesting	51.9	23.9
Controlled spraying	31.5	14.5
Public sensitization	9.3	4.3
Afforestation	18.5	8.5
Cultivation at home gardens	74.1	34.2
Establishment of medicinal plant conservation areas	14.8	6.8
Good implementation of government policies on forest management	13.0	6.0
Good stakeholders' involvement in the management of natural forests	3.7	1.7

4.2 PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS

The proceeding section focuses on phytochemical analysis, **subsection 4.2.1** presents preliminary phytochemical analysis, **subsection 4.2.2** presents quantitative analysis, and **subsection 4.2.3** presents GC-MS results. Three plants, as detailed in Table 4.8 below, were chosen for this study. The criteria for selection were rooted in the extensive usage of these plants, coupled with their status of being either unstudied or inadequately studied in terms of their chemical composition and toxicity.

Table 4.8: List of medicinal plants selected for phytochemicals and toxicity analysis

Scientific name	Local name	Parts analysed	Disease treated in this study	Other reports
Ficus saussureana DC.	Omuwo	Stem bark	Diabetes	Diabetes, fallopian tube blockage, HIV/AIDS, Male infertility, syphilis, typhoid fever, and ulcers (Gang *et al*., 2023).
Clerodendrum rutundifolium Oliv.	Ekisekeseke	Leaves	Diabetes and Hypertension	Malaria, intestinal parasites, induction of labor in childbirth, stomach aches, and deworming (Adia *et al*., 2016).
Microglossa pyrifolia (Lam.) O. Ktze.	Kafugankande	Leaves and Stem	Hypertension	Malaria, abdominal disorders, cough and chest pain, convulsions, skin allergy, and syphilis (Adia *et al*., 2016).

4.2.1 Qualitative phytochemical analysis

This study revealed the presence of most of the analysed phytochemicals, which include among them alkaloids, steroids, phenols, tannins, flavonoids, coumarins, and terpenoids (Table 4.9). Resins were absent in all of the extracts except in *Microglossa pyrifolia* Methanolic extract (MPM), and glycosides were recorded in all of the extracts except in *Clerodendrum rutundifolium* Methanolic extract (CRM). Saponins were present in the four extracts except in the *Ficus saussureana* Methanolic extract (FSM) and MPM.

Table 4.9: Qualitative phytochemical analysis of *Ficus saussureana*, *Microglossa pyrifolia*, and *Clerodendrum rutundifolium*

GROUPS	TEST	DETECTION	EXTRACTS					
			FSM	FSA	MPM	MPA	CRM	CRA
Alkaloids	Hager's test	Yellow precipitate	+	+	+	+	+	+
Saponins	Foam test	Stable froth	-	+	-	+	+	+
Steroids	Extract + chloroform + H_2SO_4	Red colour	+	+	+	+	+	+
Phenols	Ferric chloride test	Blue-black colour	+	+	+	+	+	+
Tannins	Braymer's test	Dark blue colour	+	+	+	+	+	+
Flavonoids	Shinoda test	Red fluorescence	+	+	+	+	+	+
Resins	Precipitate test	Green precipitate	-	-	+	-	-	-
Glycosides	Salkowski's test	Red ppt	+	+	+	+	-	+
Coumarins	1 N NaOH	Greenish-yellow fluorescence	+	+	+	+	+	+
Terpenoids	Salkowski's test	Reddish-brown layer	+	+	+	+	+	+

FSM= *Ficus saussureana* Methanol**, FSA=** *Ficus saussureana* Aqueous, **MPM=***Microglossa pyrifolia* Methanol, **MPA=** *Microglossa pyrifolia* Aqueous**, CRM=***Clerodendrum rutundifolium* Methanol and **CRA=***Clerodendrum rutundifolium* Aqueous**.** + Detected, - Not detected.

4.2.2 Quantitative phytochemical analysis

From qualitative phytochemical analysis of the six plant extracts, quantitative analysis was done on the major phytochemicals such as saponins, alkaloids, tannins, flavonoids, and polyphenols. Quantitative analysis depicted the amount of polyphenols such as 217 mg/g, highest in FSM, followed by CRM 174mg/g, MPM 152.05mg/g, FSP 44.73mg/g, MPP 40mg/g, and CRP 29.04mg/g (Table 4.10). For flavonoids amount was measured in mg of RE/g of extract, the highest amount was depicted in CRM (28.75mg/g), followed by CRP 12.33mg/g, MPM 11.88mg/g, FSM 7.62mg/g, MPP 3.72mg/g and least in FSP 0.78mg/g. Tannins were measured in mg of GAE/g of extract, the highest amount was recorded in FSM 179.77mg/g, followed by CRM 67.47mg/g, and the least in MPP 3.8mg/g. For saponins highest amount was depicted in CRM 225.07mg/g, followed by FSM 92.36mg/g, and the least in CRP 29mg/g. And for alkaloids, the highest amount was depicted as in the order of saponins except that the least amount was depicted in FSP 29.55mg/g.

Table 4.10: Quantitative phytochemical analysis of *Ficus saussureana*, *Microglossa pyrifolia*, and *Clerodendrum rutundifolium*

PLANT EXTRACT	Total Polyphenols (GAE) mg/g	Total Flavonoids (RE) mg/g	Total Tannins (GAE) mg/g	Total Saponins (mg/g)	Total Alkaloids (mg/g)
FSP	44.73 ± 2.51	0.78 ± 0.02	32.9 ± 3.3	46.92 ± 3.38	29.55 ± 0.52
FSM	217 ± 25.05	7.62 ± 0.22	179.75 ± 3.44	92.36 ± 2.65	79.99 ± 2.73
MPP	40 ± 8.9	3.72 ± 0.09	3.8 ± 0.2	40.77 ± 5.1	32.19 ± 2.33
MPM	152.05 ± 5.45	11.88 ± 0.44	43.02 ± 5.6	63.08 ± 7.12	59.69 ± 5.59
CRP	29.04 ± 1.43	12.33 ± 0.88	6.27 ± 0.43	29 ± 1.64	44.4 ± 5.67
CRM	174.15 ± 7.2	28.75 ± 0.98	67.47 ± 0.62	225.07 ± 4.11	116.15 ± 3.73

FSP= *Ficus saussureana* Powder**, FSM=** *Ficus saussureana* Methanol, **MPP=***Microglossa pyrifolia* Powder, **MPM=** *Microglossa pyrifolia* Methanol**, CRP=***Clerodendrum rutundifolium* Powder and **CRM=***Clerodendrum rutundifolium* Methanol

4.2.3 Gas Chromatography-Mass Spectrometry Profiles

The findings from the GC-MS analysis of *Ficus saussureana*, *Clerodendrum rutundifolium*, and *Microglossa pyrifolia* are presented in Tables 4.11, 4.12, and 4.13, respectively. The compounds identified are systematically arranged in accordance with their RT. Additionally, a thorough literature search was conducted to elucidate the biological (pharmacological) activities associated with the identified biomolecules for each plant.

4.2.3.1 Gas Chromatography Mass Spectrometry analysis of *Ficus saussureana*

The GCMS results of methanolic extract of *Ficus saussureana* revealed the presence of fourteen compounds which included Tridecanoic acid, 12-methyl-,methyl ester (7.7%), Tetradecanoic acid (29.9%), Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester (0.9%), Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-,(6R,13R,25R)- (1.2%), 9, 12-Octadecadienoic acid (Z,Z)- (5.5%), Oleic Acid (8.0%), Lup-20(29)-en-3-ol, acetate, (3.beta.)- (1.3%), Squalene (5.3%), Vitamin E (1.0%), 17.beta.-Acetoxy-1',1'-dicarboethoxy-1.beta., 2.beta.-dihydrocycloprop[1,2]-5.alpha.-androst-1-en-3-one (4.4%), 7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-,2,4,7,10,11-pentaacetate (1.2%), .alpha.-Amyrin (0.4%), Betulinaldehyde (0.8%), and Lupeol (7.5 %) Table 4.11.

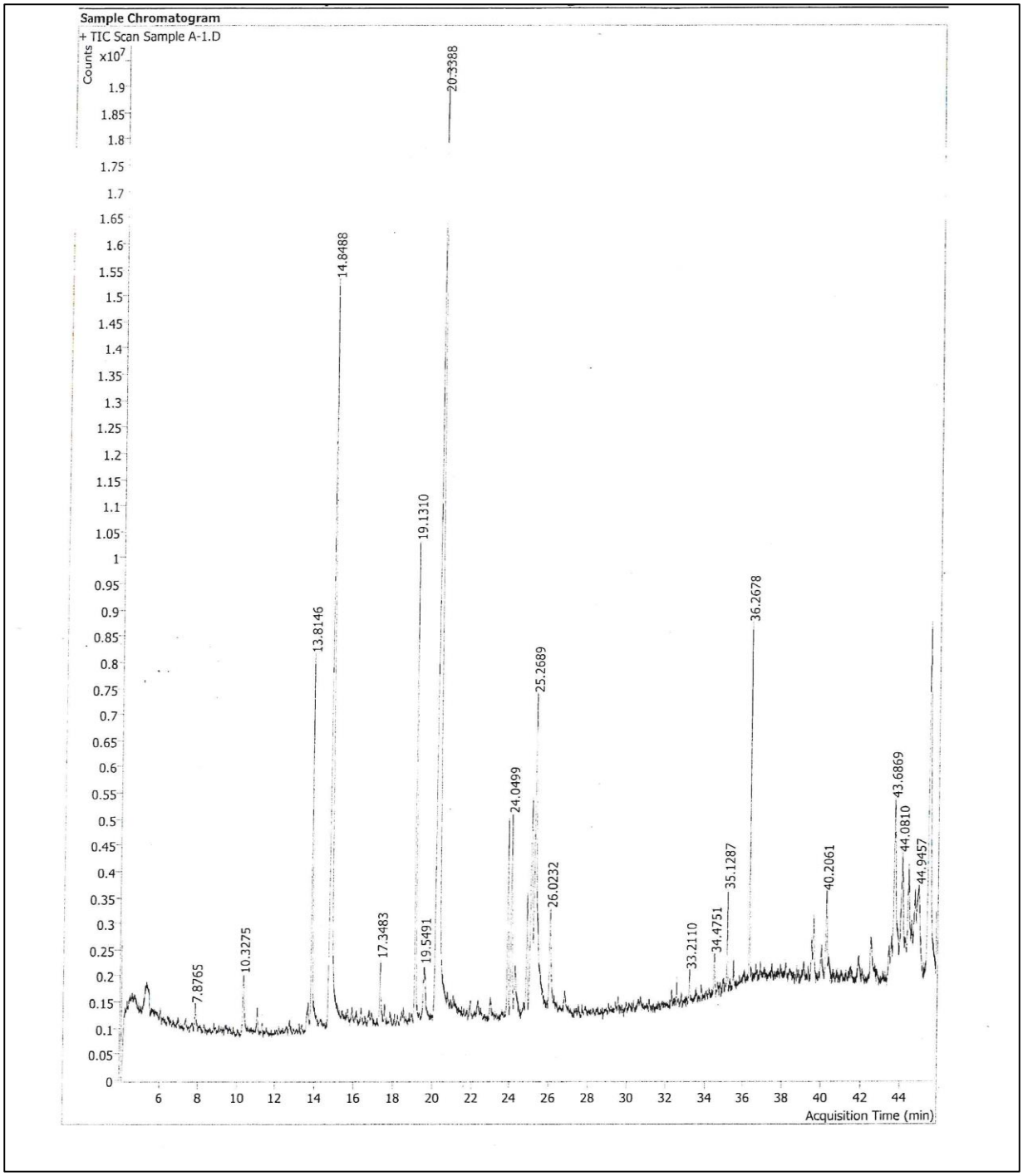

Figure 4.8: The Gas Chromatography –Mass Spectrometry chromatogram of *Ficus saussureana* showing the compounds detected

Table 4.11: Gas Chromatography Mass Spectrometry analysis of *Ficus saussureana* showing the chemical compounds identified

Retention Time (RT)	Compound Name	Molecular Formula	% Area	Biological activities	Reference
13.8146	Tridecanoic acid, 12-methyl-,methyl ester	$C_{15}H_{30}O_2$	7.67586	Not known	No report
14.8488	Tetradecanoic acid	$C_{14}H_{28}O_2$	29.8613	**Antioxidant**** Larvicidal and repellent activity, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017) (Vijayalingam & Rajesh, 2019)
19.5491	Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester	$C_{35}H_{68}O_5$	0.86858	Lubricants, emollients. Acidifiers, acidulants, and arachidonic acid inhibitors, increase aromatic amino acid decarboxylase activity and inhibit the production of uric acid	(Sivakumaran *et al*., 2019) (Vijayalingam & Rajesh, 2019)
24.2421	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5-(hydroxyimino)-25-(1-methylethyl)-,(6R,13R,25R)-	$C_{33}H_{46}ClNO_7$	1.17701	Not known	No report
25.0654	9, 12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	5.52681	**Antioxidant**** Antiarthritic, Antieczemic, Hepatoprotective, Anti-inflammatory, Anticane, Nematicide, anti-cancer, Insectifuge, Antihistaminic	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017)
25.2689	Oleic Acid	$C_{18}H_{34}O_2$	7.9841	**Antidiabetic****. Antibacterial	(Vassiliou *et al*., 2009)
35.1287	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	1.25243	**Antidiabetic**.** Anticancer, anti-inflammatory, antituberculosis, antimalarial, antimicrobial, antinociceptive	(Javaid *et al*., 2021) (Amankwaah *et al*., 2023)

Table 4.11: GC MS result of *Ficus saussureana* showing the chemical compounds identified (**Continued)**

36.2678	Squalene	$C_{30}H_{50}$	5.29692	**Hypoglycaemic, Antioxidant**.** Antibacterial, cancer-preventive, immunostimulant, Antitumor, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypolipidemic effects, Sedative action, Antihistaminic, Hepatoprotective activities, lipoxygenase-inhibitor, perfumery, pesticide, sunscreen	(Ingole, 2016) (Arora & Meena, 2017)
40.2061	Vitamin E	$C_{29}H_{50}O_2$	0.97196	**Antidiabetic and Antioxidant**.** Hypocholesterolemic, hepatoprotective Anti-inflammatory, anticancer, anti-coronary, antiulcerogenic, antidermatitic, antiaging, analgesic, antidermatitic, antileukemia, antitumor, vasodilator, antispasmodic, anti-bronchitic.	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017)
43.6869	17.beta.-Acetoxy-1',1'-dicarboethoxy-1.beta., 2.beta.-dihydrocycloprop[1,2]-5.alpha.-androst-1-en-3-one	$C_{28}H_{40}O_7$	4.41163	Not known	No report
44.081	7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-,2,4,7,10,11-pentaacetate	$C_{30}H_{44}O_{11}$	1.21305	Immune system enhancement and antimicrobial property	(Verma *et al*., 2021)
44.3854	.alpha.-Amyrin	$C_{30}H_{50}O$	0.38601	Antimicrobial, anti-inflammatory	(Priyadarshini *et al.*, 2017)

Table 4.11: GC MS result of *Ficus saussureana* showing the chemical compounds identified (**Continued**)

45.4779	Lupeol	$C_{30}H_{50}O$	7.54766	**Antihyperglycemic, Antioxidant**.** Anti-cancer, anti-arthritic, Anti-inflammatory, anti-mutagenic, Antiviral, Anti-HIV, Antitumor, Antihyperlipidemic, Anti-flue, Prostaglandin-synthesis and Topoisomerase II-inhibitor, Antimalarial, Pesticide, Cytotoxic	(Arora & Meena, 2017) (Perumal, *et al*., 2021)

4.2.3.2 Gas Chromatography Mass Spectrometry analysis of *Clerodendrum rutundifolium*

Clerodendrum rutundifolium methanolic extract showed the presence of thirty chemical compounds which included among them, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- **(49.37 %),** 5-Hydroxymethylfurfural **(5.04 %),** Octadecanoic acid **(4.29 %),** Tetradecanoic acid **(1.36 %),** Benzene, 1,2-dichloro- **(1.31 %),** Squalene **(1.23 %),** Phytol **(1.06 %),** 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,acetate, [R-[R*,R*-E)]]- **(1.03 %),** Stigmasterol **(0.94%),** Heptadecanoic acid **(0.72 %),** 9,12-Octadecadienoic acid (Z,Z)- **(0.58 %),** Betulinaldehyde **(0.49 %),** Vitamin E **(0.42 %),** .psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy **(0.29 %),** and 2-Methoxy-4-vinyl phenol **(0.23 %).** Details of all other compounds detected in *Clerodendrum rutundifolium* are presented in Table 4.12 and Figure 4.9 below.

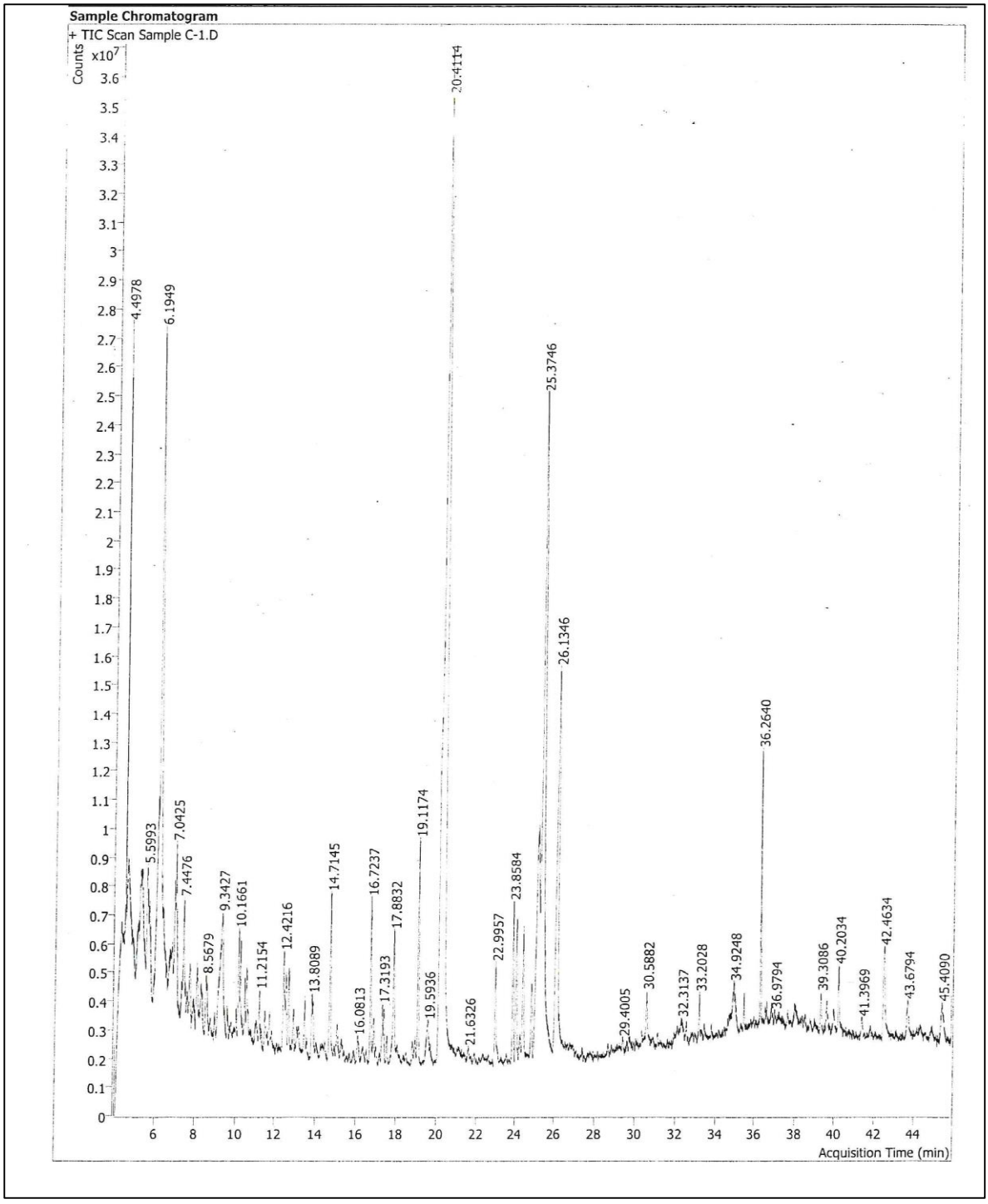

Figure 4.9: The Gas Chromatography Mass Spectrometry chromatogram of *Clerodendrum rutundifolium* showing the compounds detected

Table 4.12: Gas Chromatography Mass Spectrometry analysis of *Clerodendrum rutundifolium* showing the compounds identified

Retention Time (RT)	Compound Name	Molecular Formula	% Area	Biological activities	Reference
4.4978	Benzene, 1,2-dichloro-	$C_6H_4CI_2$	1.31	Fumigants, Insecticides	(Suganthy & Gajendra, 2020)
6.1949	5-Hydroxymethylfurfural	$C_6H_6O_3$	5.04	**Antioxidant ****. Anti-proliferative activity. It is reported to stop neuron apoptosis (Hai)	(Gu et al., 2013) (Nandhini *et al*., 2021)
6.979	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	0.23	**Antioxidant** ***. Antimicrobial & anticancer	(Kim et al., 2019) (Nandhini *et al*., 2021)
10.0578	1b,4a-Epoxy-2H- cyclopenta [3,4]cycloundec[1,2-b]oxiren-5(1aH)-one,2,7,10-tetrakis(acetyloxy)decahydro-3,6,8,8,10a-pentamethyl-	$C_{28}H_{38}O_{11}$	0.09	Not known	No report
10.1661	Homovanillyl alcohol	$C_9H_{12}O_3$	0.66	Not known	No report
10.2669	Dodecanoic acid	$C_{12}H_{24}O_2$	0.35	Antimicrobial	(Anbuselvi *et al*., 2012)
12.4216	Androsta-1,4-dien-3-one,17-hydroxy-17-methyl-, (17. alpha.)-	$C_{20}H_{28}O_2$	0.97	Not known	No report
12.8959	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{18}$	0.23	Not known	No report
13.4598	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	$C_{13}H_{22}O_2$	0.39	Not known	No report
13.8089	Tridecanoic acid , 12-methyl-,methyl ester	$C_{15}H_{30}O_2$	0.34	Not known	No report

Table 4.12: GC MS analysis of *Clerodendrum rutundifolium* showing the chemical compounds identified (**Continued**)

14.7145	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.36	**Antioxidant. **** Nematicidal and repellent activity, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic	(Arora & Meena, 2017) (Vijayalingam & Rajesh, 2019)
13.8631	2-Cyclohexen-1-one,3-(3-hydroxybutyl)-2,4,4-trimethyl-	$C_{13}H_{22}O_2$	0.31	Not known	No report
16.7237	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,acetate, [R-[R*,R*-E)]]-	$C_{22}H_{42}O_2$	1.03	Not known	No report
16.8861	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	$C_{34}H_{64}O_2$	0.02	Not known	No report
17.3193	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.25	Lubricants, Adhesive agents	(Arora & Meena, 2017)
22.9957	Heptadecanoic acid	$C_{17}H_{34}O_2$	0.72	**Antioxidant.** Anti-fungal, surfactant	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017)
24.2468	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	0.14	Anti-spasmodic and immune modulators	(Al-marzoqi *et al.*, 2016)
24.3943	Phytol	$C_{20}H_{40}O$	1.06	**Antioxidant**. ** Anticancer, Antimicrobial, Deceases the autoimmune response and ameliorates both acute and chronic phases of arthritis, Anti-inflammatory, Diuretic	(Arora & Meena, 2017) (Vijayalingam & Rajesh, 2019)

Table 4.12: GC MS analysis of *Clerodendrum rutundifolium* showing the chemical compounds identified (**Continued**)

25.0983	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	0.58	**Antioxidant. **** Hepatoprotective, Hypocholesterolemic, antiarthritic, anti-inflammatory, anti-arteriosclerotic, anti-anaphylactic, antieczemic, Cancer preventive, anti-prostatic, Metastatic, Nematicide	(Ponnamma & Manjunath, 2012)
25.3746	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	49.37	**Antidiabetic and Antihypertensive **.** Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, insectifuge	(Vijayalingam & Rajesh, 2019) (Rao *et al.*, 2019) (Kavitha, 2021)
26.1346	Octadecanoic acid	$C_{18}H_{36}O_2$	4.29	Hypocholesterolemic, Antimicrobial, Antifungal, Antitumor, Antibacterial, Cosmetic, Flavor, Lubricant, Perfumery, Propecic, Suppository	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017)
30.5882	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	0.29	**Antioxidant.** ** Nutrient, Cytotoxic activity	(Kavitha, 2021)

Table 4.12: GC MS analysis of *Clerodendrum rutundifolium* showing the chemical compounds identified (**Continued**)

32.3137	4H- Cyclopropa[5',6']benz[1'2':7,8]azuleno[5,6-b]oxiren-4-one, 8,8a-bis(acetyloxy)-2a-(acetyloxy)methyl]-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro- 6b- hydroxy-3a-methoxy-1,1,5,7-tetramethyl-[1aR(1a.alpha.,1b.beta.,1c.alpha.,2a.alpha.,3a.alpha.,6a.alp	$C_{27}H_{36}O_{10}$	0.02	Not known	No report
33.8191	2,4,6,8,10-Tetradecapentaenoic acid, 9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1H-cyclopropa[3,4]benz[1,2-e]azulen-9-yl ester, [1aR-(1a.alpha.,1b.beta.,7a.alpha.,7b.alpha.,8.alp	$C_{36}H_{46}O_8$	0.05	Not known	No report
36.264	Squalene	$C_{30}H_{50}$	1.23	**Antioxidant.** ** Antibacterial, antitumor, chemo-preventive, immunostimulant, lipoxygenase-inhibitor, perfumery, pesticide, sunscreen	(Ingole, 2016) (Zayed & Samling, 2016)
36.5778	Hexadecanoic acid, 1a,2,5,5a,6,9, 10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-[1a.alpha.,2.alpha.,5.beta.,5a.beta.,8a.alpha.,9.alpha., 10a.alpha.)]-	$C_{36}H_{56}0_6$	0.06	Not known	No report
39.9549	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,3,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-,[1aR-(1a.alpha.,1b.beta.,2,alpha.,3.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha	$C_{28}H_{37}ClO_{11}$	0.14	Not known	No report

Table 4.12: GC MS analysis of *Clerodendrum rutundifolium* showing the chemical compounds identified (**Continued**)

40.2034	Vitamin E	$C_{29}H_{50}O_2$	0.42	**Antidiabetic, Antioxidant. **** Hypocholesterolemic, anti-inflammatory, antiaging, anti-coronary, antiulcer, hepatoprotective, antidermatitic, antileukemia, antiaging, anti-alzheimeran, antitumor, anticancer, immunostimulant, analgesic, vasodilator, antispasmodic, anti-bronchitic.	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017)
42.4634	Stigmasterol	$C_{29}H_{48}O$	0.94	**Hypoglycemic effects, antioxidant. **** Antihypercholesterolemic, thyroid inhibitory, antiperoxidative, Precursor of progesterone, acts as intermediate in the biosynthesis of androgens and estrogens, anti-osteoarthritic, cytotoxic, antitumor, antimutagenic, anti-inflammatory, analgesic	(Sivakumaran et al., 2019) (Perumal, *et al.*, 2021)
45.409	Betulinaldehyde	$C_{30}H_{48}O_2$	0.49	Anti-tumor	(Huang *et al*., 2023)

4.2.3.3 Gas Chromatography Mass Spectrometry analysis of *Microglossa pyrifolia*

GCMS analysis of *Microglossa pyrifolia* methanolic extract displayed the presence of thirty compounds, which include Benzene,1,2-dichloro- (**15.70 %),** n-Hexadecanoic acid (**14.50 %),** 1,2-Benzenedicarboxylic acid (**8.50 %),** 5-Hydroxymethylfurfural (**6.10 %),** Catechol (**5.40 %),** Tetradecanoic acid (**4.70 %),** Hexadecanoic acid, methyl ester (**3.50 %), and** Oleic Acid (**2.00 %**) 1,2-Benzenediol,3-methyl- **(1.80 %),** Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl- **(1 %)**, 1-Heptatriacotanol **(0.70 %)**, and psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- **(0.40 %).** Details of all other compounds in *Microglossa pyrifolia* are presented in Table 4.13.

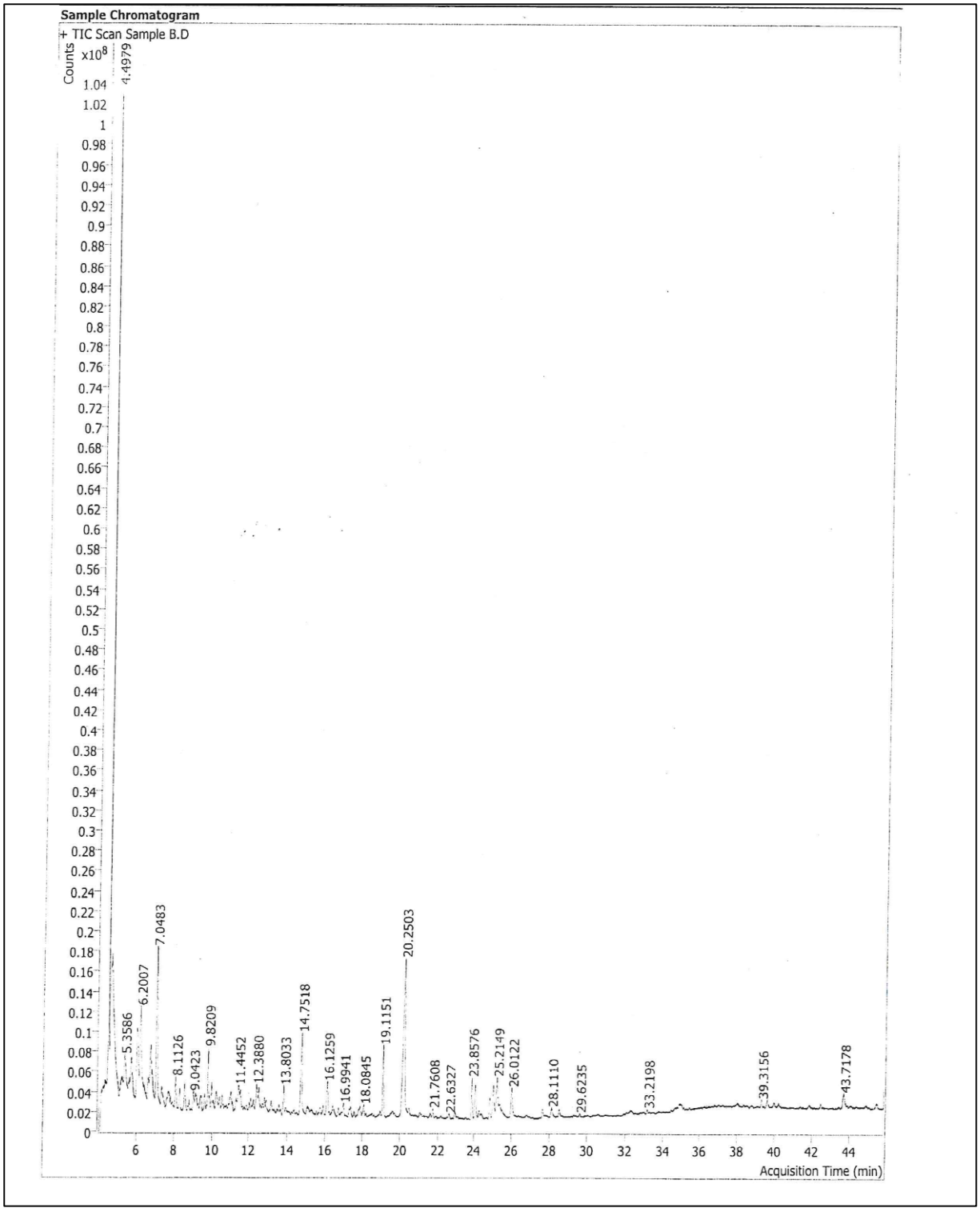

Figure 4.10: The Gas Chromatography Mass Spectrometry chromatogram of *Microglossa pyrifolia* showing the compounds detected

Table 4.13: Gas Chromatography Mass Spectrometry analysis of *Microglossa pyrifolia* showing the chemical compounds identified

Retention Time	Compound Name	Molecular Formula	% Area	Biological activities	Reference
4.4979	Benzene,1,2-dichloro-	$C_6H_4CI_2$	15.7	Fumigants, Insecticides	(Suganthy & Gajendra, 2020)
5.7133	Cyclobutane-1,1-dicarboxamide, NN'-di-benzoyloxy-	$C_{20}H_{18}N_2O_6$	0.9	Anaphylactic, antitumor, arylamine-n-acetyltransferase-inhibitor, downregulate nuclear and cytosol androgens, GABAergic, increase natural killer cell activity, Myo-neuro Stimulant, NADH oxidase inhibitor, NADH-ubiquinone-oxidoreductase-inhibitor, anticancer, increase NK cell activity, inhibit TNF activity, decrease norepinephrine production	(Prabhu *et al*., 2020)
6.0177	Catechol	$C_6H_6O_2$	5.4	Not known	No report
6.2007	5-Hydroxymethylfurfural	$C_6H_6O_3$	6.1	**Antioxidant **.** Anti-proliferative activity. It is reported to stop neuron apoptosis (Hai)	(Gu et al., 2013) (Nandhini *et al*., 2021)
6.7645	1,2-Benzenediol,3-methyl-	$C_7H_8O_2$	1.8	**Antioxidant activity ****	(Naksing *et al*., 2021)
7.0483	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	8.5	Antimicrobial and Antifouling	(Ingole, 2016)
8.1126	1,3-Isobenzofuradione, 4-methyl-	$C_9H_6O_3$	1.3	Not known	No report
8.331	Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	$C_{15}H_{24}$	1	**Antioxidant**.** Antihyperlipidemic, Antimicrobial, anti-inflammatory	(Prakasia & Nair, 2015)
8.57	4-Methylphthalic anhydride	$C_9H_6O_3$	1.3	Not known	No report

Table 4.13: GC MS analysis of *Microglossa pyrifolia* showing the chemical compounds identified (**Continued)**

9.0423	Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene-	$C_{15}H_{24}$	0.2	Not known	No report
9.8209	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7dimethyl-1-(1-methylethyl)-,(1S-cis)-	$C_{15}H_{24}$	2.4	Not known	No report
10.0132	Ylangene	$C_{15}H_{24}$	1.1	Anti-inflammatory	Mohammed *et al.*, 2022)
11.0176	1-Heptatriacotanol	$C_{37}H_{76}O$	0.7	**Antioxidant**.** Anti-hypercholesterolemic, Antibacterial lupeol, Anticancer, anti-inflammatory properties, antimalarial, Anti-flu, antiviral, antiprotozoal, anti-peroxidant, antitumor, enzyme inhibitor	(Kotteswari *et al*., 2020)
12.08	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]91ndecane-5.beta.-ol	$C_{15}H_{24}O$	0.3	Not known	No report
12.5106	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1carboxaldehyde	$C_{23}H_{32}O$	0.2	Not known	No report
13.1516	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-,[1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	$C_{20}H_{28}O_6$	0.5	Not known	No report
14.3559	Rhodopin	$C_{40}H_{58}O$	0.2	**Antioxidant ****	(Tamilselvan & Rajeswari, 2015)
14.7518	Tetradecanoic acid	$C_{14}H_{28}O_2$	4.7	**Antioxidant.** ** Hypercholesterolemic, Chemo-preventive, Larvicidal and repellent activity, Lubricant, Cosmetic	(Ponnamma & Manjunath, 2012) (Arora & Meena, 2017)

Table 4.13: GC MS analysis of *Microglossa pyrifolia* showing the chemical compounds identified (**Continued)**

16.1259	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	2.1	Ethanol absorption inhibitor, Ethanolytic	(Perumal *et al*., 2021)
19.1151	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	3.5	**Antioxidant.** ** hypocholesterolemic, Antibacterial and Antifungal	(Ali *et al.*, 2017)
20.2503	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	14.5	**Antioxidant.** ** Anti-inflammatory, Anti-cancer, Hemolytic, Antifibrinolytic, Pesticide, Flavor, 5-Alpha reductase inhibitor, Lubricant, and Anti-alopecic	(Nandhini *et al*., 2021)
23.8576	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	2.2	Cancer preventive, hypocholesterolemic, Anti-inflammatory anti-genic, anti-coronary, antiarthritic, anti-histaminic, insectifuge, nematicide, anti-androgenic	(Khanday & Sharma, 2021)
24.0443	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	2	Antimicrobial, Nematicidal	(Ali *et al.*, 2017)
24.2385	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.3	Not known	No report
25.0152	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	1.7	Antimicrobial	(Uka *et al.*, 2022)

Table 4.13: GC MS analysis of *Microglossa pyrifolia* showing the chemical compounds identified (**Continued)**

25.2149	Oleic Acid	$C_{18}H_{34}O_2$	2	**Antidiabetic ****. Antibacterial	(Vassiliou *et al*., 2009)
26.0122	Octadecanoic acid	$C_{18}H_{36}O_2$	2.1	Antimicrobial Antifungal, Antitumor, Antibacterial	(Arora & Meena, 2017)
28.111	1H,Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-,5,9,9a-triacetate,[1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	$C_{26}H_{36}O_8$	0.6	Not known	No report
39.6257	.psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	0.4	**Antioxidant **.** Nutrient, Cytotoxic activity	(Kavitha, 2021)
43.7178	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol,3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-,5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	$C_{28}H_{38}O_{10}$	0.5	Not known	No report

4.3 TOXICITY ASSESSMENT OF SELECTED MEDICINAL PLANTS

This last section of the presentation of the results focuses on toxicity analysis, subsection one presents weight changes, haematology, and biochemical results for Acute toxicity. Subsection two presents weight changes, haematology, and biochemical results for Subacute toxicity.

4.3.1 Acute toxicity

4.3.1.1 Effect of extracts on behaviour and weight of experimental animals

Physical examination of the rats revealed no discernible effects on observed parameters such as skin and fur condition, eye appearance, discharges, presence of diarrhoea, coma, or salivation. However, polyuria was observed in groups treated with *Clerodendrum rutundifolium* (CR) and *Microglossa pyrifolia* (MP) extracts. Furthermore, the administration of the extracts did not result in any mortality and all rats survived up to the last day of experiment termination. Table 4.14 below shows the effects of each extract on the weight of rats. The findings indicate that the administration of *Ficus saussureana* (FS), CR, and MP extracts reduced the weight gain of rats when compared to that of the control group. Furthermore, it was observed that the administration of 5000 mg/kg of FS and CR extracts significantly caused weight loss in the rats treated. Albeit the lower dose of 2000 mg/kg of these two extracts did not cause weight loss but the mean weight gain was significantly lower when compared to the control group. This was also similar for both doses of MP extract.

Table 4.14: Alterations in the body weight of rats administered with *Ficus*, *Clerodendrum*, and *Microglossa* extracts

	CONTROL	**FS EXTRACT**		**CR EXTRACT**		**MP EXTRACT**	
	0 mg/kg	**2000 mg/kg**	**5000 mg/kg**	**2000 mg/kg**	**5000 mg/kg**	**2000 mg/kg**	**5000 mg/kg**
Day 0	84.56 ± 1.57	96.23 ± 0.38	95.73 ± 1.27	98.03 ± 0.98	101.63 ± 0.84	92.06 ± 1.01	102.43 ± 1.02
Day 7	99.03 ± 1.28	97.33 ± 3.41	96.73 ± 4.2	105.73 ± 0.92	106.86 ± 4.11	87.7 ± 3.85	106.9 ± 4.45
Day 14	113.06 ± 4.64	97.06 ± 3.07	94.8 ± 4	107.33 ± 0.58	101 ± 6.94	93.96 ± 3.53	110.56 ± 4.16
Mean weight change	**28.5 ± 3.2**	**0.83 ± 2.69****	**-0.93 ± 2.84*****	**9.3 ± 0.4***	**-0.63 ± 7.69*****	**1.9 ± 4.4****	**8.13 ± 3.67***

FS-*Ficus saussureana,* **CR**-*Clerodendrum rutundifolium,* **MP-** *Microglossa pyrifolia*. * P < 0.05, ** P < 0.01, *** P < 0.001

4.3.1.2 Effect of extracts on haematological parameters of rats

After 14 days of the experiment, blood was collected for haematological analysis and the results are presented in Table 4.15. Administration of FS extract at 5000 mg/kg dose to the rats significantly elevated the WBC ($P<0.01$), in contrast, the value was significantly lower in the rats that received a 5000 mg/kg dose of MP ($P<0.05$). A significant reduction in the NEUT value was observed across all treatment groups in comparison to the control group. LYMP was significantly higher in the groups administered with 2000mg/kg and 5000mg/kg doses of FS extract and 5000mg/kg dose of CR extract. Similarly, the EO value was significantly higher in the CR and MP groups both at 5000mg/kg dose. There was also a remarkable change in the BASO values in all the treatment groups when compared to the control. HCT and HGB values were significantly higher in the rats treated with 5000 mg/kg dose of CR and MP extracts. MCV was significantly lower in rats that received a 5000 mg/kg dose of FS. CR extract at both doses significantly elevated the PLT value, however there was a decrease in the value in rats that received a 5000 mg/kg dose of MP extract. MPV was significantly lower in rats that received a 5000mg/kg dose of CR extract. PLCR was significantly lower in all groups except in rats treated with a 2000mg/kg dose of FS. However, the FS, CR, and MP extracts at both doses caused no significant changes on the other tested haematological parameters such as IG, RBC, MCH, MCHC, RDW-CV, PDW, and PCT (P-value >0.05).

Table 4.15: Response of haematological parameters in rats administered with *Ficus*, *Clerodendrum*, and *Microglossa* extracts

Haematological parameters	CONTROL	FS EXTRACT		CR EXTRACT		MP EXTRACT	
		2000 mg/kg	5000 mg/kg	2000 mg/kg	5000 mg/kg	2000 mg/kg	5000 mg/kg
WBC (10^3/µL)	5.06 ± 0.18	6.08 ± 0.25	7.37 ± 0.32**	6.28 ± 0.97	6.74 ± 0.07	6.68 ± 0.06	3.28 ± 0.09*
NEUT (10^3/µL)	2.73 ± 0.18	0.44 ± 0****	0.63 ± 0.01****	2 ± 0.16**	1.88 ± 0.17**	1.2 ± 0.09****	0.94 ± 0.02****
LYMP (10^3/µL)	3.11 ± 0.55	5.09 ± 0.33*	6.68 ± 0.63***	3.66 ± 0.27	5.31 ± 0.5**	4.21 ± 0.11	2.22 ± 0.01
MONO (10^3/µL)	0.41 ± 0.02	0.59 ± 0.24	0.77 ± 0.09	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0	1 ± 0**
EO (10^3/µL)	0.07 ± 0	0.04 ± 0.01	0.05 ± 0	0.03 ± 0.01	0.15 ± 0.01***	0.04 ± 0	0.12 ± 0***
BASO (10^3/µL)	0.19 ± 0	0.02 ± 0****	0.01 ± 0****	0.14 ± 0.01*	0.31 ± 0.02****	0.14 ± 0*	0.05 ± 0****
IG (10^3/µL)	0.01 ± 0	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.01 ± 0	0.01 ± 0
RBC (10^3/µL)	7.72 ± 0.31	8.07 ± 0.07	8.31 ± 0.01	7.09 ± 0.77	7.87 ± 0.42	7.3 ± 0.56	8.31 ± 0.03
HGB (g/dL)	12.76 ± 0.23	12.7 ± 0	12.56 ± 0.12	12 ± 0.5	15.26 ± 0.61***	12.4 ± 0.2	14.53 ± 0.23*
HCT (%)	41.76 ± 0.72	43.23 ± 0.2	42.06 ± 0.31	42.8 ± 0.66	50.8 ± 0.95****	43.8 ± 0.46	48.33 ± 0.2****
MCV (fL)	55.5 ± 1.15	54.5 ± 0.45	49.7 ± 0.81*	58.63 ± 1.73	56.06 ± 1.04	56.56 ± 1.02	56.16 ± 1.15
MCH (pg)	15.83 ± 0.03	15.93 ± 0.17	14.76 ± 0.4	15.47 ± 0.82	16.56 ± 0.13	16.56 ± 0.18	16.66 ± 0.06
MCHC (g/dL)	29.23 ± 0.31	29.43 ± 0.65	29.16 ± 0.12	30.43 ± 1.79	29.13 ± 0.03	28.46 ± 0.44	29.13 ± 0.03
RDW-SD (fL)	27.03 ± 1.73	26.66 ± 0.44	23.63 ± 0.52	25.8 ± 0.45	26.23 ± 0.74	25.4 ± 0.73	32.33 ± 0.18**
RDW-CV (%)	16.4 ± 0.65	17.56 ± 0.32	17.63 ± 0.32	16.03 ± 0.58	17.13 ± 0.57	18.2 ± 0.05	16.1 ± 0.86
PLT (10^3/µL)	517 ± 3.21	507.3 ± 14.6	517 ± 4.16	704 ± 15.9****	617 ± 10.11****	530.3 ± 8.08	473 ± 8.62*
PDW (fL)	7.56 ± 0.72	7.86 ± 0.38	7.66 ± 0.21	7.73 ± 0.72	7.4 ± 0.55	6.96 ± 0.12	6.86 ± 0.14
MPV (fL)	7.16 ± 0.08	7.23 ± 0.08	7.1 ± 0.05	7 ± 0.05	6.4 ± 0.3**	7.26 ± 0.03	7.1 ± 0.05
P-LCR (%)	9.8 ± 1.72	6.7 ± 0.32	6.13 ± 0.23*	5.13 ± 0.88**	4 ± 0.2***	5.9 ± 0.17*	6.13 ± 0.33*
PCT (%)	0.41 ± 0.03	0.38 ± 0	0.35 ± 0.03	0.47 ± 0	0.46 ± 0.02	0.47 ± 0.03	0.4 ± 0.07

WBC-White Blood Cells, **NEUT**-Neutrophils, **LYMPH**-Lymphocyte, **MONO**-Monocytes, **EO**-Eosinophils, **BASO**-Basophils, **IG**-Immunoglobulin, **RBC**-Red Blood Cells, **HGB**-Haemoglobin, **HCT**-Haematocrit, **MCV**-Mean Corpuscular Volume, **MCH**-Mean Corpuscular Haemoglobin, **MCHC**-Mean Corpuscular Haemoglobin Concentration, **RDW-SD**-Standard Deviation in Red Cell Distribution Width, **RDW-CV**-Coefficient of Variation in Red Cell Distribution Width, **PLT**-Platelet, **PDW**-Platelet Distribution Width, **MPV**-Mean Platelet Volume, **P-LCR**-Platelet Larger Cell Ratio, and **PCT**-Procalcitonin. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

4.3.1.3 Effect of extracts on biochemical results

Table 4.16 provides the findings regarding the impact of extracts on the biochemical parameters, including the Liver Function Test (LFT) and Renal Function Test (RFT), within the experimental groups. Results revealed a significant increase in ALB in rats administered with a 5000 mg/kg dose of MP when compared to that of a control group. The administration of FS extract at 5000mg/kg dose and MP extract at both 2000 and 5000 mg/kg doses significantly elevated the ALT values of the rats when compared to the control. AST and GGT values were significantly higher in FS and CR groups treated with 5000 mg/kg dose. FS extract at 5000 mg/kg dose elevated CRE significantly when compared to that of the control group. ALP value was significantly higher in rats treated with FS and MP extracts at both doses and significantly lower in rats treated with a 2000mg/kg dose of CR extract. However, there was no significant change in other tested biochemical parameters such as TP, BILD, UREA, $Na^{+,}$ and K^{+} in rats treated with FS, CR, and MP extracts.

Table 4.16: Response of biochemical parameters in rats administered with *Ficus*, *Clerodendrum*, and *Microglossa* extracts

Biochemical parameters	**CONTROL**	**FS EXTRACT**		**CR EXTRACT**		**MP EXTRACT**	
		2000 mg/kg	**5000 mg/kg**	**2000 mg/kg**	**5000 mg/kg**	**2000 mg/kg**	**5000 mg/kg**
ALB (g/L)	35.1 ± 0.66	30.23 ± 0.18	31.7 ± 0.65	29.06 ± 0.96	34.9 ±0.81	29.26 ± 1.88	43.26 ± 3.38*
TP (g/L)	67.4 ± 3.3	56.93 ± 6.03	65.33 ± 2.43	66.03 ± 5.71	57.36 ±2.94	57.83 ± 2.74	63.33 ± 2.33
ALT (U/L)	78.66 ± 1.68	79.96 ± 2.9	107.8 ± 3.04***	67.2 ± 3.21	83.03 ±4.62	141.06 ± 4.9****	130.7 ± 2.56****
AST (U/L)	168.7 ± 5.14	153.86 ± 3.97	213.5 ± 9.77***	154.03 ± 3.47	108.63 ±6.56****	149.63 ± 2.82	179.2 ± 1.41
GGT (U/L)	6.26 ± 0.37	5.33 ± 0.88	10.56 ± 0.99*	6.4 ± 0.3	9.8 ±0.92*	6.33 ± 1.45	6 ± 0
BILT (umol/L)	2.4 ± 0.19	2.53 ± 0.31	1.73 ± 0.17	1.56 ± 0.12	1.76 ±0.61	2.16 ± 0.03	1.73 ± 0.17
BILD (umol/L)	0.2 ± 0.05	0.32 ± 0.05	0.36 ± 0.08	0.1 ± 0.05	0.36 ±0.06	0.4 ± 0.1	0.36 ± 0.03
CRE (umol/L)	32.73 ± 0.93	27.5 ± 2	45.26 ± 4.57**	37.86 ± 2.11	33.73 ±1.96	39.66 ± 1.2	39.33 ± 0.88
UR (mmol/L)	5.2 ± 0.11	4.8 ± 0.3	5.1 ± 0.25	5.06 ± 0.4	5.1 ±0.36	4.76 ± 0.46	5.16 ± 0.29
ALP (U/L)	106.53 ± 3.09	142.33 ± 2.4****	171.4 ± 4.82****	90.96 ± 2.13*	102.5 ±1.89	126.03 ± 1.7**	123.3 ± 3.28**
Na^+ (mmol/L)	143.33 ± 1.33	144 ± 1.15	146.6 ± 1.59	145.8 ± 3.8	146.2 ±1.11	141.66 ± 0.88	143 ± 0.57
K^+ (mmol/L)	6.11 ± 0.21	5.36 ± 0.14	6.05 ± 0.2	4.56 ± 0.43	5.47 ±0.23	5.5 ± 0.1	5.86 ± 0.12
Cl^- (mmol/L)	101.9 ± 1.26	103.4 ± 0.89	103.16 ± 1.09	104.06 ±0.67	104.83 ± 2.51	104.26 ± 0.08	103.6 ± 0.79

ALB-Albumin, **TP**-Total protein, **ALT**-Alanine aminotransferase, **AST**-Aspartate aminotransferase, **GGT**-Gamma-Glutamyl Transferase, **BILT**-Total Bilirubin, **BILD**-Direct Bilirubin, **CRE**-Creatinine, **UR**-UREA **ALP**-Alkaline phosphatase, Sodium (**Na+**), Potassium (**K+**) and Chloride (**Cl-**). ***** P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

4.3.2 Subacute toxicity

4.3.2.1 Effect of extracts on weight of experimental animals

Table 4.17 shows the effects of each extract on the weight changes of rats. The results revealed that the repeated administration of extracts reduced the mean weight gain of rats when compared to the mean weight change of the control group except for the rats administered with 500 mg/kg dose of FS. Generally, FS extract at both tested doses did not cause weight loss and the mean weight change was not significantly different from that of the control group ($P>0.05$). This was also similar for the lowest dose of MP extract, although the mean weight gain of rats treated with 1000 mg/kg (high dose) was significantly lower when compared to control ($P<0.05$). A significant weight loss was observed in rats treated with both doses of CR extract ($P<0.0001$). The repeated dose treatment did not result in any mortality and all rats survived up to the end of the experiment. Similarly, there were also no changes in the overall behaviour of the rats however polyuria was also observed in the rats treated with CR and MP extracts at both doses.

Table 4.17: Alterations in the body weight of rats administered with repeated doses of *Ficus*, *Clerodendrum*, and *Microglossa* extracts

	CONTROL	**FS EXTRACT**		**CR EXTRACT**		**MP EXTRACT**	
	0 mg/kg	**500 mg/kg**	**1000 mg/kg**	**500 mg/kg**	**1000 mg/kg**	**500 mg/kg**	**1000 mg/kg**
DAY 0	81.3 ± 0.47	85.55 ± 0.81	92.47 ± 2.59	96 ± 0.82	104.46 ± 0.64	94.04 ± 1.9	94.77 ± 1.01
DAY 7	84.97 ± 0.57	93.12 ± 2.76	97.7 ± 2.76	93.52 ± 1.42	101.64 ± 1.69	95.9 ± 2.14	95.77 ± 1.05
DAY 14	90.87 ± 1.92	101.02 ± 4.19	102.72 ± 4.16	96.02 ± 2.71	103.8 ± 1.8	101.34 ± 3.08	102.15 ± 1.01
DAY 21	104.95 ± 3.83	113.15 ± 5.89	109.4 ± 8.34	90.1 ± 4.49	102.18 ± 1.98	110.12 ± 3.75	110.27 ± 2.44
DAY 28	113.22 ± 3.52	123.3 ± 6.62	112.72 ± 9.97	90.76 ± 5.22	103.9 ± 2.67	118.82 ± 3.94	109.07 ± 3.09
Mean Weight change	**31.92 ± 3.6**	**37.75 ± 5.84**	**20.25 ± 7.55**	**-5.24 ± 4.84******	**-0.56 ± 2.34******	**24.78 ± 2.25**	**14.3 ± 3.34***

* P < 0.05, **** P < 0.0001

4.3.2.2 Effect of *Ficus*, *Clerodendrum*, and *Microglossa* extracts on haematological parameters

The results revealed changes in some of the haematological parameters after repeated dosage administration. WBC and LYMPH values were significantly higher in groups administered with a 1000 mg/kg dose of FS and MP extracts and in groups treated with 500mg/kg and 1000mg/kg doses of CR. The repeated administration of 1000mg/kg dose of FS to the rats significantly elevated the MONO while 1000mg/kg dose of CR extract elevated the BASO. The extract of FS at 1000 mg/kg dose, and CR at both 500 mg/kg and 1000 mg/kg doses significantly elevated the RBC of the treated rats when compared to the control group. HGB values of the rats treated with FS and CR extracts were significantly elevated. HCT value was significantly higher in the groups treated with 1000mg/kg of FS extract, 500mg/kg and 1000mg/kg of CR extract, and 1000mg/kg dose of MP extract. The administration of a 1000mg/kg dose of FS extract to the rats significantly elevated the PLT and significantly decreased the MPV and P-LCR. Repeated administration of FS, CR, and MP to the rats at the two dose levels did not significantly change the other tested haematological parameters such as NEUT, EO, IG, MCV, MCH, RDW-SD, RDW-CV, PDW, and PCT ($P>0.05$) (Table 4.18).

Table 4.18: Response of haematological parameters in rats administered with repeated doses of *Ficus*, *Clerodendrum*, and *Microglossa* extracts

Haematological parameters	CONTROL	FS EXTRACT		CR EXTRACT		MP EXTRACT	
		500 mg/kg	1000 mg/kg	500 mg/kg	1000 mg/kg	500 mg/kg	1000 mg/kg
WBC (10^3 /µL)	6.37 ± 0.71	6.31 ± 0.2	15.09 ± 1.71****	14.18 ± 0.84****	12.95 ± 1.21***	8.29 ± 0.48	12.63 ± 0.86***
NEUT (10^3 /µL)	2.25 ± 0.52	1.21 ± 0.32	1.02 ± 0.23	3.46 ± 0.39	3.06 ± 0.48	1.18 ± 0.31	1.17 ± 0.17
LYMPH (10^3 /µL)	3.23 ± 0.37	4.61 ± 0.53	12.14 ± 1.61****	9.28 ± 0.8**	9.04 ± 1.2**	6.18 ± 0.75	10.58 ± 0.82***
MONO (10^3 /µL)	0.25 ± 0.04	0.44 ± 0.13	1.57 ± 0.71*	0.97 ± 0.32	0.58 ± 0.12	0.56 ± 0.23	0.69 ± 0.16
EO (10^3 /µL)	0.05 ± 0.02	0.07 ± 0.04	0.18 ± 0.1	0.04 ± 0.02	0.13 ± 0.01	0.09 ± 0.02	0.18 ± 0.02
BASO (10^3 /µL)	0.16 ± 0.01	0.22 ± 0.05	0.17 ± 0.07	0.34 ± 0.02	0.42 ± 0.1*	0.2 ± 0.06	0.24 ± 0.01
IG (10^3 /µL)	0.01 ± 0	0 ± 0	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0	0.03 ± 0.02	0.04 ± 0.01
RBC (10^3 /µL)	7.71 ± 0.03	8.61 ± 0.38	8.84 ± 0.25*	9.25 ± 0.13**	9.28 ± 0.45**	8.42 ± 0.28	8.2 ± 0.15
HGB (g/dL)	12.75 ± 0.12	14.32 ± 0.43*	14.52 ± 0.3*	14.82 ± 0.13**	14.85 ± 0.74**	13.3 ± 0.42	13.8 ± 0.23
HCT (%)	45.17 ± 0.83	49.35 ± 1.24	51.62 ± 1.26**	50.82 ± 0.74*	52.52 ± 2.04**	48.75 ± 1.15	50.3 ± 1.2*
MCV (fL)	58.6 ± 0.88	57.42 ± 1.25	58.4 ± 0.93	54.97 ± 1.54	56.65 ± 0.6	57.9 ± 1.08	61.02 ± 0.53
MCH (pg)	16.62 ± 0.11	16.65 ± 0.27	16.45 ± 0.27	16.02 ± 0.3	16 ± 0.1	16.57 ± 0.2	16.82 ± 0.08
MCHC (g/dL)	28.25 ± 0.29	29.02 ± 0.17	28.15 ± 0.23	29.22 ± 0.59	28.25 ± 0.34	27.27 ± 0.32	27.47 ± 0.2
RDW-SD (fL)	29.32 ± 1.27	26.15 ± 0.75	27.97 ± 0.63	25.92 ± 0.61	25.85 ± 0.36	30.4 ± 2.65	32.55 ± 0.51
RDW-CV (%)	16.52 ± 0.53	16.5 ± 0.88	17.37 ± 0.29	18.22 ± 0.43	17.55 ± 0.68	18.17 ± 0.72	17.92 ± 0.27
PLT (10^3 /µL)	568.9 ± 47.15	634.75 ± 72.23	829.62 ± 19.2**	643.5 ± 22.39	616 ± 3.67	484 ± 73.38	705.75 ± 53.93
PDW (fL)	8.3 ± 0.63	8.6 ± 0.23	8.22 ± 0.3	8.75 ± 0.15	8.72 ± 0.27	9.37 ± 0.24	9.1 ± 0.24
MPV (fL)	8.15 ± 0.02	7.82 ± 0.08	7.55 ± 0.09*	7.87 ± 0.06	7.82 ± 0.2	8.1 ± 0.14	8.25 ± 0.15
P-LCR (%)	12.25 ± 0.49	10.07 ± 0.53	8.12 ± 0.64**	10.9 ± 0.52	9.42 ± 0.86	11.97 ± 1.08	13.37 ± 1.12
PCT (%)	0.49 ± 0.03	0.55 ± 0.09	0.48 ± 0.05	0.47 ± 0.03	0.47 ± 0	0.38 ± 0.05	0.57 ± 0.04

WBC-White Blood Cells, **NEUT**-Neutrophils, **LYMPH**-Lymphocyte, **MONO**-Monocytes, **EO**-Eosinophils, **BASO**-Basophils, **IG**-Immunoglobulin, **RBC**-Red Blood Cells, **HGB**-Haemoglobin, **HCT**-Haematocrit, **MCV**-Mean Corpuscular Volume, **MCH**-Mean Corpuscular Haemoglobin, **MCHC**-Mean Corpuscular Haemoglobin Concentration, **RDW-SD**-Standard Deviation in Red Cell Distribution Width, **RDW-CV**-Coefficient of Variation in Red Cell Distribution Width, **PLT**-Platelet, **PDW**-Platelet Distribution Width, **MPV**-Mean Platelet Volume, **P-LCR**-Platelet Larger Cell Ratio, and **PCT**-Procalcitonin. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

4.3.2.3 Effect of *Ficus*, *Clerodendrum*, and *Microglossa* extracts on biochemical parameters

Results revealed changes in some of the analysed biochemical blood parameters. TP was significantly elevated in the group treated with a 1000 mg/kg dose of CR extract (Table 4.19). ALT value was higher in all the treated groups but was only significant in the rats treated with CR and MP extracts. Repeated dosages of 1000 mg/kg of FS and MP extracts significantly elevated the AST of the treated rats. BILT was highly elevated in the group that received a 500 mg/kg dose of CR extract. The administration of 1000 mg/kg of FS and MP extracts to the rats led to a significant decrease in the blood UREA levels. CRE and K^+ were high in all experimental groups but were only significant in the group administered with 1000 mg/kg of FS extract. Nevertheless, the repeated doses of FS, CR, and MP extracts to the rats did not significantly alter the other tested biochemical parameters such as ALB, BILD, CREJ, $Na^{+,}$ and Cl^- ($P>0.05$).

Table 4.19: Response of biochemical parameters in rats administered with repeated doses of *Ficus*, *Clerodendrum*, and *Microglossa* extracts

Biochemical		**FS EXTRACT**		**CR EXTRACT**		**MP EXTRACT**	
parameters	**CONTROL**	**500 mg/kg**	**1000 mg/kg**	**500 mg/kg**	**1000 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALB (g/L)	38.08 ± 1.45	40.4 ± 0.8	37.07 ± 1.94	35.65 ± 0.72	40.2 ± 1.36	38.4 ± 0.92	39.37 ± 0.52
TP (g/L)	57.17 ± 1.42	61.55 ± 2.12	63.22 ± 2.46	61.2 ± 0.77	67.77 ± 2.06***	60.27 ± 0.56	61.45 ± 1.03
ALT (U/L)	81.82 ± 5.98	127.4 ± 23.95	110.65 ± 8.71	151.2 ± 19.48*	161.7 ± 4.36**	172.3 ± 20.51**	151.33 ± 4.74*
AST (U/L)	166.87 ± 5.46	238.65 ± 20.12	303.97 ± 40.9*	266.85 ± 25.16	198.4 ± 23.75	223.35 ± 21.69	324.35 ± 59.8*
BILT (umol/L)	2.65 ± 0.35	3.67 ± 1.01	3.67 ± 0.62	5.27 ± 0.14*	4.7 ± 0.32	3.05 ± 0.55	2.42 ± 0.13
BILD (umol/L)	0.75 ± 0.02	0.52 ± 0.26	0.72 ± 0.3	0.55 ± 0.11	1.12 ± 0.33	0.3 ± 0.1	0.37 ± 0.17
CRE (umol/L)	33.75 ± 1.1	38.5 ± 0.64	50.75 ± 2.65*	46.25 ± 4.21	46.75 ± 2.95	39.5 ± 2.06	43.25 ± 6.48
UR (mmol/L)	7.57 ± 1.22	6.2 ± 0.37	5 ± 0.1*	7.32 ± 0.24	6.27 ± 0.75	5.25 ± 0.37	4.55 ± 0.18**
Na^+ (mmol/L)	139.5 ± 1.84	135 ± 1.63	135 ± 2.51	131.25 ± 3.19	139.75 ± 4.09	136.5 ± 1.84	135.87 ± 4.73
K^+ (mmol/L)	7.93 ± 2.03	17.86 ± 2.35	18.35 ± 2*	16.57 ± 1.71	11.53 ± 4.64	14.2 ± 1.14	16.75 ± 2.45
Cl^- (mmol/L)	98.97 ± 1.52	98.17 ± 2.44	99.85 ± 1.48	94.15 ± 2.65	99.67 ± 2.63	98.4 ± 0.77	96.12 ± 0.27

ALB-Albumin, **TP**-Total protein, **ALT**-Alanine aminotransferase, **AST**-Aspartate aminotransferase, **BILT**-Total Bilirubin, **BILD**-Direct Bilirubin, **CRE**-Creatinine, **UR**-UREA, Sodium (**Na+**), Potassium (**K+**) and Chloride (**Cl-**). ***** $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

CHAPTER FIVE

DISCUSSION

5.1 Ethnobotanical Indigenous Knowledge

5.1.1 Socio-demographic of respondents

The study findings indicated that most of the TH were older individuals, primarily females, with a basic level of education. The age factor among traditional healers has been a consistent observation, as evidenced by studies conducted in Kenya (Kamau *et al*., 2016) and Nalbari District, Assam, India (Chakravarty & Kalita, 2012). These studies affirm that traditional healers tend to be older individuals. This is supported by the fact that older individuals possess more experience and knowledge gained through long-term interactions with plants, consequently dominating the field of TH practices (Tugume *et al*., 2016). In contrast, the prevalence of older traditional healers suggests that younger generations may be reluctant to embrace and adopt this traditional healing culture, potentially influenced by exposure to modern knowledge and a subsequent loss of interest in traditional healing and cultural practices (Tugume *et al*., 2016). Furthermore, it signifies that the transfer of this knowledge to younger generations is still limited (Kamau *et al*., 2016).

In terms of educational background, my findings align with the research conducted by Abubakar *et al.* (2017) in Nigeria, which similarly reported a prevalence of basic education among traditional healers. However, my study diverges from the findings of Mrabti *et al.* (2021) in Morocco, where the majority of healers were found to be illiterate, with only 12% having completed primary education.

In the realm of traditional healing and its intersection with gender, my research aligns with other investigations, such as the ones conducted in Morocco, that revealed that a significant majority of THs or users were females (Jouad *et al*., 2001; Eddouks *et al*., 2002; Bousta *et al*.,

2014; Mrabti *et al*., 2021). The predominance of female healers can be attributed to several factors, as highlighted by Jouad *et al*. (2001) and Eddouks *et al*. (2002). These include factors such as analphabetism, the ease of information transmission among women, and their strong attachment to traditional knowledge. Additionally, the responsibility that falls on women for the overall health care of their families is a significant contributing factor, as emphasized by Mrabti *et al*. (2021). However, my findings are in contrast with other research, including studies conducted in Nigeria (Abo *et al*., 2008; Abubakar *et al*., 2017), which indicated a predominant presence of male traditional healers. Most of the healers practiced healing as a primary and agriculture as a secondary occupation. This pattern was also observed in other studies such as the one conducted in Togo (Karou *et al*., 2011).

5.1.2 Causes, symptoms, and diagnosis methods for diabetes and hypertension

The results of the current study revealed that herbalists were able to elucidate key symptoms, causes, and diagnostic methods employed for the two targeted diseases. Similar studies conducted in different regions, such as in Togo (Karou *et al*., 2011), as well as in Kenya (Kamau *et al.*, 2017), also revealed that traditional healers could identify crucial symptoms. The use of traditional diagnosis methods has been also reported in other studies, for instance, the recognition of sweet urine attracting insects as a diagnostic indicator for diabetes was consistent across studies (Karou *et al.*, 2011b; Zain-ul-abidin *et al*., 2018).

In the context of diabetes and hypertension patients, respondents said the majority were females, a finding that diverges from a study conducted in Kenya by Kamau *et al*. (2017), where the prevalence was reported to be higher among males. In Uganda, this variance could be attributed to two main factors. Firstly, according to the UBoS National Household Survey, the incidences of NCD diseases were reported to be higher in women than males (Kakudidi *et al*., 2017). This may offer an additional explanation for the observed gender distribution as reported by respondents. Furthermore, it has also been shown that women exhibit a higher

susceptibility to diabetes compared to men, a susceptibility attributed to hormonal shifts induced by menopause, as well as the onset of gestational diabetes during pregnancy (Hoda *et al*., 2019). Secondly, women are recognized as the primary dependents and users of medicinal plants in Uganda, as observed in previous studies (Kamatenesi-Mugisha & Oryem-Origa, 2006), thus corroborating the findings of this study.

5.1.3 Medicinal plants used to treat diabetes and hypertension

This study identified 120 plants used to treat diabetes and hypertension diseases in the Mpigi district in Uganda. In the case of diabetes, a previous study conducted in selected districts in central Uganda identified eighteen plants used to treat Type II diabetes (Ssenyange *et al.*, 2015), with eight of these plants also being referenced in my current study. In the review paper authored by Gang *et al*. (2023), which synthesized information from previous studies in Uganda, a compilation of herbs used for the treatment of diabetes was presented. This compilation identified 46 plant species, and notably, 23 of these plants overlap with those mentioned in this study.

Some of the plants that were highlighted in this study for their potential benefits in managing diabetes have also been cited in other regions worldwide, although some were similar by genera names but with different species names. For example, in Morocco, *Ficus, Citrus, Eurphobia, Artemisia, Eucalyptus, Aloe*, and *Allium sativum, Allium cepa L*, *Persea americana* Mill., were prominently genera and species featured for their relevance to diabetes, a correlation that aligns with the findings of this study (Eddouks *et al*., 2002; Bousta *et al*., 2014). In India, research has been conducted on plants with anti-diabetic properties, some plants are also cited in this study including *Mangifera indica* L., *Allium cepa* L., *Allium sativum* L., *Azadirachta indica* A.Juss., *Catharanthus roseus* (L.) G. Don., *Lantana camara* L., *Moringa oleifera* Lam., *Aloe vera* (L.) Burm.f, and *Centella asiatica*(L.)Urban. Other genera were also important such as

Cucumis, Ficus, Annona, Albizia, and Clerodendrum (Chakravarty & Kalita, 2012; Kumar *et al*., 2019).

In Togo *Catharanthus roseus* (L.) G. Don., *Vernonia amygdalina* Del, *Persea americana* Mill, *Allium cepa* L. *Allium sativum* L. *Aloe vera* (L.) Burm.f., *Moringa oleifera* Lam., *Stachytarpheta angustifolia* (Mill.) Vahl, have been recognized for their potential as antidiabetic plants (Karou *et al*., 2011). These plants are also mentioned in this study. In Pakistan *Azadirachta indica* A.Juss, and *Syzygium cumini* were mentioned plants, and *Datura, Solanum, Ficus, Momordica,* and *Artemisia* among the genera with species also identified in this study (Zain-ul-abidin *et al*., 2018). In Kenya *Allium cepa* L., *Warbugia ugandensis Azardiracta indica, Solanum incanum,* and *Momordica foetida* were also mentioned to treat diabetes (Keter & Mutiso, 2012; Kamau *et al*., 2017). Among the widely utilized plants in Limpopo, South Africa, Moringa oleifera was also highlighted in this study (Semenya *et al*., 2012). *Cinnamomum verum* was also widely used for managing diabetes in Iran and has been mentioned in this study (Bahmani, *et al*., 2014; Baharvand-Ahmadi *et al.*, 2016). Asteraceae was also mentioned as one of a family with many plant species in treating diabetes in South-West Pakistan having 8 species similar to this study followed by Solanaceae (Zain-ul-abidin *et al*., 2018). Asteraceae was also the second most cited family in treating DM in the Taza region in Morocco (Mrabti *et al*., 2021).

5.1.4 Plant parts used

In this study, leaves emerged as the predominantly utilized plant parts, closely followed by the bark and whole plants. This finding aligns with similar studies conducted on diabetes, such as the one conducted in Nalbari District, Assam, India (Chakravarty & Kalita, 2012), as well as studies in Morocco (Mrabti *et al*., 2021), Iran (Bahmani *et al*., 2014), and South-West Pakistan (Zain-ul-abidin *et al*., 2018). Another study in Guinea reported 97 plant species for

hypertension and leaves were also the highly used part (Traore *et al*., 2022). The high utilization of leaves can be attributed to their easy accessibility and harvestability. Additionally, the abundance of phytochemicals in leaves enhances their therapeutic potential, further contributing to their high usage (Zain-ul-Abidin *et al*., 2018). The extensive utilization of leaves, on the other hand, holds significant importance as it is less destructive (Tugume *et al*., 2016). This practice plays a crucial role in preserving and ensuring the long-term survival of these medicinal plants (Traore *et al*., 2022). However, it's essential to be cautious about potential consequences, as excessive harvesting of leaves may impede the natural regeneration process, limiting the transformation from vegetative to reproductive states (Tugume *et al*., 2016).

In contrast to my study, other research findings diverged. For instance in South Africa, roots were identified as the most utilized part, followed by the whole plant, while leaves were reported as the least used (Oyedemi *et al.*, 2010). In Kenya, a similar trend was observed, with roots being highly utilized, followed by leaves (Kamau *et al*., 2017).

5.1.5 Threats facing medicinal plants and conservation strategies

The threats to medicinal plants identified in this study are reiterated in other studies, encompassing issues such as charcoal burning, overexploitation, and deforestation observed in Amboseli, Thika, and Nairobi in Kenya (Kiringe, 2005; Njoroge, 2012). Unresponsive harvesting strategies were noted in Simanjiro, northern Tanzania (Mbinile *et al*., 2020), while overexploitation was observed in the cold desert of Ladakh (Chauhan *et al.*, 2020), as well as in Morocco (Bouiamrine *et al*., 2017). Overharvesting, overexploitation, deforestation, and human activities were also reported as concerns in natural areas, specifically in Kashmir Himalaya in India (Ganie *et al*., 2019). In general, these threats can result in significant consequences, including the local and global extinction of medicinal plant species, a reduction in the genetic pool due to the impact on genetic diversity, diminished regeneration potential,

and the complete eradication of medicinal plants in various locations (IUCN, 2006). Therefore, ensuring sustainable harvesting practices and implementing conservation measures becomes crucial for the survival of these plants and for the well-being of present and future generations.

5.2 Phytochemical analysis

The efficacy of medicinal plants in treating human ailments is attributed to the presence of secondary metabolites or phytochemicals, which induce physiological responses in the human body (Edeoga *et al*., 2006). This research has identified crucial phytochemical groups in the analysed medicinal plants. Certain compounds identified in this study play vital roles in the management and alleviation of diabetes and hypertension.

5.2.1 Qualitative and quantitative phytochemical analysis

This study revealed the presence of alkaloids, steroids, phenols, tannins, flavonoids, coumarins, terpenoids, resins, glycosides, and saponins in the plant extracts. In contrast to the study by Adia *et al*. (2016), which previously reported the absence of alkaloids and flavonoids in MP extracts, and alkaloids in CR extract, this study detected their presence in both methanolic and aqueous extracts. In the process of quantification, noteworthy concentrations of total phenols and tannins were detected in FSM. Conversely, elevated levels of total flavonoids, saponins, and alkaloids were observed in CRM. When compared to the study conducted in Kenya by Odhiambo *et al.* (2019), this study detected the highest amount of Flavonoids and Phenols in CRM.

Many of these phytoconstituents possess properties that are effective in managing diabetes and hypertension. Flavonoids and phenolic compounds are identified as the primary components responsible for reducing blood glucose levels (Baharvand-Ahmadi *et al*., 2016). For example, phenolic compounds have the ability to augment glucose uptake by facilitating the translocation of GLUT 4 (Zhao *et al*., 2019). Flavonoids control hyperglycaemia by increasing

blood insulin levels, promoting insulin production, and inhibiting aldolase reductase (Bahmani *et al.*, 2014; Zhao *et al*., 2019). Terpenoids possess diverse mechanisms to manage diabetes, including reducing serum glucose levels, enhancing glycogen synthesis, lessening the breakdown of glycogen into glucose, and impeding the aldose reductase enzyme (Zhao *et al*., 2019). The antihypertensive properties of steroidal alkaloids make them vital in the treatment of chronic diseases (Li *et al.*, 2006; Gunaherath & Gunatilaka, 2014). It has also been documented that plants containing tannins, cardiac glycosides, and alkaloids are highly effective in the management of hypertension. Additionally, flavonoids serve as antioxidants and scavengers of free radicals, playing a preventive role against oxidative cell damage (Tyagi & Agarwal, 2017).

5.2.2 Gas Chromatography Mass spectrometry profiles; antidiabetic and antihypertension potentials

The GC-MS analysis of *Ficus saussureana* (FS) indicated the presence of fourteen compounds. The methanolic extract of *Clerodendrum rutundifolium* (CR) contained thirty chemical compounds, while the extract from *Microglossa pyrifolia* (MP) revealed thirty compounds. Most of the identified compounds in these three extracts have been also detected in other medicinal plants such as *Justicia Wynaadensis* (Ponnamma & Manjunath, 2012), *Ceropegia bulbosa* (Arora & Meena, 2017), *Cymodocea serrulata*, *Syringodium isoetifolium* and *Enhalus acoroides* (Vijayalingam & Rajesh, 2019).

Some of the identified compounds in this study such as stigmasterol, Squalene, Vitamin E, Lupeol, and Oleic acid have been investigated for their pharmacological potential. Stigmasterol has been also detected in the leaves of *Pseuderanthemum palatiferum* (Nualkaew *et al*., 2015) and *Bridelia duvigneaudii* (Credo *et al*., 2018). This compound has hypoglycaemic potentials such as the ability to lower blood glucose levels (Nualkaew *et al*., 2015; Credo *et al*., 2018),

the ability to augment glucose uptake, the capacity to alleviate insulin resistance, and improve oral glucose tolerance (Wang *et al*., 2017).

Squalene has been also detected in other medicinal plants such as *Syzygium polyanthum* (Widyawati *et al.*, 2023). This compound possesses noteworthy potential in diabetes and hypertension treatment such as its ability to reduce Fasting Blood Glucose levels, and antioxidant capability (Widyawati *et al.*, 2023). The study by Shreenithi *et al*., (2019) explored the therapeutic potential of lupeol in diabetes treatment. The study unveiled that lupeol has the capacity to regulate insulin receptors and GLUT 4 protein expression (Shreenithi *et al*., 2019).

Studies have uncovered the therapeutic benefits of Vitamin E in diabetes management. It is apparent that supplementing with Vitamin E plays a crucial role in delaying the onset of diabetic complications and slowing down their progression, lowering fasting blood sugar, post-prandial blood sugar, and total cholesterol levels (Jain & Jain, 2012). Vitamin E has been detected in other medicinal plants such as *Justicia Wynaadensis* (Ponnamma & Manjunath, 2012), and *Ceropegia bulbosa* (Arora & Meena, 2017). This study's findings indicated the presence of oleic acid which has been reported to possess antidiabetic potentials such as increasing insulin production, reversing the repressive effect of insulin production, and ability to decrease glucose levels (Vassiliou *et al.*, 2009). These compounds validate the potential of analysed plants in treating diabetes and hypertension therefore vindicating their traditional use.

5.2.3 Gas Chromatography Mass Spectrometry profiles; antioxidant potentials

This study detected key phytochemicals with antioxidant activities such as Vitamin E, Squalene, Lupeol, and 5-Hydroxymethylfurfural. Studies have assessed the value of plant-based antioxidants, offering potential benefits for disorders linked to oxidative stress like diabetes and hypertension (Rahimi *et al*., 2005). Examples of these notable compounds include Vitamin E, which prevents lipid peroxidation and protects cells against oxidative damage,

contributing to diabetes prevention (Khan *et al*., 2015; Rajendiran *et al.*, 2018). Squalene, a hydrophilic natural antioxidant, showed effectiveness in reducing heart tissue lipid peroxidation (Amarowicz, 2009). Lupeol has the ability to reduce protein levels in the pancreas, as well as increase protein levels under diabetic conditions indicating its ability to decrease oxidative stress and in preventing oxidative protein damage associated with diabetes respectively. Lupeol also has the ability to improve pancreatic antioxidants and reduce lipid peroxidation (Gupta *et al*., 2012). 5-Hydroxymethylfurfural which was detected in this study has novel antioxidant activities (Zhao *et al*. 2013) with its ability to scavenge ABTS and DPPH radicals, inhibit AAPH-induced haemolysis, ability to reduce ROS and MDA, and enhanced SOD, CAT, and GPx enzyme activities, showcasing its ability to prevent peroxidation and protect erythrocytes (Zhao *et al.*, 2013).

Highlighted above is just a snapshot of a few compounds detected by GCMS in the three extracts with antioxidant potentials. Other compounds have also been reported to possess antioxidant activities such as 2-Methoxy-4-vinyl phenol (Nandhini *et al*., 2021), Tetradecanoic acid (Vijayalingam & Rajesh, 2019), Heptadecanoic acid (Ponnamma & Manjunath, 2012), Phytol (Arora & Meena, 2017), .psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- (Kavitha, 2021), Stigmasterol (Perumal *et al.*, 2021), 1,2-Benzenediol,3-methyl- (Naksing *et al.*, 2021), Bicyclo[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl- (Prakasia & Nair, 2015), 1-Heptatriacotanol (Kotteswari *et al.*, 2020), Hexadecanoic acid, methyl ester (Ali *et al.*, 2017), and n-Hexadecanoic acid (Nandhini *et al.*, 2021).

5.2.4 Gas Chromatography Mass Spectrometry profiles; other potentials

Many additional compounds were identified in the methanolic extracts of the three examined plants. In addition to their potential for treating diabetes and hypertension, these compounds exhibit various pharmacological activities, playing crucial roles in addressing diverse human ailments. Comprehensive details of the pharmacological activities of each compound, along

with citations, are provided in Tables 4.11, 4.12, and 4.13. Briefly, some of them possessed hypercholesterolemic activities, Anticancer/ Chemo-preventive, Antimalaria potential, Anti-inflammatory bioactivity, Antibacterial bioactivity, and Antimicrobial. They also possess other key pharmacological potentials such as hepatoprotective, anti-HIV/AIDS, anti-arthritic, anti-tuberculosis, antitumor, anti-aging, antifungal, and immune system enhancement, etc.

5.3 Toxicity assessment

5.3.1 Effects of extracts on behaviour and weight

In this study, a single administration of extracts of these three plants up to a dose of 5000mg/kg, and repeated administration up to 1000mg/kg dose did not result in any mortality and behavioural change to the animals treated except polyuria was observed in some groups. Since no animal died, it could be concluded that the lethal dose of ethanolic extracts of FS, CR, and MP is above 5000 mg/kg bwt. A lethal dose exceeding 5000 mg/kg has been reported in several studies. For example, the lethal dose of *Combretum micranthum* (Kpemissi *et al.*, 2020), *Lonicera japonica* Thunb (Thanabhorn *et al*., 2006), Cassia occidentalis L. (Silva *et al*., 2011), and *Marsdenia tenacissima* (Silva *et al*., 2011) was found to be well above 5000 mg/kg. Although the lethal dose exceeded the highest tested dose, the administration of certain extracts had noticeable effects on the weight gain of the rats in both acute and subacute toxicity tests. Specifically, weight loss was observed in the groups treated with CR extract and high doses of FS. The weight loss of treated animals has been also reported in other studies (Oliveira De *et al*., 2010; Bello *et al*., 2016; Ghelani *et al*., 2016). It has been shown that weight loss is one of the preliminary indicators of the harmful effects of drugs (Sureshkumar *et al*., 2018), and is usually caused by either the loss of appetite (anorexia) as the result of drug administration or disturbances of the fat, protein, and carbohydrate metabolism (Ghelani *et al.*, 2016; Alelign *et al*., 2020).

5.3.2 Effects on haematological parameters

Acute and Subacute toxicity study of the three extracts resulted in a change in some key haematological parameters especially those pertaining to WBC such as WBC counts, Neutrophils, Lymphocytes, Monocytes, Eosinophils, and Basophils. This could be attributed to the impact of these extracts on hematopoietic cells and/or their influence on circulating white blood cells (Tchoumtchoua *et al.*, 2014). Furthermore, changes in these blood parameters, particularly in cell counts, may suggest cytolytic or cytotoxic effects (Elufioye *et al*., 2009). This was reinforced by the detection of .psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy- in MP and CR extracts, stigmasterol in CR, and Lupeol in FS extract (Tables 4.11, 4.12, and 4.13). These compounds have been previously reported to exhibit cytotoxic activity (Arora & Meena, 2017; Sivakumaran *et al*., 2019; Kavitha, 2021; Perumal *et al*., 2021).

An elevation in Red Blood Cells count, Haemoglobin, Haematocrit, and Mean corpuscular volume, was consistently observed in nearly all rats subjected to repeated doses of FS, CR, and MP extracts. This pattern was also evident in certain groups within the acute experimental setup, particularly those subjected to the highest doses of these extracts. The results imply that the administration of the extracts may have influenced erythropoiesis, the process of red blood cell formation. Moreover, the noted elevation in these haematological parameters may result from an imbalance between their synthesis and catabolism (Mugisha *et al*., 2014). Additionally, elevated levels of RBC, HGB, and HCT may serve as potential indicators of dehydration (Halim *et al.*, 2011). This is reinforced by the observation that these animals especially those treated with CR and MP extracts exhibited polyuria throughout the entire duration of the experiment, specifically on the Subacute setup. Furthermore, the notable rise in WBC and LYMPH levels is a positive indicator, suggesting that these extracts may harbour phytochemicals capable of boosting the immune system (Mugisha *et al*., 2014). The results of this study align with the findings observed in other studies (Mugisha *et al*., 2014; Tchoumtchoua *et al*., 2014).

In the acute experimental setup of this study, an altered PLT count was observed, marked by a reduced percentage of P-LCR in rats across all three extracts. This trend was similar to the response seen in rats subjected to a repeated dose of 1000mg/kg of FS extract. The alteration in PLT count suggests that these extracts could potentially induce thrombocytopenia in rats. This effect may be ascribed to their influence on platelet production, either enhancing or suppressing it or their impact on the circulating levels of platelets (Mukinda & Eagles, 2010; Tchoumtchoua *et al*., 2014; Kpemissi *et al*., 2020). These results closely aligned with the results of the study conducted by Tchoumtchoua *et al*. (2014). Although certain haematological parameters displayed notable changes following the administration of these extracts, other parameters did not exhibit significant alterations. Furthermore, specific parameter changes were observed exclusively in either acute or subacute settings.

5.3.3 Effects on biochemical parameters

The study results revealed significant changes in hepatic and renal biomarkers following the administration of extracts to the rats. Single and repeated doses of FS, CR, and MP resulted in significant changes in the levels of liver serum biomarker enzymes such as AST, ALT, and ALP. These findings were consistent with the outcomes reported in the study of Mugisha *et al*. (2014) and Bello *et al*. (2016). Elevated levels of ALT are an initial indicator of cellular damage within the liver (Sureshkumar *et al*., 2018). Similarly, increased AST levels is a marker for liver injury or myocardial infarction (Mugisha *et al.*, 2014). Moreover, fluctuations in AST and ALT levels serve as markers for hepatocyte necrosis (Tchoumtchoua *et al*., 2014). Hence, the findings suggest that these plants have the potential to impact hepatic functions, as evidenced by the increased levels of liver enzymes. Based on this observation, it is crucial to deduce that the utilization of these plants should be approached cautiously to prevent adverse effects on the liver.

The administration of a single dose of 5000mg/kg of MP extract resulted in a significant elevation in the ALB levels in the experimental animals. Additionally, repeated administration of a 1000mg/kg dose of CR led to a noteworthy elevation of the TP levels in the treated animals. The liver plays another crucial role in producing plasma proteins, therefore, changes in TP and ALB levels are an indication of liver dysfunction (Prasanth *et al*., 2015). Although the factors leading to an increase in ALB levels remain unclear but studies suggest a potential association with dehydration, which in turn is linked to renal function (Oliveira *et al.*, 2010). Dehydration might be attributed to the observed polyuria in treated rats, suggesting a potential cause-and-effect relationship. The elevated levels may suggest liver toxicity resulting from the administration of high doses of these two extracts.

A singular as well as repeated administration of the highest doses of FS led to a notable increase in serum creatinine levels in rats in this study. It is suggested that an increased level of creatinine is only evident when there is impairment to the operational nephrons (Mukinda & Eagles, 2010; Sureshkumar *et al*., 2018), suggesting a consequential impact on renal function. Moreover, the repeated administration of the maximum doses of both FS and MP caused a significant decrease in blood urea levels. This reduction may be attributed to heightened activities of urea enzymes, potentially indicating damage to the liver or kidneys (Ajagbonna *et al*., 1977). Also, repeated administration of the highest dose of FS extract remarkably elevated the amount of K electrolyte in the blood of rats. The elevation of K levels may indicate impaired kidney function, as the kidneys play a crucial role in regulating K excretion. However, this cannot be termed a definitive conclusion regarding kidney function considering that K is primarily an intracellular electrolyte (Onifade & Suleiman, 1977).

Administering the CR extract at a low dose repeatedly resulted in a notable rise in BILT levels in the rats. BILT is a byproduct resulting from the breakdown of haemoglobin, that serves as

an indicator of hepatobiliary damage (Tchoumtchoua *et al*., 2014). Elevated serum BILT levels are linked to heightened occurrences of haemolysis, liver damage, or cholestasis (Tchoumtchoua *et al*., 2014). However, it should be noted that this effect of extracts on the BILT of treated rats in this study may be considered incidental, as it did not exhibit a dose-dependent relationship.

Generally, harmful impacts of medicinal plants are thought to arise from their chemical makeup (Adeneye *et al*., 2006). Apart from the negative impacts associated with xenobiotics, additional factors contributing to plant toxicity include soil contaminants such as heavy metals, aflatoxins, and pathogenic microbes that may be introduced during the extraction process (Alelign *et al.*, 2020).

5.4 Limitation of the study

Achieving a comprehensive understanding of the safety profile of plants necessitates the inclusion of histopathological analysis. Unfortunately, the current study faces a limitation due to a shortage of research funds, rendering it unfeasible to conduct the necessary histopathological examinations. Notwithstanding this challenge, it is pertinent to note that the preserved tissues are available at the Toxicological Laboratory of Makerere University.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Interviews with traditional herbalists resulted in the identification of 120 plants used for the management of these two targeted diseases. This research demonstrates the significant role of plants and traditional wisdom in effectively managing DM and HTN. However, certain medicinal plants identified in the study are under threat, as reported by herbalists. This underscores the necessity for adopting sustainable harvesting practices and implementing conservation measures crucial for the preservation of these plants and the well-being of both current and future generations.

The efficacy of medicinal plants in treating human health issues is ascribed to the presence of secondary metabolites or phytochemicals. The findings of this research substantiate the assertions of traditional herbalists regarding these three selected plants, revealing compounds with potential antidiabetic and antihypertensive properties, along with antioxidant capabilities. It could be concluded that *F. saussureana*, *C. rotundifolium*, and *M. pyrifolia* are effective in managing diabetes and hypertension due to their pharmacologically active compounds. However, further research is needed to investigate their bioactivity and fully understand their

mechanisms of action. Additionally, the study validates the claims of these plants in treating other significant human ailments, as reported in previous studies.

Given the diverse therapeutic potentials of FS, CR, and MP it was imperative to establish a safety profile for these plants. The findings, including the absence of mortality and LD_{50} values exceeding 5000 mg/kg for the ethanolic extracts of FS, CR, and MP, indicate that these plants are deemed safe according to experimental evidence, thereby affirming their traditional usage. Nevertheless, it is crucial to note that their utilization, particularly in prolonged and high doses, has demonstrated the potential to alter certain haematological and biochemical parameters in the model animals. Consequently, this study recommends exercising caution in their usage, particularly when administered in high doses and extended periods. Furthermore, this study recommend exploring innovative collaborations between traditional herbalists and pharmacological experts to develop sustainable and precise methods for extracting active ingredients. The goal is to enhance both the efficacy and safety profiles of herbal remedies while preserving their traditional value.

6.2 Recommendations

Drawing from the findings and conclusions, this study recommends the undertaking of comparable studies in diverse regions of the country to document the utilization of plants to treat these two targeted diseases within different communities and cultures. This study emphasizes the imperative for sustainable utilization of the identified plants and advocates for conservation measures to ensure their long-term survival. Additionally, the findings underscore the significance of traditional healing practices in managing human ailments. This highlights the necessity for government strategies to incorporate traditional healing into the national healthcare system.

This study also advocates for additional scientific investigations into the plants documented in this study to explore their pharmacological potentials, efficacy, phytochemical compositions, antioxidants, toxicity profiles, and other essential categories that remain unstudied. This will be a key step in providing scientific proof of treatment potentials, discovery of drugs, and the safety of these plants for human health.

This study offers valuable insights into the acute and subacute toxicity of these three plant extracts. However, this study suggests conducting additional toxicity assessments using different model species and extraction methods as well as histopathological analysis to determine the effect of extracts on organs. As a preclinical trial, the study recommends further clinical investigations to assess the chronic toxicity of these plants and complete their safety profile. Furthermore, this study identified some phytochemicals reported to possess toxicity elements but further investigation should be done to identify other compounds with toxicity effects.

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APPENDICES

Appendix I: Ethical Clearance Letter

UGANDA CHRISTIAN UNIVERSITY
A Centre of Excellence in the Heart of Africa

03/05/2023

To: SILAS NNKO

KYAMBOGO UNIVERSITY
+256-783591927 +255-762895395

Type: Initial Review

Re: UCUREC-2023-504: ETHNOBOTANICAL SURVEY AND PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANTS USED IN THE MANAGEMENT OF DIABETES AND HYPERTENSION DISEASES IN MPIGI DISTRICT, UGANDA

I am pleased to inform you that the Uganda Christian University REC, through expedited review held on **27/04/2023** approved the above referenced study.
Approval of the research is for the period of **03/05/2023** to **03/05/2024.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **03/05/2024** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Uganda Christian University REC:

No.	Document Title	Language	Version Number	Version Date
1	Data collection tools	Luganda	Final	2023-04-
2	Protocol	English	Final, Approved.	2023-03-
3	Data collection tools	English	Final	2023-04-13

Yours Sincerely

Peter Waiswa
For: Uganda Christian University REC

Appendix II: Introductory Letter

KYAMBOGO UNIVERSITY

P. O. BOX 1 KYAMBOGO
Tel: 041 - 4286792 Fax: 256-41-220464
Website :www.kyu.ac.ug, Email: drgt@kyu.ac.ug

Directorate of Research and Graduate Training

Office of the Director

Date:

TO WHOM IT MAY CONCERN

RE: SILAS SANGITO NNKO

Dear Sir/Madam,

This is to introduce to you the above named student Reg: No 2100814614 pursuing MSC IN CONSERVATION AND NATURAL RESOURCE MANAGEMENT, Department of BIOLOGICAL SCIENCES, Kyambogo University.

She/he intends to carry out research on MEDICINAL PLANTS USED TO MANAGE TYPE II DIABETES AND HYPERTENSION

in partial fulfillment of the requirements for the award of MASTER OF SCIENCE DEGREE IN CONSERVATION AND NATURAL RESOURCE MANAGEMENT

The purpose of this letter therefore is to request you to grant him/her permission to carry out his/her study in your institution.

Any assistance rendered to him/her will be highly appreciated.

Yours sincerely,

Prof. Bosco Bua
AG. DIRECTOR

KYAMBOGO UNIVERSITY
04 APR 2023
DIRECTOR
DIRECTORATE OF RESEARCH AND GRADUATE TRAINING

Appendix III: Pictorial presentation of data collection methods

I. Photos showing plants selected for phytochemicals and toxicity assessment

Microglossa pyrifolia (Kafugankande)

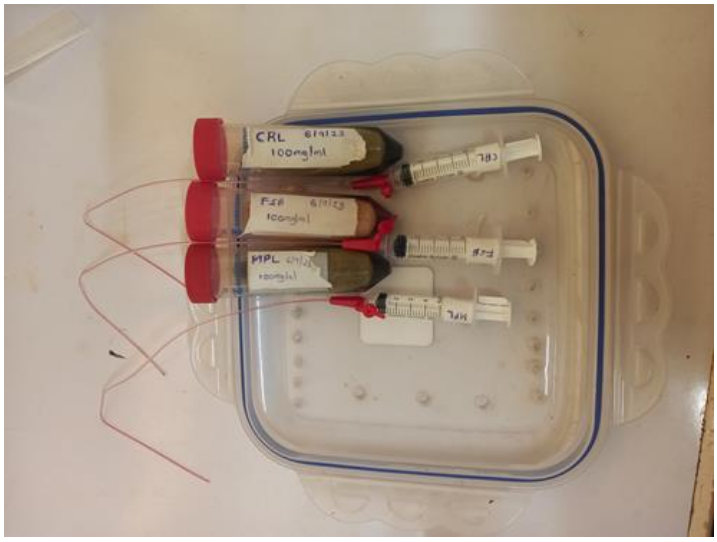
Clerodendrum rutundifolia (Ekisekeseke)

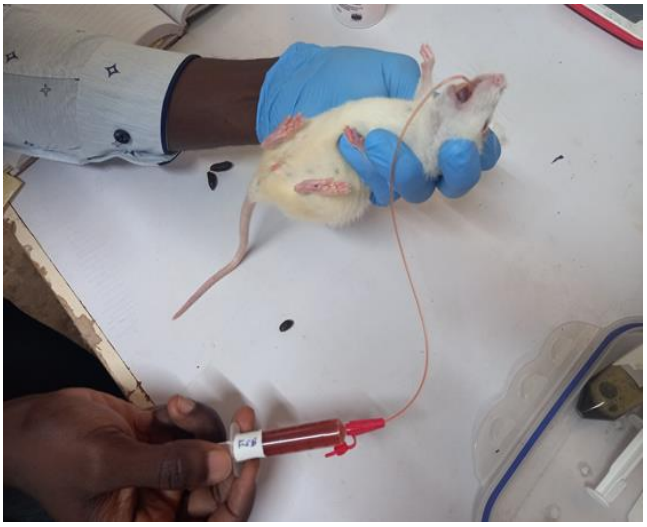
Ficus saussureana (Omuwo)

Researcher interviewing a traditional herbalist

I. Photos showing plants drying and final extracts

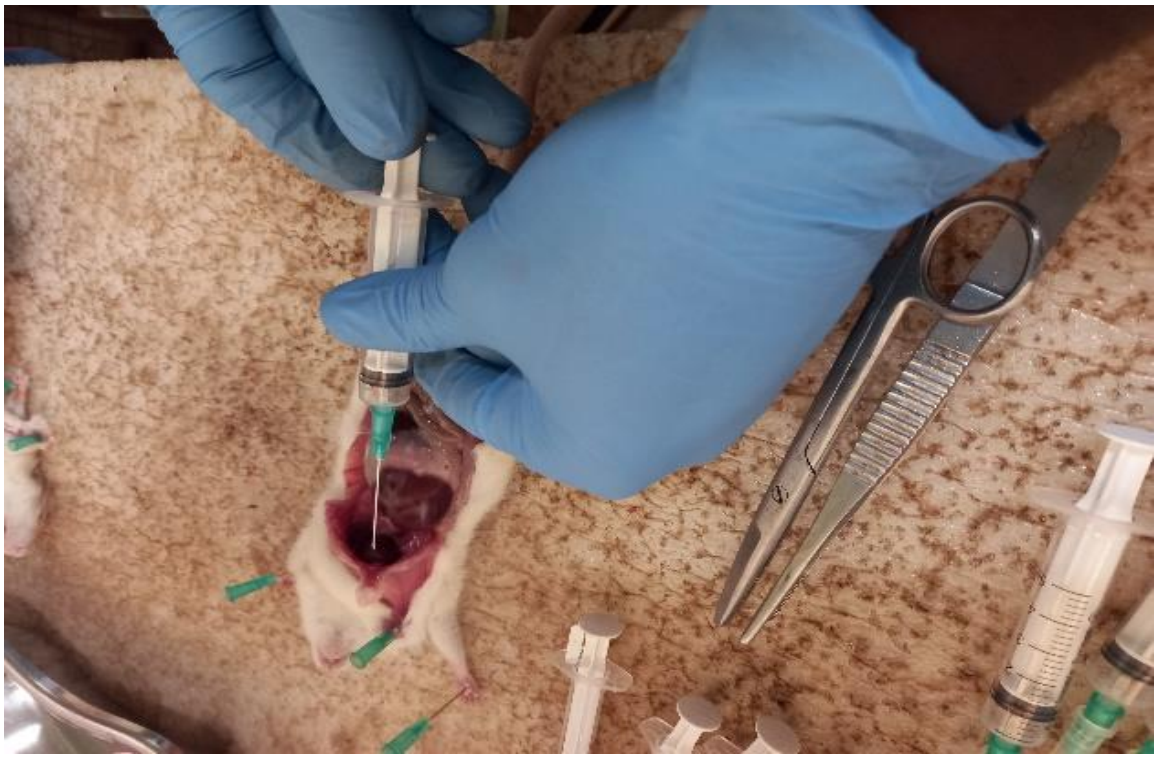
II. Photos showing test substances, animal dosing and serum collection





Dissolved test substances Rats dosing

Blood collection for haematology and biochemical analysis



Appendix IV: Interview guiding questions

Dear respondents welcome to this interview,

My name is Silas Sangito Nnko, a student at Kyambogo University pursuing a Master of Science degree (Conservation and Natural Resources Management), conducting this interview as part of my studies at Kyambogo University. This ethnobotanical study aims to gather information from herbalists about the medicinal plants used to manage diabetes and hypertension diseases. Your participation in this study is entirely voluntary. Your openness and

honesty will be greatly appreciated. The responses you provide will be treated very confidentially only for the purpose of this study.

1. Demographic information; Subcounty…… Village…… Household GPS coordinates…… Tribe…… Herbalist phone number……..	**2. Sex;** **3. Age** **4. Marital status; Level of education;** **5. Residence duration;** **6. Occupational;** **7. Are you a traditional herbal healer?**

8. Where did you attain traditional knowledge in treating diseases?
    1. Self-experience
    2. Learned from grandparents
    3. Learned from my parents
    4. Learned from other herbalists
    5. Acquired through formal education
    6. Other; …….
9. Do you share this knowledge?
    1. Yes
    2. No
10. Which people do you share your knowledge with?.....
11. How do you share this knowledge? ……
12. Which age groups do you normally share this information with?.....
13. Which plant types do you use to treat diabetes and hypertension diseases?.....
14. How long have you been treating diabetes and hypertension? …….
15. How many diabetes/hypertension patients do you treat per day/month?.....
16. Which gender forms the majority of diabetes/hypertension patients?.....
17. What is the most frequent age group of diabetes/hypertension patients? ……
18. Do you use a mixture of herbs or only one plant? ……
19. What are the main products, are they only plants or others?.....
20. What do you think are the causes of diabetes and hypertension?.....
21. How do you diagnose the patients to confirm diabetes/hypertension?
    1. Interviewing the patients

2. Signs shared by the patients
3. Patients that have been diagnosed already come seeking for help
4. Others, (Please specify) …….

22. Please can you mention some of the diabetes symptoms that you treat/you know?.....
23. Why do you think patients prefer the use of plants to treat the two diseases?...
24. Which plants do you use to treat,

A. Diabetes (Table 4.10)

B. Hypertension (Table 4.10)

25. Where do you harvest these plants from?
    1. Wild
    2. Cultivated/home gardens
    3. Others (Specify)……..
26. How far is it from your home place?

    ……

27. At what time of the day do you harvest these plants?
    1. Early morning
    2. Mid-morning
    3. Afternoon
    4. Late evening
28. Are there any threats facing these medicinal plants?
    1. Yes 2. No
29. If yes, what

    threats?.....

30. How do you keep and ensure the plants stay for a long time?

    ……...

31. What strategies do you think can be adopted to conserve these

    plants?.....

Appendix V: Informed consent form

Title of Research: Ethnobotanical survey and phytochemical analysis of medicinal plants used in the management of diabetes and hypertension diseases in Mpigi district, Uganda

Principle Investigator: Silas Sangito Nnko; Tel. contact +256-783591927, +255-762895395
Affiliated to Kyambogo University, Department of Biological Sciences, P. O. Box 1, Kampala, Uganda.

1. Introduction and Purpose of the Study

As part of my studies at Kyambogo University, am conducting this study in partial fulfillment of the requirement for the award of Masters of Science degree (Conservation and Natural Resources Management). This ethnobotanical survey aims to identify and document medicinal plants used by traditional herbalists in the management of diabetes and hypertension diseases

in the Mpigi district, as well as study the phytochemical constituents of the highly used plants. The information you give me will be confidential and only used for the purposes of this study. In the process of report writing, your name will never be used and so everything you tell me will remain anonymous. I will also not use any information in any report I may publish that would make it possible to identify you. I shall ask questions about the traditional knowledge that you use to manage diabetes and hypertension diseases including the plants that you use. If you do not want to respond to a particular question, you can simply say so, and I will not insist.

2. Description of the Research

This is a cross-sectional design survey aiming to document medicinal plants used to manage diabetes and hypertension diseases by traditional herbalists in the Mpigi district.

3. Subject Participation

Participants for this study will be Traditional Herbalists and other knowledgeable local communities both aged above 18 years.

4. Potential Risks and Discomforts

This is a social survey involving a conversation between the researcher and the respondents on traditional knowledge of managing the two targeted diseases. Therefore, very minimal risk is expected.

5. Potential Benefits

This will enhance the current understanding of utilizing plants for the treatment of human diseases, with a specific focus on chronic/non-communicable diseases (such as diabetes and hypertension). Additionally, it plays a crucial role in the preservation and conservation of biological resources, particularly medicinal plants as well as indigenous knowledge.

6. Confidentiality

Your participation in this study is entirely voluntary. The responses you provide will be treated very confidentially only for the purpose of this study.

7. Authorization

By endorsing this document, you grant permission for me to use the information from this research; for example, for Education and evidence interventions in relation to the treatment of human diseases using indigenous traditional knowledge.

8. Participation

Your participation in this research is entirely voluntary. If you choose not to participate, it will have no impact on you in any way.

9. Withdrawal from the Study and/or Withdrawal of Authorization

You are entitled to refrain from answering any questions that make you uncomfortable. Additionally, you have the right to withdraw entirely from the interview at any point if you decide not to continue.

10. Reimbursements

Reimbursement of fifteen thousand Ugandan Shillings (15,000 UGX) has been budgeted for you.

11. Whom to contact in case of ethical-related concerns

The Uganda Christian University Research Ethics Committee (UCU-REC) approved and the Uganda National Council for Science and Technology (UNCST) cleared this study. For any ethical concerns or inquiries, please contact the UCU-REC chairperson, Prof. Peter Waiswa, at 0772 405 357 or pwaiswa@musph.ac.ug, or the UCU-REC Secretariat, Mr. Osborn Ahimbisibwe, at 0775737627 or oahimbisibwe@ucu.ac.ug.

I voluntarily agree to participate in this research program; to tick appropriately

Yes **No.**

I understand that I will be given a copy of this signed Consent Form.

Name of Participant (Optional): ……

Signature: …… Date: …….

Name of Researcher: …….

Signature: ……. Date: ……