IDENTIFICATION OF PHYTOCHEMICALS, ADULTERANTS AND CONTAMINANTS IN SELECTED COMMERCIALLY-AVAILABLE ANTICANCER AND ANTIDIABETIC HERBAL MEDICINES IN KENYA

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Identification of Phytochemicals, Adulterants and Contaminants in Selected Commercially-Available Anticancer and Antidiabetic Herbal Medicines in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Signature…… Date……

Caroline Nyambura Maina

This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

I dedicate this thesis to my family and friends. Special gratitude to my mum, dad, and brother who believed in my dreams from childhood. Special mention to my life-time friend, Jacqueline Thuku whose constant support and encouragement got me through this part of my academic journey.

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ABBREVIATIONS AND ACRONYMS

amu	Atomic mass unit
CDC	U.S. Centers of Disease Control and Prevention
ESI	Electron spray ionization
eV	Electron volt
ID	Internal diameter
GC	Gas chromatography
HPLC	High performance liquid chromatography
ICH	International Council for Harmonization
IDF	International Diabetes Federation
KEMRI	Kenya Medical Research Institute
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
m/z	Mass to charge ratio
MRM	Multiple reaction monitoring
MS	Mass spectrometry
NATHEPA	National Traditional Health Practitioners Association
SPE	Solid phase extraction
THP	Traditional health practitioner
TIC	Total ion chromatogram
TLC	Thin layer chromatography
WHO	World Health Organization

ABSTRACT

The number of people using herbal medicines to treat chronic conditions is increasing worldwide due to the perception that these medicines are safer than conventional pharmaceuticals. However, several studies have established that herbal medicines can contain synthetic and toxic natural compounds, undermining patient safety. Nonetheless, most countries, including Kenya, have a weak regulatory framework for herbal medicines despite their potential to harm consumers. In Kenya, most commercially-available herbal medicines have not undergone laboratory testing to determine if they contain synthetic contaminants, adulterants, or phytochemicals with relevant reported biological activities. This study addresses this gap by evaluating herbal medicines sold for diabetes and cancer treatment in Nairobi and Uasin Gishu Counties for phytochemicals, adulteration and contamination with synthetic compounds. 24 herbal medicines (9 anticancer and 15 antidiabetic) were collected from herbal clinics, herbal product manufacturers, herbalists, local retailers (supermarkets and nutrition stores), and hawkers/ street vendors in Uasin Gishu and Nairobi Counties. Gas chromatography- mass spectrometry (GC-MS) was used to determine the phytochemical profile of each herbal medicine. The GC-MS results revealed that all the sampled herbal medicines contained a wide range of phytochemicals with reported biological activities useful to diabetes and cancer treatment. They also highlighted the presence of toxic phytochemicals, including prunasin-d, 5-keto-d-fructose, and ketone, 7-methoxy-2-benzofuranyl methyl. GC-MS was also used to identify synthetic contaminants or adulterants present in the sampled herbal medicines. The results highlighted the presence of several synthetic compounds, including pharmaceutical salts, pesticide residue, and phthalates, which pointed to potential safety issues. Thus, this study developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination, identification and quantification of four commonly used antidiabetics – metformin, gliclazide, glimepiride, and glibenclamide - in herbal medicines. The developed method showed detection and quantification limits ranging from 2.86 to 7.67 ng/mL and 8.64 to 23.24 ng/mL, respectively. The precision of the developed method ranged from 8.5% to 18.1%, while accuracy was above 80% for all analytes except metformin (52%). This method was then applied to analyse the 24 sampled herbal medicines. Metformin was detected in 17% of the samples, at concentrations ranging from 900 ng/g to 1969 ng/g. Gliclazide, glimepiride, and glibenclamide were not detected in any sample. Overall, the study findings provide proof of adulteration of herbal medicines with pharmaceuticals. This adulteration undermines patient safety due to the potential for adverse herb-drug interactions. Therefore, market surveillance of herbal medicines sold in the country for toxicity, contamination and

adulteration with synthetic compounds should be conducted and legislation that outlaws adulteration enacted.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The prevalence of chronic conditions, such as cancer, respiratory illnesses, diabetes and cardiovascular diseases, has been rising in many countries around the globe, accounting for 74 percent of all deaths worldwide in 2022 (WHO, 2023). According to the United Nations News (2023), it is projected that this prevalence will continue rising and chronic conditions will contribute to 86% of the deaths every year by 2030, which represents a 90% increase in total numbers from 2019. Currently, most of these deaths occur in middle- and low-income countries and are caused by cardiovascular diseases, followed by cancer and diabetes (WHO, 2023).

1.1.1 Diabetes

The rise in the prevalence of diabetes worldwide is a major public health concern, with about 463 million adults living with this condition worldwide and over 24 million adults living with the disease in Africa in 2020 (IDF, 2021). The number of individuals living with diabetes worldwide by region is shown in Figure 1.1. The rise in diabetes prevalence is linked to social and demographic changes such as a growing aging population, increased adoption of unhealthy lifestyle behaviours, urbanization, and globalization. In Kenya, the International Diabetes Federation (IDF) estimates that 4.56% of the adult population was living with diabetes in 2021, with about 2.7% of rural dwellers and 10.7% of urban dwellers living with the condition (IDF, 2022). However, this figure is suspected to be an underestimation given that about 60% of

Kenyans with diabetes are undiagnosed as they present to healthcare facilities with unrelated complaints (Ministry of Health Kenya, 2020). Additionally, approximately 14% of the Kenyan population is estimated to have impaired glucose intake.

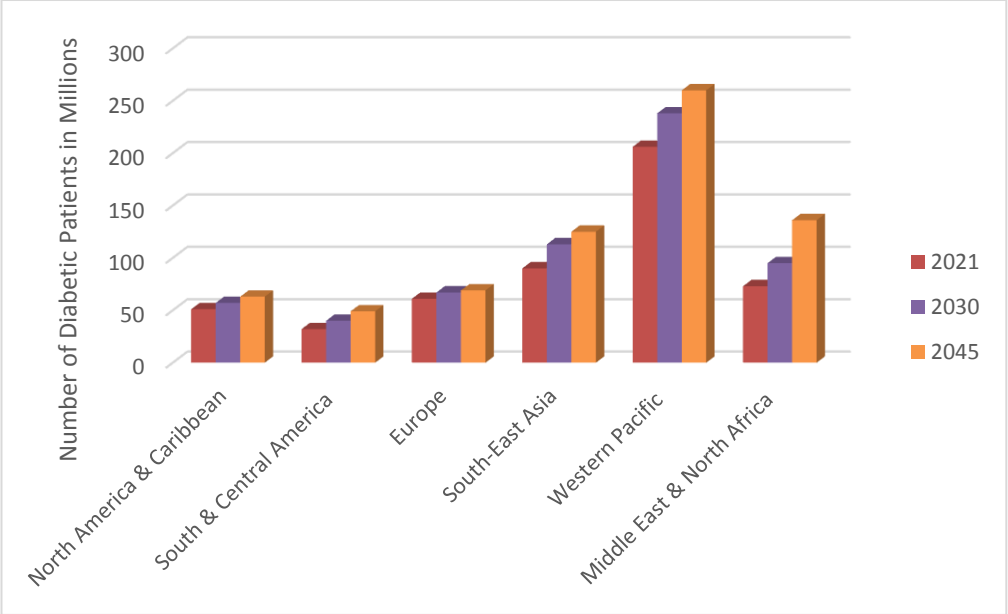


Figure 1.1: The Number of Individuals Living with Diabetes Worldwide (IDF, 2022)

Diabetes is a chronic condition characterized by unfavourably high blood glucose levels due to either poor utilization of insulin or absence of insulin (WHO, 2022a). According to the Centres of Disease Control and Prevention (CDC) (2023), the major types of diabetes based on their clinical presentations and aetiologies include type 1 diabetes, gestational diabetes, and type 2 diabetes. Type 1 diabetes is an autoimmune condition wherein the pancreas stops producing insulin. Thus, people with type 1 diabetes are treated with daily insulin doses. Only about 5 to 10% of people with diabetes have this type (WHO, 2022a). Type 2 diabetes involves the body cells having an inadequate response to insulin (CDC, 2023). It is the most commonly

diagnosed diabetes type, with 90 to 95% of individuals with diabetes having this type (WHO, 2022a). Gestational diabetes involves insufficient production of insulin during pregnancy and usually disappears after the child's birth (CDC, 2023).

Several classes of synthetic drugs, including biguanides, meglitinides, sulfonylureas, alpha-glucosidase inhibitors, and thiazolidinediones, have been developed for diabetes treatment (Tran *et al*., 2020). The chemical structures of common biguanides (metformin), meglitinides (repaglinide and nateglinide), alpha-glucosidase inhibitors (miglitol and voglibose), sulfonylureas (glipizide) and thiazolidines (pioglitazone and rosiglitazone) used in diabetes treatment are provided in Figure 1.2. However, none of these drugs cure diabetes. Hence, most diabetic patients take synthetic drugs for the rest of their life to manage their condition and associated complications (Ngugi *et al*., 2015). Unfortunately, this continuous use of synthetic drugs causes adverse side effects, which has increased the demand for non-toxic drugs for diabetes management (Kumar *et al*., 2021). As a result, the use for herbal medicines for diabetes treatment has increased in recent years. Over 800 medicinal plants worldwide are used in diabetes treatment because they are relatively inexpensive, effective, and have fewer side effects (Bilal *et al*., 2018; Kumar *et al*., 2021).

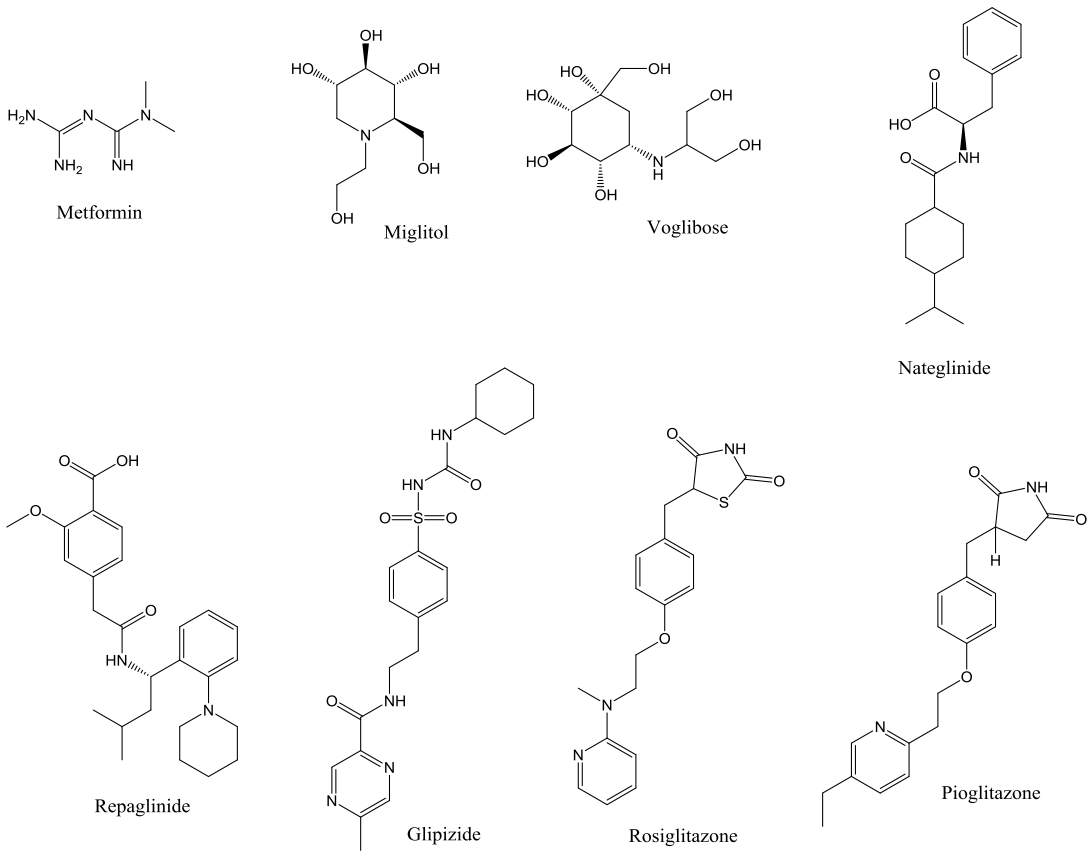


Figure 1.2: Chemical Structures of Some Approved Antihyperglycemic Pharmaceuticals (Tran *et al*., 2020)

Achieving optimal diabetes control is difficult because of the complexity of the disease (CDC, 2023). However, only a few herbal plants and medicines have undergone laboratory testing to determine their efficacy and safety. Uncontrolled diabetes is associated with several major complications – such as hyperglycaemia, hyperlipidaemia, angiopathy, nephropathy, retinopathy, nephropathy, and ketoacidosis – which result in reduced life expectancy and disability (Ngugi *et al*., 2015). Hyperglycaemic condition is the most common complication in both Type 1 and Type 2 diabetic patients (Papatheodorou *et al*., 2018). It involves the blood sugar concentrations remaining higher than the normal range despite the patient taking an antidiabetic medication, necessitating the prescription of additional medications to

correct it (Tomic *et al*., 2022). Additionally, poor glycaemic control often leads to organ damage, necessitating specialized treatment (Ministry of Health Kenya, 2020). Therefore, it is important to subject herbal plants and medicines used for diabetes treatment to laboratory testing to determine their efficacy and safety to prevent the development of diabetes-related complications (Kumar *et al*., 2021).

1.1.2 Cancer

The prevalence of cancer is also rising globally, with over 19 million new cases and about 10 million deaths being reported in 2020 (International Agency for Research on Cancer, 2020). Notably, cancer is among the primary causes of mortality and morbidity globally, making it a major public health concern (Ministry of Health Kenya, 2017). The estimations are grimmer for middle- and low-income countries, where cancer diseases account for about 70% of deaths. In Sub-Saharan Africa, 520,158 cancer-related deaths were reported in 2020, representing an 87.2% mortality rate (Globocan, 2020). In Kenya, cancer causes seven out of a hundred deaths, with a 21.5% survival rate (Ministry of Health Kenya, 2017). In 2020, approximately 42,116 new cancer cases were diagnosed in Kenya, with 27,092 cancer-related deaths occurring in the same year (Globocan, 2020). The three most commonly diagnosed cancer cases among Kenyan males and females in 2020 are presented in Figure 1.3.

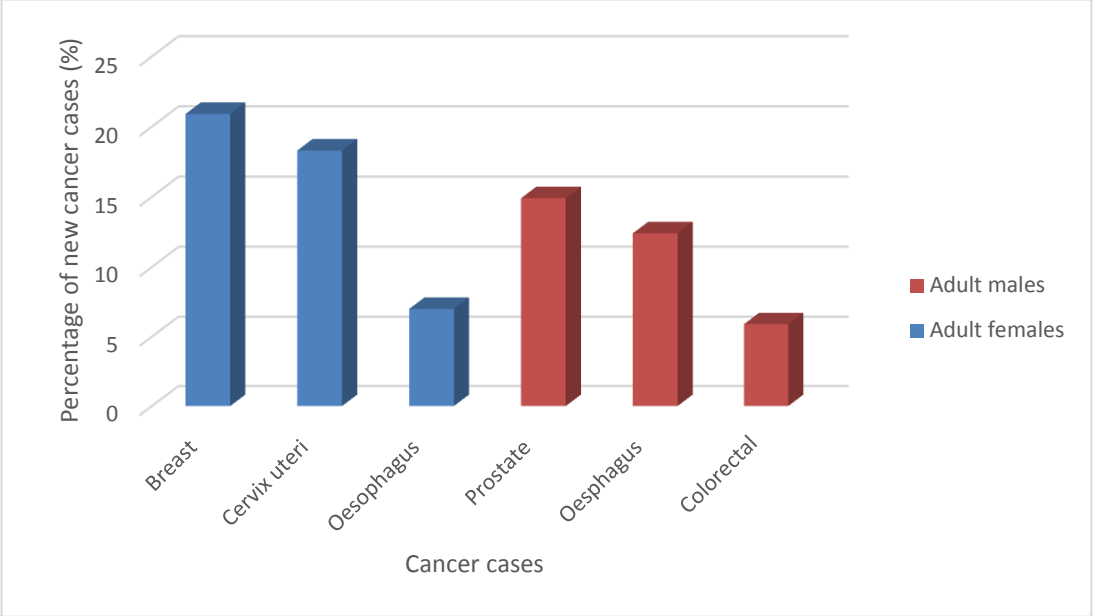


Figure 1.3: Incidence of Cancer Cases in Kenya, 2020 (Globocan, 2020)

Cancer is a chronic condition that results when normal cells are transformed into tumour cells in multiple stages, generally progressing from pre-cancerous lesions to malignant tumours (WHO, 2022b). A wide range of environmental, behavioural, and genetic factors cause and promote cancer (National Cancer Institute, 2023). For example, tobacco use, air pollution, physical inactivity, unhealthy diet, and alcohol consumption. The cancer care process from diagnosis to treatment is illustrated in Figure 1.4. Current conventional cancer treatments include surgery, radiation, gene therapy, chemotherapy, antibody-based immunotherapy, and combination therapy, depending on the stage or type of the disease (Ong'udi *et al*., 2019). Chemotherapy is the most widely used treatment modality for cancer because it is the most effective one when used alone or together with radiotherapy (Debela *et al*., 2021).

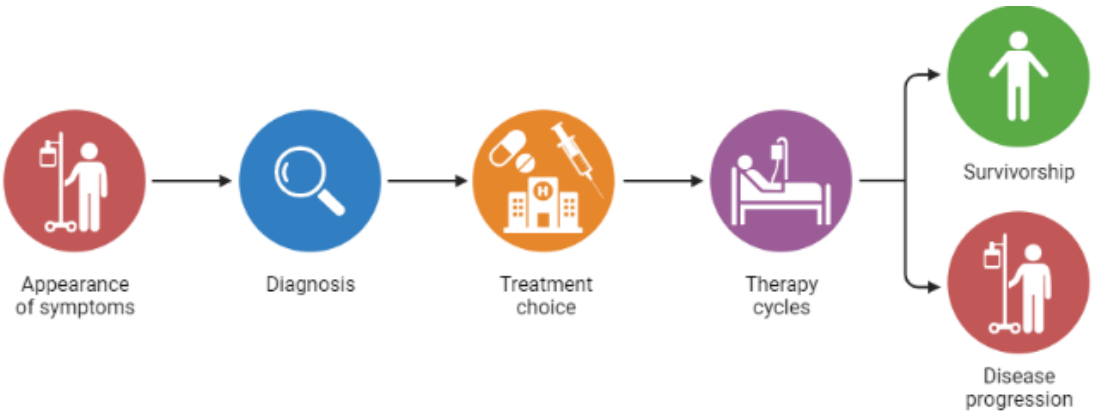


Figure 1.4: The Cancer Care Process

However, the cost of cancer therapies is high, causing people in developing countries to depend on herbal medicines for their treatment because of their affordability (Wambalaba *et al*., 2019; Ochwang'i *et al*., 2018). Additionally, increased cases of failed chemotherapy due to the development of drug resistance and adverse side effects have led researchers to look at herbal medicines as an alternative treatment therapy for cancer (Omosa *et al*., 2016). Herbal medicines are perceived to be more biologically friendly because they are less toxic to normal cells and have alternative ways of promoting the apoptosis of cancerous cells (Seca & Pinto, 2018). Hence, a huge increase in patients using herbal remedies to manage cancer is being experienced worldwide. However, most of these commercially-available herbal remedies have not been subjected to laboratory testing to determine their safety and efficacy.

1.2 Problem Statement

The increased use of herbal medicines by diabetes and cancer patients poses several quality control issues due to weak regulatory mechanisms for herbal medicines in

Kenya. Herbal medicines, unlike pharmaceuticals, are not subjected to routine analytical analysis. The first issue is that herbal medicines sold in the country might be adulterated with synthetic compounds, including pharmaceuticals, without the knowledge of patients and regulatory bodies. Herbal medicines are sometimes adulterated with pharmaceuticals to enhance their efficacy. However, this practice is very dangerous due to the high likelihood of adverse herb-drug interactions. Secondly, some herbal medicines may contain phytochemicals (secondary metabolites) that are toxic to humans, leading to adverse patient events. Many anticancer and antidiabetic herbal medicines are advertised as 100% safe despite not being subjected to safety tests. Thus, some herbal medicines may contain phytochemicals with reported toxicity to humans without the knowledge of the herbalist or vendor. Third, some herbal medicines may lack bioactive phytochemicals useful in diabetes and cancer treatment. Lastly, some herbal medicines might be contaminated during the processing and packaging process. Therefore, analysis of antidiabetic and anticancer herbal medicines sold in Kenya for phytochemicals, contaminants and adulterants is necessary to ensure that they are both safe and efficacious

1.3 Justification

The high prevalence of diabetes and cancer in Kenya and the increased use of herbal remedies to treat them justify the analysis of antidiabetic and anticancer herbal medicines sold in the country for phytochemicals to determine their efficacy (IDF, 2021; Globocan, 2020; WHO, 2019). Additionally, several studies have established that herbal remedies are adulterated with synthetic compounds, including

pharmaceuticals, leading to several adverse patient events (Guo et al., 2020; Al Lawati et al., 2017; Chen et al., 2009). Moreover, various phytochemicals found in medicinal plants have reported toxicity to humans when consumed. For example, pyrrolizidine alkaloids, which occur in a variety of medicinal plants, cause significant hepatoxicity when consumed by animals and humans. Therefore, analysing anticancer and antidiabetic medicines sold in Kenya for phytochemicals and synthetic compounds present as adulterants is justified to ensure these medicines are safe and efficacious.

1.4 Null Hypothesis

There are no phytochemicals, contaminants, or adulterants present in selected herbal remedies sold for diabetes and cancer treatment in Uasin Gishu and Nairobi Counties, Kenya.

1.5 Objectives

1.5.1 General Objective

To determine the phytochemicals, contaminants, and adulterants present in selected herbal remedies sold for diabetes and cancer treatment in Uasin Gishu and Nairobi Counties, Kenya.

1.5.2 Specific Objectives

i. To determine the phytochemical profiles of selected commercially-available herbal remedies used for diabetes and cancer treatment in Uasin Gishu and Nairobi Counties, Kenya using GC-MS.

ii. To determine the synthetic compounds present as adulterants or contaminants in the sampled herbal remedies using GC-MS.

iii. To validate an LC-MS/MS method to be used to test for adulteration of herbal medicines with selected locally-available antidiabetic pharmaceuticals.

iv. To determine the antidiabetic pharmaceuticals present as adulterants in the sampled herbal remedies using the validated LC-MS/MS method.

1.6 Study Limitations

The study faced several limitations that limited its scope. The first limitation involved a low number of herbal medicines available in the Kenyan market being specifically indicated for diabetes and cancer treatment, which limited the number of herbal medicines sampled. The second limitation was the inaccessibility of analytical and internal standards of many pharmaceuticals, including anticancer pharmaceuticals, for researchers due to funding challenges. As a result, the study did not evaluate the sampled herbal medicines for adulteration with anticancer pharmaceuticals. The last limitation was software limitations. The LC-MS/MS instrument used did not have a pre-installed spectral library, thus could not be used for phytochemical screening. Consequently, the study was limited to the identification of volatile phytochemicals in the herbal remedies sampled.

CHAPTER TWO

LITERATURE REVIEW

An upsurge in patients using herbal medicines to treat their chronic illnesses is being experienced globally. Approximately 80% of the global population uses herbal products for their primary healthcare needs (WHO, 2022a). This upsurge is due to herbal remedies being perceived as safe, efficacious, affordable, and accessible (Kamau *et al*., 2016). Like other African countries, Kenya is composed of many communities that utilize traditional herbal medicines to manage their health due to the rich traditional ethnobotanical knowledge that these communities possess (Wambalaba *et al*., 2019). However, the regulation of herbal medicines is less robust than that of pharmaceuticals – which are regulated by the Pharmacy and Poisons Board. As such, most of the herbal medicines sold in the country have not been scientifically tested for their efficacy and safety, which poses a great risk to consumers' health (Okumu *et al*., 2017). Additionally, many herbalists practice without being registered by the National Traditional Health Practitioners Association (NATHEPA) and operate clinics not licensed by the Ministry of Sports, Culture, and Arts (Kenya News Agency, 2019). As such, most of the herbal medicines sold in the country have not been scientifically tested for their efficacy and safety, which poses a great risk to consumers' health (Okumu *et al.*, 2017).

2.1 Known Plants Used for Diabetes Treatment

Numerous plants are used by different communities globally to treat diabetes, but most have not been evaluated for their efficacy (Kumar *et al*., 2021; Tran *et al*., 2020, Ngugi *et al*., 2015). These plants are classified into four primary types: those

that act like insulin, those that increase insulin production by preserving the functioning of the β-cells, those that improve glucose utilization, and those that have hypoglycaemic activity by miscellaneous mechanisms (Gupta *et al*., 2018). Some of the reported biological activities of these plants include hypoglycaemic activity, α-amylase inhibitory, antiglucosidase activity, hypolipidemic activity, lipid peroxidation inhibition, anti-inflammatory activity, and antioxidant activity (Kamau *et al*., 2016). Antidiabetic activities emanate from a wide variety of mechanisms, and some common mechanisms responsible for these activities are shown in Figure 2.1.

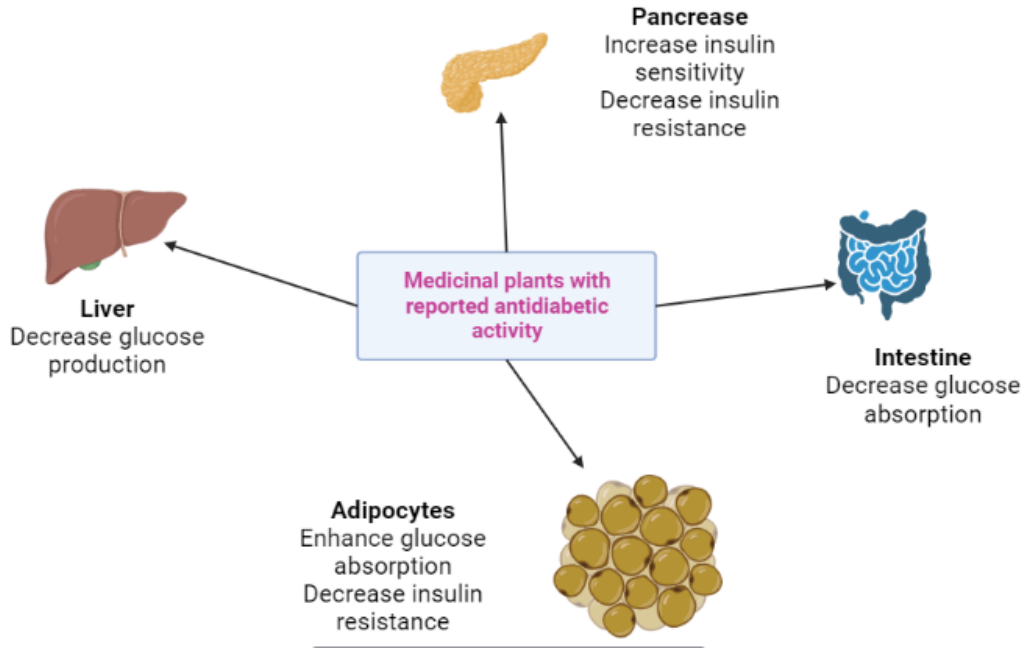


Figure 2.1: Common Therapeutic Mechanisms of Herbal Plants with Antidiabetic Activity (Tran *et al*., 2020)

In Kenya, a few ethnobotanical publications document traditional medicinal plants used in diabetes management. A study by Keter and Mutiso (2012) on medicinal plants used to manage diabetes in Lower Eastern Province reported that thirty-nine

different species were used by traditional health practitioners (THPs) to manage diabetes in the region. Another study by Kamau *et al*. (2016) on diabetes management using medicinal plants in Nyeri County reported that thirty different plant species were used in the region to manage diabetes. Table 2.1 lists some plants, their families, and parts commonly used for diabetes treatment in Kenya and their relevant pharmacological activity (Kamau *et al*., 2016; Keter & Mutiso, 2012).

Table 2.1: Plant Species Commonly Used by THPs to Treat Diabetes in Kenya

Scientific name	Family	Parts used	Reported biological activity
Zanthoxylum chalybeum Engl.	Rutaceae	Stem bark	None
Azadirachta indica A. Juss.	Meliaceae	Stem bark	Antioxidant and antihyperglycemic activities
Cassia abbreviata Oliv.	Fabaceae	Leaves and pods	Antioxidant activity and α-glucosidase inhibition of stem bark extracts
Senna singueana (Delile) Lock	Fabaceae	Leaves	None
Cactus spp.	Cactaceae	Leaves	Antidiabetic activity
Momordica foetida Schumach	Cucurbitaceae	Leaves	Hypoglycaemic activity
Lactuca inermis	Asteraceae	Leaves	Antibacterial activity
Mangifera indica	Anacardiaceae	Leaves	Antihyperglycemic, antibacterial, and antidiabetic activities
Sonchus asper	Asteraceae	Leaves and roots	Antioxidant, hepaprotective, and antibacterial activities. Enhances cognitive performance
Sonchus oleraceus	Asteraceae	Roots	Antibacterial and antioxidant activities
Launaea cornuta	Asteraceae	Whole plant: leaves, stems	Antidiabetic and antimalarial activities

2.2 Known Plants Used for Cancer Treatment

Similarly, numerous medicinal plants are used to treat cancer in Kenya. An ethnopharmacological review of medicinal plants used for cancer treatment in Kenya reported 63 species of trees, 40 species of herbs and shrubs, and 2 species of climbers were used (Onyancha *et al*., 2019). Most of these plants were from the Lamiaceae, Rubiaceae, Fabaceae, Euphorbiaceae, Asteraceae, Solanaceae, Rutaceae, Apocynaceae, and Malvaceae families. The leaves, stem bark, and roots were the most commonly used plant parts. Decoction, infusion, and poultices were the most commonly used preparation methods. The proposed therapeutic mechanisms of these plants used in cancer treatment is shown in Figure 2.2.

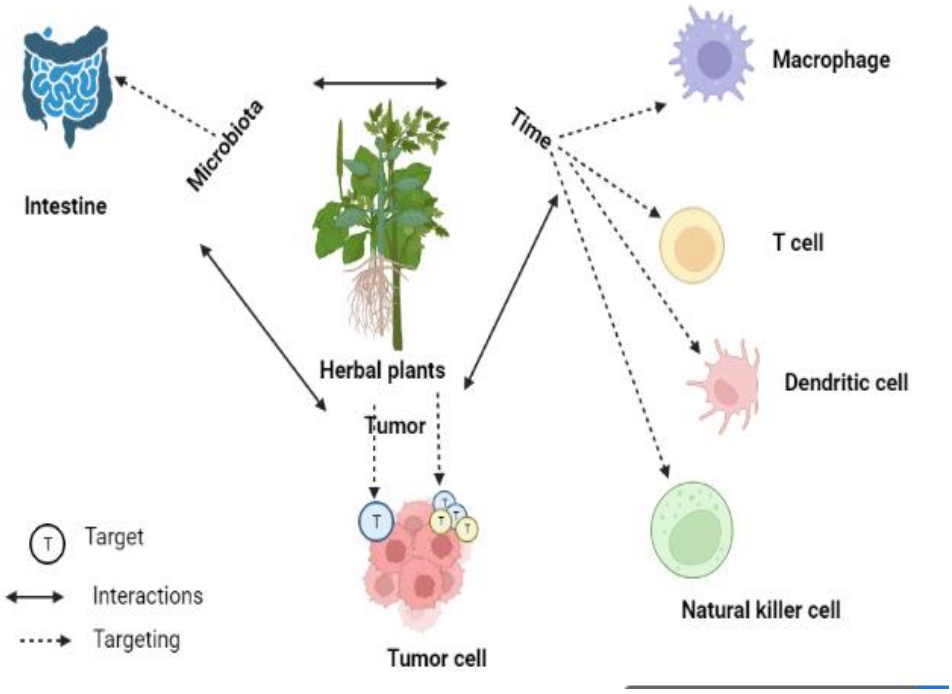


Figure 2.2: Proposed Therapeutic Mechanisms of Herbal Plants Used in Cancer Treatment (Huang *et al*., 2021)

Few studies have investigated the *in vitro* anticancer activity of the medicinal plants used to treat cancer in Kenya. Omosa *et al*. (2016) analysed 91 medicinal plants used to treat cancer-related symptoms and cancer by indigenous communities in Kenya. They reported that 12 extracts, which are listed in Table 2.2, showed over 50 percent leukaemia cell inhibition. The highest cytotoxicity was exhibited by S. *aculeastrum*'s berries and A. *schimperiana*'s stem bark. Another study by Onyancha *et al*. (2018) analysed the *in vitro* cytotoxicity of 8 medicinal plants against the HCC 1395 breast cancer cell line. The highest cytotoxicity was exhibited by methanol and water extracts of *Fagaropsis angolensis*, *Hydnora abyssinica*, and *Combretum tanaense*. However, 36% of the extracts were cytotoxic to normal cells. Moreover, Kimani *et al*. (2018) reported that the extract of *Dichrostachys cinereal* had the highest *in vitro* cytotoxicity against 22Rv1 prostate cancer cell lines.

Table 2.2: Some of the Plants Used to Treat Cancer in Kenya with Significant *in vitro* Cytotoxicity

Scientific Name	Family	Part Used
Erythrina sacleuxii	Fabaceae	Root bark
Albizia gummifera	Fabaceae	Leaves
Zanthoxylum gilletii	Rutaceae	Stem bark
Croton sylvaticus	Euphorbiaceae	Stem bark
Albizia schimperiana	Fabaceae	Stem bark
Erythrina burttii	Fabaceae	Root bark
Microglossa pyrifolia	Asteraceae	Leaves, root bark, and stem bark
Bridelia micrantha	Euphorbiaceae	Leaves, roots, and stem bark
Prunus africana	Rosaceae	Roots and stem bark
Phyllanthus fischeri	Euphorbiaceae	Stem bark and leaves
Bridelia micrantha	Euphorbiaceae	Stem bark
Shirakiopsis elliptica	Euphorbiaceae	Leaves and bark

2.3 Common Bioactive Phytochemicals in Herbal Products Used to Treat Diabetes

Secondary metabolites of medicinal plants used to treat diabetes are usually responsible for their antidiabetic activities. For plants that have been proven to have antidiabetic activity, the common types of secondary metabolites found include alkaloids, phenolic acids, terpenes, saponins, anthraquinones, and flavonoids (Kamau, 2018). Some secondary metabolites that have been isolated and proven to exert significant antidiabetic activities include curcumin (phenolic acid), genistein (isoflavone), catechin (flavanol), acacetin (flavone), rutin (flavonol), mahanimbine (alkaloid), limonene (terpene), gymnemic acid (saponin), emodin (anthraquinone) (Madariaga-Mazón *et al*., 2021; Shanmugam *et al*., 2021; Tran *et al*., 2020; Lankatillake *et al*., 2019). Their structures are provided in Figure 2.3.

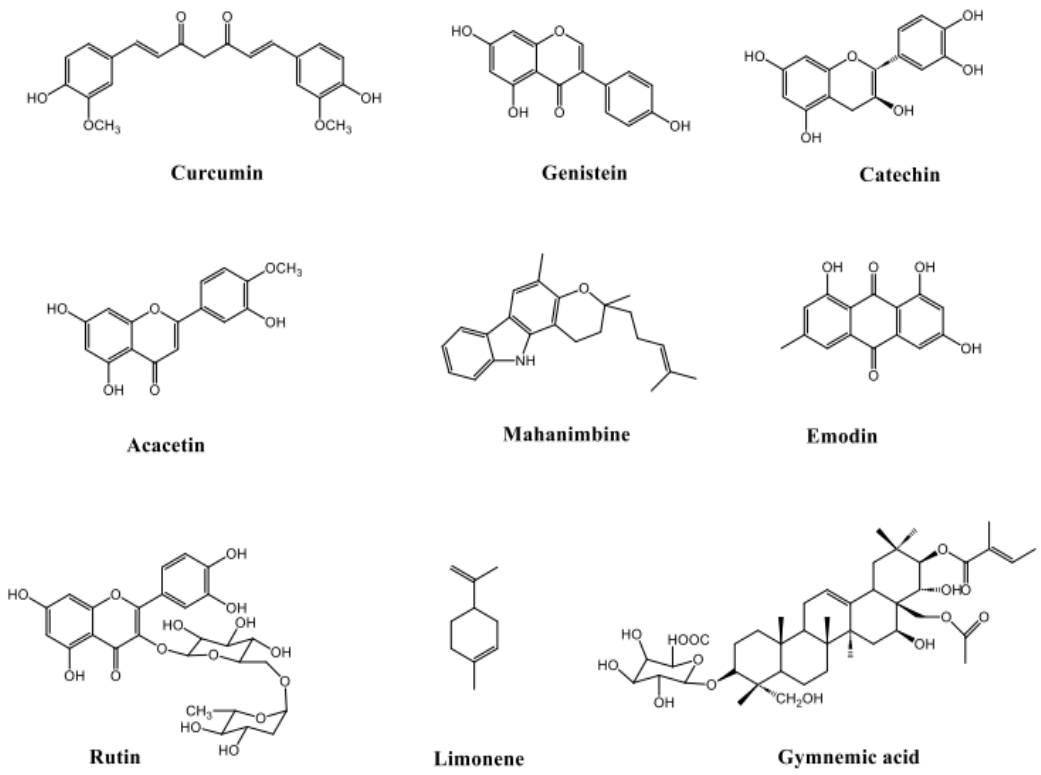


Figure 2.3: Chemical Structures of Selected Bioactive Phytochemicals with Reported Antidiabetic Activities

Flavonoids' antidiabetic activity stems from their ability to improve insulin secretion, inhibit α-glucosidase and α-amylase activity, inhibit intestinal glucose uptake, inhibit glucose-induced insulin secretion, prevent beta-cell apoptosis, and hypoglycaemic effect (Ota & Ulrih, 2017; Mohan & Nandhakumar, 2014; Pinent *et al*., 2008). Terpenes' antidiabetic effect is associated with inhibited α-amylase activity and amelioration of cardiomyopathy alterations (Kamau *et al*., 2016). The antidiabetic effect of alkaloids is linked to their hypolipidemic and hypoglycaemic activity (Zhou *et al*., 2012). The antidiabetic activity of saponins is associated with their anti-obesity, hypoglycaemic, hypocholesterolaemic, antiglycation, and antioxidant effects (Elekofehinti *et al*., 2014; Chen *et al*., 2011). However, no study was found that

gave the phytochemical profile of commercially-available antidiabetic herbal medicines in Kenya to determine if they contained bioactive compounds with reported antidiabetic activity.

2.4 Common Bioactive Phytochemicals in Herbal Products Used to Treat Cancer

Over 3000 plants globally are claimed to have anticancer activity; however, only a few have been scientifically proven to be effective in cancer treatment (Seca & Pinto, 2018). Therefore, some herbal medicines sold for cancer treatment may lack anticancer activity as claimed since they have not undergone laboratory testing. For Kenyan plants used to treat cancer, Ochwang'I *et al*. (2016) found several phytochemicals with significant anticancer activity, including alkaloids, anthraquinones, xanthines, valepotriates, cardioactive glycosides, flavonoids, essential oils, coumarins, lignans, saponins, and arbutin compounds. The distribution of these phytochemicals is shown in Figure 2.4. However, no study was found that gave the phytochemical profile of commercially-available anticancer herbal medicines in Kenya to confirm if they contained these important bioactive phytochemicals.

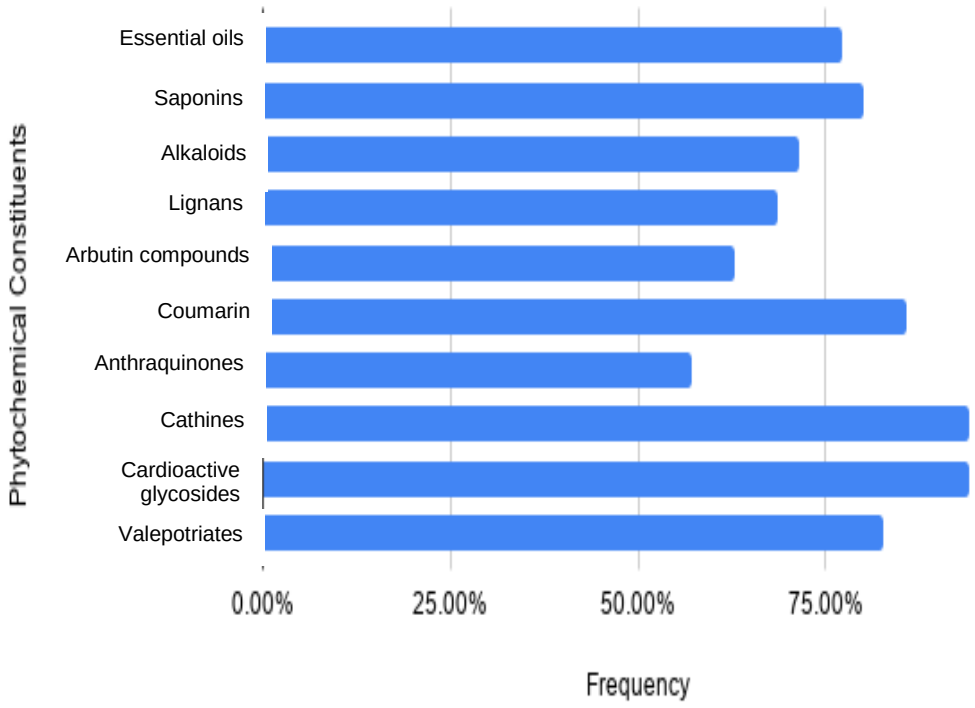


Figure 2.4: Distribution of Phytochemicals Present in Medicinal Plants Used to Treat Cancer in Kakamega County (Ochwang'I *et al*., 2016)

Flavonoids have been proven to have antiproliferative and antioxidant effects against breast cancer, antimutagenic activity, and strong anticancer activities (Salah *et al*., 1995). Flavonoids also prevent the expression of proteins necessary for the survival, angiogenesis, and proliferation of cancer cells (Greenwell & Rahman, 2015). Coumarins have been proven to possess *in vivo* anticancer activity and inhibit cyclin D1 release (Lacy & O'Kennedy, 2004). Lignans exhibit antimitotic, antiviral, and antitumor activities, as well as specific enzyme inhibition (Ochwang'I *et al*., 2016). Alkaloids are proven to possess antibacterial and cytotoxic activity (Omara *et al*., 2022; Omosa *et al*., 2016). Anthraquinones have been shown to inhibit cell proliferation, prevent metastasis, and induce apoptosis (Huang *et al*., 2007).

Saponins have anti-inflammatory activity and antitumorigenic properties (Francis *et al*., 2002). They can also selectively inhibit cancer cells' growth *in vitro* by induction of apoptosis and reduction of the multiplicity and incidence of tumours (Hanausek *et al*., 2001). Cardioactive glycosides increase the immunogenicity of damaged cancer cells by exerting antineoplastic effects (Menger *et al*., 2013). Valepotriates have been proven to have moderate cytotoxicity toward prostate cancer cell lines (Xu *et al*., 2007). Some essential oil components, such as α-humulene, α-Cadinol, and β-elemene, exhibit cytotoxic activity toward cancer lines (Sylvestre *et al*., 2006). However, some of these phytochemicals are cytotoxic to normal cells, undermining the safety of anticancer medicinal plants (Onyancha *et al*., 2019). The structures of selected secondary metabolites of Kenyan medicinal plants with reported anticancer activities are given in Figure 2.5 (Omara *et al*., 2022).

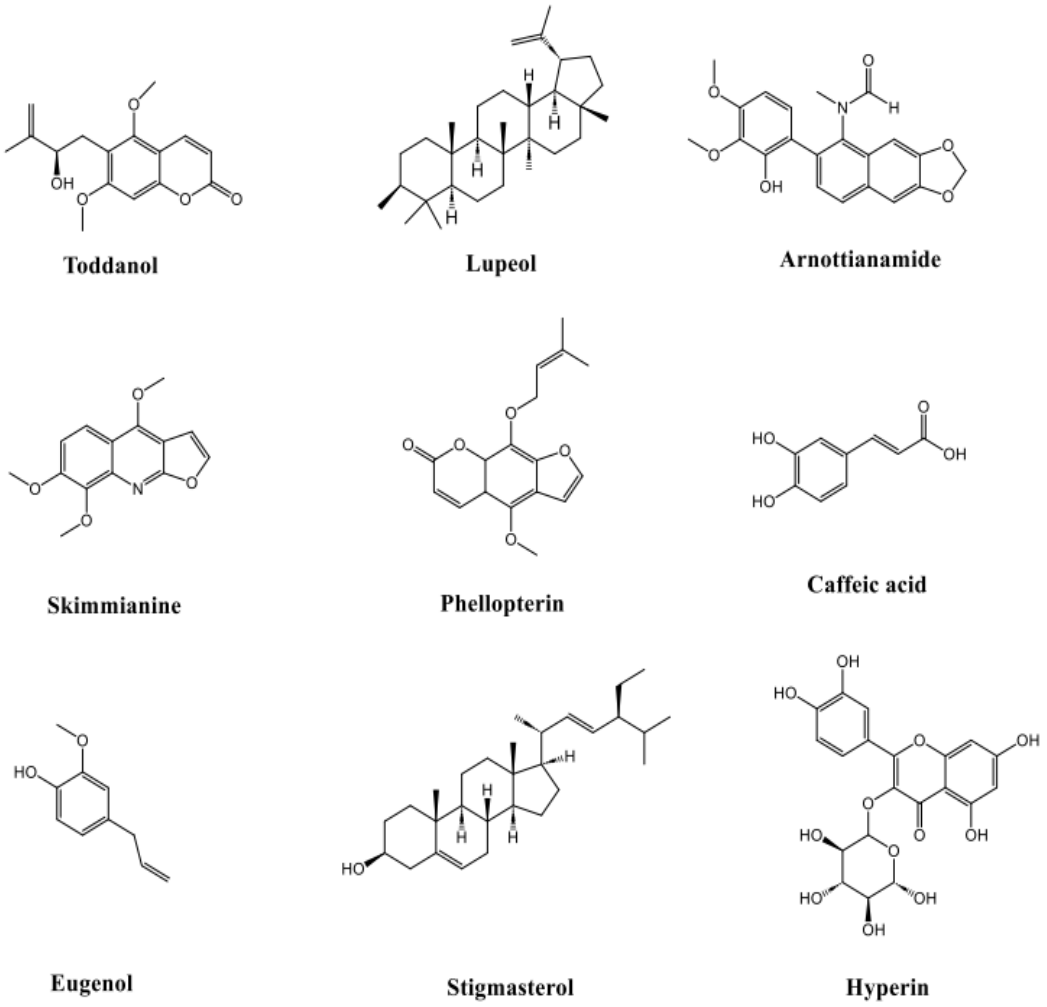


Figure 2.5: Chemical Structures of Selected Bioactive Phytochemicals with Reported Anticancer Activities

2.5 Adulteration and Contamination of Herbal Products with Synthetic Compounds

2.5.1 Contamination of Herbal Products with Phthalates

Several studies have reported contamination of herbal products with phthalates (Andjelković *et al*., 2021; Russo *et al*., 2015). Phthalates are phthalic acid esters extensively used as plasticizers in a wide variety of polymer materials to make them

more soluble, adhesive, and flexible (Wang & Qian, 2021). Some materials that incorporate phthalates include gels, hairsprays, plastic bottles, paper coatings, and paints. Hence, phthalates are suspected to migrate from plastic herbal and food packaging into the food or herbal formulation (Andjelković *et al*., 2021; Russo *et al*., 2015).

Additionally, phthalates have been detected in several species of medicinal plants, including *Calotropis gigantea*, *Pongamia pinnata*, *Brassica napus*, *Triticum aestivum*, *Zea mays*, and *Raphanus salivas* (Saeidnia & Abdollahi, 2013). Interestingly, plants absorb phthalates from contaminated soils via their roots (Kumari & Kaur, 2019). Tan *et al*. (2016) established that using wastewater to irrigate soils contaminates agricultural soils with phthalates, which plants then absorb. This determination of phthalates in food and herbal substances is a major public health concern because of their high potential to disrupt the endocrine system (Giuliani *et al*., 2020; Ma *et al*., 2018).

The chemical structures of commonly reported phthalates with endocrine-disrupting potential in herbal matrices are shown in Figure 2.6. As a result of this potency, these phthalates have been labelled as possible carcinogens by various international government agencies, including the National Institute of Environmental Health Sciences and the Food and Drug Administration (Dutta *et al*., 2020). Numerous countries, including China, the European Union and the United States, have implemented regulations to limit the use of phthalates in food packaging and consumer products (Monti *et al*., 2022).

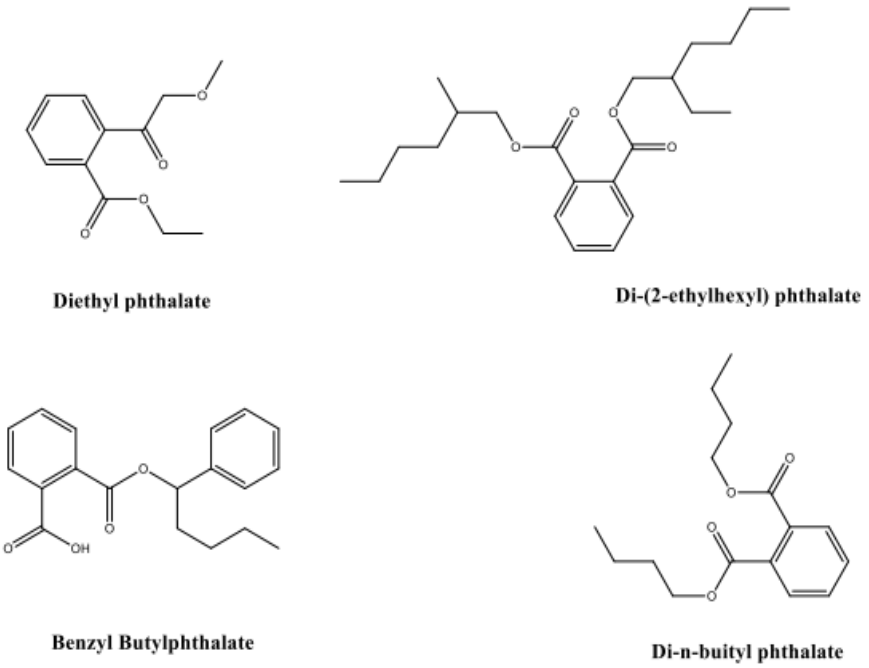
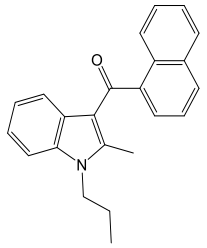


Figure 2.6: Chemical Structures of Phthalates Commonly Reported in Herbal Matrices

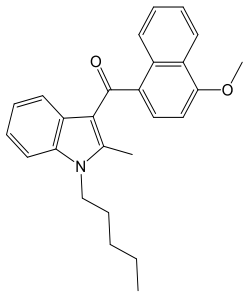
However, the determination of phthalates is highly controversial, even when using their analytical standards, because substantial peaks can be obtained even when they are not present in the sample due to the widespread use of these phthalates in the laboratory environment (Russo *et al*., 2015). Phthalates are found in glassware, reagents, and surfaces. As such, phthalates, particularly di-(2-ethyl-hexyl)phthalate and di-butyl phthalate, are commonly reported as contaminants of food and herbal samples (Andjelković *et al*., 2021; Russo *et al*., 2015). Subsequently, it is challenging to establish confidence that a determination of phthalates is accurate.

2.5.2 Adulteration of Herbal Products with Synthetic Cannabinoids

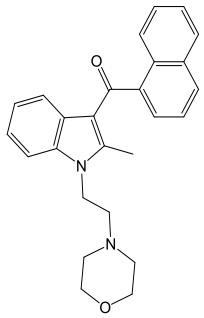
Several studies have reported the adulteration of herbal mixtures with synthetic cannabinoids (Moosmann *et al*., 2012; Hudson & Ramsey, 2011; Penn *et al*., 2011; Uchiyama *et al*., 2010). This adulteration is problematic due to scientific evidence suggesting that synthetic cannabinoids are more potent compared to traditional cannabis, leading to increased dependence. Additionally, synthetic cannabinoid use has been associated with sudden cardiac death, myocardial infarction, psychosis, seizures, arrhythmias, acute tubular necrosis, suicidal ideation, and intracranial hemorrhage (Alipour *et al*., 2019). The structures of some of the commonly reported synthetic cannabinoids in herbal products are provided in Figure 2.7.



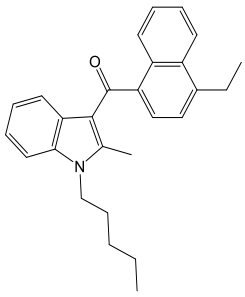
(2-methyl-1-propyl-1H-indol-3-yl)(naphthalen-1-yl)methanone (JW-015)



(4-methoxynaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone (JW-081)



{1-[2-(morpholin-4-yl)ethyl]-1H-indol-3-yl}(naphthalen-1-yl)methanone (JWH-200)



(4-ethylnaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone (JWH-210)

Figure 2.7: Chemical Structures of Synthetic Cannabinoids Commonly Determined in Herbal Products

2.5.3 Adulteration of Herbal Products with Pharmaceuticals

Adulterating herbal medicines with pharmaceuticals has become a common practice in recent years despite its potential to cause fatalities due to adverse drug-herb interactions. Both unapproved and approved synthetic drugs have been used to adulterate herbal medicines (Al Lawati *et al*., 2017). Several pharmaceuticals, including antidepressants, anticoagulants, antidiabetic, antihypertensive, anticancer, and cardiovascular drugs, have been proven to interact with herbal medicines (Zhang *et al*., 2011). The proposed mechanisms of these interactions are shown in Figure 2.8. Some pharmacodynamic interactions led to adverse health effects, including hypoglycaemia, massive gastrointestinal bleeding, lactic acidosis, hypertension, Cusing's syndrome, arrhythmia, somnolence, poisoning cases, and death in some cases (Chen *et al*., 2011; Ernst, 2002). For example, the death of thirty individuals in Cambodia due to the consumption of Artesunate, which was adulterated with pyrimethamine and sulphadoxine (Chowdry, 2018).

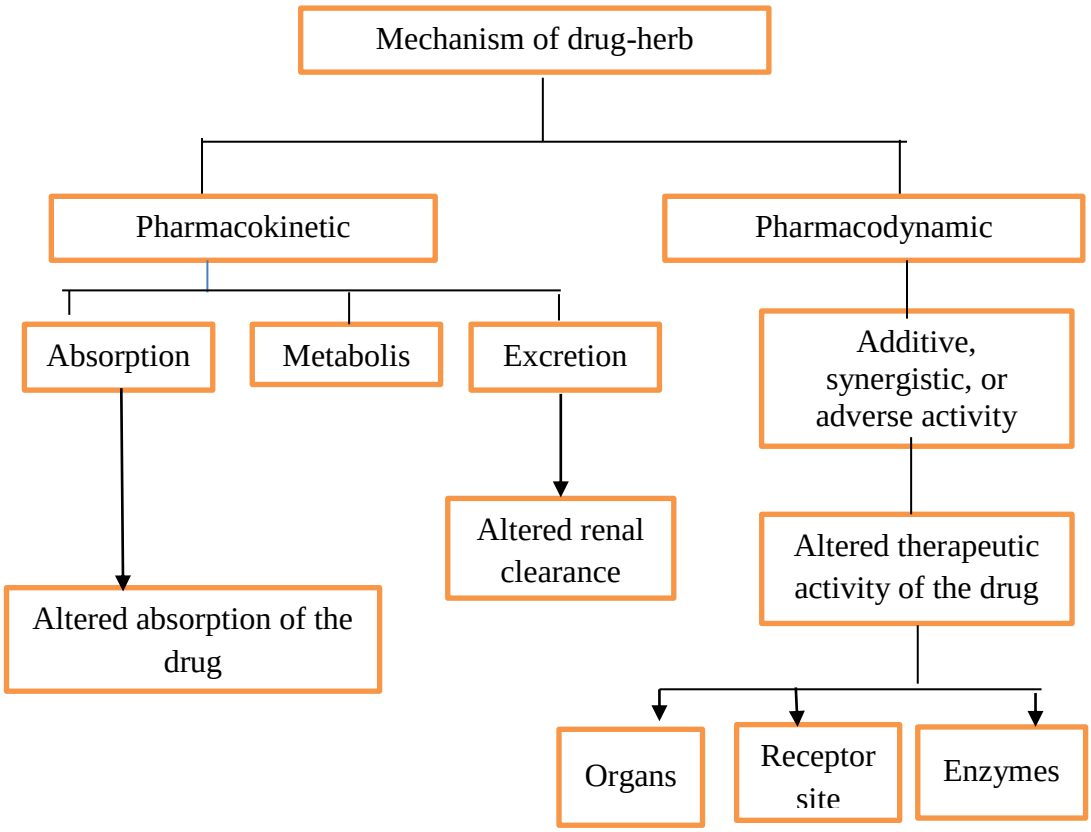


Figure 2.8: Proposed Mechanisms of Drug-Herb Interactions (Zhang *et al.*, 2011)

Various analytical techniques have been utilized to identify and quantify pharmaceuticals used to adulterate herbal remedies, including capillary electrophoresis, infrared spectroscopy, high performance liquid chromatography (HPLC), GC, and TLC (Minh *et al.*, 2019; Fejos *et al.*, 2014; Viana *et al.*, 2013; Cianchino *et al.*, 2008; Verbitski *et al.*, 2008; Ku *et al.*, 2003). Hyphenated techniques, particularly LC-MS/MS or LC-MS, are most commonly used to analyse for adulteration of herbal products with pharmaceuticals because they meet the majority of the essential requirements of analysis for food samples and herbal products (Al Lawati *et al.*, 2017).

Several studies have reported adulteration of antidiabetic herbal medicines with oral antidiabetic pharmaceuticals, including metformin, rosiglitazone, and glibenclamide. Kumar *et al*. (2011) reported that antidiabetic herbal medicines sourced from a local THP had 93.1 milligrams of metformin hydrochloride despite being labelled as containing only herbal ingredients. Chowdhury (2018) analysed twenty-five herbal medicines for adulteration in Bangladesh and found that 64% were adulterated with both glimepiride and metformin. Steyn *et al*. (2018) also found glimepiride and metformin in an antidiabetic herbal medicine prescribed to a woman in India who had been experiencing hypoglycaemia. These studies show that adulteration of antidiabetic herbal medicines is a common occurrence, necessitating their assessment for pharmaceuticals present as adulterants.

In Kenya, gliclazide, metformin, glimepiride, glibenclamide, and insulin injections are the most commonly used antidiabetic pharmaceuticals (Ministry of Health, 2019). Thus, herbal antidiabetic drugs sold in Kenya should be analysed for possible adulteration using these pharmaceuticals and others reported in literature. The chemical structures of metformin, glibenclamide, gliclazide, and glimepiride are shown in Figure 2.9.

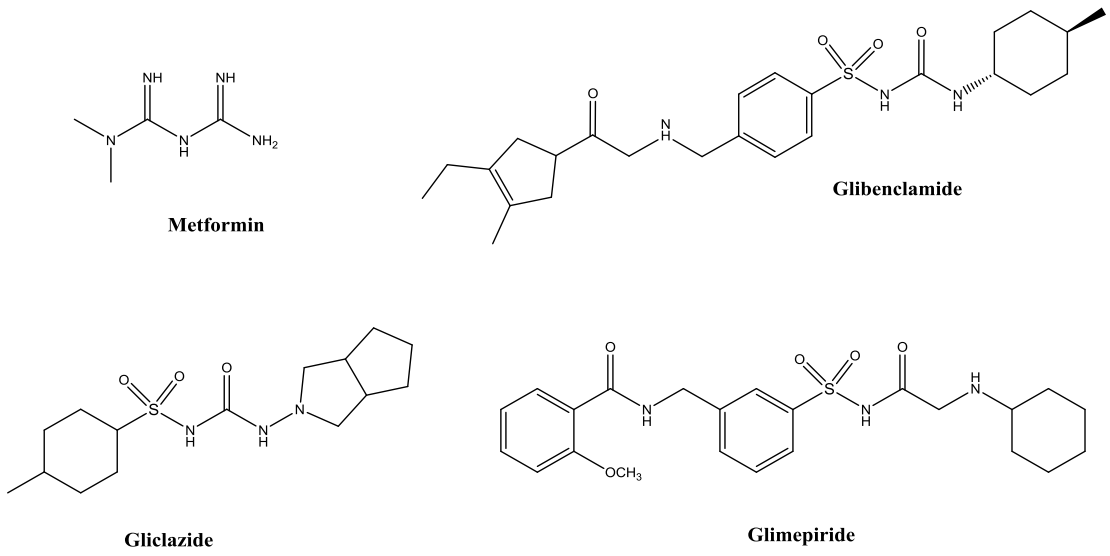


Figure 2.9: Chemical Structures of the Most Commonly Used Antidiabetic Pharmaceuticals in Kenya

For cancer treatment, the Kenya Essential Medicines List (KEML) includes alendronic acid, allopurinol, asparaginase, bleomycin, calcium folinate, capecitabine, carboplatin, chlorambucil, cisplatin, and cyclophosphamide (Ministry of Health, 2019). However, these drugs are not easily accessible over the counter as most are administered in hospitals, limiting the likelihood of THPs adulterating their anticancer herbal medicines using these pharmaceuticals. Additionally, the high cost of these drugs limits their use as adulterants in herbal therapies used for cancer treatment. Moreover, the formulation of these drugs is complex, limiting their use as adulterants.

2.6 Analytical Techniques for Analysis of Herbal Products

2.6.1 Solid Phase Extraction (SPE)

SPE is a separation technique used to extract analytes of interest from complex matrices during sample preparation. The use of SPE in the clean-up and preconcentration of herbal samples prior to GC-MS analysis has been increasing in recent years (Lim *et al*., 2020; Ji *et al*., 2019; Hu *et al*., 2016; Lehotay *et al*., 2016; Cui *et al*., 2015; Wulandari *et al*., 2015). The unique advantages of this analytical technique that have driven this use include:

- Consumes low amounts of solvents and is easy to use (Cui *et al*., 2015)
- Avoids solvent emulsification (Hu *et al*., 2021)
- SPE cartridges, particularly C_{18} SPE cartridges, adsorb a wide variety of organic compounds with different polarities from herbal and food samples (Andrade-Eiroa *et al*., 2016b)
- High sample throughput and high analyte-enrichment factors, enabling the analysis of small volumes of sample solutions (Andrade-Eiroa *et al*., 2016a)

A typical SPE procedure involves four steps:

1. Conditioning the SPE adsorbent by passing an organic solvent, usually the incoming sample matrix (Andrade-Eiroa *et al*., 2016a).
2. Loading the sample solution using an appropriate flow rate to obtain adequate analyte adsorption (Cui *et al*., 2015). Andrade-Eiroa *et al*. (2016b) recommend loading the sample using gravity for optimal analyte adsorption.
3. Washing the cartridge by flushing a solvent that is weaker than the matrix of the loaded sample to remove any adsorbed interferences, then drying the cartridge using nitrogen or air (Hu *et al*., 2021).

4. Elution of the retained analytes using a suitable solvent, evaporating the solvent, and reconstituting the residue in a suitable solvent.

2.6.2 Gas Chromatography coupled with Mass Spectrometry (GC-MS)

GC-MS combines gas chromatography (GC) to separate compounds from a gaseous mixture with mass spectrometry (MS) to identify the separated components.

2.6.2.1 Phytochemical Screening of Herbal Products Using GC-MS

GC-MS has widely been utilized for phytochemical analysis of medicinal plants, herbs, and herbal drugs (Bhalla *et al*., 2021; Yang *et al*., 2021; Shaikh & Patil, 2020; Shettar *et al*., 2017; Saraswati *et al*., 2016; Chandel & Kumar, 2015). Notably, a review of phytochemical studies on herbal tea in China by Zhao *et al*. (2013) reported that 90% of the studies used GC-MS. Some reasons for this wide use of GC-MS in phytochemical studies include rapid analysis, high sensitivity, less solvent consumption, and less analysis time (Yang *et al*., 2021). Additionally, GC-MS has several MS libraries that can be installed to identify the volatile compounds in different extracts through comparisons with reference mass spectra. As such, GC-MS can be used for the phytochemical screening of herbal extracts to guide the isolation of identified bioactive compounds.

In phytochemical screening, the cleaned-up herbal extract solution is injected into the GC system via a heated injector, which vaporizes the sample components instantly (Hübschmann, 2015). The gaseous sample is transported into a heated column by the

carrier gas (mobile phase). The temperature of the column is varied to separate the sample components based on the strength of their interactions with the stationary phase (column adsorbent). The separated components flow through a transfer line to the MS.

Once the components are in the MS, they are bombarded by a stream of fast-moving electrons from the ion source at 70 eV and become ionized. The resultant ions are then accelerated to the mass analyser that separates them based on their mass-to-charge ratio (m/z). The detector counts the ions and produces an electric signal which is processed to provide a mass spectrum. The ion with the highest intensity is called the base peak and is assigned 100% intensity, and the other ion peaks are expressed relative to it (Olivia *et al*., 2021). The mass spectra of the unknown detected components are then compared to reference mass spectra in the MS libraries, and the most similar spectrum is used to identify each compound (Stein, 2012).

However, GC-MS phytochemical analysis is usually limited to identifying volatile compounds because it is a gas-phase technique. While non-volatile compounds can be derivatized to improve their volatility and make them amenable to this technique, derivatization is complex and time-intensive (Yang *et al*., 2021). As such, using GC-MS for phytochemical screening usually provides a profile of only volatile phytochemicals present in herbal extracts if derivatization is not performed.

Nevertheless, a wide range of volatile phytochemicals have useful biological activities; thus, GC-MS phytochemical screening still generates useful insights about the potential treatment effectiveness of a given medicinal plant or herb. While thin layer chromatography (TLC) can be used to separate and identify non-volatile

phytochemicals in plant extracts, the TLC fingerprint can only be used to identify the types of phytochemicals such as flavonoids, but not the actual compounds (Gad *et al*., 2018; Qureshi *et al*., 2011). As such, it is usually combined with GC-MS to enable the identification of the actual bioactive compounds.

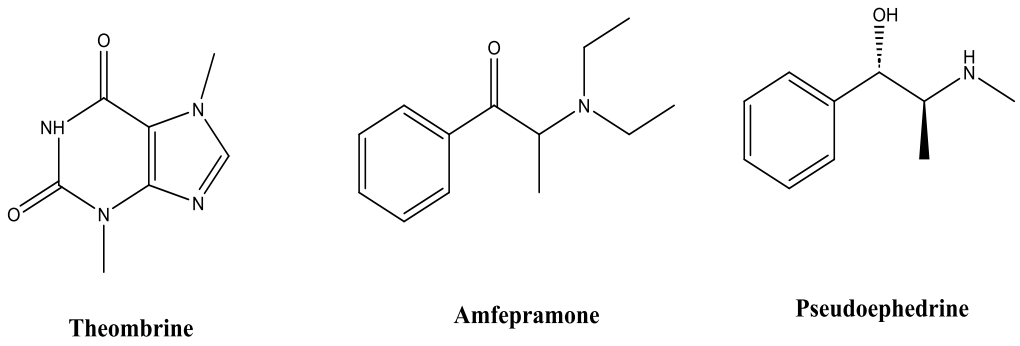
Another limitation of GC-MS phytochemical analysis is the need to purchase standards for qualitative and quantitative analysis of phytochemicals present in herbal extracts. Although the GC-MS libraries can be used to identify unknown compounds based on similarity scores, determining the confidence that a given identification is accurate is difficult (Stein, 2012). The mass spectrum of a compound can differ significantly from its library spectrum for several reasons, including background noise, decomposition in the ionizer, low signal, and collision energy variation (Valdez *et al*., 2018).

This variation in the mass spectrum reduces confidence in the identification in the absence of standards. Additionally, it introduces a risk of false positive results. To increase the probability that the compound identified using the library search is present in the sample, Stein (2012) recommends looking for references to the compound in relevant literature and citing these studies. Therefore, GC-MS is useful for phytochemical screening to identify potential bioactive compounds in herbal extracts. A GC-MS method for qualitative and quantitative analysis can then be developed and validated using standards based on the results of the screening.

2.6.2.2 Determination of Synthetic Compounds in Herbal Matrices Using GC-MS

In addition to phytochemical screening, GC-MS has been applied to identify synthetic compounds present in food and herbal matrices. One common application of GC-MS is the identification of phthalates in food and herbal matrices (Russo *et al*., 2015). Another application is the determination of pharmaceuticals, including depressants, anxiolytics, diuretics, laxatives, and anorexics, in herbal samples (Khazan *et al*., 2014). Lastly, GC-MS has been used to determine the adulteration of herbal mixtures with synthetic cannabinoids (Alipour *et al*., 2019).

Although standards are required for qualitative analysis, the GC-MS analysis of herbal samples can reveal the presence of miscellaneous synthetic compounds. Khazan *et al*. (2014) used the GC-MS library to screen for miscellaneous synthetic compounds present in herbal supplements for weight loss. They reported the presence of theobromine, amfepramone, and pseudoephedrine. The presence of these compounds was later confirmed using standards. The structures of these compounds are provided in Figure 2.10. Therefore, GC-MS can be valuable in screening for miscellaneous synthetic compounds in herbal samples. The screening results can then guide the purchase of standards for qualitative and quantitative analysis.



Theombrine **Amfepramone** **Pseudoephedrine**

Figure 2.10: Chemical Structures of Some Synthetic Compounds Detected in Herbal Supplements

2.6.3 Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS)

LC-MS/MS allows for a wide range of potential adulterants to be analysed and quantified simultaneously in one run using multiple reaction monitoring (MRM). This technique has high selectivity and sensitivity for pharmaceuticals, which allows for trace analysis (Bottoni & Caroli, 2019; Rocha *et al*., 2016). As such, LC-MS/MS has extensively been used to determine herbal products adulterated with pharmaceuticals (Jin *et al*., 2020; Liu *et al*., 2017; Zhong *et al*., 2017; Chen *et al*., 2009; Liang *et al*., 2006).

A sample solution is injected into the LC system via the injector system maintained at ambient temperature. The components in the solution are separated based on the strength of their interactions with the stationary phase (column) (Corradini, 2010). Components with a similar polarity to the stationary phase are retained longer in the column, while those with a different polarity elute first from the column. As such, the components are partitioned between the stationary and mobile phases, and the difference in this partitioning facilitates their separation in the column.

The column effluent with the separated components passes through the interface, where they are vaporized, and the mobile phase is discarded. The ions enter the MS system, where they are separated based on their m/z ratio in the mass analyser before being counted by the detector. The detector gives a signal which is processed to give a tandem mass spectrum, which can then be compared to standard tandem mass spectra of known compounds in MS/MS libraries for the identification of unknown compounds (Wohlfarth *et al*., 2013). MS/MS mass spectral libraries contain over 120,000 spectra for over 15,000 ions of over 7,000 compounds of environmental and biological significance (Simón-Manso *et al*., 2013).

A key limitation of LC-MS/MS methods is the substantial variation of LC-MS/MS results between laboratories due to varying method performance, necessitating regular method validation (Vogeser & Stone, 2020). Jansen *et al*. (2005) compared the mass spectra obtained from different tandem mass spectrometers after standardizing them using a reference compound. They reported that tandem mass spectra did not provide good inter-instrument reproducibility. Thus, there is a significant risk for library searching algorithms misidentifying compounds. As such, validation of developed LC-MS/MS methods, particularly quantitative methods, to determine accuracy, reliability, matrix effects, recoveries, and precision using standard reference materials is important (Simón-Manso *et al*., 2013).

However, the number of standard reference materials is limited due to the complexity of the certification process, forcing analysts to use analytical standards of high purity (>95%) and previously-determined blank samples to develop and validate LC-MS/MS methods for determination of pharmaceutical adulterants in herbal samples

(Jin *et al*., 2020; Liu *et al*., 2017; Zhong *et al*., 2017; Chen *et al*., 2009; Liang *et al*., 2006). One of the most widely used protocols for validating analytical procedures for pharmaceutical analysis is provided by the International Council for Harmonization (ICH) (ICH, 2022). This protocol enables the development of valid and reliable LC-MS/MS methods for determining pharmaceuticals in different matrices. Therefore, this protocol can be used to develop and validate an LC-MS/MS method for the simultaneous determination of selected antidiabetic pharmaceuticals in herbal remedies sold in the country.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study area was Nairobi and Uasin Gishu Counties, Kenya. Nairobi County was chosen because it is a cosmopolitan area with various communities having diverse cultures and customs (Kenya National Bureau of Statistics, 2020). Hence, a wide range of herbal products ends up in this area. These herbal products are then distributed to other parts of the country, making the results of this study generalizable to the wider Kenyan market. Uasin Gishu County was chosen because the region has a wealth of ethnobotanical knowledge, and many communities in this region primarily rely on herbal medicines for treatment (Chebii *et al*., 2020). Additionally, previous studies on herbal products in Nairobi and Uasin Gishu Counties have reported contamination of herbal products (Hassan, 2019; Kariuki, 2012). The map of Kenya showing the geographical positions of Uasin Gishu and Nairobi Counties is given in Figure 3.1.

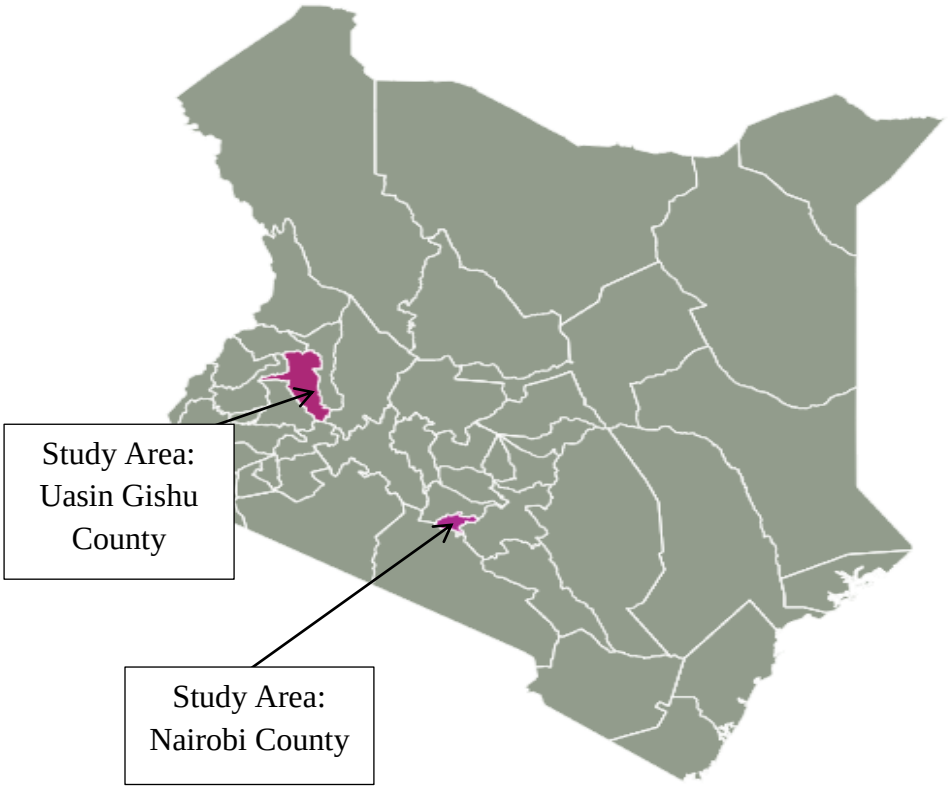


Figure 3.1: Map of Kenya Showing the Geographical Positions of Uasin Gishu and Nairobi Counties (Kenya Magazine, 2023)

Nairobi County is the capital city of Kenya. It covers a total area of 696.1 km^2. It borders Kiambu County to the North, Machakos County to the East and Kajiado County to the South and West. It has 17 sub-counties: Langata, Kibra, Roysambu, Kasarani, Ruaraka, Embakasi South, Embakasi North, Embakasi Central, Embakasi East, Embakasi West, Makadara, Kamkunji, Starehe, Mathare, Westlands, Dagoretti North and Dagoretti South. The map of Nairobi showing its 17 sub-counties is given in Figure 3.2.

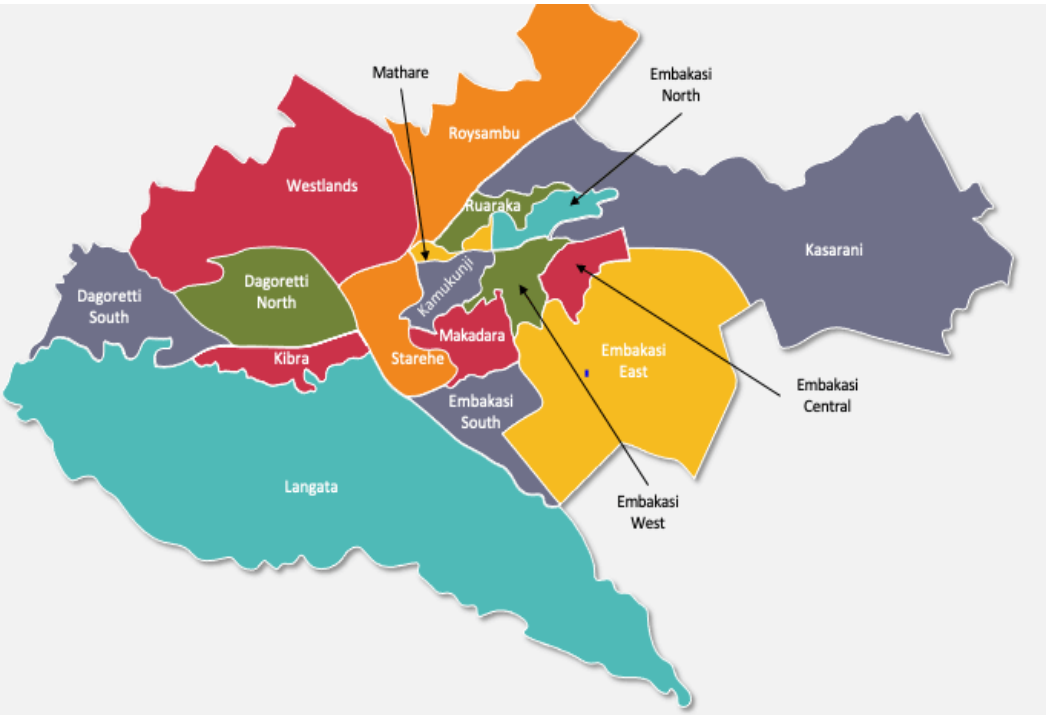


Figure 3.2: Map of Nairobi County (Maphill, 2023)

Uasin Gishu County covers a total area of 2,955.3 km^2. It borders Elgeyo Marakwet County to the east, Trans Nzoia to the North, Kericho to the South, Baringo to the South East, Nandi to the South West and Bungoma to the West. Administratively, the county is divided into six sub-counties: Soy, Turbo, Moiben, Ainabkoi, Kapseret and Kesses. The map of Uasin Gishu County is illustrated in Figure 3.3.

Figure 3.3: Map of Uasin Gishu County (Maphill, 2023)

3.2 Research Design

An experimental research design was used for this study. Samples were randomly collected from designated sampling sites in Nairobi and Uasin Gishu Counties, Kenya. Questionnaires were administered to the vendors and THPs to determine pertinent information about the constituents, preparation, storage, and consumption of the purchased herbal medicines. They were then transported to the laboratory, where they were coded and processed using standard laboratory protocols (Pang *et al*., 2009). The samples were then extracted using organic solvents and the extract solution subjected to GC-MS and LC-MS/MS analyses to determine their

phytochemical profile and synthetic compounds present as contaminants and adulterants.

3.3 Sampling Procedure

3.3.1 Determination of Appropriate Sample Size

The sample size was estimated such that the study had a 95% chance of detecting anticancer activity in approximately 10% of the herbal remedies used to treat cancer. It was determined that a minimum of 30 anticancer herbal remedies was required, as shown in equation 1 (Gogtay, 2010):

$$n = \log \beta / \log p = \log 0.05 / \log 0.9 = 28.4 \; rounded\; off\; to\; 30 \quad (1)$$

Where

1-β = Power of the test (0.95)

p = Proportion of anti-cancer herbal remedies with no anticancer activity (0.9 assumed)

The same sample size calculation was also applied to the antidiabetic herbal remedies. Similarly, a minimum of 30 antidiabetic herbal remedies was required.

3.3.2 Sample Collection

Although the study was projected to use 60 samples (30 antidiabetic and 30 anticancer herbal medicines), only 24 samples were used due to the limited availability of herbal products specifically indicated for cancer and diabetes treatment. The herbal medicines were randomly purchased from herbal clinics, herbal product manufacturers, herbalists, local retailers (supermarkets and nutrition stores), and hawkers/ street vendors in Uasin Gishu and Nairobi Counties. A total of

4 anticancer and 8 antidiabetic herbal medicines from Nairobi County and 5 anticancer and 7 antidiabetic herbal medicines from Uasin Gishu County were sampled. The samples were coded using a combination of letters and numbers that identified the treatment indication of the herbal medicine and sampling site.

The sample collector posed as a patient seeking treatment for either diabetes or cancer and administered the questionnaire to the vendor or herbalist. A questionnaire previously tested by the Kenya Institute of Medical Research (KEMRI) was used to get pertinent details about the herbal medicines from the vendors and THPs. The questionnaire assessed how the herbal medicines were prepared, the dosage prescribed, recommended storage practices, and the medicinal plant(s) used to prepare the remedies, as shown in Appendix I.

3.4 Equipment

Shimadzu GCMS-QP 2010 SE was used to screen for phytochemicals and synthetic compounds present in each sample extract. A Waters Alliance 2975 LC system (LC, Milford, MA, USA) coupled with a Waters Quattro Micro mass spectrometer (Micromass, UK) was used to determine the adulteration of herbal samples using selected antidiabetic pharmaceuticals. The system was operated using Masslynx 4.1 software. A rotary evaporator was used to concentrate the samples extracts. Mi-Vac 23050-A00 DNA pre-concentrator was used to evaporate the solvent from SPE sample eluates. An SPE manifold with a vacuum pump was used to load sample extracts onto SPE cartridges and elute the analyte of interests from the cartridge after removal of interfering species.

3.5 Chemicals and Reagents

HPLC-grade formic acid, acetonitrile, hexane, and methanol were purchased from Merck (Germany). Ultrapure water was obtained from a Milli-Q water apparatus. Analytical standards of metformin were donated by the Kenya National Quality Control Laboratory, while those of gliclazide, glibenclamide, and glimepiride were donated by Cosmos Limited, Kenya. All standards had >99% purity.

3.6 Ethical Considerations

Ethical clearance for collection of antidiabetic and anticancer herbal medicines was granted by KEMRI Independent Scientific and Ethical Review Unit (SERU): (KEMRI/SERU/CTMDR/CSC068/3566).

3.7 GC-MS Screening of Samples for Phytochemicals and Synthetic Compounds

3.7.1 Organic Solvent Extraction of Samples

At least 10 g of the powdered herbal sample was mixed using a vortex mixer to gain a uniform composition. The homogenised sample was extracted sequentially by cold maceration using 300 mL of hexane, acetonitrile, and methanol, in the order of increasing polarity for three days at room temperature. Cold maceration is widely utilized to extract plant materials because it prevents the thermal decomposition of thermolabile compounds (Abubakar & Haque, 2020). Additionally, it is simple to use, although it takes a long time due to its low extraction efficiency (Zhang *et al*., 2018). The extract solutions for each day were filtered using a Whatman No.1 filter

paper, and the filtrate was stored in 500-mL glass flat-bottomed flasks. The filtrates for the three days were combined and concentrated to about 10 mL using a rotatory evaporator set at 40 ºC to avoid the decomposition of thermolabile metabolites (Mlozi *et al*., 2022).

3.7.2 Solid Phase Extraction

Silica-based C_{18} SPE cartridges (500 mg, 3 mL) and DSC-NH_2 SPE cartridges (500 mg, 3 mL) were used to clean up about 1 mL of the concentrated extracts from the extraction step. DSC-NH_2 cartridges were used to clean up the hexane extracts while the C_{18} cartridges were used to clean up the methanol/ acetonitrile extracts. The workflow diagram for the SPE process is shown in Figure 3.4.

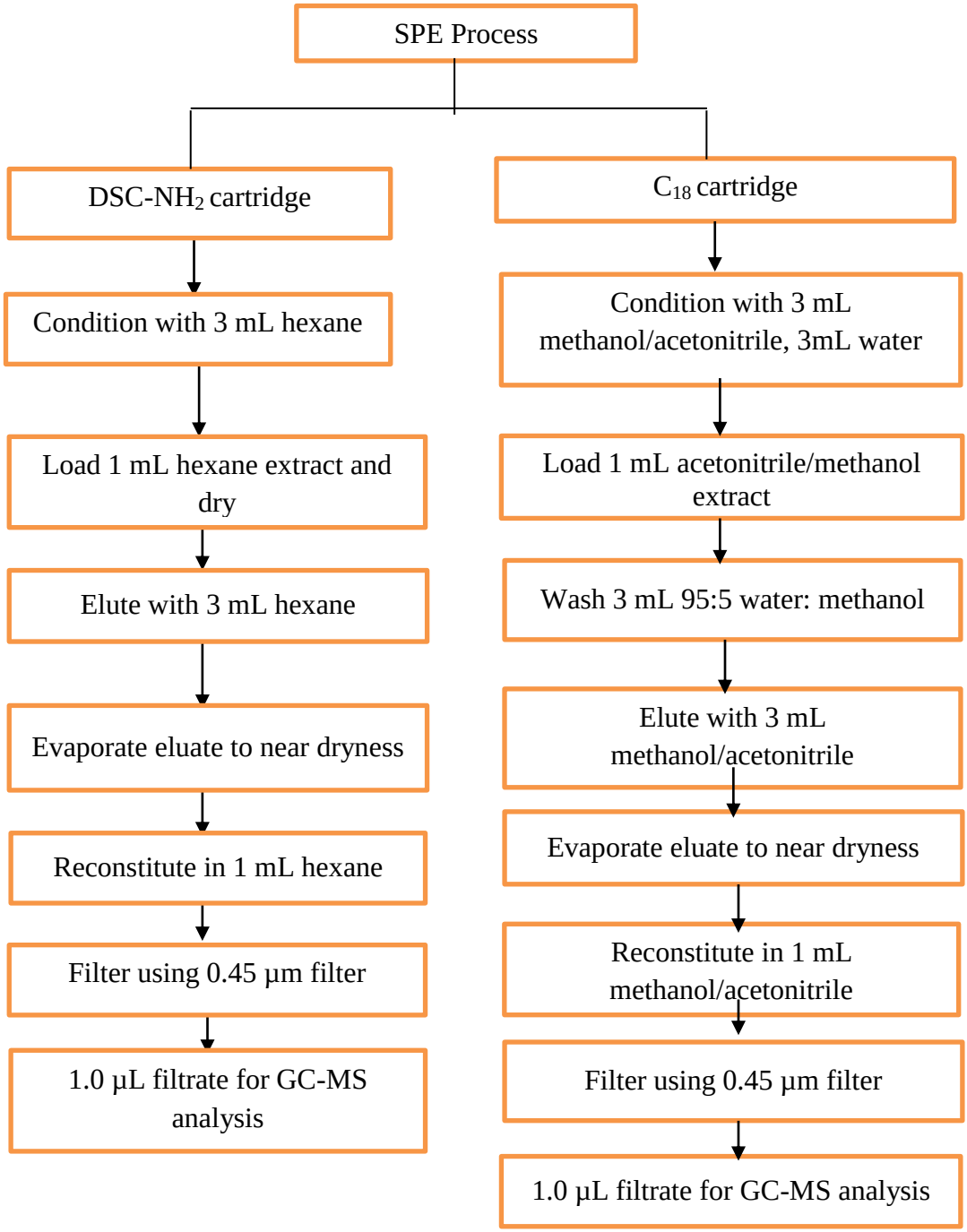


Figure 3.4: Workflow Diagram of the SPE Process Used

3.7.3 GC Operating Conditions

Helium was used as the carrier gas with a flow rate of 1 mL/min in the split mode (10:1). Separation was performed on an Agilent DB-5MS (30 m x 0.25 mm x 0.25

μm). The oven temperature program, detector temperature, and injection temperature were varied to optimize the separation conditions. The optimal interface and injector temperatures were set to 250℃ and 200℃, respectively. The optimal temperature program was determined to be: 50℃ held for 1 min before being ramped to 200℃ at a rate of 10ºC/min, without holding, then raised to 280℃ at a rate of 5℃/min and held for 6 mins. The total analysis time was 38 mins.

3.7.4 MS Operating Conditions

The ion source was maintained at a temperature of 200℃. Electron ionization (EI) at 70 eV was used to ionize the volatile compounds in the gas flow from the GC and obtain their mass spectra. The MS was operated in scan mode from 45 amu to 450 amu (atomic mass units).

3.7.5 Data Processing

The mass spectra obtained were compared with known spectra of various compounds stored in the NIST database, which has over 62,000 reference spectra (Olivia *et al*., 2021). The compounds were identified based on their molecular formula, retention time, and probability of their mass spectrum being similar to those found on the NIST database. The relative percentage content of each identified compound was calculated by comparing the average peak area to the total area of all compounds present (Mlozi *et al*., 2022).

3.8 LC-MS/MS Method Development and Validation for Analysis of Antidiabetic Pharmaceuticals present as Adulterants in Herbal Remedies

3.8.1 Preparation of Working Standards

The standard preparation technique employed by Pang *et al*. (2009) in the analysis of antidiabetic herbal remedies for synthetic drugs was used to prepare the standards for gliclazide, metformin, glimepiride, and glibenclamide. Individual stock solutions were prepared by dissolving accurately weighed standards in methanol to make 1mg/mL concentrations. These stock solutions were refrigerated at -20℃, awaiting further analysis. A mixed standard solution of 0.02 mg/mL concentration was prepared in methanol and refrigerated at -20℃. Working standard solutions were prepared by diluting the mixed standard solution with 5% acetonitrile in water (initial mobile phase composition) to solutions in the concentration range 5–250 ng/mL.

3.8.2 Optimization of the LC-MS/MS Operating Conditions

MRM parameters were optimized using direct infusion, whereby a syringe pump was used to directly inject 400 ng/mL standard solutions into the mass spectrometer. For each standard, both the negative and positive electrospray ionization (ESI) modes were tested. The most abundant m/z for each compound was chosen as the precursor ion, and the intensity of the resultant signal was optimized by changing the cone voltage. The cone voltage with the most intense signal was selected. The collision energies were optimized for each transition, and the two most intense product ions were chosen for qualification and quantification, respectively.

For the organic mobile phase, acetonitrile and methanol acidified with 0.1% formic acid were tested to determine which gave better chromatographic separation. Different compositions of the mobile phase were evaluated to improve chromatographic separation. Different separation temperatures (30℃ to 40℃) and flow rates (0.35 mL/min to 0.45 mL/min) were tested to optimize peak shapes, chromatographic resolution, and analysis time.

3.8.3 Optimization of the Sample Preparation Process

The sample preparation process involved three key steps: extraction, evaporation, and filtration. Optimization of the extraction step involved spiking the blank sample to 50 ng/mL using the mixed standard solution, adding different concentrations of methanol, then sonicating or shaking the mixture for 20 mins. Optimization of the evaporation step involved spiking the extract of the blank sample to 50 ng/mL prior to evaporation. Evaporation was carried out under a gentle stream of nitrogen. Optimization of the filtration step involved spiking the extracted blank sample extract to 50 ng/mL using the mixed standard solution after the evaporation step then filtering the extract using a 0.22-micron cellulose and PTFE filter. The absolute recoveries for each step and the entire process were evaluated to determine the optimal conditions. The absolute recoveries were determined by comparing the mean peak area of the spiked blanks (50 ng/mL) with that of the neat standards of similar concentration, as shown in equation 2 (Ngumba *et al*., 2016). A previously-analysed herbal product with no detectable levels of the targeted pharmaceuticals was used as the blank sample.

$$Absolute\ recovery\ (\%) = \frac{mean\ peak\ area_{spiked\ blank}}{mean\ peak\ area\ _{neat\ standard}} * 100 \qquad (2)$$

3.8.4 Validation of the Developed LC-MS/MS Method

The performance of the developed method was validated with reference to the ICH guidelines for analytical techniques (ICH, 2022). Parameters evaluated during method validation were tested as explained below.

3.8.4.1 Linearity

Linearity was determined by constructing calibration curves and determining the coefficient of correlation (Kairigo *et al*., 2020). Calibration curves were developed by injecting a set of standard solution of different concentrations (5-250 µg/L).

3.8.4.2 Accuracy

Accuracy was calculated by comparing the peak area of the blank spiked to 50 ng/mL prior to extraction using the mixed standard solution to that of the blank spiked to 50 ng/mL using the mixed standard solution prior to injection into the LC-MS/MS for analysis.

3.8.4.3 Precision

Precision was determined by making three consecutive injections of the mixed standard solution at 10 ng/mL, 100 ng/mL, and 200 ng/mL and calculating the relative standard deviation (RSD) of the mean peak areas obtained.

3.8.4.4 Detection Limits

The limit of detection (LOD) was calculated as three times the ratio of the unspiked blank's standard deviation, approximated using the y-intercept's standard deviation,

to the slope of the calibration curve (Ouma *et al*., 2021). The limit of quantification (LOQ) was calculated as ten times the ratio of the unspiked blank's standard deviation to the slope of the calibration curve.

3.8.4.5 Matrix Effect

The matrix effect was measured by comparing the mean peak area of the blank extract spiked using the mixed standard solution to a concentration of 50 ng/mL after filtration to that of the neat standard of similar concentration (Ngumba *et al*., 2016).

3.8.5 Data Analysis

Microsoft Windows Excel 2019 was used in data entry and analysis. An independent two-tailed t-test was performed to compare the experimental values for the different extraction and filtration conditions tested during the sample optimization process. A confidence interval of 95% (p= 0.05) was used. A p-value of less than 0.05 was considered to be significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Details of the Herbal Medicines Sampled

The distribution of the collected samples is given in Table 4.1. A summary of the sample information captured by questionnaire responses is given in Appendix II. Nine (38%) of the 24 herbal medicines sampled were indicated for cancer treatment, while the other 15 (62%) were indicated for diabetes treatment. The country of origin of 15 (63%) of the sampled herbal medicines was indicated as Kenya, while 5 (21%) were imported from China. The country of origin of the other 4 sampled products was not indicated.

Table 4.1: Distribution of the Herbal Samples

Location	Type	Herbalist	Hawker	Herbal Clinic	Local Retailers	Total
Nairobi	Anticancer	1	1	1	1	4
	Antidiabetic	1	2	2	3	8
Uasin	Anticancer	2	1	2	0	5
Gishu	Antidiabetic	2	2	3	0	7

Only 4 (17%) of the sampled herbal products had a brand name, indicated conditions, and a list of ingredients on the product label. The other 20 sampled medicines (73%) did not have a list of ingredients, indicated conditions, or a brand name indicated on the product label. These products were mainly purchased from herbalists and herbal clinics. The high proportion of samples without a list of ingredients, indicated

conditions, or brand name underscores the secrecy surrounding the herbal medicine practice in the country (Chebii *et al*., 2020; Kigen *et al*., 2019). Mislabelling or inadequate labelling of herbal medicines is common due to weak regulation and poor monitoring of these products in most countries (Kaggwa *et al*., 2022; Chebii *et al*., 2020; Ekor, 2014).

Jordan *et al*. (2023) reported that 60% of the Ayurvedic herbal medicines sold in the U.S. did not comply with the established labelling requirements. This high level of noncompliant labelling has been associated with various adverse patient events (Ekor, 2014). For example, an Australian man died from kidney failure after consuming a toxic preparation that contained *Aristolochia fangchi*, which was not declared on the label (Chau *et al*., 2011). Many countries, including Australia, have banned importation of herbal medicines containing plants of the *Aristolochia* genus because they contain aristolochic acids, which can cause liver failure (Han *et al*., 2019). However, herbal medicines containing these toxic plants are mislabeled and imported into many countries.

Most of the sampled herbal medicines were made from multiple medicinal plants based on the information obtained from the product labels and questionnaire responses, as highlighted in Figure 4.1. However, no information on the names of the plants used was provided, consistent with the secretive practices of herbalists and herbal clinics in Kenya. The reluctance of traditional health practitioners (THPs) to identify the ingredients of their herbal medicines is due to fear that someone will copy their formulations and undermine their practice (Thipanyane *et al*., 2022; Chebii *et al*., 2020). However, this secrecy undermines patient safety and health

because one cannot determine the potential of adverse reactions without knowing the medicinal plants used to prepare the herbal formulation (Ekor, 2014). Additionally, it provides a loophole for quack THPs to infiltrate the herbal medicine market (Chebii *et al*., 2020; Kigen *et al*., 2019).

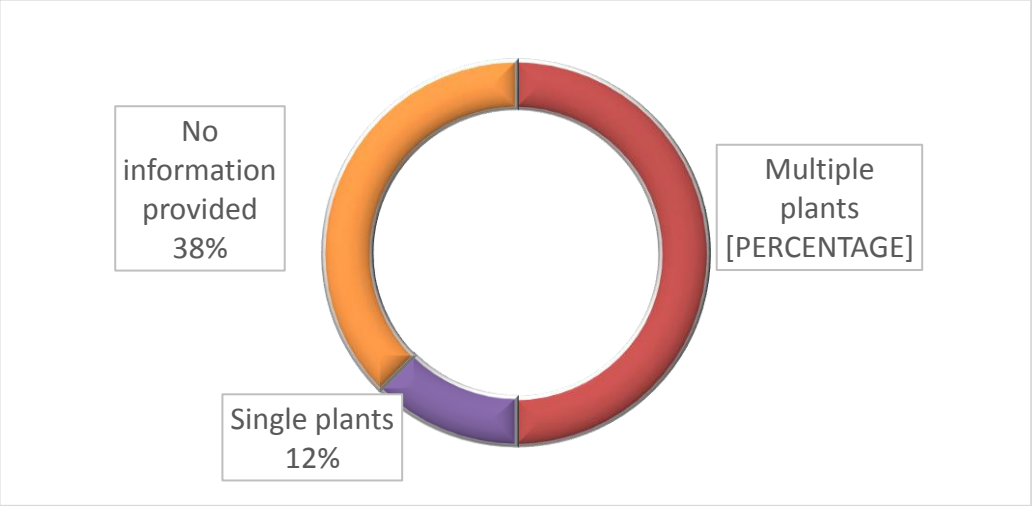


Figure 4.1: Composition of the Herbal Medicines Sampled

The most common method of administration was ingestion (23 of the 24 sampled herbal medicines). Only 1 sampled product was meant to be inhaled. Eleven of the sampled products were to be prepared by hot infusion, 6 by decoction, and 1 by cold infusion, as shown in Figure 4.2. Similar studies in other parts of the country have reported hot infusion and decoction as the primary method of preparation for herbal medicines and plants (Omara *et al*., 2022; Chebii *et al*., 2020; Onyancha *et al*., 2019; Keter & Mutiso, 2012). Decoction involves boiling the herbal formulation in water for about 20 minutes and drinking the mixture after it cools while hot infusion involves pouring warm or hot water on the herbal material then letting the mixture

cool (Tugume & Nyakoojo, 2019). The method of preparation of the other sampled medicines was not provided.

Regarding contraindications, only 4 (17%) of the 24 sampled herbal medicines required the patient to stop taking pharmaceutical drugs. 10 (42%) of the sampled products could be taken with pharmaceutical drugs while no instructions were provided on possible contradictions for the remaining 10 (42%). It was surprising that although the ingredients of most of the sampled medicines were not identified, many of them did not require the patient to stop conventional treatment despite the high probability of adverse herb-drug interactions (Okaiyeto & Oguntibeju, 2021).

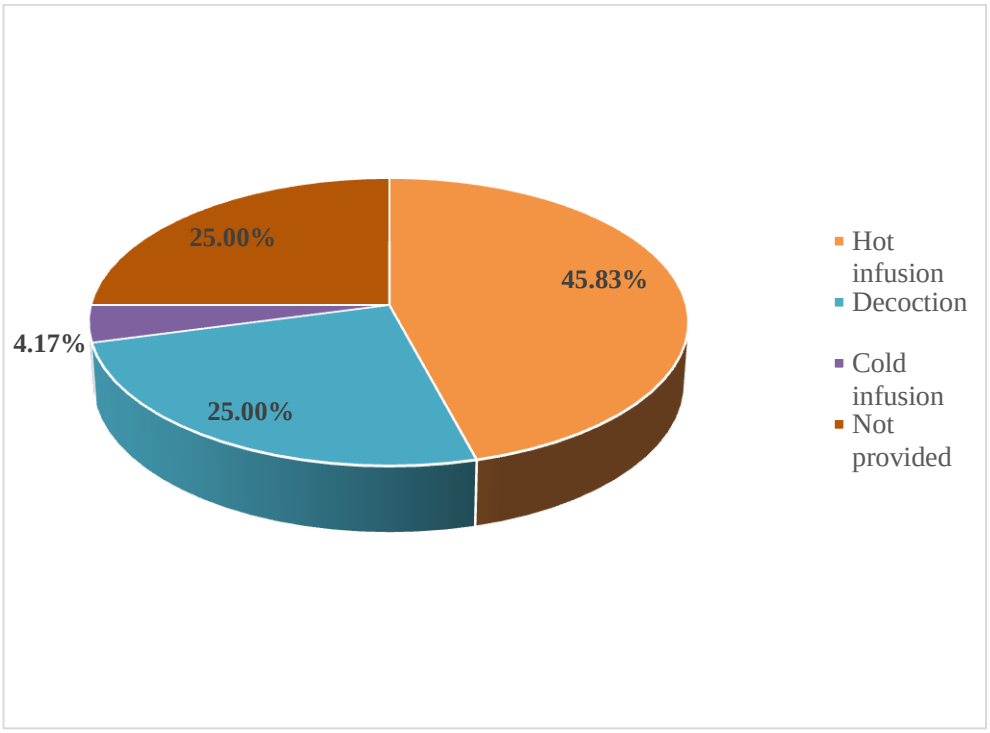


Figure 4.2: Indicated Method of Preparation for the Herbal Medicines Sampled

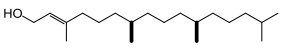
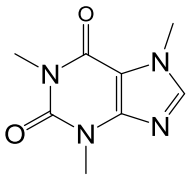
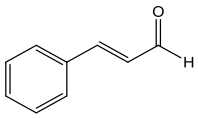
4.2 Phytochemical Profiles of the Herbal Medicines Sampled

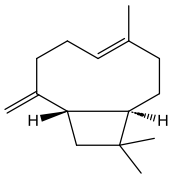
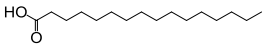
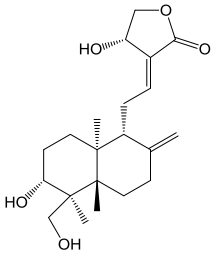
A wide range of phytochemicals with different biological activities were determined from the GC-MS results of the sampled herbal medicines. Over 30 different phytochemicals were identified in each of the sampled herbal medicine.

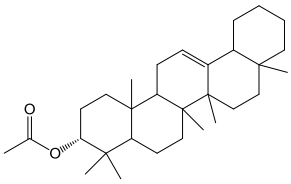
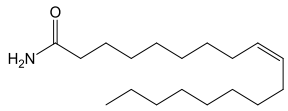
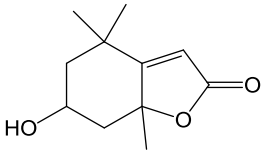
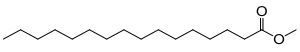
4.2.1 Bioactive Phytochemicals Identified in the Herbal Medicines Sampled

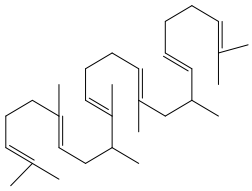
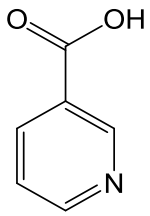
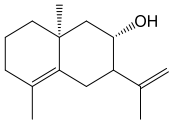
The GC-MS results of the hexane, acetonitrile, and methanol crude extracts of the 8 antidiabetic herbal medicines sampled from Nairobi County revealed various volatile bioactive compounds with reported antidiabetic and other useful activities. Table 4.2 lists some of the compounds in each sampled herbal medicine with reported biological activities useful to diabetes treatment identified. The total ion chromatograms (TICs) for selected extracts of the sampled herbal medicines that were determined to have these compounds are provided in Appendix III.

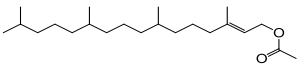
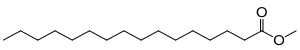
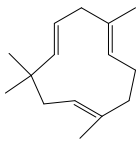
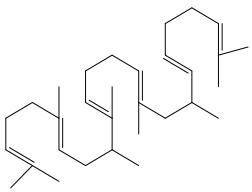
Table 4.2: Selected Bioactive Phytochemicals Identified in Antidiabetic Herbal Samples from Nairobi County

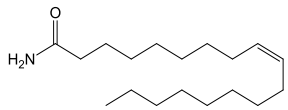
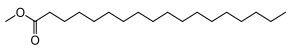
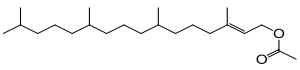
Sample Code	Compound Name	Classification	Reported Biological Activities
D12	Phytol 	Terpene	Apoptotic and cytotoxic activity (Thakor *et al*., 2016) Antimicrobial and antiradical activities (Pejin *et al*., 2014) Antioxidant, antidiabetic, immunomodulatory, immunoadjuvant, anti-teratogenic, anticancer, anticonvulsant, and antitumor activities (Islam *et al*., 2016, 2018)
	Caffeine 	Xanthine	Antidiabetic activity(Fu *et al*., 2017) Antioxidant activity (Vieira *et al*., 2020) Antimicrobial activity (Nonthakaew *et al*., 2015) Antitumor activity (Allen *et al*., 1985)
	Cinnamaldehyde 	Flavonoid	Antibacterial activity (Yossa *et al*., 2014) Antifungal activity (Cheng *et al*., 2008) Anti-inflammatory activity (Gunawardena *et al*., 2015) Antimicrobial activity (He *et al*., 2019) Antidiabetic effect (Guo *et al*., 2017) Antioxidant activity (Subash-Babu *et al*., 2014)
D14	Caryophyllene	Sesquiterpene	Antihyperglycemic activity (Basha & Sankaranarayanan, 2014)

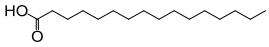
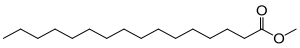
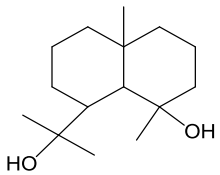
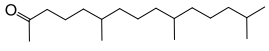
Sample Code	Compound Name	Classification	Reported Biological Activities
			Anticancer and antioxidant activities (Legault & Pichette, 2007) Anti-inflammatory and analgesic activities (Chavan *et al*., 2010) Antibacterial activity (Moo *et al*., 2020)
	Palmitic acid 	Fatty acid	Immunomodulatory and anticancer activities (Boubaker *et al*., 2018) Antimicrobial and antifungal activities (Liu *et al*., 2008) Antidiabetic activity (Kadan *et al*., 2016)
	Andrographolide 	Terpene	Immunomodulatory and anticancer activities (Wanandi *et al*., 2020) Anti-inflammatory activity (Jayakumar *et al*., 2013) Antidiabetic activity (Subramanian *et al*., 2008) Antiviral activity (Panraksa *et al*., 2017) Hepatoprotective activity (Maiti *et al*., 2010) Antihyperlipidemic and hypoglycaemic activities (Nugroho *et al*., 2012)
D18	Olean-12-en-3.beta.-ol, acetate	Terpene	Antibacterial activity (Lazreg-Aref *et al*., 2011) Antimalarial activity (Nafiu *et al*., 2021) Antioxidant and cytotoxic

Sample Code	Compound Name	Classification	Reported Biological Activities
			activities (Fabiyi *et al*., 2012) Antidiabetic activity (Nair *et al*., 2014) Anticholinesterase activity (Alves & Cruz, 2011) Anticancer activity on cervical, ovarian, and breast cancer (Afshar *et al*., 2021)
	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer and antioxidant activities (Wisitpongpun *et al*., 2020)
	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one 	Sesquiterpene	Anti-inflammatory activity against LPS induced inflammation (Jayawardena *et al*., 2019) Antiplasmodial activity (Gustav, 2016) Antidiabetic activity (Tatipamula *et al*., 2021)
D17	Palmitic acid, methyl ester 	Fatty acid methyl ester	Anti-angiogenic activity (Seow *et al*., 2011) Antibacterial activity (Shaaban *et al*., 2021) Anticancer and antioxidant activity (Wei *et al*., 2011)

Sample Code	Compound Name	Classification	Reported Biological Activities
	Squalene 	Terpene	Antifungal activity (Abubacker & Deepalakshmi, 2013) Antimicrobial and antioxidant activity (Davoodbasha *et al*., 2018) Anti-inflammatory activity (Wu *et al*., 2021) Antileishmanial activity (Delgado-Altamirano *et al*., 2019) Antigastric carcinogenic activity (Palaniyandi *et al*., 2018) Antioxidant activity (Kraujalis & Venskutonis, 2013) Anti-tumour, chemo-preventative, anti-cancer, and antimicrobial activities (Ezhilan & Neelamegam, 2012) Antidiabetic activity (Nachar *et al*., 2015)
	Niacin 	Pyridinecarboxylic acids	Anti-inflammatory, antidyslipidemic, antihyperlipidemic, anti-apoptotic, antioxidant, and antidiabetic activities (Gasperi *et al*., 2019)
XD72P	Eudesm-4(14)-en-11-ol 	Sesquiterpene	Anti-angiogenic activity (Tshering *et al*., 2021) Antibacterial activity (Ahmed *et al*., 2017)
	Phytol, acetate	Fatty acid	Apoptotic and cytotoxic

Sample Code	Compound Name	Classification	Reported Biological Activities
			activity (Thakor *et al*., 2016) Antimycobacterial activity (Rajab *et al*., 2007) Antimicrobial and antiradical activities (Pejin *et al*., 2014) Antioxidant, antidiabetic, immunomodulatory, immunoadjuvant, anti-teratogenic, anticancer, anticonvulsant, and antitumor activities (Islam *et al*., 2016, 2018)
	Palmitic acid 	Fatty acid	Antibacterial activity (Barbara *et al*., 2002) Immunomodulatory and anticancer activity (Boubaker *et al*., 2018) Antimicrobial and antifungal activity (Liu *et al*., 2008) Antidiabetic activity (Kadan *et al*., 2016)
MD43P	Humulene 	Sesquiterpene	Anti-inflammatory activity (Ajiboye *et al*., 2016) Antibacterial activity (Jang *et al*., 2020) Anti-tumor activity (El Hadri *et al*., 2010) Antidiabetic activity (Zaccai *et al*., 2020)
	Squalene 	Terpene	Antigastric carcinogenic activity (Palaniyandi *et al*., 2018) Antioxidant activity (Kraujalis & Venskutonis, 2013) Anti-tumor, chemo-preventative, anti-cancer,

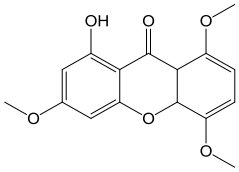
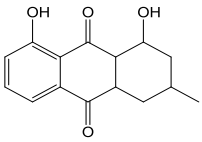
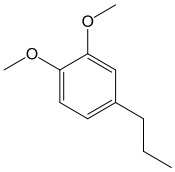
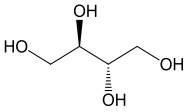
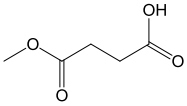
Sample Code	Compound Name	Classification	Reported Biological Activities
			and antimicrobial activities (Ezhilan & Neelamegam, 2012) Antidiabetic activity (Nachar *et al*., 2015)
	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer and antioxidant activities (Wisitpongpun *et al*., 2020)
MD53P	Methyl stearate 	Fatty acid methyl ester	Anti-inflammatory activity (Razi *et al*., 2015) Anti-bacterial and anti-oxidant activity (Sudharsan *et al*., 2011)
	Phytol, acetate 	Fatty acid	Apoptotic and cytotoxic activity (Thakor *et al*., 2016) Antimycobacterial activity (Rajab *et al*., 2007) Antimicrobial and antiradical activities (Pejin *et al*., 2014) Antioxidant, antidiabetic, immunomodulatory, immunoadjuvant, anti-teratogenic, anticancer, anticonvulsant, and antitumor activities (Islam *et al*., 2016, 2018)
	Palmitic acid	Fatty acid	Antibacterial activity (Barbara *et al*., 2002)

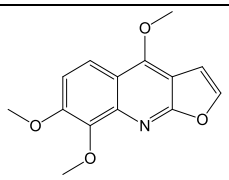
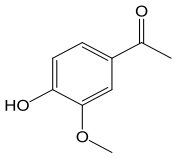
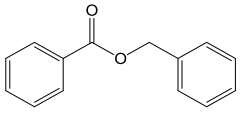
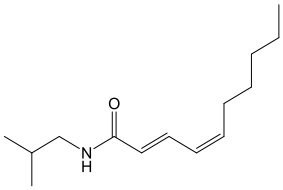
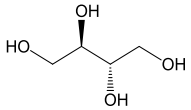
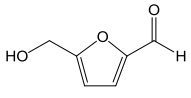
Sample Code	Compound Name	Classification	Reported Biological Activities
			Immunomodulatory and anticancer activities (Boubaker *et al*., 2018) Antimicrobial and antifungal activities (Liu *et al*., 2008) Antidiabetic activity (Kadan *et al*., 2016)
RD32P	Palmitic acid, methyl ester 	Fatty acid methyl ester	Anti-angiogenic activity (Seow *et al*., 2011) Antibacterial activity (Shaaban *et al*., 2021) Anticancer and antioxidant activity (Wei *et al*., 2011) Antifungal activity (Abubacker & Deepalakshmi, 2013) Antimicrobial and antioxidant activity (Davoodbasha *et al*., 2018) Anti-inflammatory activity (Wu *et al*., 2021)
	Cryptomeridiol 	Sesquiterpene	Antioxidant, anti-microbial, antispasmodic, and anti-inflammatory activities (Tong *et al*., 2018)
	2-Pentadecanone, 6,10,14-trimethyl- 	Sesquiterpene	Antimicrobial and anti-inflammatory activities (Adeosun *et al*., 2017) Antidiabetic activity (Bahadori *et al*., 2017)

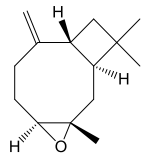
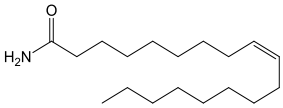
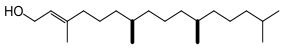
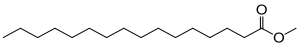
Similarly, the GC-MS results of the hexane, acetonitrile, and methanol crude extracts of the 7 antidiabetic herbal medicines sampled from Uasin County revealed

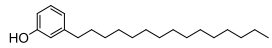
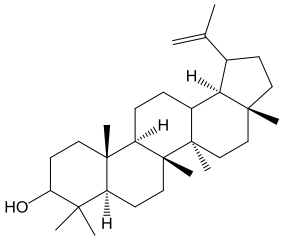
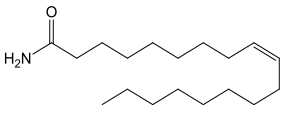
numerous volatile bioactive phytochemicals with reported antidiabetic and other useful biological activities. Some of the compounds identified in each sample herbal medicines with reported antidiabetic activities are summarized in Table 4.3. The TICs of some of the extracts of the sampled herbal medicines that were determined to have these compounds are provided in Appendix III.

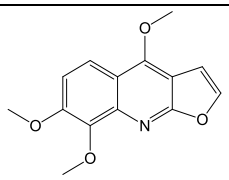
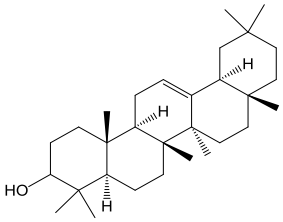
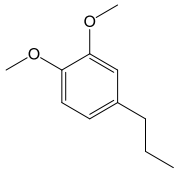
Table 4.3: Selected Phytochemicals Identified in Antidiabetic Herbal Samples from Uasin Gishu County

Sample Code	Compound Name	Classification	Reported Biological Activities
ELD20P	Xanthen-9-one, 1-hydroxy-3,5,8-trimethoxy- 	Xanthone	Antimalaria, hepatoprotective, and blood sugar lowering activities (Kshirsagar *et al*., 2019)
	1,8-Dihydroxy-3-methyl-anthraquinone 	Anthraquinone	Anticancer, antibacterial, antiviral, anti-proliferative, and antioxidant activities (Abdelfattah, 2009; Shafiq *et al*., 2020)
	cis-Methyl isoeugenol 	Terpene	Antidepressant and anxiolytic activities (Fajemiroye *et al*., 2014) Antimicrobial and antifungal activity (Liu *et al*., 2008) Antidiabetic activity (Kadan et al., 2016)
ELD28P	Erythritol 	Sugar alcohol	Antioxidant, antibacterial, and antidiabetic activities (Rzechonek *et al*., 2017)
	Butanedioic acid, monomethyl ester 	Dicarboxylic acid ester	Antidiabetic activity (Eizirik *et al*., 1994; Vicent *et al*., 1994)
	Skimmianine	Alkaloid	Antibacterial, antiviral,

			antiplasmodia activities (Guetchueng *et al*., 2022) Anticancer and anticholinesterase activities (Kozioł *et al*., 2019)
ELD47P	Vanillin 	Phenol	Antinociceptive, anti-acetylcholinesterase, anticancer, anti-hyperlipidaemic, anti-hyperglycaemic, anti-inflammatory, antimicrobial, antioxidant, and cardio-protective, activities (Olatunde *et al*., 2022)
	Ascabin 	Benzoic acid ester	Acaricidal activity (Ohno *et al*., 2008) Antihypertensive and Immunobiological activities (Sang *et al*., 2005)
	(E,E)-N-Isobutyl-2,4-decadienamide 	Fatty amide	Antidiabetic, analgesic, immunomodulatory, antimugenic, and anti-inflammatory activities (Elufioye *et al*., 2020)
ELD/D/77P	Erythritol 	Sugar alcohol	Antioxidant and antibacterial activities; low insulin index (Rzechonek *et al*., 2017)
	5-Hydroxymethylfurfural 	Phenol	Antioxidant and antiproliferative activities (Manganyi *et al*., 2019) Antimicrobial activities (L.

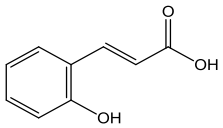
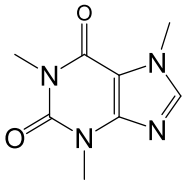
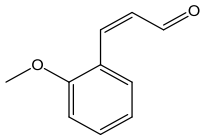
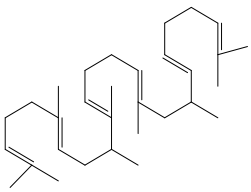
			Zhao *et al*., 2013)
	Caryophyllene oxide 	Sesquiterpene	Anticancer and analgesic activity (Fidyt *et al*., 2016) Anti-inflammatory activity (Chavan *et al*., 2010) Antioxidant and antidiabetic activities (Gyrdymova & Rubtsova, 2021)
ELD/D/75P	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer and anticancer activities (Wisitpongpun *et al*., 2020)
	Phytol 	Diterpene alcohol	Apoptotic and cytotoxic activities (Thakor *et al*., 2016) Antimycobacterial activity (Rajab *et al*., 2007) Antimicrobial and antiradical activities (Pejin *et al*., 2014) Antioxidant, antidiabetic, immunomodulatory, immunoadjuvant, anti-teratogenic, anticancer, anticonvulsant, and antitumor activities (Islam *et al*., 2016, 2018)
	Palmitic acid, methyl ester 	Fatty acid methyl ester	Anti-angiogenic activity (Seow *et al*., 2011) Antibacterial activity

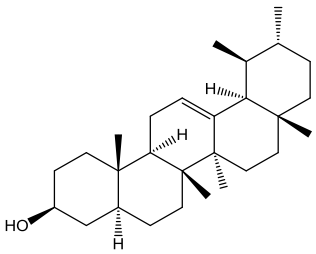
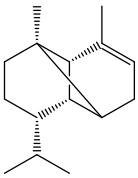
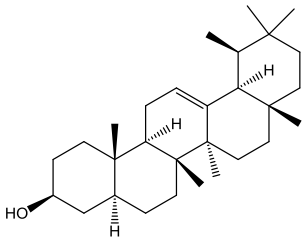
			(Shaaban *et al*., 2021) Anticancer and antioxidant activities (Wei *et al*., 2011) Antifungal activity (Abubacker & Deepalakshmi, 2013) Antimicrobial and antioxidant activities (Davoodbasha *et al*., 2018) Anti-inflammatory activity (Wu *et al*., 2021)
ELD/D/76P	Phenol, 3-pentadecyl- 	Phenol	Anti-obesity, cytotoxic, antibacterial, and antioxidant properties (Cieślik-Boczula *et al*., 2009)
	Lup-20(29)-en-3.beta.-ol 	Terpene	Antidiabetic activity (Na *et al*., 2009) Antimicrobial activity (Andrade *et al*., 2020) Anti-inflammatory and antioxidant properties (Jin *et al*., 2012)
	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer and antioxidant activities (Wisitpongpun *et al*., 2020)
JD15P	Skimmianine	Alkaloid	Anti-inflammatory activity (Ratheesh *et al*., 2013) Antileishmanial, anti-

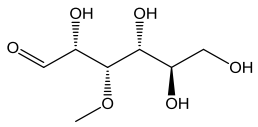
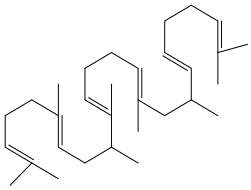
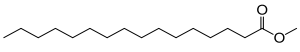
		acetyl-cholinesterase, and antimicrobial activities (Shang *et al*., 2018)
beta.-Amyrone	Terpene	Anti- acetyl-cholinesterase activity (Olasehinde *et al*., 2019) Anti-fungal activity (Elfadil *et al*., 2015)
		
cis-Methyl isoeugenol	Terpene	Antidepressant and anxiolytic activities (Fajemiroye *et al*., 2014) Antimicrobial and antifungal activity (Liu *et al*., 2008) Antidiabetic activity (Kadan et al., 2016)
		

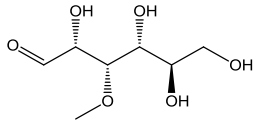
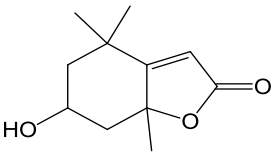
The GC-MS results of the hexane, acetonitrile, and methanol crude extracts of the 4 anticancer herbal medicines sampled from Nairobi County revealed numerous bioactive compounds with anticancer and other useful biological activities. Some of the volatile compounds identified in each sampled herbal medicine with reported anticancer activities are summarized in Table 4.4. The TICs of some of the extracts of the sampled herbal medicines that were determined to have these compounds are provided in Appendix III.

Table 4.4: Selected Bioactive Phytochemicals Identified in Anticancer Herbal Samples from Nairobi County

Sample Code	Compound Name	Classification	Reported Biological Activities
C09	Trans-o-coumaric acid 	Phenolic acid	Antimicrobial and anti-inflammatory activities (Pham *et al*., 2021) Anticancer and antioxidant activities (Kianmehr *et al*., 2020)
	Caffeine 	Xanthine	Antidiabetic activity (Fu *et al*., 2017) Antioxidant activity (Vieira *et al*., 2020) Antimicrobial activity (Nonthakaew *et al*., 2015) Antitumor activity (Allen *et al*., 1985)
	(Z)-2-methoxycinnamaldehyde 	Flavonoid	Anti-inflammatory and antioxidant activities (Hwa *et al*., 2012) Anticancer activity (Perng *et al*., 2016) Antitumor activity (Jin & Kim, 2017)
GC82P	Squalene 	Terpene	Antileishmanial activity (Delgado-Altamirano *et al*., 2019) Antigastric and carcinogenic activities (Palaniyandi *et al*., 2018) Antioxidant activity (Kraujalis & Venskutonis, 2013) Anti-tumor, chemo-preventative, anti-cancer, antimicrobial,

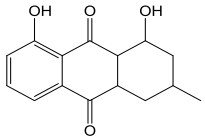
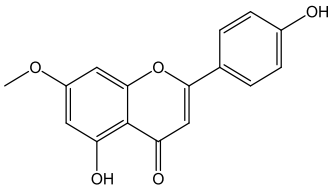
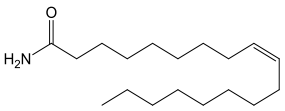
			and sun-screen activities (Ezhilan & Neelamegam, 2012) Antidiabetic effect (Nachar *et al*., 2015)
	alpha.-Amyrin 	Terpene	Anti-inflammatory activity (Júnior *et al*., 2019) Antidepressant and anxiolytic activities (Aragão *et al*., 2006) Anticonvulsant activity(Aragão *et al*., 2015) Gastoprotective (Oliveira *et al*., 2004) and hepatoprotective activity (Oliveira *et al*., 2005) Anticancer activity (Abu-Lafi et al., 2019)
	Copaene 	Aliphatic hydrocarbon	Antioxidant and nticancer activities, not genotoxic (Türkez *et al*., 2014) Antibacterial activity (Landoulsi *et al*., 2020)
GC81P	beta.-Amyrin 	Terpene	Anti-inflammatory activity (Júnior *et al*., 2019) Antidepressant and anxiolytic activity (Aragão *et al*., 2006) Anticonvulsant activity (Aragão *et al*., 2015) Gastoprotective and hepatoprotective activities (Oliveira *et al*., 2005) Anticancer activity (Wen *et al*., 2018)

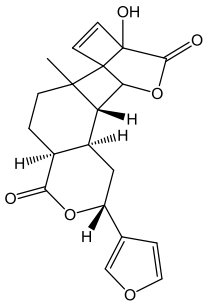
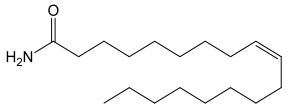
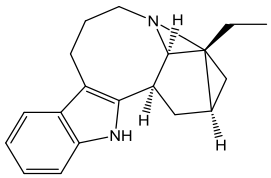
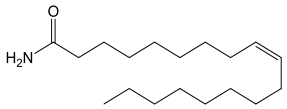
	3-O-Methyl-d-glucose 	Monosaccharide	Reduces toxicity of streptozotocin and enhances antitumor activity of streptozotocin (Anandan *et al*., 2011)
	Squalene 	Terpene	Antileishmanial activity (Delgado-Altamirano *et al*., 2019) Antigastric and carcinogenic activities (Palaniyandi *et al*., 2018) Antioxidant activity (Kraujalis & Venskutonis, 2013) Anti-tumor, chemo-preventative, anti-cancer, antimicrobial, and sun-screen activities (Ezhilan & Neelamegam, 2012) Antidiabetic effect (Nachar *et al*., 2015)
C13	Palmitic acid, methyl ester 	Fatty acid methyl ester	Anti-angiogenic activity (Seow *et al*., 2011) Antibacterial activity (Shaaban *et al*., 2021) Anticancer and antioxidant activities (Wei *et al*., 2011) Antifungal activity (Abubacker & Deepalakshmi, 2013) Antimicrobial and antioxidant activities (Davoodbasha *et al*., 2018) Anti-inflammatory activity (Wu

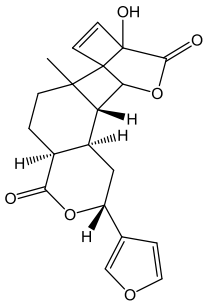
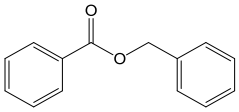
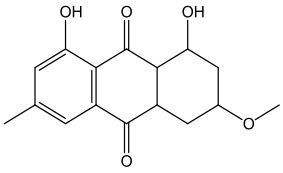
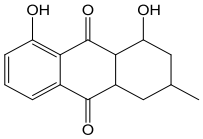
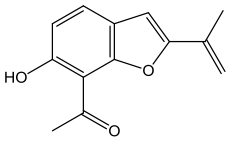
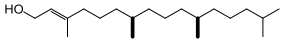
		et al., 2021)
3-O-Methyl-d-glucose 	Monosaccharide	Reduces toxicity of streptozotocin and enhances antitumor activity of streptozotocin (Anandan *et al*., 2011)
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one 	Sesquiterpene	Anti-inflammatory activity against LPS induced inflammation (Jayawardena *et al*., 2019) Antidiabetic and antioxidant activities (Tatipamula *et al*., 2021)

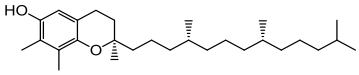
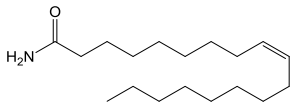
The GC-MS results of the hexane, acetonitrile, and methanol crude extracts of the 5 anticancer herbal medicines sampled from Uasin Gishu County revealed numerous volatile bioactive compounds with anticancer and other useful activities. Some of the compounds identified in each sampled herbal medicine with reported anticancer activities are summarized in Table 4.5. The TICs of some of the extracts of the sampled herbal medicines that were determined to have these compounds are provided in Appendix III.

Table 4.5: Selected Bioactive Phytochemicals Identified in Anticancer Herbal Samples from Uasin Gishu County

Sample Code	Compound Name	Classification	Reported Biological Activities
EIC21P	Anthraquinone, 1,8-dihydroxy-3-methyl- 	Anthraquinone	Antioxidant, antimicrobial, and tumor promoting activities (Shafiq *et al*., 2020; Zeid *et al*., 2000; Digiovanni & Boutwell, 1983)
	Genkwanin 	Flavonoid	Anti-inflammatory activity (Gao *et al*., 2014) Immunomodulatory and antitumor activities (Wang *et al*., 2015) Anti-neurotoxicity (Li *et al*., 2021) Anti-proliferative activity (Nasr *et al*., 2016)
	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer activity (Wisitpongpun *et al*., 2020)
EIC26P	Columbin	Terpene	Anti-inflammatory and chemopreventative activities (Ibrahim *et*

			*al*., 2012; Kohno *et al*., 2002; Majhi, 2020)
	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer activity (Wisitpongpun *et al*., 2020)
	Ibogamine 	Alkaloid	Anticancer and antioxidant activities (Zhang *et al*., 2016)
EIC27P	9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer activity (Wisitpongpun *et al*., 2020)
	Columbin	Terpene	Anti-inflammatory and chemopreventative activities (Majhi,

			2020; Ibrahim *et al*., 2012; Kohno *et al*., 2002)
	Ascabin 	Benzoic acid ester	Acaricidal activity (Ohno *et al*., 2008) Antihypertensive and immunobiological activities (Sang *et al*., 2005)
EIC35P	Anthraquinone, 1,8-dihydroxy-3-methoxy-6-methyl- 	Anthraquinone	Antitumor and anti-leishmanial activities (Alasmary *et al*., 2018; Awaad *et al*., 2013)
	Anthraquinone, 1,8-dihydroxy-3-methyl- 	Anthraquinone	Antioxidant, antimicrobial, and tumour promoting activities (Shafiq *et al*., 2020; Zeid *et al*., 2000; Digiovanni & Boutwell, 1983;)
	1-(6-Hydroxy-2-isopropenyl-1-benzofuran-7-yl)ethenone 	Benzofuran	Immunomodulatory and antioxidant activities (Sajjadi-Ghotbabadi *et al*., 2017; Khaleghi *et al*., 2014)
ELD/C/64P	Phytol 	Terpene	Apoptotic and cytotoxic activity (Thakor *et al*., 2016) Antimycobacterial

		activity (Rajab *et al*., 2007) Antimicrobial and antiradical activities (Pejin *et al*., 2014) Antioxidant, antidiabetic, immunomodulatory, immunoadjuvant, anti-teratogenic, anticancer, anticonvulsant, and antitumor activities (Islam *et al*., 2016, 2018)
(+)-.gamma.-Tocopherol, O-methyl- 	Phenol	Antioxidant and anti-inflammatory activities (Birringer & Lorkowski, 2019)
9-Octadecenamide, (Z)- 	Fatty amide	Anti-inflammatory activity and prevents against Alzheimer's (Ano *et al*., 2015) Hypogenic activity (Huitrón-Reséndiz *et al*., 2001) Anti-allergic activity (Yang *et al*., 2016) Anticancer activity (Wisitpongpun *et al*., 2020)

4.2.2 Potentially toxic phytochemicals identified in the herbal medicines sampled

The GC-MS analysis revealed the presence of several phytochemicals with reported toxicity, including prunasin-d (1), 5-keto-d-fructose (2), and ketone, 7-methoxy-2-benzofuranyl methyl (3). Their chemical structures are given in Figure 4.3.

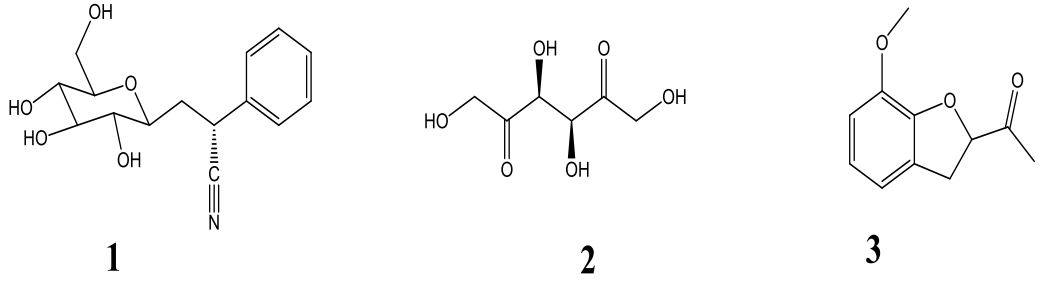


Figure 4.3: Chemical Structures of Potentially Toxic Phytochemicals Identified in Some of the Herbal Medicines Sampled

4.2.2.1 Prunasin-D

Prunasin is a natural cyanogenic glycoside found in the seeds of various edible plants, including peaches, almonds, apricot, and other Rosacease family members (Madrera & Valles, 2021). It was detected in one of the antidiabetic samples from Uasin Gishu County (ELD/D/75P), and its TIC is shown in Figure 4.4. When ingested, prunasin releases hydrogen cyanide, which is poisonous to humans at high concentrations (Deng *et al.*, 2021). Clinical manifestations of acute cyanide poisoning include mental confusion, diarrhoea, vomiting, dizziness, headache, low blood pressure, rapid pulse, and rapid respiration (Cressey & Reeve, 2019). Additionally, several neurological disorders have been linked to chronic cyanide exposures due to cyanide inhibiting oxidative phosphorylation, which impairs vital functions, metabolic acidosis, and hypoxia (Schrenk *et al*., 2019).

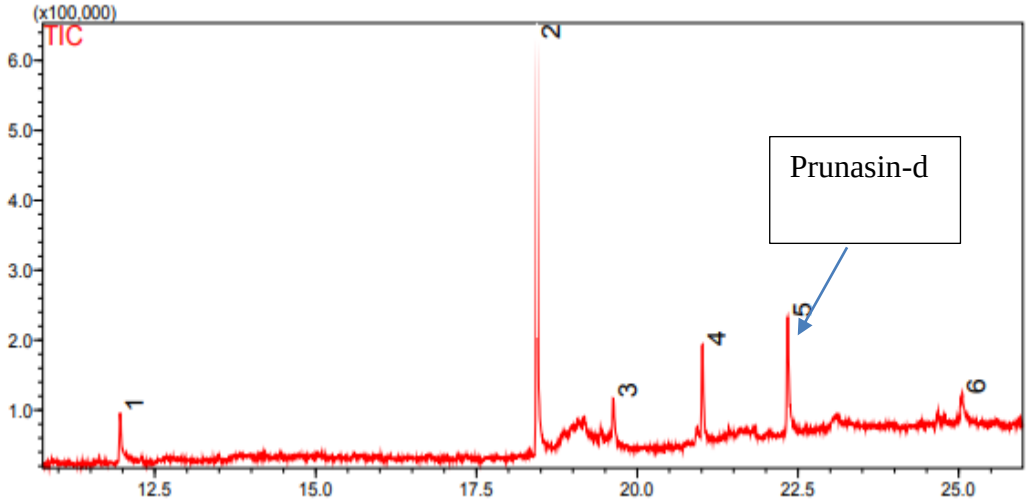


Figure 4.4: TIC of the ELD/D/75P Extract Containing Prunasin-D

Several plants containing 1-2.5% of prunasin and other cyanogenic glycosides are considered toxic, including *Prunus laurocerasus* (Malaspina *et al*., 2022). Notably, The European Food Safety Authority Panel on Contaminants in the Food Chain set the limit of dietary exposure to cyanides at 20 µg/kg body weight (Schrenk *et al*., 2019). However, this study could not determine the amount of dietary exposure to prunasin in the prescribed dosage, which prevented risk assessment. Malaspina *et al*. (2022) assert that prunasin and other cyanogenic glycosides rarely result in serious illnesses when ingested at low concentrations because the human body does not activate cyanogenic glycosides at these low concentration levels. Nevertheless, the presence of prunasin, whose relative peak area was 25%, might point to the herbal medicine being unfit for human consumption.

4.2.2.2 5-Keto-D-Fructose

5-keto-d-fructose is a natural diketone found in several *Gluconobacter* strains (Siemen *et al*., 2018). *Gluconobacter* strains include acetic acid bacteria that use

membrane-associated dehydrogenases to oxidize a wide range of alcohols and carbohydrates to ketones, aldehydes, and organic acids (Battling *et al*., 2020). 5-keto-d-fructose is formed when the fructose dehydrogenase complex bound to the membrane of some *Gluconobacter* strains catalyses the oxidation of D-fructose to 5-keto-D-fructose (Hövels *et al*., 2022). As such, it is a unique compound produced from fructose or mannitol by *Gluconobacter* strains (Nguyen *et al*., 2021). Therefore, the presence of 5-keto-d-fructose in one of the anticancer herbal samples from Nairobi County (C13) indicates the presence of *Gluconobacter* strains in the sample. The TIC of the C13 extract containing 5-keto-d-fructose is shown in Figure 4.5.

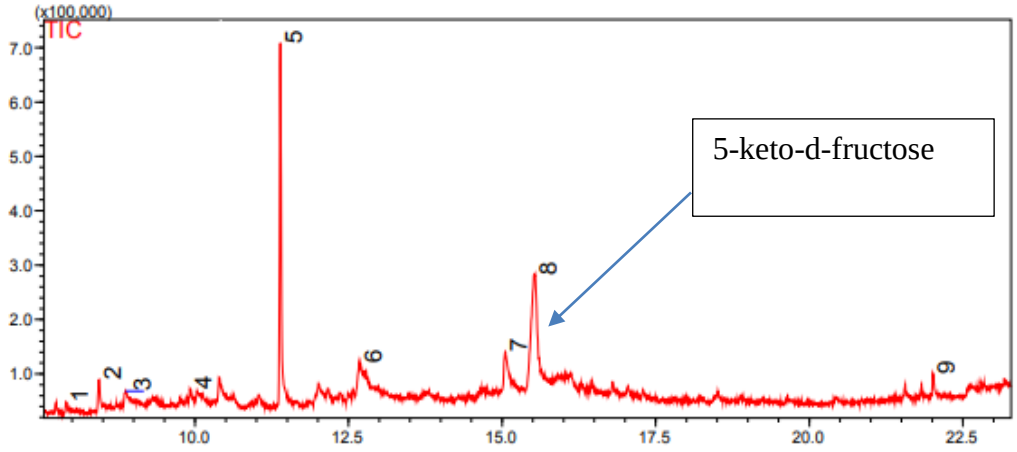


Figure 4.5: TIC of the C13 Extract Containing 5-Keto-D-Fructose

Although 5-keto-D-fructose is not toxic, studies have established that *Gluconobacter* spp. can cause colonization of other acetic acid bacteria and infections in patients with indwelling devices or chronic diseases (Alauzet *et al*., 2010). For example, Bassetti *et al*. (2013) reported a case of a female patient diagnosed with endocarditis caused by *Gluconobacter* spp. Therefore, the consumption of this anticancer sample

might undermine the patient's health due to the presence of *Gluconobacter* spp - an opportunistic human pathogen.

4.2.2.3 Ketone, 7-Methoxy-2-Benzofuranyl Methyl

Ketone, 7-methoxy-2-benzofuranyl methyl is a secondary metabolite derived from fungi that grows on mangrove plants – *Sprothix* spp. (Liao *et al*., 2019; Xu, 2014; Wen *et al*., 2010). It was detected in one of the antidiabetic herbal samples from Uasin Gishu County (ELD47P), which points to the presence of *Sprothix* spp. in the sample. The TIC of the ELD47P extract with ketone, 7-methoxy-2-benzofuranyl methyl is shown in Figure 4.6.

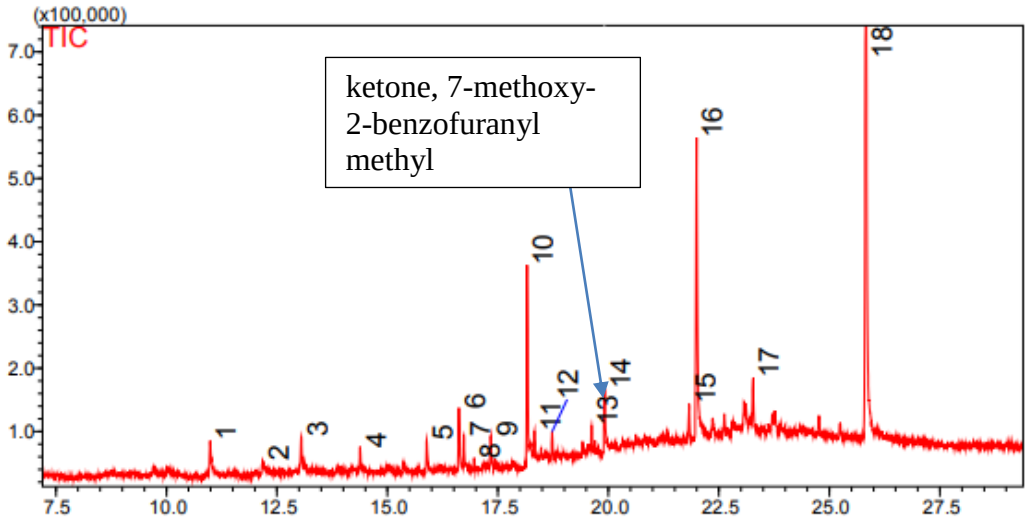


Figure 4.6: TIC of the ELD47P Extract Containing Ketone, 7-Methoxy-2-Benzofuranyl Methyl

Sprothix spp. is common in subtropical and tropical regions and is associated with sporotrichosis outbreaks in these regions (de Lima-Barros *et al*., 2011). Most cases of sporotrichosis have been reported in South Africa, Asia, and South America (Fernandes *et al*., 2018). Humans get infected when *Sprothix* spp. from contaminated surfaces inoculates itself on the human skin, causing lesions (Barros *et al*., 2011).

Once infected, one can spread the disease to other people through contact with the wounds or herbal/indegenous topical medication (Mahajan, 2014). Therefore, although ketone, 7-methoxy-2-benzofuranyl methyl has no reported toxicity, its presence points to possible microbial contamination of the sample – posing a safety risk.

4.3 Synthetic Compounds Identified in the Herbal Medicines Sampled

4.3.1 Phthalates

The GC-MS analysis of the acetonitrile and methanol extracts revealed the presence of several phthalates in most of the herbal samples. Notably, 13 (54%) of the 24 sampled herbal medicines contained at least one phthalate. The phthalates determined in the sampled herbal medicines included diisobutyl phthalate, diisooctyl phthalate, diethyl phthalate, and dibutyl phthalate, whose chemical structures are provided in Figure 4.7. The distribution of these phthalates in the 13 herbal samples is highlighted in Figure 4.8, and their proposed fragmentation patterns that confirmed their presence are provided in Figures 4.9–4.11. Notably, most of the samples contained more than one phthalate.

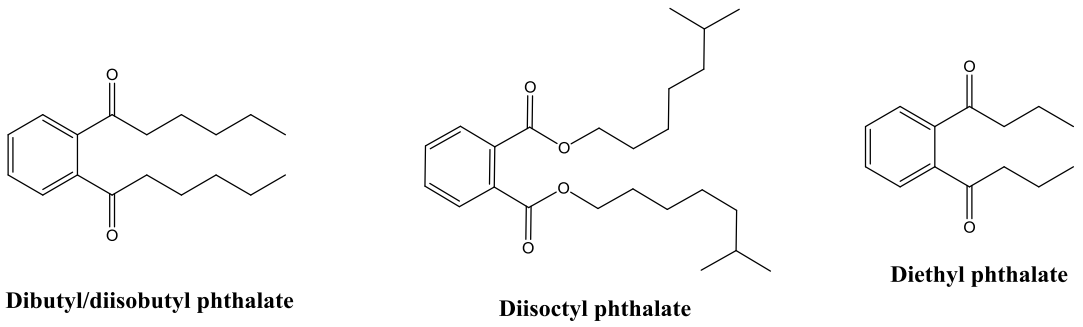


Figure 4.7: Chemical Structures of Phthalates Identified in the Sampled Herbal Medicines

Di-n-butyl phthalate, diisooctyl phthalate, and diethyl phthalate are widely used to make plastics, pesticides, coatings, and personal care products (Baranenko *et al*., 2022; Giuliani *et al*., 2020). These phthalates have been detected in a wide range of food products, and it is suspected that they easily leach from plastic packaging into food products (Giuliani *et al*., 2020). As such, the phthalates detected in this study could have come from the plastics used to package the herbal formulation or the medicinal plants used to make the formulations. However, the confidence of the phthalate determination is low due to the high likelihood of false positive results (Andjelković *et al*., 2021; Russo *et al*., 2015). Efforts were taken to reduce the phthalate contamination of the samples, like using only glassware throughout the process, but even laboratories that are phthalate free still record false positives (Fan *et al*., 2019; He *et al*., 2015).

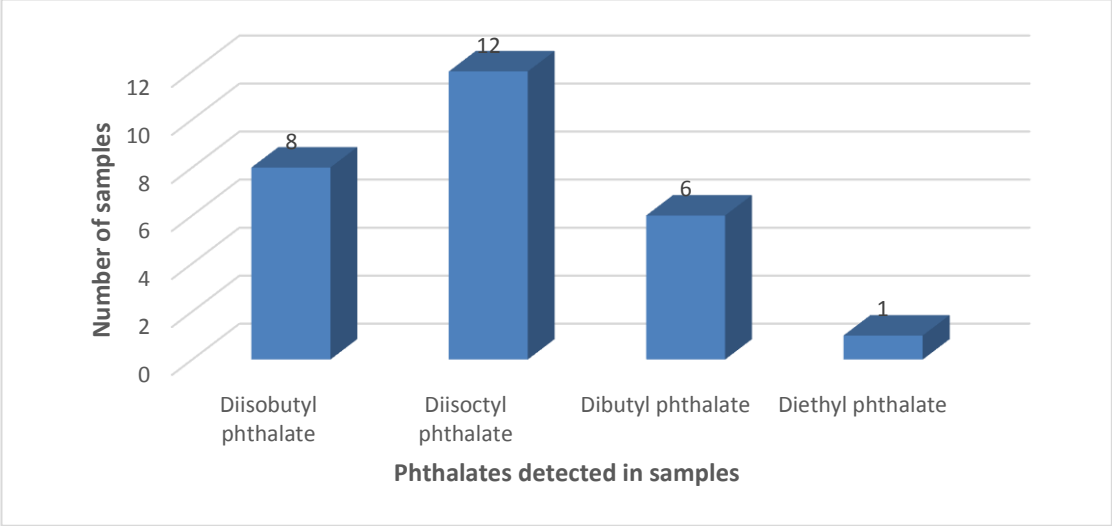


Figure 4.8: Distribution of Phthalates Detected in the Sampled Herbal Medicines

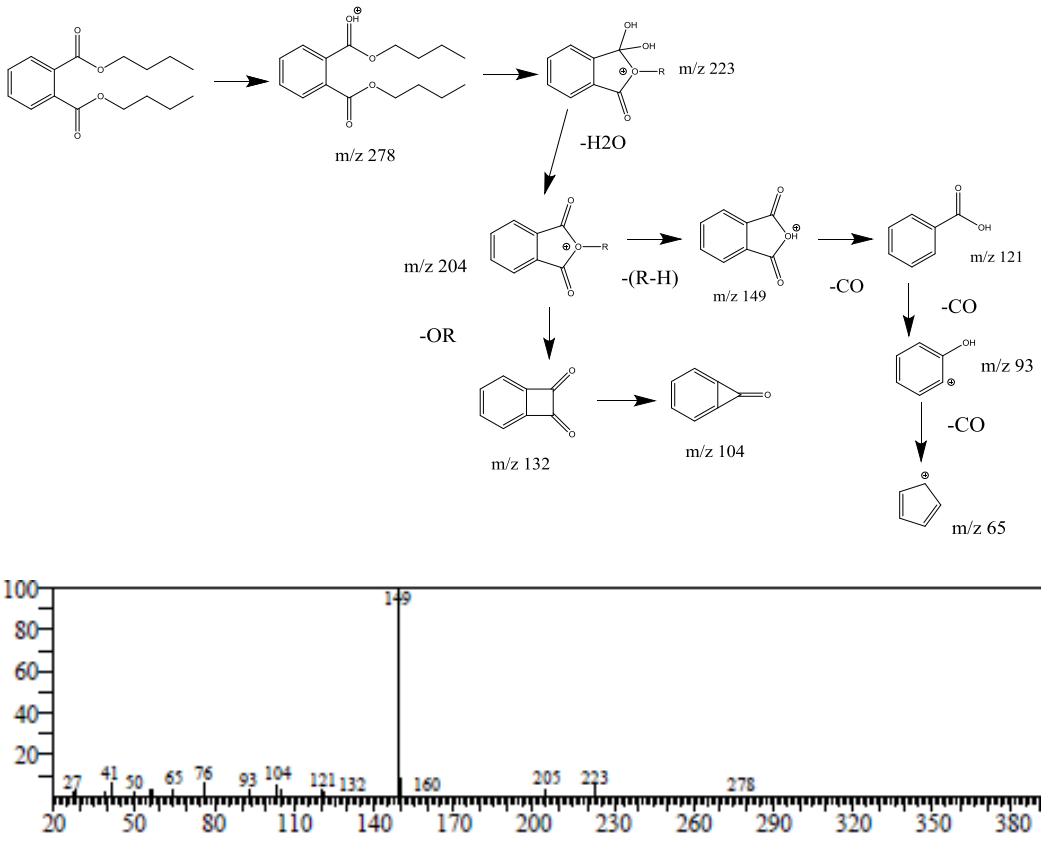


Figure 4.9: Proposed Fragmentation Pattern of Dibutyl/Diisobutyl Phthalate (Russo *et al*., 2015)

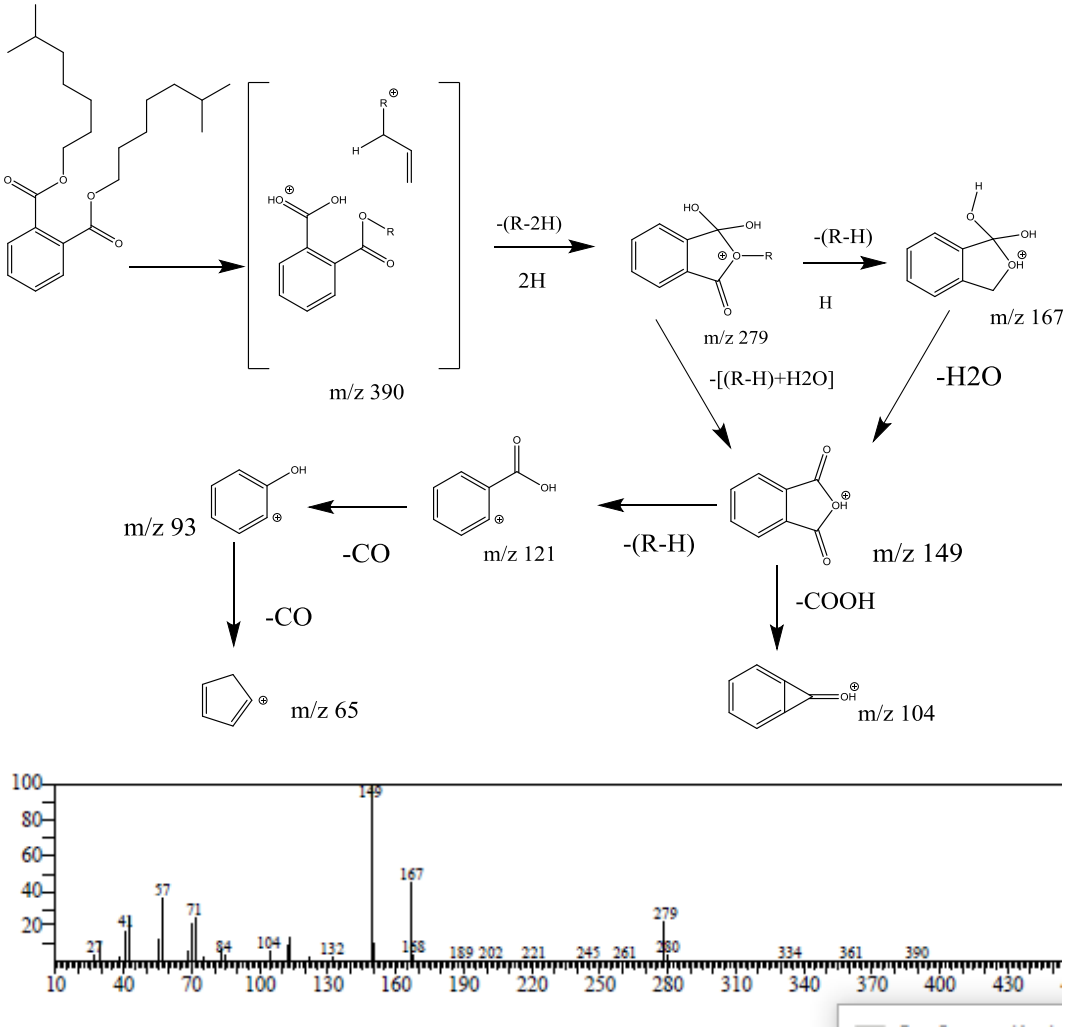


Figure 4.10: Proposed Fragmentation Pattern of Diisoctyl Phthalate (Russo *et al.*, 2015)

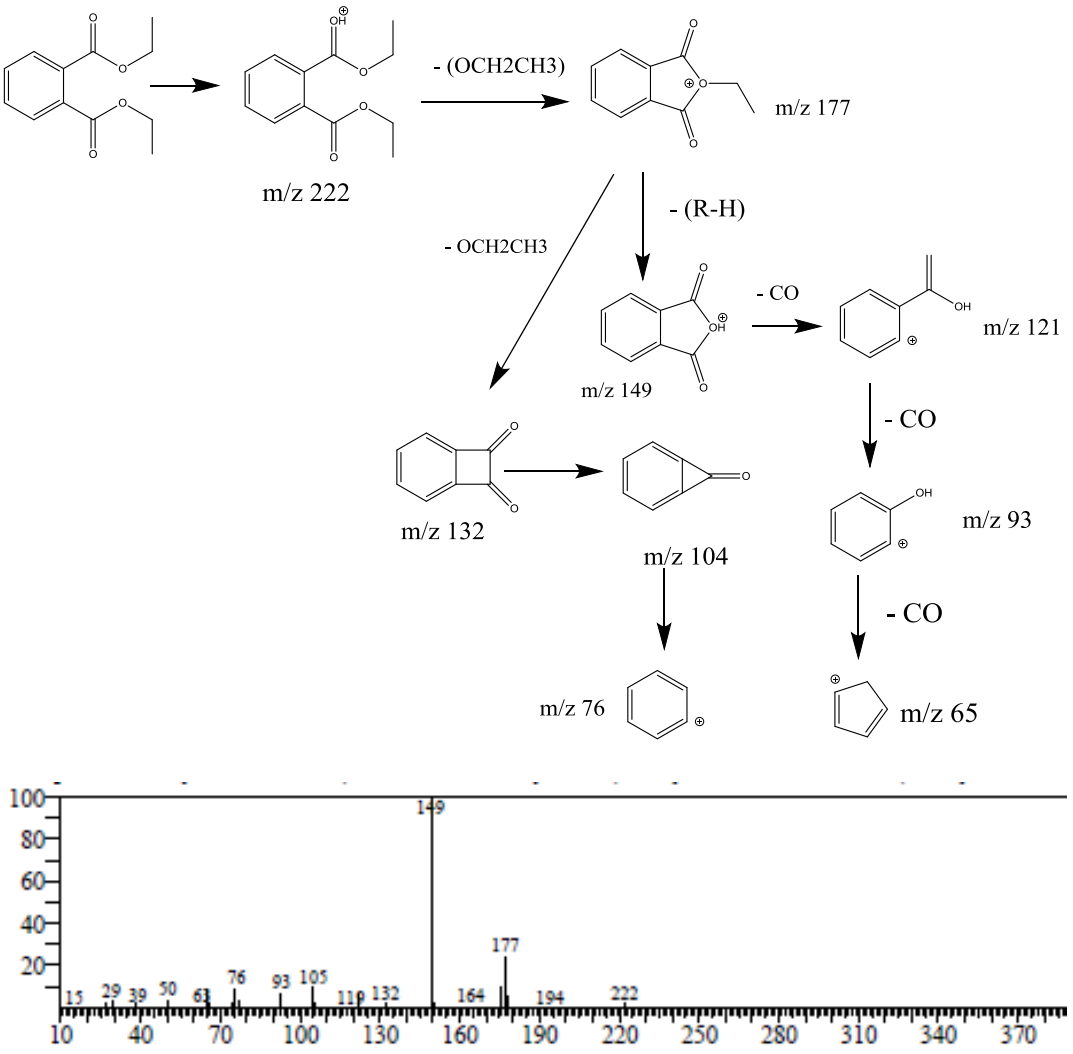


Figure 4.11: Proposed Fragmentation Pattern of Diethyl Phthalate (Russo *et al.*, 2015)

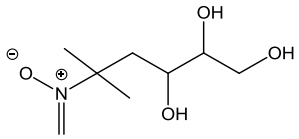
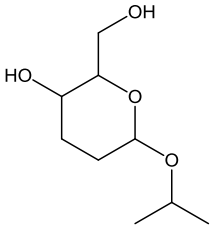
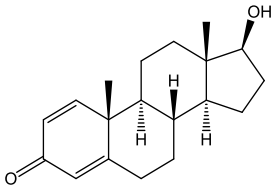
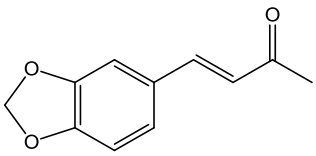
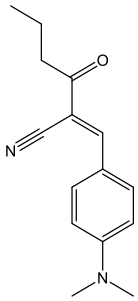
Food intake is the primary route of phthalate exposure in humans. Once ingested, diester phthalates are quickly metabolized by hydrolysis and then conjugated (Baranenko *et al.*, 2022). This hydrolysis of diester phthalates into monoester phthalates occurs in the parenchyma and the intestines and is catalysed by lipases and esterase. These monoester phthalates are highly bioactive and are associated with a wide range of illnesses. Studies have established that phthalates might disrupt the endocrine system when ingested, adversely impact the male reproductive system, and act as carcinogens – causing ovarian, cervix, skin, and breast cancer (Giuliani *et al.*,

2020; Ma *et al*., 2018). This exposure has also been correlated with nephrotoxicity, hepatoxicity, cardiotoxicity, autism spectrum disorders, type II diabetes, altered foetal development, sex anomalies, early puberty, and endometriosis (Wang & Qian, 2021; Saeidnia & Abdollahi, 2013; Saillenfait *et al*., 2013). Therefore, the determination of phthalates in 54% of the sampled herbal medicines in this study could point to potential safety issues with herbal medicines sold in Kenya.

4.3.2 Pharmaceutical and Pesticide Salts and Adjuncts in the Herbal Medicines Sampled

Several synthetic pharmaceutical and pesticide salts and adjuncts were detected in the GC-MS results of some samples. Adulterating herbal medicines with pharmaceuticals poses various health risks, especially to people with chronic illnesses because they use these medicines for a long time (Wang *et al*., 2020). The distribution of the detected pharmaceuticals can be found in Table 4.6.

Table 4.6: The Distribution of the Pharmaceutical Salts and Adjuncts Detected

Compound Name	Uses	Sample Code	Herbal Medicine Indication
2-nitroisobutyl-glycerol 	Vasodilator	ELD/D/76P D14 MD43P EIC27P	Diabetes Diabetes Diabetes
Isopropyl 2,3-dideoxyhex-2-enopyranoside 	Antitubercular	ELD/D/76P	Diabetes
Boldenone 	Anabolic steroid	D14	Diabetes
3,4-methylenedioxybenzylidene acetone 	Pesticides	ELD47P	Diabetes
ethyl 2-cyano-3-[4-(dimethylamino)phenyl]acrylate 	Synthesis of antibiotic pharmaceuticals	EIC21P ELD20P	Cancer Diabetes

4.3.2.1 2-Nitroisobutyl-Glycerol

2-nitroisobutyl-glycerol, a prodrug, was among the compounds detected in the methanol extracts of 3 herbal medicines, 2 from Uasin Gishu County (ELD/D/76P and EIC27P) and 2 from Nairobi County (D14 and MD43P). The TICs of the extracts of ELD/D/76 and D14 containing 2-nitroisobutyl-glycerol are provided in Figure 4.12 and 4.13, respectively.

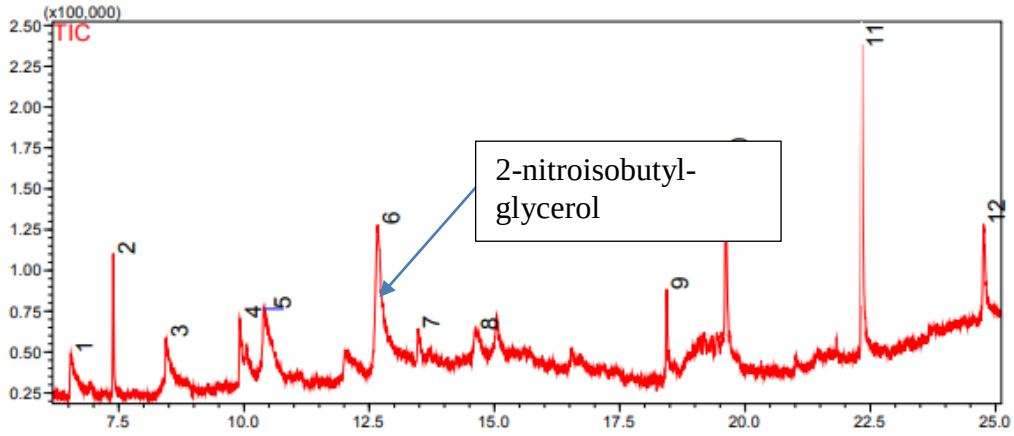


Figure 4.12: TIC of the ELD/D/76P Extract Containing 2-Nitroisobutyl-Glycerol

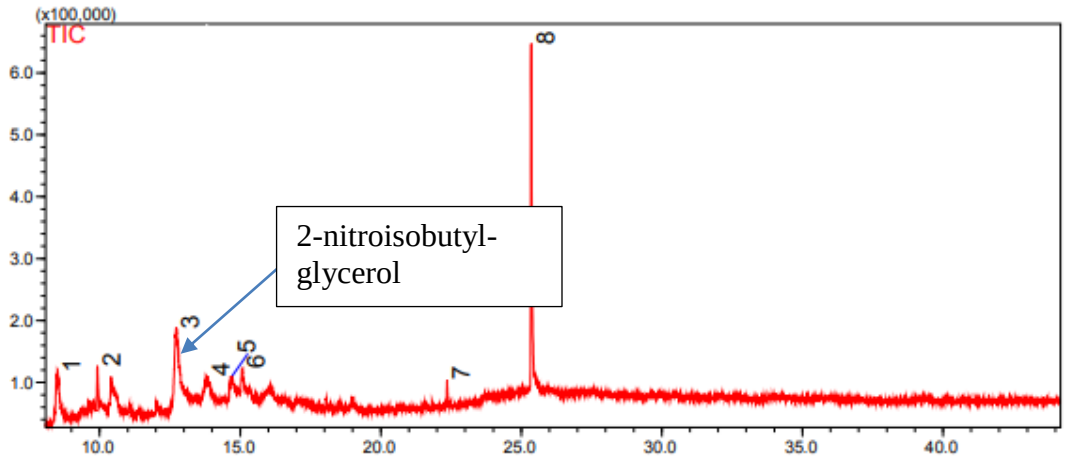


Figure 4.13: TIC of the D14 Extract Containing 2-Nitroisobutyl-Glycerol

2-nitroisobutyl-glycerol is used as a vasodilating hypotensive agent to improve cardiac function in patients with cardiovascular conditions (Bibli *et al*., 2019; Huh *et al*., 2019). It activates guanylyl cyclase and inhibits calcium uptake by myocytes, which relaxes the filaments and causes dilation of arterioles, arteries, and veins (Li *et al*., 2020). Synthetic antihypertensive drugs are among the synthetic drugs most commonly used to adulterate herbal medicines (Wang *et al*., 2020). For instance, Guo *et al*. (2020) reported that nine batches of herbal medicines labelled as "all natural" products were adulterated with antihypertensive pharmaceuticals, with amounts ranging from 2.8 mg/g to 27.9 mg/g.

4.3.2.2 Isopropyl 2,3-Dideoxyhex-2-Enopyranoside

Another pharmaceutical identified was isopropyl 2,3-dideoxyhex-2-enopyranoside. Isopropyl 2,3-dideoxyhex-2-enopyranoside was detected in 1 of the 4 herbal medicines from Uasin Gishu County (ELD/D/76P), and its TIC is shown in Figure 4.14.

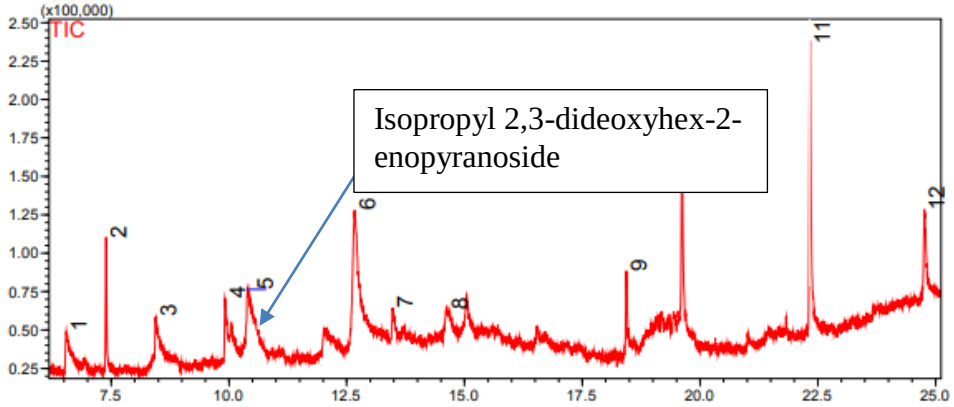


Figure 4.14: TIC of the ELD/D/76P Extract Containing Isopropyl 2,3-Dideoxyhex-2-Enopyranoside

Isopropyl 2,3-dideoxyhex-2-enopyranoside compound is among the group of hex-2-enopyranosides that are synthesized using the Ferrier reaction (Gomez *et al.*, 2015). Hex-2-enopyranosides are commonly used as intermediates in the synthesis of pharmaceuticals because they have an unsaturated site and are small in size (Saquib *et al.*, 2011). They have also been used as antitubercular agents (Gupta, 2010). Therefore, the presence of this compound in ELD/D/76P, which also contained 2-nitroisobutyl-glycerol, indicates the adulteration of this sample with multiple pharmaceuticals.

4.3.2.3 Boldenone

Boldenone, an anabolic steroid, was detected in one antidiabetic herbal medicine from Nairobi County (D14). The TIC of the D14 extract containing boldenone is shown in Figure 4.15.

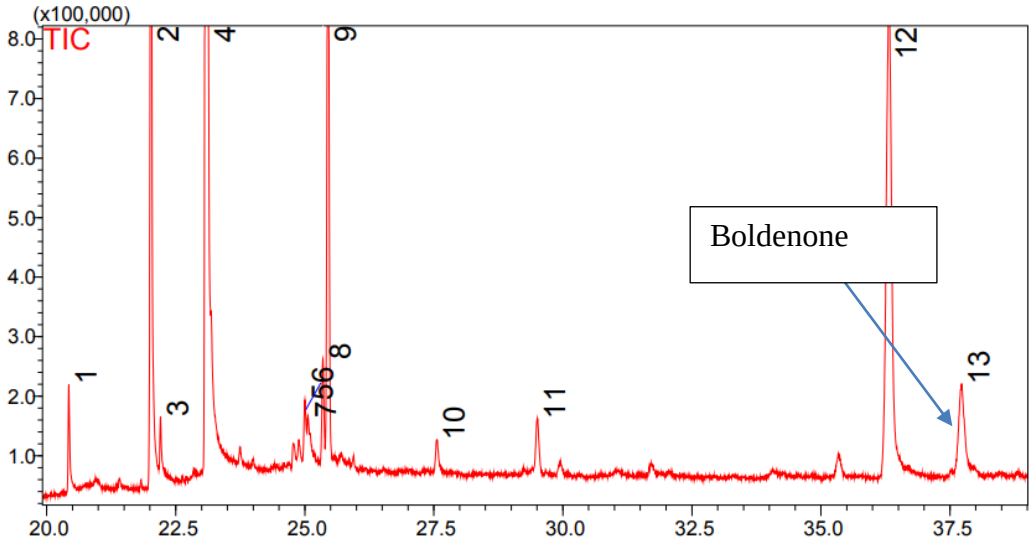


Figure 4.15: TIC of the D14 Extract Containing Boldenone

Boldenone is a synthetic derivative of testosterone that has been altered to improve its anabolic activity instead of its androgenic activity (Oda & El-Ashmawy, 2012). Hence, it is used to enhance the durability and strength of human, canine, and equine athletes and promote the growth of animals. Several studies have reported the adulteration of herbal supplements with boldenone and other anabolic steroids (Deconinck *et al*., 2021; Micalizzi *et al*., 2021; Rocha *et al*., 2016). This adulteration is dangerous because anabolic steroid use is associated with irreversible organ damage, blood clotting, hypertension, reduced fertility, and atherosclerosis (Behairy *et al*., 2021; Gagliano-Jucá & Basaria, 2019; Oda & El-Ashmawy, 2012).

4.3.2.4 3,4-Methylenedioxybenzylidene Acetone

3,4-methylenedioxybenzylidene acetone, a modified form of the natural compound piperonyl acetone, was detected in one herbal sample – the antidiabetic medicine from Uasin Gishu County (ELD47P) that contained ketone, 7-methoxy-2-

benzofuranyl methyl, a fungi secondary metabolite. The TIC of the ELD47P extract containing 3,4-methylenedioxybenzylidene acetone is shown in Figure 4.16.

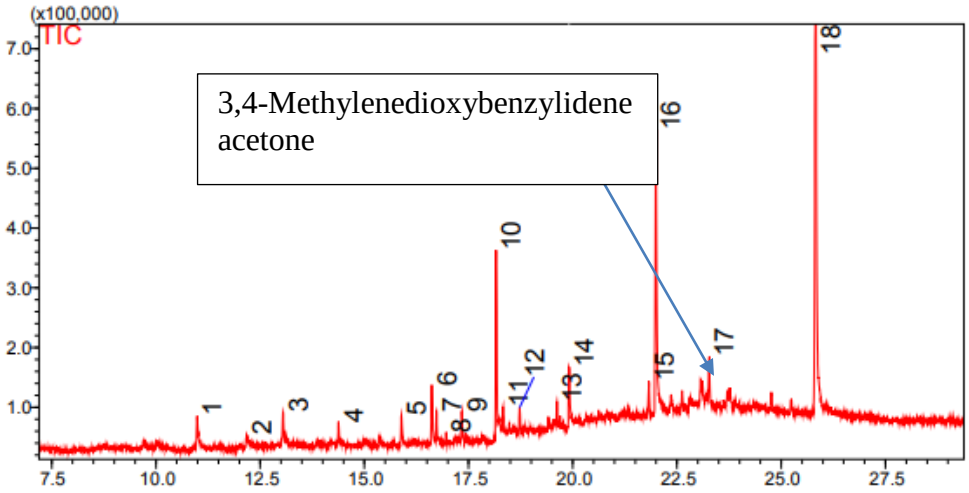


Figure 4.16: TIC of the ELD47P Extract Containing 3,4-Methylenedioxybenzylidene Acetone

3,4-methylenedioxybenzylidene acetone is used in pesticide formulations. Thus, its presence indicates possible contamination of the sample with pesticides. Various studies have reported contamination of herbal samples with pesticide residue above the recommended limits, which poses significant health risks when consumed (Shaban *et al*., 2016; Harris *et al*., 2011; Zhang *et al*., 2012). Some adverse health effects associated with consumption of pesticide-contaminated foods include tremor, convulsions, discoordination, dizziness, headache, and paraesthesia (Thompson & Darwish, 2019; Shaban *et al*., 2016). As a result, the WHO has set limits and recommends screening medicinal plant materials for pesticide residue to ensure they are safe for human consumption (WHO, 2007). However, determining the amount of pesticide residue present was beyond the scope of this study.

4.3.2.5 Ethyl 2-Cyano-3-[4-(Dimethylamino)Phenyl]Acrylate

Lastly, ethyl 2-cyano-3-[4-(dimethylamino)phenyl]acrylate was detected in 2 antidiabetic herbal medicines from Uasin Gishu County (EIC21P and ELD20P). The TICs of ELD20P and EIC21P extracts containing ethyl 2-cyano-3-[4-(dimethylamino)phenyl]acrylate are shown in Figures 4.17 and 4.18, respectively.

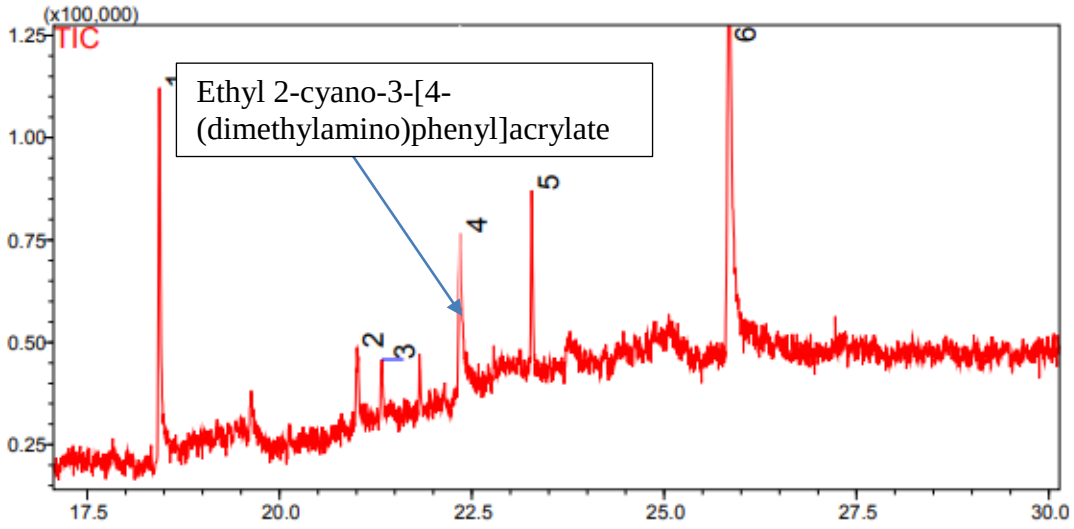


Figure 4.17: TIC of the ELD20P Extract Containing Ethyl 2-Cyano-3-[4-(Dimethylamino)Phenyl]Acrylate

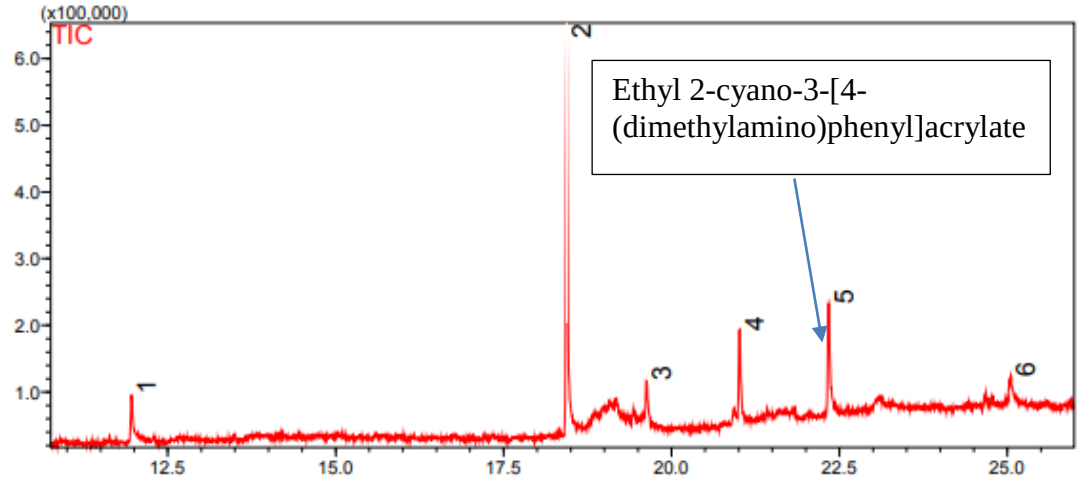


Figure 4.18: TIC of the EIC21P Extract Containing Ethyl 2-Cyano-3-[4-(Dimethylamino)Phenyl]Acrylate

Ethyl 2-cyano-3-[4-(dimethylamino)phenyl]acrylate is a compound used in the synthesis of a wide range of products (Bekhit *et al*., 2012; Medyouni *et al*., 2013). It is commonly used to synthesize quinolone drugs, which are used to treat a wide range of bacterial infections (Al-Hazmi, 2022). As such, the presence of ethyl 2-cyano-3-[4-(dimethylamino)phenyl]acrylate in these herbal samples points to

possible adulteration with antibiotic pharmaceuticals. Mwankuna *et al*. (2023) reported that 20% of herbal product samples from Tanzania in their study were adulterated using conventional antibiotics.

4.4 Development and Validation of LC-MS/MS Method

4.4.1 Optimized Operating Conditions

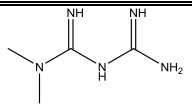
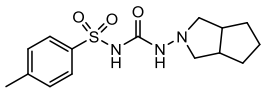
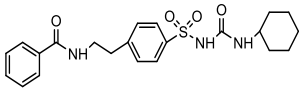
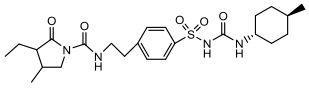
Separation was conducted on an XbridgeTM C18 column (100 mm * 2.5 mm, 5 µm particle size) fitted with a Vanguard ® pre-column (2.1 mm x 5 mm). The collision and desolvation gases were argon and nitrogen, respectively. The source temperature was set at 120℃ while the desolvation temperature was set at 350℃. The cone and desolvation gas flow rates were 50 and 700 L/min, respectively. The mass spectrometer was operated in the MRM mode.

The optimal mobile phase included 0.1% formic acid in ultrapure water as solvent A, and 0.1% formic acid in acetonitrile as solvent B. 0.1% formic acid in acetonitrile gave sufficient analyte retention, adequate response, and ideal peak shapes for all the antidiabetics, except metformin that had a slightly broad peak as illustrated in Figure 4.19. Conversely, methanol as the organic mobile phase resulted in a broader peak for metformin and insufficient response for the other analytes, consistent with the findings of similar studies (Shah & Shrivastav, 2018; Shah *et al*., 2017; Heinig & Bucheli, 2004).

The separation was conducted at a flow rate of 0.40 mL/min and temperature of 40℃, as the peaks were better resolved for all the analytes at these conditions. The following gradient program was used: 0-0.5 min (5% B); 0.5-1.5 min (increase to

30% B); 1.5-2.0 min (increase to 40% B); 2.0-6.0 min (increase to 60% B); 6.0-7.0 min (60% B); 7.0-8.0 min (decrease to 40% B); 8.0-9.0 min (40% B); 9.0-9.5 min (5% B); 9.5-10.0 min (5% B). The positive ESI mode was used because it gave optimal results for all the analytes. The MRM transitions for each analyte were optimized, as shown in Table 4.7.

Table 4.7: Optimized MRM Parameters for the 4 Antidiabetic Pharmaceuticals

Compound Name	Structure	Precursor ion	Product ion	Cone voltage (V)	Collision energy (eV)
Metformin		130	71[q] 60	23	22 24
Gliclazide		324	127[q] 110	30	28 28
Glibenclamide		494	369[q] 169	22	20 38
Glimepiride		491	352[q] 126	22	17 39

[q] Indicates the product ion used for quantification

The TIC of the four antidiabetic pharmaceuticals obtained under these MRM conditions for the analytes at 50 ng/mL concentration is shown in Figure 4.19.

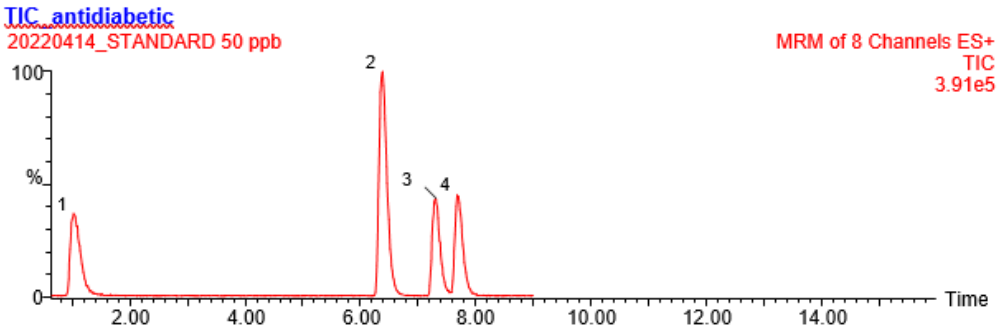


Figure 4.19: TIC of the Chosen Antidiabetic Pharmaceuticals (1 – Metformin, 2 - Gliclazide, 3 - Glibenclamide, And 4 - Glimepiride)

4.4.2 Optimization of Sample Preparation Steps

4.4.2.1 Optimized Extraction Step

Methanol (20 mL) at different concentrations (30%, 60%, 90%) in ultrapure water was used to extract 2 g of the blank sample spiked using the mixed standard solution to a final analyte concentration of 50 ng/mL. The extraction was conducted using a shaker at 200 rpm and a sonicator at 40℃ for 20 minutes each, and the absolute recoveries compared, as shown in Figure 4.20. 90% methanol in water as the extraction solvent using a sonicator had significantly higher absolute recoveries for glibenclamide (73.4% vs 47.5%, p=0.03) and glimepiride (78.1% vs 27.9%, p=0.002) compared to when a shaker was used. It also had higher absolute recoveries of metformin (52.0% vs 42.4%, p=0.11) and gliclazide (129.7% vs 125.2%, p=0.72), but the differences were not statistically significant as only p-values of less than 0.05 were considered to be significant.

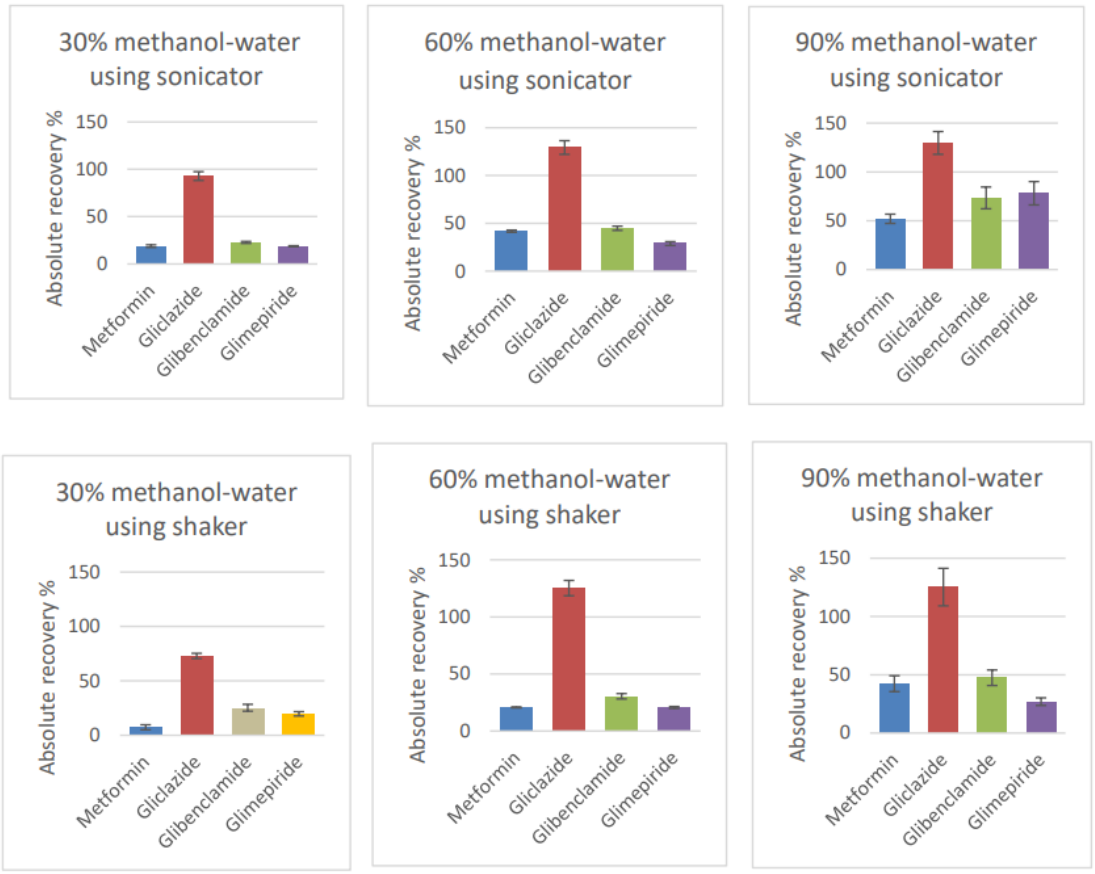


Figure 4.20: Absolute Recoveries of the Selected Analytes Using Different Sample Extraction Conditions

Although the absolute recoveries of gliclazide were the highest, they were within the acceptable range of 70% to 130% for routine analysis of products with complex matrices, such as herbal products (Steiner *et al*., 2020). One possible explanation of the absolute recoveries of gliclazide being so high is the presence of matrix influences, particularly ion enhancement, as gliclazide is the only analyte in the study whose matrix effect was above 100%. Similar studies on gliclazide have reported high recoveries (Pamidimarri *et al*., 2019; Pang *et al*., 2009; Bogusz *et al*., 2006). Hence, it is recommended that the pre-and post-spiking approach is used when optimizing the sample preparation process to ensure that the method accounts for

matrix influences. As for metformin, several studies have reported low recoveries due to its high polarity (van de Ven *et al*., 2016; Heinig & Bucheli, 2004).

Varying concentrations of formic acid (0.05% and 0.02% formic acid) were added to lower the pH of the extracting solvent and improve the absolute recoveries of metformin since metformin is a strong base. However, the addition of formic acid to the extraction solvent resulted in lower extraction efficiencies for all the analytes, as shown in Figure 4.21. Sonicating using 90% methanol in water with 0.05% formic acid as the extracting solvent compared to using 90% methanol in water significantly reduced the absolute recoveries of metformin (42.7% vs 52.0%, $p=0.03$), gliclazide (104.4% vs 129.7%, $p=0.04$), glibenclamide (40.9% vs 73.4%, $p=0.01$), and glimepiride (26.9% vs 78.1%, $p=0.001$). Similarly, but to a greater extent, using 90% methanol in water with 0.02% formic acid as the extracting solvent compared to using 90% methanol in water significantly reduced the absolute recoveries of metformin (42.0% vs 52.0%, $p=0.03$), gliclazide (97.4% vs 129.7%, $p=0.02$), glibenclamide (11.3% vs 73.4%, $p<0.001$).

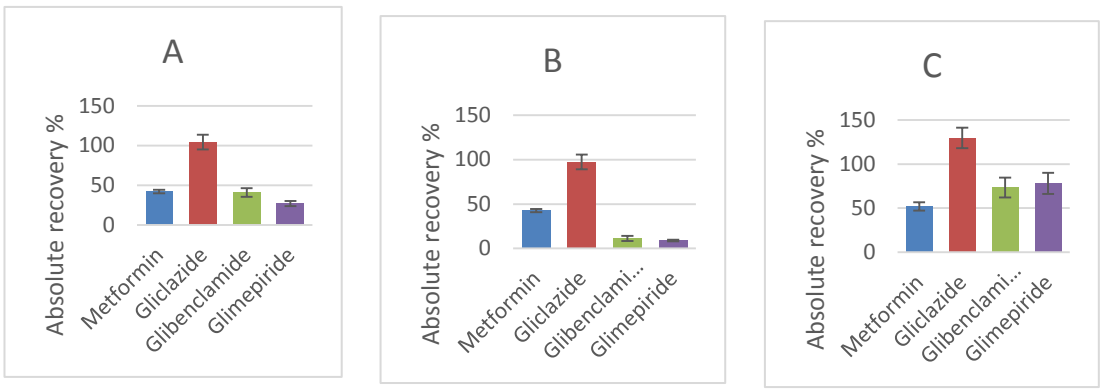


Figure 4.21: Effect of Adding Formic Acid to the Extraction Solvent on the Absolute Recoveries of the Selected Antidiabetic Pharmaceuticals (A - 0.05%; B - 0.02%; And C - No Formic Acid)

This decreased extraction efficiency can be attributed to formic acid lowering the pH of the extraction solvent, which subsequently reduced the solubility of the analytes. Gliclazide, glibenclamide, and glimepiride are sulfonylurea drugs; thus, their solubility is poor in weakly acidic media because they are weakly acidic (Steiner *et al*., 2020; Shah *et al*., 2017). As such, sonicating the samples with 90% methanol in water for 20 mins was determined to be the optimal conditions for extracting the selected analytes.

4.4.2.2 Optimized Evaporation Step

The use of 90% methanol in water to extract the blank herbal sample necessitated the switching of the extraction solvent with water to optimize chromatographic separation (van de Ven *et al*., 2016). Consequently, the extraction solvent was evaporated to near dryness under a gentle nitrogen stream at 40ºC in a nitrogen evaporator (N-EVAP^{TM}111, model 5085, USA), and the residue was reconstituted in water. The absolute recoveries for the analytes were calculated in this step to determine the effectiveness of this solvent-switching process. The blank sample was

spiked with the mixed standard solution to a final analyte concentration of 50 ng/mL after extraction prior to evaporation. The absolute recoveries were determined to be 68% for metformin, 107% for gliclazide, 96% for glibenclamide, and 85% for glimepiride.

4.4.2.3 Optimized Filtration Step

Samples need to be filtered using a 0.22-micron filter before they are injected into the LC for analysis to protect the column from damage, prolonging its lifetime (Li *et al*., 2020). However, some studies have reported the adsorption of pharmaceutical compounds on the filter (Chen *et al*., 2019; Mompelat *et al*., 2013; Buchberger, 2011). Hence, the absolute recoveries for the 0.22-micron cellulose and PTFE filters were calculated to determine their potential to adsorb the four antidiabetics, as illustrated in Figure 4.22. Using a 0.22-micron PTFE filter compared to a 0.22-micron cellulose filter had significantly better recoveries for gliclazide (108% vs 58%, $p<0.001$), glibenclamide (88% vs 4%, $p<0.001$), and glimepiride (74% vs 7%, $p<0.001$), as p-values of less than 0.05 were considered to be significant. Several studies have established cellulose materials have a high adsorption capacity for sulfonylurea drugs and herbicides (Zhang *et al*., 2021; Cara *et al*., 2017). Hence, the 0.22-micron PTFE filter was used.

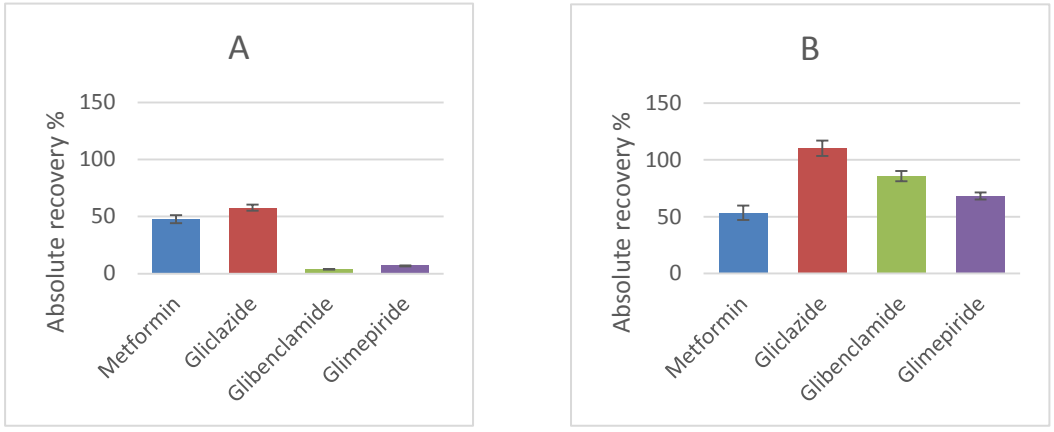


Figure 4.22: Absolute Recoveries of the Selected Antidiabetic Pharmaceuticals for the Filtration Step (A = 0.22-Micron Cellulose Filter and B = 0.22-Micron PTFE Filter)

4.4.3 Optimized Sample Preparation Process

The optimized sample preparation process was as follows. About 1 gram of the powdered sample were accurately weighed into a 50-mL centrifuge tube and vortexed for 2 mins for a uniform composition. The homogenous sample was then dissolved in 90% methanol in water (10 mL) in a ratio of 1:10 and vortex-mixed for 1 min, followed by sonication at 40 ºC for 20 minutes before centrifuging at 4500 rpm for 5 mins. The extraction was done in duplicate and the supernatants combined. One millilitre of the combined supernatant was transferred into a 10 mL vial, and the extraction solvent evaporated to almost dryness under a gentle stream of nitrogen gas at room temperature. It was then reconstituted in 1 mL of Milli-Q water, transferred into a 1.5 mL Eppendorf tube and centrifuged at 15000 rpm for 5 mins. One millilitre of the supernatant was filtered using a 0.22-micron PTFE syringe filter and diluted

ten times using Milli-Q water. One millilitre of the filtrate was then placed in an HPLC vial, and 10 µL injected in triplicate into the LC system for analysis.

4.4.4 Validation of Method Performance

Linearity calibration curves in 5–200 ng/mL range were determined for glimepiride and glibenclamide and 5–250 ng/mL for metformin and gliclazide, as shown in Appendix IV. The coefficients of correlation (r) were 0.9981, 0.9997, 0.9978, and 0.9985 for metformin, gliclazide, glibenclamide, and glimepiride, respectively. The r values of all the standards indicated good linearity as they were within the acceptable limits of > 0.990 (Ouma *et al*., 2021). The LOQs and LODs for all the compounds are indicated in Table 4.8. The precision ranged between 8.5% and 16.1%, with the lowest variability at the highest concentration level and vice versa. The deviations for precision were within the acceptable level of variation recommended by most guidelines (Sveshnikova *et al*., 2019).

The accuracy for the analytes, expressed as mean relative recovery, was at least 80% for all the analytes, except for metformin (52%). The acceptable level of variation for accuracy recommended by most of the guidelines is ± 20% (Sveshnikova *et al*., 2019). However, several studies have also reported low accuracies for metformin due to its high polarity (Shah & Shrivastav, 2018; Shah *et al*., 2017; van de Ven *et al*., 2016; Heinig & Bucheli, 2004). The matrix effect for each analyte can be found in Table 4.8. Only gliclazide had a matrix effect of over 100%, indicating ion enhancement. The other analytes – metformin, glibenclamide, and glimepiride – had a matrix effect of less than 100%, indicating ion suppression.

Table 4.8: Method Validation Parameters Determined for the Selected Antidiabetic Pharmaceuticals

Compound	Linear Range (ng/mL)	r	Accuracy (%)	LOD (ng/mL)	LOQ (ng/mL)	Matrix Effect (%)
Metformin	5 - 250	0.9981	76.42	7.67	23.24	68.41
Gliclazide	5 -250	0.9997	120.22	2.84	8.62	106.96
Glibenclamide	5 - 200	0.9978	92.52	7.11	21.63	96.08
Glimepiride	5 - 200	0.9985	102.24	5.96	18.07	79.97

4.5 Application of Validated LC-MS/MS Method to Herbal Medicine Sampled

After the method was validated, it was used to analyse 24 herbal medicines purchased from Nairobi and Uasin Gishu Counties for possible adulteration. Gliclazide, glibenclamide, and glimepiride were not detected in any of the analysed samples. Metformin was detected in 17 % of the samples (n = 4) at concentrations shown in Table 4.9. Three of the adulterated medicines were from Nairobi County (D14, JD15P and D17) while the other one was from Uasin Gishu County (EIC35P).

Table 4.9: Concentrations (mean ±SD) of Metformin in Sampled Herbal Products Adulterated with Metformin (n = 4)

Sample No.	Sample Code	Concentration (ng/g)
Sample 5	D14	1781 ± 116
Sample 10	EIC21P	1969 ± 86
Sample 18	JD15P	1385 ± 174
Sample 22	D17	903 ± 265

The TICs of all the adulterated samples are provided in Figure 4.23. Notably, EIC35P was indicated for cancer treatment, but had been adulterate with metformin. Although metformin is typically an antidiabetic drug, clinical data shows that it has significant anticancer effect in various patient populations (Wu *et al*., 2022; Kasznicki *et al*., 2014). Several studies have reported adulteration of herbal drugs with metformin (Chowdry, 2018; Steyn *et al*., 2018; Ching *et al*., 2012; Kumar *et al*., 2011). Metformin is readily available nationwide because it is the least costly antidiabetic pharmaceutical (Ongarora *et al*., 2019; Rockers *et al*., 2019; Hailu *et al*., 2018), which could explain why it was the only antidiabetic detected in the samples.

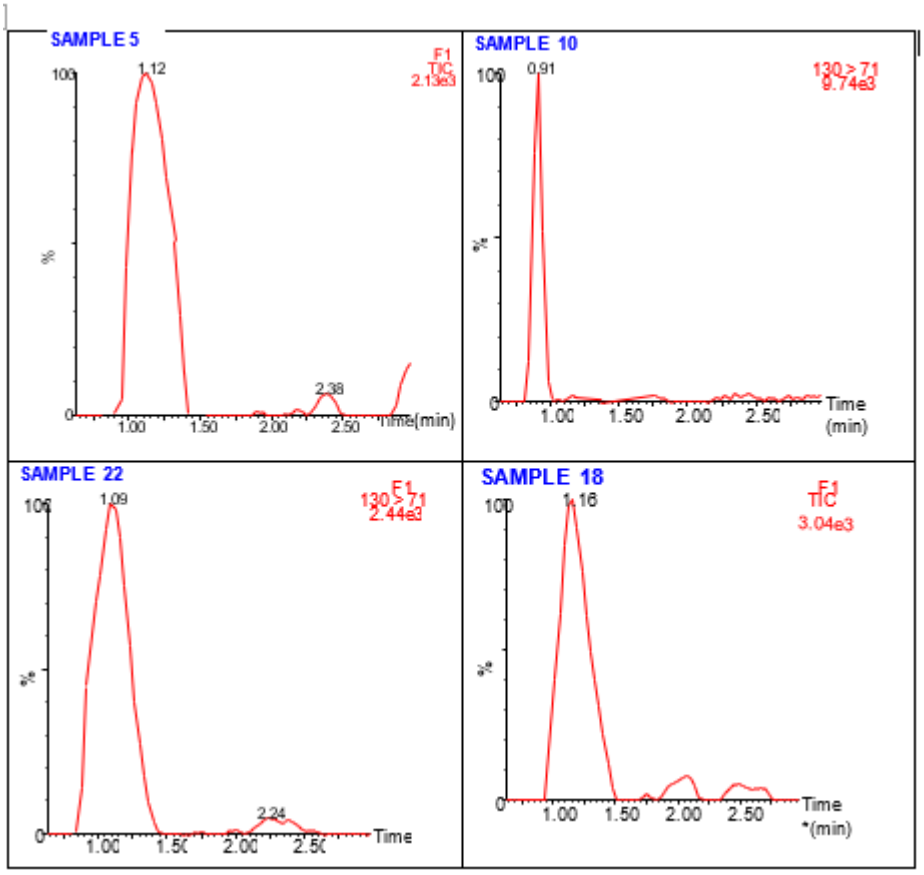


Figure 4.23: The TICs of the Samples Adulterated with Metformin

Adulterating herbal products with metformin is dangerous because of its high potential for adverse drug-herb interactions. Several studies have reported mild hypoglycaemia in diabetic patients taking metformin with various herbal plants such as melatonin, ginseng, salvia, gobo, and nopal (Carella, 2017; Holloway *et al*., 2007). Additionally, some herbs, including *Gymnema sylvestre*, reduce metformin's bioavailability when taken together, leading to increased blood glucose levels (Gupta *et al*., 2017). Moreover, the dosage that the patient would take based on the quantification results would be way below the recommended initial dose of 500 mg once daily. Taking suboptimal dose of metformin is associated with development of comorbidities, increased complication rates, poor glucose control, and early

progression of diabetes (Al-Waeli *et al*., 2022; Christofides, 2019). Therefore, this adulteration poses a significant risk to consumers' health.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Phytochemical Profiles of Herbal Medicines Sampled

The GC-MS screening of the sampled antidiabetic and anticancer herbal medicines revealed a wide variety of phytochemicals with reported antidiabetic and anticancer activities, respectively. The presence of these phytochemicals highlights that the antidiabetic and anticancer herbal medicines sold in the country might be effective in treating their indicated diseases. However, the presence of these phytochemicals does not guarantee that the herbal medicines have *in vivo* or *in vitro* activity against diabetes or cancer. This screening also revealed the presence of three potentially toxic phytochemicals – prunasin-d, 5-keto-d-fructose, and ketone, 7-methoxy-2-benzofuranyl methyl, in 12.5% of the samples indicates that some of the commercially-available herbal medicines are unsafe for human consumption.

5.1.2 Profiles of Synthetic Compounds Identified in Herbal Medicines Sampled

The GC-MS screening of the sampled antidiabetic and anticancer herbal medicines revealed the presence of several synthetic compounds, including phthalates, pesticide residue, and pharmaceutical adjuncts and salts. The presence these synthetic compounds in 29% of the samples highlights that some of the herbal medicines sold

in Kenya for cancer and diabetes treatment are not 100% natural, as advertised. Consequently, they pose a risk to consumers' health.

5.1.3 Validated LC-MS/MS Method

The developed LC-MS/MS method showed high precision, accuracy, linearity, and sensitivity. Therefore, this method can be used to monitor antidiabetic herbal medicines sold in the country for adulteration using the four antidiabetic pharmaceuticals.

5.1.4 Adulteration of Herbal Medicines with Antidiabetic Pharmaceuticals

The presence of metformin in 17% of the samples shows that herbal medicines sold in the country are being adulterated without the knowledge of the public and regulatory bodies. Consumption of these adulterated herbal samples would undermine consumer health as taking suboptimal dosages of metformin and taking metformin with some herbal plants are associated with development of hypoglycaemia in diabetic patients. Thus, consumption of these adulterated herbal samples undermines consumer health.

5.2 Recommendations

1. Close monitoring of herbal medicines sold in the country for toxicity is recommended.
2. A GC-MS method for phthalate and pesticide residue determination in herbal products needs to be developed and validated. This method should then be used to routinely monitor herbal medicines for phthalate and pesticide residue contamination to guide the development of related regulations to promote consumer safety.

3. The developed LC-MS/MS method should be expanded to include other classes of pharmaceuticals. This method should be used for routine monitoring of herbal medicines sold in the country for adulteration with pharmaceuticals.
4. Legislation that outlaws adulteration of herbal medicines, mandates the declaration of the constituents of herbal medicines on their labels, and imposes heavy fines on those found guilty is needed to discourage the practice.
5. Public awareness efforts on the potential safety risks of consuming herbal medicines in the country is recommended to ensure that consumers and vendors of these medicines understand the risks involved.
6. More studies on adulteration of herbal medicines should be conducted in other parts of the country to obtain a more comprehensive overview of the subject to inform the development of policies and regulatory framework in the future.

REFERENCES

Abdelfattah, M. S. (2009). Mansoquinone: Isolation and structure elucidation of new antibacterial aromatic polyketides from terrestrial Streptomyces Sp. Eg5. *Nat Prod Res*, *23*(3), 212–218.

Abu-Lafi, S., Rayan, B., Kadan, S., Abu-Lafi, M., & Rayan, A. (2019). Anticancer activity and phytochemical composition of wild Gundelia tournefortii. *Oncology Letters*, *17*(1), 713–717.

Abubacker, M. N., & Deepalakshmi, T. (2013). In vitro antifungal potentials of bioactive compound methyl ester of hexadecanoic acid isolated from Annona muricata Linn. (Annonaceae) leaves. *Biosciences Biotechnology Research Asia*, *10*(2), 879–884.

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy & Bioallied Sciences*, *12*(1), 1-13.

Afshar, A., Khoradmehr, A., Baghban, N., Mohebbi, G., Barmak, A., Khatami, M., Daneshi, A., Bargahi, A., Azari, H., Ullah, M., Anvari, M., & Tamadon, A. (2021). Anti-cancer activity of Avicennia marina. *Research Square*. 1-9.

Ahmed, S. S., Ibrahim, M. E., Khalid, A. K., & El-Sawi, S. A. (2017). Phytochemicals, volatile oil and biological activities of Triumfetta flavescens. *International Food Research Journal*, 24(5), 2102-2106.

Ajiboye, T. O., Mohammed, A. O., Bello, S. A., Yusuf, I. I., Ibitoye, O. B., Muritala, H. F., & Onajobi, I. B. (2016). Antibacterial activity of Syzygium aromaticum seed: Studies on oxidative stress biomarkers and membrane permeability.

Microbial Pathogenesis, *95*, 208–215.

Al-Hazmi, G. H. (2022). Synthesis and cytotoxicity screening of some novel 1-substituted 2-oxo-1h-quinoline-3-carboxylic acid derivatives against breast carcinoma cell line. *Russian Journal of Organic Chemistry*, *58*(3), 433–438.

Al-Waeli, D., Mohammed, A., Tahir, I., Al-Saeedi, A., Razzaq, K., & Abodhurais, A. (2022). Barriers against the use of an optimal dose of metformin among patients with T2DM in Thi-Qar province, Iraq. *Journal of Medicine and Life*, *15*(4), 567–572.

Al Lawati, H. A. J. J., Al Busaidi, I., Kadavilpparampu, A. M., & Suliman, F. O. (2017). Determination of common adulterants in herbal medicine and food samples using core-shell column coupled to tandem mass spectrometry. *Journal of Chromatographic Science*, *55*(3), 232–242.

Alasmary, F. A. S., Awaad, A. S., Kamal, M., Alqasoumi, S. I., & Zain, M. E. (2018). Antitumor activity of extract and isolated compounds from Drechslera rostrata and Eurotium tonophilum. *Saudi Pharmaceutical Journal*, *26*(2), 279–285.

Alauzet, C., Teyssier, C., Jumas-Bilak, E., Gouby, A., Chiron, R., Rabaud, C., Counil, F., Lozniewski, A., & Marchandin, H. (2010). Gluconobacter as well as Asaia species, newly emerging opportunistic human pathogens among acetic acid bacteria. *Journal of Clinical Microbiology*, *48*(11), 3935-3943.

Alipour, A., Patel, P. B., Shabbir, Z., & Gabrielson, S. (2019). Review of the many faces of synthetic cannabinoid toxicities. *The Mental Health Clinician*, *9*(2), 93, 8-17.

Allen, T. E., Aliano, N. A., Cowan, R. J., Grigg, G. W., Hart, N. K., Lamberton, J.

A., & Lane, A. (1985). Amplification of the antitumor activity of phleomycins and bleomycins in rats and mice by caffeine. *Cancer Research*, *45*(6), 2516-2521.

Alves, R., & Cruz, S. (2011). Anticholinesterasic activity of Manilkara subsericea (Mart.) Dubard triterpenes. *American Journal of Pharmacy*, *30*(8)1631-1634.

Anandan, A., Eswaran, R., Doss, A., Sangeetha, G., & Anand, S. (2011). Chemical compounds investigation of cassia auriculata leaves – A potential folklore medicinal plant. *Bulletin of Environment, Pharmacology & Life Sciences*, *1*(1), 20–23.

Andjelković, T., Bogdanović, D., Kostić, I., Kocić, G., Nikolić, G., & Pavlović, R. (2021). Phthalates leaching from plastic food and pharmaceutical contact materials by FTIR and GC-MS. *Environmental Science and Pollution Research*, *28*(24), 31380–31390.

Andrade-Eiroa, A., Canle, M., Leroy-Cancellieri, V., & Cerdà, V. (2016a). Solid-phase extraction of organic compounds: A critical review. part ii. *Trends in Analytical Chemistry*, *80*, 655–667.

Andrade-Eiroa, A., Canle, M., Leroy-Cancellieri, V., & Cerdà, V. (2016b). Solid-phase extraction of organic compounds: A critical review (Part I). *Trends in Analytical Chemistry*, *80*, 641–654.

Andrade, E. M. J., Teixeira, J. S., Silva, D. K. C., Dos Santos, T. B., Korn, M. G. A., Brandão, H. N., Ferraz, C. G., Meira, C. S., Soares, M. B. P., Guimarães, E. T., Neta, L. C. S., & Júnior, A. F. S. (2020). Phytochemical analysis, multi-element composition and biological activities of extracts and lupenone from Albizia inundata (. *Article J. Braz. Chem. Soc*, *31*(9), 1825–1837.

Ano, Y., Ozawa, M., Kutsukake, T., Sugiyama, S., Uchida, K., Yoshida, A., & Nakayama, H. (2015). Preventive effects of a fermented dairy product against Alzheimer's disease and identification of a novel oleamide with enhanced microglial phagocytosis and anti-inflammatory activity. *PLOS ONE*, *10*(3), 1-13.

Aragão, G. F., Carneiro, L. M. V., Frota, A. P., Bandeira, P. N., De Lemos, T. L. G., & De Barros Viana, G. S. (2015). Alterations in brain amino acid metabolism and inhibitory effects on PKC are possibly correlated with anticonvulsant effects of the isomeric mixture of α- and β-amyrin from Protium heptaphyllum. *Pharmaceutical Biology*, *53*(3), 407–413.

Aragão, G. F., Carneiro, L. M. V., Junior, A. P. F., Vieira, L. C., Bandeira, P. N., Lemos, T. L. G., & Viana, G. S. d. B. (2006). A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from Protium heptaphyllum (Aubl.) March. *Pharmacology Biochemistry and Behavior*, *85*(4), 827–834.

Awaad, A. S., Al-Zaylaee, H. M., Alqasoumi, S. I., Zain, M. E., Aloyan, E. M., Alafeefy, A. M., Awad, E. S. H., & El-Meligy, R. M. (2013). Anti-leishmanial Activities of Extracts and Isolated Compounds from Drechslera rostrata and Eurotium tonpholium. *Phytotherapy Research*, 28(5), 774-780.

Baranenko, D., Boulkrane, M. S., Borisova, I., Astafyeva, B., Lu, W., & Abd El-Aty, A. M. (2022). Translocation of Phthalates From Food Packaging Materials Into Minced Beef. *Frontiers in Nutrition*, *8*(1), 3-9.

Barbara, T. S., Lindsey, K. L., Taylor, M. B., Erasmus, D. G., Jäger, A. K., Yff, B. T. S., Lindsey, K. L., Taylor, M. B., Erasmus, D. G., & Jäger, A. K. (2002). The

pharmacological screening of Pentanisia prunelloides and the isolation of the antibacterial compound palmitic acid. *Journal of Ethnopharmacology*, *79*(1), 101–107.

Basha, R. H., & Sankaranarayanan, C. (2014). β-Caryophyllene, a natural sesquiterpene, modulates carbohydrate metabolism in streptozotocin-induced diabetic rats. *Acta Histochemica*, *116*(8), 1469–1479.

Bassetti, M., Pecori, D., Sartor, A., Londero, A., Villa, G., Cadeo, B., Brillo, F., Bongiorno, D., Campanile, F., & Stefani, S. (2013). First report of endocarditis by Gluconobacter spp. in a patient with a history of intravenous-drug abuse. *The Journal of Infection*, *66*(3), 285–287.

Battling, S., Wohlers, K., Igwe, C., Kranz, A., Pesch, M., Wirtz, A., Baumgart, M., Büchs, J., & Bott, M. (2020). Novel plasmid-free Gluconobacter oxydans strains for production of the natural sweetener 5-ketofructose. *Microbial Cell Factories*, *19*(1), 4-9.

Behairy, A., Mohamed, W. A. M., Ebraheim, L. L. M., Soliman, M. M., Abd-Elhakim, Y. M., El-Sharkawy, N. I., Saber, T. M., & El Deib, M. M. (2021). Boldenone undecylenate-mediated hepatorenal impairment by oxidative damage and dysregulation of heat shock protein 90 and androgen receptors expressions: Vitamin C preventive role. *Frontiers in Pharmacology*, *12*(2), 11-16.

Bekhit, A. A., Hymete, A., Damtew, A., Mohamed, A. M. I., & Bekhit, A. E. D. A. (2012). Synthesis and biological screening of some pyridine derivatives as anti-malarial agents. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *27*(1), 69–77.

Bhalla, N., Ingle, N., Patri, S. V., & Haranath, D. (2021). Phytochemical analysis of

Moringa Oleifera leaves extracts by GC-MS and free radical scavenging potency for industrial applications. *Saudi Journal of Biological Sciences*, *28*(12), 6915–6928.

Bibli, S. I., Papapetropoulos, A., Iliodromitis, E. K., Daiber, A., Randriamboavonjy, V., Steven, S., Brouckaert, P., Chatzianastasiou, A., Kypreos, K. E., Hausenloy, D. J., Fleming, I., & Andreadou, I. (2019). Nitroglycerine limits infarct size through S-nitrosation of cyclophilin D: A novel mechanism for an old drug. *Cardiovascular Research*, *115*(3), 625–636.

Bilal, M., Iqbal, M. S., Shah, S. B., Rasheed, T., & Iqbal, H. M. N. (2018). Diabetic complications and insight into antidiabetic potentialities of ethno- medicinal plants: A review. *Recent Patents on Inflammation & Allergy Drug Discovery*, *12*(1), 7–23.

Birringer, M., & Lorkowski, S. (2019). Vitamin E: Regulatory role of metabolites. *IUBMB Life*, *71*(4), 479–486.

Bogusz, M. J., Hassan, H., Al-Enazi, E., Ibrahim, Z., & Al-Tufail, M. (2006). Application of LC–ESI–MS–MS for detection of synthetic adulterants in herbal remedies. *Journal of Pharmaceutical and Biomedical Analysis*, *41*(2), 554–564.

Bottoni, P., & Caroli, S. (2019). Fake pharmaceuticals: A review of current analytical approaches. *Microchemical Journal*, *149*, 53-69.

Boubaker, J., Toumia, I. Ben, Sassi, A., Bzouich-Mokded, I., Mazgar, S. G., Sioud, F., Bedoui, A., Skhiri, S. S., Ghedira, K., & Chekir-Ghedira, L. (2018). Antitumoral potency by immunomodulation of chloroform extract from leaves of nitraria retusa, tunisian medicinal plant, via its major compounds β-sitosterol and palmitic acid in BALB/c mice bearing induced tumor. *Nutrition and*

Cancer, *70*(4), 650–662.

Buchberger, W. (2011). Current approaches to trace analysis of pharmaceuticals and personal care products in the environment. *Journal of Chromatography A, 1218*(4), 603–618.

Cara, I. G., Rusu, B. G., Raus, L., & Jitareanu, G. (2017). Sorption potential of alkaline treated straw and a soil for sulfonylurea herbicide removal from aqueous solutions: An environmental management strategy. *Chemosphere, 186*, 360–366.

Carella, A. M. (2017). Hypoglycemia by ginseng in type 2 diabetic patient: Case report. *Journal of Obesity & Metabolic Syndrome*, 12(1), 1-12.

CDC. (2023). *What is diabetes?*. Retrieved from https://www.cdc.gov/diabetes/basics/diabetes.html

Chandel, E., & Kumar, B. (2015). Antimicrobial activity and phytochemical analysis of cynodon dactylon: A review. *World Journal of Pharmacy and Pharmaceuticals*, *4*(11), 515–530.

Chau, W., Ross, R., Li, J. Y. Z., Yong, T. Y., Klebe, S., & Barbara, J. A. (2011). Nephropathy associated with use of a Chinese herbal product containing aristolochic acid. *The Medical Journal of Australia*, *194*(7), 367–368.

Chavan, M. J., Wakte, P. S., & Shinde, D. B. (2010). Analgesic and anti-inflammatory activity of Caryophyllene oxide from Annona squamosa L. bark. *Phytomedicine*, *17*(2), 149–151.

Chebii, W. K., Muthee, J. K., & Kiemo, K. (2020). The governance of traditional medicine and herbal remedies in the selected local markets of Western Kenya. *Journal of Ethnobiology and Ethnomedicine*, *16*(1), 1–24.

Chen, L., Lin, H., Li, H., Wang, M., Qiu, B., & Yang, Z. (2019). Influence of filtration during sample pretreatment on the detection of antibiotics and non-steroidal anti-inflammatory drugs in natural surface waters. *Science of The Total Environment*, *650*, 769–778.

Chen, Y.-F., Roan, H.-Y., Lii, C.-K., Huang, Y.-C., & Wang, T.-S. (2011). Relationship between antioxidant and antiglycation ability of saponins, polyphenols, and polysaccharides in Chinese herbal medicines used to treat diabetes. *Journal of Medicinal Plants Research*, *5*(11), 2322–2331.

Chen, Y., Zhao, L., Lu, F., Yu, Y., Chai, Y., & Wu, Y. (2009). Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC-MS/MS. *Food Additives & Contamints Part A*, *26*(5), 595–603.

Cheng, S. S., Liu, J. Y., Chang, E. H., & Chang, S. T. (2008). Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi. *Bioresource Technology*, *99*(11), 5145–5149.

Chowdry, A. T. (2018). *Determination of synthetic drugs as adulterant in herbal anti-diabetic medicines by HPLC*. BRAC University.

Christofides, E. A. (2019). Practical insights into improving adherence to metformin therapy in patients with type 2 diabetes. *Clinical Diabetes*, *37*(3), 234–241.

Cianchino, V., Acosta, G., Ortega, C., Martínez, L. D., & Gomez, M. R. (2008). Analysis of potential adulteration in herbal medicines and dietary supplements for the weight control by capillary electrophoresis. *Food Chemistry*, *108*(3), 1075–1081.

Cieślik-Boczula, K., Küpcü, S., Rünzler, D., Koll, A., & Köhler, G. (2009). Effects of the phenolic lipid 3-pentadecylphenol on phospholipid bilayer organization.

Journal of Molecular Structure, *919*(1–3), 373–380.

Corradini, D. (2010). *Handbook of HPLC (2nd. ed.)*. 2–718.

Cressey, P., & Reeve, J. (2019). Metabolism of cyanogenic glycosides: A review. *Food and Chemical Toxicology*, *125*, 225–232.

Cui, Z., Ge, N., Zhang, A., Liu, Y., Zhang, J., & Cao, Y. (2015). Comprehensive determination of polycyclic aromatic hydrocarbons in Chinese herbal medicines by solid phase extraction and gas chromatography coupled to tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *407*(7), 1989–1997.

Davoodbasha, M. A., Edachery, B., Nooruddin, T., Lee, S. Y., & Kim, J. W. (2018). An evidence of C16 fatty acid methyl esters extracted from microalga for effective antimicrobial and antioxidant property. *Microbial Pathogenesis*, *115*, 233–238.

de Lima-Barros, M. B., de Almeida Paes, R., & Schubach, A. O. (2011). Sporothrix schenckii and Sporotrichosis. *Clinical Microbiology Reviews*, *24*(4), 633-645.

Debela, D. T., Muzazu, S. G. Y., Heraro, K. D., Ndalama, M. T., Mesele, B. W., Haile, D. C., Kitui, S. K., & Manyazewal, T. (2021). New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Medicine*, *9*(2), 1-12.

Deconinck, E., Vanhee, C., Keizers, P., Guinot, P., Mihailova, A., Syversen, P. V., Li-Ship, G., Young, S., Blazewicz, A., Poplawska, M., Al-Sayed, J. L., Stengelshøj Olsen, L., el-Atma, O., Leist, R., Jönsson, K. H., Afxentiou, M., Barrios, M. M., Diaz, I. D., Zemser, M., … Bertrand, M. (2021). The occurrence of non-anatomical therapeutic chemical-international nonproprietary name molecules in suspected illegal or illegally traded health products in

Europe: A retrospective and prospective study. *Drug Testing and Analysis*, *13*(4), 833–840.

Delgado-Altamirano, R., López-Palma, R. I., Monzote, L., Delgado-Domínguez, J., Becker, I., Rivero-Cruz, J. F., Esturau-Escofet, N., Vázquez-Landaverde, P. A., & Rojas-Molina, A. (2019). Chemical constituents with leishmanicidal activity from a pink-yellow cultivar of Lantana camara var. aculeata (L.) collected in Central Mexico. *International Journal of Molecular Sciences, 20*(4), 872-883.

Deng, P., Cui, B., Zhu, H., Phommakoun, B., Zhang, D., Li, Y., Zhao, F., & Zhao, Z. (2021). Accumulation pattern of amygdalin and prunasin and its correlation with fruit and kernel agronomic characteristics during apricot (Prunus armeniaca L.) kernel development. *Foods*, *10*(2), 1–19.

Digiovanni, J., & Boutwell, R. K. (1983). Tumor promoting activity of 1,8-dihydroxy-3-methyl-9-anthrone (chrysarobin) in female SENCAR mice. *Carcinogenesis*, *4*(3), 281–284. 1

Dutta, S., Haggerty, D. K., Rappolee, D. A., & Ruden, D. M. (2020). Phthalate exposure and long-term epigenomic consequences: A review. *Frontiers in Genetics*, *11*(3), 405-417.

Eizirik, D. L., Welsh, N., Sener, A., & Malaisse, W. J. (1994). Protective Action of succinic acid monomethyl ester against the impairment of glucose-stimulated insulin release caused by glucopenia or starvation: Metabolic determinants. *Biochemical Medicine and Metabolic Biology*, *53*(1), 34–45.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology*, *4*(2), 177-186.

El Hadri, A., Ángeles Gómez Del Río, M., Sanz, J., Coloma, A. G., Idaomar, M., Ozonas, B. R., Benedí González, J., Isabel, M., & Reus, S. (2010). Cytotoxic activity of α-humulene and transcaryo-phyllene from Salvia officinalis in animal and human tumor cells. *An R Acad Nac Farm*, *76*(3), 343–356.

Elekofehinti, O. O., Omotuyi, I. O., Kamdem, J. P., Ejelonu, O. C., Alves, G. V., Adanlawo, I. G., & Rocha, J. B. T. (2014). Saponin as regulator of biofuel: Implication for ethnobotanical management of diabetes. *Journal of Physiology and Biochemistry 2014 70:2*, *70*(2), 555–567.

Elfadil, H., Fahal, A., Kloezen, W., Ahmed, E. M., & Van De Sande, W. (2015). The in vitro antifungal activity of sudanese medicinal plants against Madurella mycetomatis, the eumycetoma major causative agent. *PLoS Neglected Tropical Diseases*, 9(3), 5-17.

Elufioye, T. O., Habtemariam, S., & Adejare, A. (2020). Chemistry and Pharmacology of Alkylamides from Natural Origin. *Revista Brasileira de Farmacognosia*, *30*(5), 622–640.

Ernst, E. (2002). Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review. *Journal of Internal Medicine*, *252*(2), 107–113.

Ezhilan, B. P., & Neelamegam, R. (2012). GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. *Pharmacognosy Research*, *4*(1), 11-23.

Fabiyi, O. A., Olubunmi, A., Adeyemi, O. S., & Olatunji, G. A. (2012). Antioxidant and cytotoxicity of β-amyrin acetate fraction from bridelia ferruginea leaves. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), S981–S984.

Fan, J. C., Ren, R., Jin, Q., He, H. L., & Wang, S. T. (2019). Detection of 20

phthalate esters in breast milk by GC-MS/MS using QuEChERS extraction method. *Food Additives & Contaminants Part A, 36*(10), 1551–1558.

Fejos, I., Neumajer, G., Béni, S., & Jankovics, P. (2014). Qualitative and quantitative analysis of PDE-5 inhibitors in counterfeit medicines and dietary supplements by HPLC–UV using sildenafil as a sole reference. *Journal of Pharmaceutical and Biomedical Analysis*, *98*, 327–333.

Fernandes, B., Caligiorne, R. B., Coutinho, D. M., Gomes, R. R., Rocha-Silva, F., Machado, A. S., Santrer, E. F. R. La, Assunção, C. B., Guimarães, C. F., Laborne, M. S., Nunes, M. B., Vicente, V. A., & de Hoog, S. (2018). A case of disseminated sporotrichosis caused by Sporothrix brasiliensis. *Medical Mycology Case Reports*, *21*, 34-45.

Fidyt, K., Fiedorowicz, A., Strządała, L., & Szumny, A. (2016). β-caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. *Cancer Medicine*, *5*(10), 3007–3017.

Francis, G., Kerem, Z., Makkar, H. P. S., & Becker, K. (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, *88*(6), 587–605.

Fu, Q.-Y., Li, Q.-S., Lin, X.-M., Qiao, R.-Y., Yang, R., Li, X.-M., Dong, Z.-B., Xiang, L.-P., Zheng, X.-Q., Lu, J.-L., Yuan, C.-B., Ye, J.-H., & Liang, Y.-R. (2017). Antidiabetic effects of tea. *Molecules*, *22*(5), 849-860.

Gagliano-Jucá, T., & Basaria, S. (2019). Abuse of anabolic steroids: A dangerous indulgence. *Current Opinion in Endocrine and Metabolic Research*, *9*, 96–101.

Gao, Y., Liu, F., Fang, L., Cai, R., Zong, C., & Qi, Y. (2014). Genkwanin inhibits proinflammatory mediators mainly through the regulation of miR-101/MKP-

1/MAPK pathway in lps-activated macrophages. *PLOS ONE*, *9*(5), 3-8.

Gasperi, V., Sibilano, M., Savini, I., & Catani, M. V. (2019). Niacin in the central nervous system: an update of biological aspects and clinical applications. *International Journal of Molecular Sciences*, *20*(4), 974-981.

Giuliani, A., Zuccarini, M., Cichelli, A., Khan, H., & Reale, M. (2020). Critical review on the presence of phthalates in food and evidence of their biological impact. *International Journal of Environmental Research and Public Health*, *17*(16), 1–43.

Globocan. (2020). *Kenya*. Retrieved from https://gco.iarc.fr/today/data/factsheets/populations/404-kenya-fact-sheets.pdf

Gogtay, N. (2010). Principles of sample size calculation. *Indian Journal of Ophthalmology*, *58*(6), 517-523.

Gomez, A. M., Lobo, F., Miranda, S., & Lopez, J. C. (2015). A survey of recent synthetic applications of 2,3-dideoxy-hex-2-enopyranosides. *Molecules*, *20*(5), 8357-8364.

Greenwell, M., & Rahman, P. K. S. M. (2015). Medicinal plants: Their use in anticancer treatment. *International Journal of Pharmaceutical Sciences and Research*, *6*(10), 4103–4112.

Guetchueng, S. T., Nahar, L., Ritchie, K. J., & Sarker, S. D. (2022). Evaluation of the chemopreventive effect of selected medicinal plants extracts via induction of the Nrf2 in a modified model of breast cancer cells: Identification of bioactive lead compounds. *European Journal of Cancer Prevention*, *31*(1), 50–53.

Gunawardena, D., Karunaweera, N., Lee, S., Kooy, F. van Der, Harman, D. G., Raju, R., Bennett, L., Gyengesi, E., Sucher, N. J., & Münch, G. (2015). Anti-

inflammatory activity of cinnamon (C. zeylanicum and C. cassia) extracts – identification of E-cinnamaldehyde and o-methoxy cinnamaldehyde as the most potent bioactive compounds. *Food & Function*, *6*(3), 910–919.

Guo, C., Niu, C., Zhou, L., Wang, W., Nie, Y., Liu, Q., Zhang, L., Chen, Z., Wang, W., & Xu, Y. (2020). Targeted and nontargeted screening and identification of 50 antihypertensive adulterants in dietary supplements and herbal medicines using quadrupole–orbitrap high resolution mass spectrometry with compound database. *Journal of Separation Science*, *43*(13), 2529–2538.

Guo, X., Sun, W., Huang, L., Wu, L., Hou, Y., Qin, L., & Liu, T. (2017). Effect of cinnamaldehyde on glucose metabolism and vessel function. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research*, *23*(2), 3844-3849.

Gupta, M. K. (2010). Multi-Model environment as a rational approach for drug design: An experience with CP-MLR. *IUP Journal of Chemistry*,*12*(3), 1-33.

Gupta, N., Gudipati, T., & Prasad, G. B. K. S. (2018). Plant secondary metabolites of pharmacological significance in reference to diabetes mellitus: An update. *International Journal of Current Microbiology and Applied Sciences*, *7*(05), 3409–3448.

Gupta, R. C., Chang, D., Nammi, S., Bensoussan, A., Bilinski, K., & Roufogalis, B. D. (2017). Interactions between antidiabetic drugs and herbs: An overview of mechanisms of action and clinical implications. *Diabetology and Metabolic Syndrome*, *9*(1), 1–12.

Gustav, K. (2016). *Antiplasmodial compounds from Ghanaian medicinal plants* [Kwame Nkrumah University of Science and Technology].

Gyrdymova, Y. V., & Rubtsova, S. A. (2021). Caryophyllene and caryophyllene oxide: A variety of chemical transformations and biological activities. *Chemical Papers*, *76*(1), 1–39.

Han, J., Xian, Z., Zhang, Y., Liu, J., & Liang, A. (2019). Systematic overview of aristolochic acids: nephrotoxicity, carcinogenicity, and underlying mechanisms. *Frontiers in Pharmacology*, *10*(JUN), 648-653.

Hanausek, M., Ganesh, P., Walaszek, Z., Arntzen, C. J., Slaga, T. J., & Gutterman, J. U. (2001). Avicins, a family of triterpenoid saponins from Acacia victoriae (Bentham), suppress H-ras mutations and aneuploidy in a murine skin carcinogenesis model. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(20), 11551–11556.

Harris, E. S. J., Cao, S., Littlefield, B. A., Craycroft, J. A., Scholten, R., Kaptchuk, T., Fu, Y., Wang, W., Liu, Y., Chen, H., Zhao, Z., Clardy, J., Woolf, A. D., & Eisenberg, D. M. (2011). Heavy metal and pesticide content in commonly prescribed individual raw Chinese Herbal Medicines. *Science of The Total Environment*, *409*(20), 4297–4305.

Hassan, K. M. (2019). *Evaluation of Heavy Metals and Microbial Contamination in Herbal Medicines Used for Chronic Illnesses in Nairobi Metropolis* [University of Nairobi].

He, M., Yang, C., Geng, R., Zhao, X., Hong, L., Piao, X., Chen, T., Quinto, M., & Li, D. (2015). Monitoring of phthalates in foodstuffs using gas purge microsyringe extraction coupled with GC–MS. *Analytica Chimica Acta*, *879*, 63–68.

He, Z., Huang, Z., Jiang, W., & Zhou, W. (2019). Antimicrobial activity of

cinnamaldehyde on Streptococcus mutans biofilms. *Frontiers in Microbiology*, *10*(5), 2241-2247.

Hefny Gad, M., Tuenter, E., El-Sawi, N., Younes, S., El-Ghadban, E. M., Demeyer, K., Pieters, L., Vander Heyden, Y., & Mangelings, D. (2018). Identification of some bioactive metabolites in a fractionated methanol extract from Ipomoea aquatica (aerial parts) through TLC, HPLC, UPLC-ESI-QTOF-MS and LC-SPE-NMR fingerprints analyses. *Phytochemical Analysis*, *29*(1), 5–15.

Heinig, K., & Bucheli, F. (2004). Fast liquid chromatographic-tandem mass spectrometric (LC–MS–MS) determination of metformin in plasma samples. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(5), 1005–1011.

Holloway, S. W., Tran, D. H., Greyber, E. R., Kantor, S., Bush, T. M., Rayburn, K. S., Sanchez-Yamamoto, D. S., Allen, B. L., Lam, T., So, B. K., & Roth, L. W. (2007). Adverse interactions between herbal and dietary substances and prescription medications: A clinical survey. *Alternative Therapies*, *13*(2), 30–35.

Hövels, M., Gallala, N., Keriakes, S. L., König, A. P., Schiessl, J., Laporte, T., Kosciow, K., & Deppenmeier, U. (2022). 5-keto-d-fructose, a natural diketone and potential sugar substitute, significantly reduces the viability of prokaryotic and eukaryotic cells. *Frontiers in Microbiology*, 13, 4-9.

Hu, T., Chen, R., Wang, Q., He, C., & Liu, S. (2021). Recent advances and applications of molecularly imprinted polymers in solid-phase extraction for real sample analysis. *Journal of Separation Science*, *44*(1), 274–309.

Hu, Y.-L., Wang, X.-B., Chen, D.-D., Guo, X.-J., Yang, Q.-J., Dong, L.-H., & Cheng, L. (2016). Germanicol activity in human colon cancer cells 627.

JBUON, *21*(3), 626–632.

Huang, M., Lu, J. J., & Ding, J. (2021). Natural products in cancer therapy: Past, present and future. *Natural Products and Bioprospecting*, *11*(1), 5–13.

Huang, Q., Lu, G., Shen, H. M., Chung, M. C. M., & Choon, N. O. (2007). Anti-cancer properties of anthraquinones from rhubarb. *Medicinal Research Reviews*, *27*(5), 609–630.

Hübschmann, H.-J. (2015). *Handbook of GC-MS : Fundamentals and Applications.* Wiley.

Hudson, S., & Ramsey, J. (2011). The emergence and analysis of synthetic cannabinoids. *Drug Testing and Analysis*, *3*(7–8), 466–478.

Huh, J., Chung, H., & Hwang, W. (2019). Comparison of the effects of milrinone, sodium nitroprusside, and nitroglycerine for induced hypotension in elderly patients undergoing spine surgery. *Clinical Spine Surgery*, *32*(8), E366–E371.

Huitrón-Reséndiz, S., Gombart, L., Cravatt, B. F., & Henriksen, S. J. (2001). Effect of oleamide on sleep and its relationship to blood pressure, body temperature, and locomotor activity in rats. *Experimental Neurology*, *172*(1), 235–243.

Hwa, J. S., Jin, Y. C., Lee, Y. S., Ko, Y. S., Kim, Y. M., Shi, L. Y., Kim, H. J., Lee, J. H., Ngoc, T. M., Bae, K. H., Kim, Y. S., & Chang, K. C. (2012). 2-Methoxycinnamaldehyde from Cinnamomum cassia reduces rat myocardial ischemia and reperfusion injury in vivo due to HO-1 induction. *Journal of Ethnopharmacology*, *139*(2), 605–615.

Ibrahim, S., Syaed Koko, W., Mohamed Elhassan Taha, M., Mohan, S., Achoui, M., Ameen Abdulla, M., Rais Mustafa, M., Ahmad, S., Ibrahim Noordin, M., Lip Yong, C., Roslan Sulaiman, M., Othman, R., & Amir Hassan, A. (2012). In

vitro and in vivo anti-inflammatory activities of columbin through the inhibition of cycloxygenase-2 and nitric oxide but not the suppression of NF-κB translocation. *European Journal of Pharmacology*, *678*(1–3), 61–70.

ICH. (2022). *Validation of Analytical Procedures Q2(R2)*. Retrieved from https://database.ich.org/sites/default/files/ICH_Q2-R2_Document_Step2_Guideline_2022_0324.pdf

IDF. (2021). *IDF Diabetes Atlas 2021 | IDF Diabetes Atlas*. Retrieved from https://diabetesatlas.org/atlas/tenth-edition/

IDF. (2022). *IDF Africa Members*. Retrieved from https://idf.org/our-network/regions-members/africa/members/13-kenya.html

International Agency for Research on Cancer. (2020). *World*. Fact Sheet. Retrieved from https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., Chandra Shill, M., Karmakar, U. K., Yarla, N. S., Khan, I. N., Billah, M. M., Pieczynska, M. D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Banach, M., … Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, *121*, 82–94.

Islam, M. T., Sheikh, B., Rahman, M., Santos, P. S., Paz, M. F. C. J. M., Costa, J. P., Islam, T., Santos, P. S., Ferreira, P. B., Oliveira, G. L. S., Alencar, M. V. O. B., Paz, M. F. C. J. M., Ferreira, É. L. F., Feitosa, C. M., Citó, A. M. G. L., Sousa, D. P., Amelia, A., & Melo-Cavalcante, C. (2016). Evaluation of antioxidant activity of phytol using non-and pre-clinical models. *Current Pharmaceutical Biotechnology*, *17*, 1278-1286.

Jang, H. I., Rhee, K. J., & Eom, Y. Bin. (2020). Antibacterial and antibiofilm effects of α-humulene against Bacteroides fragilis. *Canadian Journal of Microbiology*, *66*(6), 389–399.

Jansen, R., Lachatre, G., & Marquet, P. (2005). LC-MS/MS systematic toxicological analysis: Comparison of MS/MS spectra obtained with different instruments and settings. *Clinical Biochemistry*, *38*(4), 362–372.

Jayakumar, T., Hsieh, C. Y., Lee, J. J., & Sheu, J. R. (2013). Experimental and clinical pharmacology of andrographis paniculata and its major bioactive phytoconstituent andrographolide. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 2-7.

Jayawardena, T. U., Kim, H. S., Sanjeewa, K. K. A., Kim, S. Y., Rho, J. R., Jee, Y., Ahn, G., & Jeon, Y. J. (2019). Sargassum horneri and isolated 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (HTT); LPS-induced inflammation attenuation via suppressing NF-κB, MAPK and oxidative stress through Nrf2/HO-1 pathways in RAW 264.7 macrophages. *Algal Research*, *40*(4), 13-19.

Ji, Y. Bin, Wang, Y. S., Fu, T. T., Ma, S. Q., Qi, Y. D., Si, J. Y., Sun, D. A., & Liao, Y. H. (2019). Quantitative analysis of pyrrolizidine alkaloids in Gynura procumbens by liquid chromatography–tandem quadrupole mass spectrometry after enrichment by PCX solid-phase extraction. *International Journal of Environmental Analytical Chemistry*, *99*(11), 1090–1102.

Jin, P., Xu, S., Xu, W., He, X., Kuang, Y., & Hu, X. (2020). Screening and quantification of fourteen synthetic antidiabetic adulterants in herbal pharmaceuticals and health foods by HPLC and confirmation by LC-Q-TOF-

MS/MS. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *37*(1), 11–18.

Jin, S. E., Son, Y. K., Min, B.-S., Jung, H. A., & Choi, J. S. (2012). Anti-inflammatory and antioxidant activities of constituents isolated from Pueraria lobata roots. *Archives of Pharmacal Research*, *35*(5), 823–837.

Jin, Y. H., & Kim, S. A. (2017). 2-Methoxycinnamaldehyde inhibits the TNF-α-induced proliferation and migration of human aortic smooth muscle cells. *International Journal of Molecular Medicine*, *39*(1), 191–198.

Jordan, C. R., Harris, C. M., Miranda, M. I., Kim, D. Y., & Hellberg, R. S. (2023). Labeling compliance and online claims for Ayurvedic herbal supplements on the U.S. market associated with the purported treatment of COVID-19. *Food Control*, *148*(5), 73-80.

Júnior, W. F. da S., de Menezes, D. L. B., de Oliveira, L. C., Koester, L. S., de Almeida, P. D. O., Lima, E. S., de Azevedo, E. P., Júnior, V. F. da V., & de Lima, Á. A. N. (2019). Inclusion complexes of β and hpβ-cyclodextrin with α, β amyrin and in vitro anti-inflammatory activity. *Biomolecules*, *9*(6), 241-253.

Kadan, S., Saad, B., Sasson, Y., & Zaid, H. (2016). In vitro evaluation of anti-diabetic activity and cytotoxicity of chemically analysed Ocimum basilicum extracts. *Food Chemistry*, *196*(1), 1066–1074.

Kaggwa, B., Kyeyune, H., Munanura, E. I., Anywar, G., Lutoti, S., Aber, J., Bagoloire, L. K., Weisheit, A., Tolo, C. U., Kamba, P. F., & Ogwang, P. E. (2022). Safety and efficacy of medicinal plants used to manufacture herbal products with regulatory approval in Uganda: A cross-sectional study. *Evidence-Based Complementary and Alternative Medicine* , *2022*, 3-11.

Kairigo, P., Ngumba, E., Sundberg, L. R., Gachanja, A., & Tuhkanen, T. (2020). Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments. *Science of The Total Environment*, *720*(2), 80-86.

Kamau, L. K. (2018). *Study of extent of use, efficacy and acute toxic effects of selected antidiabetic plants in Nyeri and Narok Counties, Kenya* [University of Nairobi].

Kamau, L. N., Mbaabu, M. P., Mbaria, J. M., Karuri, G. P., & Kiama, S. G. (2016). Knowledge and demand for medicinal plants used in the treatment and management of diabetes in Nyeri County, Kenya. *Journal of Ethnopharmacology*, *189*(13), 218–229.

Kariuki, E. (2012). *Assessment for the presence of aflatoxins and heavy metals in commercially available herbal medicines.* [University of Nairobi].

Kenya Magazine. (2023). *List Of All 47 Counties In Kenya, Counties Map and Their Headquarters - Kenyan Magazine*. Retrieved from https://kenyanmagazine.co.ke/list-of-all-47-counties-in-kenya-counties-map-and-their-headquarters/

Kenya National Bureau of Statistics. (2020). *2019 Kenya Population and Housing Census Reports*. Retrieved from https://housingfinanceafrica.org/documents/2019-kenya-population-and-housing-census-reports/

Kenya News Agency. (2019). *Herbalists association warns against treatment by illegal practitioners*. Retrieved from https://www.kenyanews.go.ke/herbalists-association-warns-against-treatment-by-illegal-practitioners/

Keter, L. K., & Mutiso, P. C. (2012). Ethnobotanical studies of medicinal plants used by traditional health practitioners in the management of diabetes in Lower Eastern Province, Kenya. *Journal of Ethnopharmacology*, *139*(1), 74–80.

Khaleghi, F., Jantan, I., Din, L. Bin, Yaacob, W. A., Khalilzadeh, M. A., & Bukhari, S. N. A. (2014). Immunomodulatory effects of 1-(6-hydroxy-2-isopropenyl-1-benzofuran-5-yl)-1-ethanone from Petasites hybridus and its synthesized benzoxazepine derivatives. *Journal of Natural Medicines*, *68*(2), 351–357.

Khazan, M., Hedayati, M., Kobarfard, F., Askari, S., & Azizi, F. (2014). Identification and determination of synthetic pharmaceuticals as adulterants in eight common herbal weight loss supplements. *Iranian Red Crescent Medical Journal*, *16*(3), 15344-15358.

Kianmehr, Z., Khorsandi, K., Mohammadi, M., & Hosseinzadeh, R. (2020). Low-level laser irradiation potentiates anticancer activity of p-coumaric acid against human malignant melanoma cells. *Melanoma Research*, 136–146.

Kigen, G., Kamuren, Z., Njiru, E., Wanjohi, B., & Kipkore, W. (2019). Ethnomedical survey of the plants used by traditional healers in Narok County, Kenya. *Evidence-Based Complementary and Alternative Medicine , 2019*, 3-16.

Kimani, P. M., Mwitari, P. G., Mwenda Njagi, S., Gakio Kirira, P., & Kiboi, D. M. (2018). In vitro anti-proliferative activity of selected plant extracts against cervical and prostate cancer cell lines. *Journal of Cancer Science & Therapy*, *10*(9), 6-14.

Kohno, H., Maeda, M., Tanino, M., Tsukio, Y., Ueda, N., Wada, K., Sugie, S., Mori, H., & Tanaka, T. (2002). A bitter diterpenoid furanolactone columbin from Calumbae Radix inhibits azoxymethane-induced rat colon carcinogenesis.

Cancer Letters, *183*(2), 131–139.

Kozioł, E., Luca, S. V., Marcourt, L., Nour, M., Hnawia, E., Jakubowicz-Gil, J., Paduch, R., Mroczek, T., Wolfender, J. L., & Skalicka-Woźniak, K. (2019). Efficient extraction and isolation of skimmianine from new Caledonian plant Medicosma leratii and evaluation of its effects on apoptosis, necrosis, and autophagy. *Phytochemistry Letters*, *30*, 224–230.

Kraujalis, P., & Venskutonis, P. R. (2013). Supercritical carbon dioxide extraction of squalene and tocopherols from amaranth and assessment of extracts antioxidant activity. *The Journal of Supercritical Fluids*, *80*, 78–85.

Kshirsagar, P. R., Jagtap, U. B., Gaikwad, N. B., & Bapat, V. A. (2019). Ethanopharmacology, phytochemistry and pharmacology of medicinally potent genus Swertia: An update. *South African Journal of Botany*, *124*, 444–483.

Ku, Y. R., Chag, L. Y., Ho, L. K., & Lin, J. H. (2003). Analysis of synthetic anti-diabetic drugs in adulterated traditional Chinese medicines by high-performance capillary electrophoresis. *Journal of Pharmaceutical and Biomedical Analysis*, *33*(2), 329–334.

Kumar, M., Mandal, V., & Hemalatha, S. (2011). Detection of metformin hydrochloride in a traditionally used Indian herbal drug for antidiabetic: A case report. *International Journal of Pharma & Biosciences*, *2*(1), 307-13.

Kumar, S., Mittal, A., Babu, D., & Mittal, A. (2021). Herbal medicines for diabetes management and its secondary complications. *Current Diabetes Reviews*, *17*(4), 437–456.

Kumari, A., & Kaur, R. (2019). Modulation of biochemical and physiological parameters in Hordeum vulgare L. seedlings under the influence of benzyl-butyl

phthalate. *PeerJ*, *7*(4), 3-12.

Lacy, A., & O'Kennedy, R. (2004). Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. *Current Pharmaceutical Design*, *10*(30), 3797–3811.

Landoulsi, A., Hennebelle, T., Bero, J., Rivière, C., Sahpaz, S., Quetin-Leclercq, J., Neut, C., Benhamida, J., & Roumy, V. (2020). Antimicrobial and light-enhanced antimicrobial activities, cytotoxicity and chemical variability of all Tunisian eryngium species. *Chemistry and Biodiversity*, *17*(4), 1–19.

Lankatillake, C., Huynh, T., & Dias, D. A. (2019). Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. *Plant Methods*, *15*(1), 1–35.

Lazreg-Aref, H., Mars, M., Fekih, A., Aouni, M., & Said, K. (2011). Pharmaceutical biology chemical composition and antibacterial activity of a hexane extract of Tunisian caprifig latex from the unripe fruit of Ficus carica. *Pharmaceutical Biology*, *50*(4), 407-412.

Legault, J., & Pichette, A. (2007). Potentiating effect of b-caryophyllene on anticancer activity of a-humulene, isocaryophyllene and paclitaxel. *JPP*, *59*, 1643–1647.

Lehotay, S. J., Han, L., & Sapozhnikova, Y. (2016). Automated mini-column solid-phase extraction cleanup for high-throughput analysis of chemical contaminants in foods by low-pressure gas chromatography—tandem mass spectrometry. *Chromatographia*, *79*(17–18), 1113–1130.

Li, Q., Zhang, P., & Cai, Y. (2021). Genkwanin suppresses MPP+-induced cytotoxicity by inhibiting TLR4/MyD88/NLRP3 inflammasome pathway in a

cellular model of Parkinson's disease. *NeuroToxicology*, *87*, 62–69.

Li, R. X., Li, M. M., Wang, T., Wang, T. L., Chen, J. Y., Francis, F., Fan, B., Kong, Z. Q., & Dai, X. F. (2020). Screening of pesticide residues in traditional Chinese medicines using modified QuEChERS sample preparation procedure and LC-MS/MS analysis. *Journal of Chromatography B*, *1152*, 5-17.

Li, X., Mikrani, R., Li, C., Naveed, M., Liu, Z., Abbas, M., Cheng, Y., Han, L., Wang, Z., & Zhou, X. (2020). An epicardial delivery of nitroglycerine by active hydraulic ventricular support drug delivery system improves cardiac function in a rat model. *Drug Delivery and Translational Research*, *10*(1), 23–33.

Liang, Q., Qu, J., Luo, G., & Wang, Y. (2006). Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. *Journal of pharmaceutical and biomedical analysis*, *40*(2), 305–311.

Liao, H. X., Shao, T. M., Mei, R. Q., Huang, G. L., Zhou, X. M., Zheng, C. J., & Wang, C. Y. (2019). Bioactive secondary metabolites from the culture of the mangrove-derived fungus Daldinia eschscholtzii HJ004. *Marine Drugs. 17*(12), 710-722.

Lim, H. H., Oh, Y. S., & Shin, H. S. (2020). Determination of N-nitrosodimethylamine and N-nitrosomethylethylamine in drug substances and products of sartans, metformin and ranitidine by precipitation and solid phase extraction and gas chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *189*(3), 60-71.

Liu, F., Zhang, X., Li, Y., Gao, H., Ling, P., Li, X., Chen, Q., Ma, A., Shao, H., Li, M., & Wang, F. (2017). Simultaneous screening and determination of eight tetracycline antibiotics illegally adulterated in herbal preparations using HPLC–

DAD combined with LC–MS–MS. *Chromatographia, 81*(2), 303–314.

Liu, S., Ruan, W., Li, J., Xu, H., Wang, J., Gao, Y., & Wang, J. (2008). Biological control of phytopathogenic fungi by fatty acids. *Mycopathologia, 166*(2), 93–102.

Lou, J., Mao, Z., Shan, T., Wang, Q., & Zhou, L. (2014). Chemical composition, antibacterial and antioxidant properties of the essential oils from the roots and cultures of Salvia miltiorrhiza. *Journal of Essential Oil Bearing Plants*, *17*(3), 380–384.

Ma, T., Zhou, W., Chen, L., Wu, L., Christie, P., & Liu, W. (2018). Toxicity of phthalate esters to lettuce (Lactuca sativa) and the soil microbial community under different soil conditions. *PLoS ONE*, *13*(12), 111-118.

Madariaga-Mazón, A., Naveja, J. J., Medina-Franco, J. L., Noriega-Colima, K. O., & Martinez-Mayorga, K. (2021). DiaNat-DB: a molecular database of antidiabetic compounds from medicinal plants. *RSC Advances*, *11*(9), 5172–5178.

Mahajan, V. K. (2014). Sporotrichosis: An overview and therapeutic options. *Dermatology Research and Practice, 2014,* 5-13.

Maiti, K., Mukherjee, K., Murugan, V., Saha, B. P., & Mukherjee, P. K. (2010). Enhancing bioavailability and hepatoprotective activity of andrographolide from Andrographis paniculata, a well-known medicinal food, through its herbosome. *Journal of the Science of Food and Agriculture, 90*(1), 43–51.

Majhi, S. (2020). Diterpenoids: Natural distribution, semisynthesis at room temperature and pharmacological aspects-a decade update. *ChemistrySelect*, *5*(40), 12450–12464.

Malaspina, P., Betuzzi, F., Ingegneri, M., Smeriglio, A., Cornara, L., & Trombetta,

D. (2022). Risk of poisoning from garden plants: Misidentification between laurel and cherry laurel. *Toxins*, *14*(11), 5-10.

Manganyi, M. C., Regnier, T., Tchatchouang, C. D. K., Bezuidenhout, C. C., & Ateba, C. N. (2019). Antibacterial activity of endophytic fungi isolated from Sceletium tortuosum L. (Kougoed). *Annals of Microbiology*, *69*(6), 659–663.

Maphill. (2023). *Kenya: Maps*. Retrieved from http://www.maphill.com/kenya/

Medyouni, R., Hamdi, N., Ben Said, R., Al-Ayed, A. S., & Zagrouba, F. (2013). Clean procedure and DFT study for the synthesis of 2-Amino-3-ethoxycarbonyl-4-(aryl)-4H-pyrano-[3,2-c]-chromene-5-ones derivatives: A novel class of potential antimicrobial and antioxidant agents. *Journal of Chemistry*, *2013*, 3-11.

Menger, L., Vacchelli, E., Kepp, O., Eggermont, A., Tartour, E., Zitvogel, L., Kroemer, G., & Galluzzi, L. (2013). Cardiac glycosides and cancer therapy. *Oncoimmunology*, *2*(2), 29-38.

Micalizzi, G., Huszti, K., Pálinkás, Z., Mandolfino, F., Martos, É., Dugo, P., Mondello, L., & Utczás, M. (2021). Reliable identification and quantification of anabolic androgenic steroids in dietary supplements by using gas chromatography coupled to triple quadrupole mass spectrometry. *Drug Testing and Analysis*, *13*(1), 128–139.

Minh, D. T. C., Thi, L. A., Huyen, N. T. T., Van Vu, L., Anh, N. T. K., & Ha, P. T. T. (2019). Detection of sildenafil adulterated in herbal products using thin layer chromatography combined with surface enhanced Raman spectroscopy: "Double coffee-ring effect" based enhancement. *Journal of Pharmaceutical and Biomedical Analysis*, *174*, 340–347.

Ministry of Health. (2019). *Kenya Essential Medicines List 2019*. Retrieved from https://www.health.go.ke/wp-content/uploads/2020/03/Kenya-Essential-Medicines-List-2019.pdf

Ministry of Health Kenya. (2017). *National Cancer Control Strategy 2017 - 2022* . Retrieved from https://repository.kippra.or.ke/bitstream/handle/123456789/2802

Ministry of Health Kenya. (2020). *Kenya National Strategy for the Prevention and Control of Non-Communicable Diseases 2021/22 - 2025/26*. Rettrieved from https://www.health.go.ke/wp-content/uploads/2021/07/Kenya-Non-Communicable-Disease-NCD-Strategic-Plan-2021-2025.pdf

Mlozi, S. H., Mmongoyo, J. A., & Chacha, M. (2022). GC-MS analysis of bioactive phytochemicals from methanolic leaf and root extracts of Tephrosia vogelii. *Scientific African*, *16*, 54-65.

Mohan, S., & Nandhakumar, L. (2014). Role of various flavonoids: Hypotheses on novel approach to treat diabetes. *Journal of Medical Hypotheses and Ideas*, *8*(1), 1–6.

Mompelat, S., Jaffrezic, A., Jardé, E., & LeBot, B. (2013). Storage of natural water samples and preservation techniques for pharmaceutical quantification. *Talanta*, *109*, 31–45.

Monti, M., Fasano, M., Palandri, L., & Righi, E. (2022). A review of European and international phthalates regulation: focus on daily use products. *The European Journal of Public Health*, *32*(Suppl 3), 7-12.

Moo, C. L., Yang, S. K., Osman, M. A., Yuswan, M. H., Loh, J. Y., Lim, W. M., Lim, S. H. E., & Lai, K. S. (2020). Antibacterial activity and mode of action of β-caryophyllene on Bacillus cereus. *Polish Journal of Microbiology*, *69*(1), 49-

56.

Moosmann, B., Kneisel, S., Girreser, U., Brecht, V., Westphal, F., & Auwärter, V. (2012). Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone using GC-MS, NMR analysis and a flash chromatography system. *Forensic Science International*, *220*(1–3), 8-15.

Mwankuna, C. J., Kiros, F., Mariki, E. E., Mabiki, F. P., Malebo, H. M., Mdegela, R. H., & Styrishave, B. (2023). Optimization of HPLC–MS/MS method for determination of antimalarial adulterants in herbal products. *Analytical Sciences*, *39*(3), 407–416.

Na, M., Kim, B. Y., Osada, H., & Ahn, J. S. (2009). Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from Sorbus commixta. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(4), 1056–1059.

Nachar, A., Saleem, A., Arnason, J. T., & Haddad, P. S. (2015). Regulation of liver cell glucose homeostasis by dehydroabietic acid, abietic acid and squalene isolated from balsam fir (Abies balsamea (L.) Mill.) a plant of the Eastern James Bay Cree traditional pharmacopeia. *Phytochemistry*, *117*, 373–379.

Nafiu, M. O., Adewuyi, A. I., Abdulsalam, T. A., & Ashafa, A. O. T. (2021). Antimalarial activity and biochemical effects of saponin-rich extract of Dianthus basuticus Burtt Davy in Plasmodium berghei- infected mice. *Advances in Traditional Medicine 2021*, 1–11.

Nair, S. A., Sabulal, B., Radhika, J., Arunkumar, R., & Subramoniam, A. (2014). Promising anti-diabetes mellitus activity in rats of β-amyrin palmitate isolated from Hemidesmus indicus roots. *European Journal of Pharmacology*, *734*(1),

77–82.

Nasr, N., Chaabane, F., Sassi, A., Chekir-Ghedira, L., & Ghedira, K. (2016). Effect of apigenin-7-glucoside, genkwanin and naringenin on tyrosinase activity and melanin synthesis in B16F10 melanoma cells. *Life Sciences*, *144*, 80–85.

National Cancer Institute. (2023). *Research Areas: Causes of Cancer*. Retrieved from https://www.cancer.gov/research/areas/causes

Ngugi, M. P., Makenzi, N., & Njagi, J. (2015). Diabetes mellitus – a devastating metabolic disorder. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *4*(40), 1–7.

Ngumba, E., Gachanja, A., & Tuhkanen, T. (2016). Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. *Science of The Total Environment*, *539*(4), 206–213.

Ngumba, E., Kosunen, P., Gachanja, A., & Tuhkanen, T. (2016). A multiresidue analytical method for trace level determination of antibiotics and antiretroviral drugs in wastewater and surface water using SPE-LC-MS/MS and matrix-matched standards. *Analytical Methods*, *8*(37), 6720–6729.

Nguyen, T. M., Goto, M., Noda, S., Matsutani, M., Hodoya, Y., Kataoka, N., Adachi, O., Matsushita, K., & Yakushi, T. (2021). The 5-ketofructose reductase of gluconobacter sp. strain CHM43 is a novel class in the shikimate dehydrogenase family. *Journal of Bacteriology*, *203*(19), 4-21.

Nonthakaew, A., Matan, N., & Aewsiri, T. (2015). Caffeine in foods and its antimicrobial activity | Request PDF. *International Food Research Journal*, *22*(1), 9–14.

Nugroho, A. E., Andrie, M., Warditiani, N. K., Siswanto, E., Pramono, S., &

Lukitaningsih, E. (2012). Antidiabetic and antihiperlipidemic effect of Andrographis paniculata (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. *Indian Journal of Pharmacology*, *44*(3), 377-386.

Ochwang'I, D. O., Kimwele, C. N., & Oduma, J. A. (2016). Phytochemical Screening of medicinal plants of the Kakamega Country, Kenya commonly used against cancer. *Medicinal & Aromatic Plants*, *5*(6), 7-16.

Ochwang'i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Kiama, S. G., & Efferth, T. (2018). Cytotoxic activity of medicinal plants of the Kakamega County (Kenya) against drug-sensitive and multidrug-resistant cancer cells. *Journal of Ethnopharmacology*, *215*(8), 233–240.

Oda, S. S., & El-Ashmawy, I. M. (2012). Adverse effects of the anabolic steroid, boldenone undecylenate, on reproductive functions of male rabbits. *International Journal of Experimental Pathology*, *93*(3), 172-177.

Ohno, O., Ye, M., Koyama, T., Yazawa, K., Mura, E., Matsumoto, H., Ichino, T., Yamada, K., Nakamura, K., Ohno, T., Yamaguchi, K., Ishida, J., Fukamizu, A., & Uemura, D. (2008). Inhibitory effects of benzyl benzoate and its derivatives on angiotensin II-induced hypertension. *Bioorganic & Medicinal Chemistry*, *16*(16), 7843–7852.

Okaiyeto, K., & Oguntibeju, O. O. (2021). African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research and Public Health*, *18*(11), 5988-5994.

Okumu, M. O., Ochola, F. O., Onyango, A. O., Mbaria, J. M., Gakuya, D. W., Kanja, L. W., Kiama, S. G., & Onyango, M. A. (2017). The legislative and regulatory framework governing herbal medicine use and practice in Kenya: a review. *The*

Pan African Medical Journal, *28*, 1937–8688.

Olasehinde, T. A., Olaniran, A. O., & Okoh, A. I. (2019). Aqueous–ethanol extracts of some South African seaweeds inhibit beta-amyloid aggregation, cholinesterases, and beta-secretase activities in vitro. *Journal of Food Biochemistry*, *43*(7), 70-78.

Olatunde, A., Mohammed, A., Ibrahim, M. A., Tajuddeen, N., & Shuaibu, M. N. (2022). Vanillin: A food additive with multiple biological activities. *European Journal of Medicinal Chemistry Reports*, *5*(1), 55-61.

Oliveira, F. A., Chaves, M. H., Almeida, F. R. C., Lima, R. C. P., Silva, R. M., Maia, J. L., Brito, G. A. A. C., Santos, F. A., & Rao, V. S. (2005). Protective effect of α- and β-amyrin, a triterpene mixture from Protium heptaphyllum (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *Journal of Ethnopharmacology*, *98*(1–2), 103–108.

Oliveira, F. A., Vieira, G. M., Chaves, M. H., Almeida, F. R. C., Santos, K. A., Martins, F. S., Silva, R. M., Santos, F. A., & Rao, V. S. N. (2004). Gastroprotective effect of the mixture of α- and β-amyrin from Protium heptaphyllum: Role of capsaicin-sensitive primary afferent neurons. *Planta Medica*, *70*(8), 780–782.

Olivia, N. U., Goodness, U. C., & Obinna, O. M. (2021). Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves. *Future Journal of Pharmaceutical Sciences, 7*(1), 1–5.

Omara, T., Odero, M. P., & Obakiro, S. B. (2022). Medicinal plants used for treating cancer in Kenya: an ethnopharmacological overview. *Bulletin of the National Research Centre*, *46*(1), 148-154.

Omosa, L. K., Midiwo, J. O., Masila, V. M., Gisacho, B. M., Munayi, R., Francisca-Kamakama, Chemutai, K. P., Elhaboob, G., Saeed, M. E. M., Hamdoun, S., Kuete, V., & Efferth, T. (2016). Cytotoxicity of 91 Kenyan indigenous medicinal plants towards human CCRF-CEM leukemia cells. *Journal of Ethnopharmacology*, *179*, 177–196.

Ong'udi, M., Mutai, P., & Weru, I. (2019). Study of the use of complementary and alternative medicine by cancer patients at Kenyatta National Hospital, Nairobi, Kenya. *Journal of Oncology Pharmacy Practice*, *25*(4), 918–928.

Onyancha, J., Gikonyo, N. K., & Wachira, S. W. (2019). An ethnobotanical survey of plants used for the treatment and management of cancer in Embu County, Kenya. *Journal of Medicinal Plant Studies*, *7*(4), 39–46.

Onyancha, J., Gikonyo, N. K, Wachira, S., Mwitari. P., & Gicheru, M. (2018). Anticancer activities and safety evaluation of selected Kenyan plant extracts against breast cancer cell lines. *Journal of Pharmacognosy and Phytotherapy*, *10*(2), 21–26.

Ota, A., & Ulrih, N. P. (2017). An overview of herbal products and secondary metabolites used for management of type two diabetes. *Frontiers in Pharmacology*, *8*(JUL), 12-19.

Ouma, J., Gachanja, A., Mugo, S., & Gikunju, J. (2021). Antibiotic residues in milk from Juja and Githurai markets in Kenya by liquid chromatography-tandem mass spectrometry. *Chemistry Africa*, *4*(4), 769–775.

Palaniyandi T, Sivaji, A., Thiruganasambandam, R., Natarajan, S., & Hari, R. (2018). In vitro antigastric cancer activity of squalene, a triterpenoid compound isolated from Rhizophora Mucronata mangrove plant leaves against AGS cell

line. *Pharmacognosy Magazine*, *14*(57), 369-373.

Pamidimarri, G., Mathaiyan, J., Manikandan, S., Rajan, S., & Batmanabane, G. (2019). Screening of herbal medicines for potential allopathic antidiabetic adulterants: An analytical study. *Ayu*, *40*(4), 262-270.

Pang, W., Yang, H., Wu, Z., Huang, M., & Hu, J. (2009). LC-MS-MS in MRM mode for detection and structural identification of synthetic hypoglycemic drugs added illegally to "natural" anti-diabetic herbal products. *Chromatographia*, *70*(9), 1353–1359.

Panraksa, P., Ramphan, S., Khongwichit, S., & Smith, D. R. (2017). Activity of andrographolide against dengue virus. *Antiviral Research*, *139*(3), 69–78.

Papatheodorou, K., Banach, M., Bekiari, E., Rizzo, M., & Edmonds, M. (2018). Complications of diabetes. *Journal of Diabetes Research*, *2018*, 67-87.

Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., Radotic, K., & Mojovic, M. (2014). Further in vitro evaluation of antiradical and antimicrobial activities of phytol. *Natural Product Research, 28*(6), 372–376.

Penn, H. J., Langman, L. J., Unold, D., Shields, J., & Nichols, J. H. (2011). Detection of synthetic cannabinoids in herbal incense products. *Clinical Biochemistry*, *44*(13), 1163–1165.

Perng, D. S., Tsai, Y. H., Cherng, J., Wang, J. S., Chou, K. S., Shih, C. W., & Cherng, J. M. (2016). Discovery of a novel anticancer agent with both anti-topoisomerase I and II activities in hepatocellular carcinoma SK-Hep-1 cells in vitro and in vivo: Cinnamomum verum component 2-methoxycinnamaldehyde. *Drug Design, Development and Therapy*, *10*(4), 141-146.

Pham, T. V., Hoang, H. N. T., Nguyen, H. T., Nguyen, H. M., Huynh, C. T., Vu, T.

Y., Do, A. T., Nguyen, N. H., & Do, B. H. (2021). Anti-inflammatory and antimicrobial activities of compounds isolated from Distichochlamys benenica. *BioMed Research International*, *2021*, 98-104.

Pinent, M., Castell, A., Baiges, I., Montagut, G., Arola, L., & Ardévol, A. (2008). Bioactivity of flavonoids on insulin-secreting cells. *Comprehensive Reviews in Food Science and Food Safety*, *7*(4), 299–308.

Qureshi, M. N., Stecher, G., Sultana, T., Abel, G., Popp, M., & Bonn, G. K. (2011). Determination of carbohydrates in medicinal plants-comparison between TLC, mf-MELDI-MS and GC-MS. *Phytochemical Analysis*, *22*(4), 296–302.

Rajab, M. S., Cantrell, C. L., Franzblau, S. G., & Fischer, N. H. (2007). Antimycobacterial activity of (e)-phytol and derivatives: A preliminary structure-activity study. *Planta Medica*, *64*(01), 2–4.

Ratheesh, M., Sindhu, G., & Helen, A. (2013). Anti-inflammatory effect of quinoline alkaloid skimmianine isolated from Ruta graveolens L. *Inflammation Research*, *62*(4), 367–376.

Razi, A., Abdullah, N., Ahmad, S., Safinar Ismail, I., & Pauzi Zakaria, M. (2015). Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. *BMC Complementary and Alternative Medicine*, 15, 1-10.

Rocha, T., Amaral, J. S., & Oliveira, M. B. P. P. (2016). Adulteration of dietary supplements by the illegal addition of synthetic drugs: A review. *Comprehensive Reviews in Food Science and Food Safety*, *15*(1), 43–62.

Rodríguez Madrera, R., & Suárez Valles, B. (2021). Analysis of cyanogenic compounds derived from mandelonitrile by ultrasound-assisted extraction and

high-performance liquid chromatography in Rosaceae and Sambucus families. *Molecules*, *26*(24), 5-11.

Russo, M. V., Avino, P., Perugini, L., & Notardonato, I. (2015). Extraction and GC-MS analysis of phthalate esters in food matrices: A review. *RSC Advances*, *5*(46), 37023–37043.

Rzechonek, D. A., Dobrowolski, A., Rymowicz, W., & Mirończuk, A. M. (2017). Recent advances in biological production of erythritol. *Critical reviews in Biotechnology*, *38*(4), 620–633.

Saeidnia, S., & Abdollahi, M. (2013). Are medicinal plants polluted with phthalates? *DARU Journal of Pharmaceutical Sciences*, *21*(1), 43-52.

Saillenfait, A. M., Sabaté, J. P., Robert, A., Cossec, B., Roudot, A. C., Denis, F., & Burgart, M. (2013). Adverse effects of diisooctyl phthalate on the male rat reproductive development following prenatal exposure. *Reproductive Toxicology*, *42*(7), 192–202.

Sajjadi-Ghotbabadi, H., Javanshir, S., & Rostami-Charati, F. (2017). Synthesis, characterization, and antioxidant evaluations of new 2-oxochromene and benzofuran derivatives catalyzed by KF/CP. *Journal of Heterocyclic Chemistry*, *54*(2), 979–985.

Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Paul Bolwell, G., & Riceevans, C. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics*, *322*(2), 339–346.

Sang, Z. C., Sang, U. C., Seong, Y. B., Suhk, N. P., & Kang, R. L. (2005). Immunobioloical activity of a new benzyl benzoate from the aerial parts of

Solidago virga-aurea var. gigantea. *Archives of Pharmacal Research*, *28*(1), 49–54.

Saquib, M., Husain, I., Sharma, S., Yadav, G., Singh, V. K., Sharma, S. K., Shah, P., Siddiqi, M. I., Kumar, B., Lal, J., Jain, G. K., Srivastava, B. S., Srivastava, R., & Shaw, A. K. (2011). 2,3-dideoxy hex-2-enopyranosid-4-uloses as promising new anti-tubercular agents: design, synthesis, biological evaluation and SAR studies. *European Journal of Medicinal Chemistry*, *46*(6), 2217–2223.

Saraswati, J. K., Katnoria, A., & Kaur, N. (2016). Analytical techniques for phytochemicals screening and bioactivities of some coleus species: A review. *Journal of Pharmaceutical Sciences and Research*, *8*(4), 227–237.

Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Leblanc, J. C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Benford, D., Brimer, L., Mancini, F. R., … Schwerdtle, T. (2019). Evaluation of the health risks related to the presence of cyanogenic glycosides in foods other than raw apricot kernels. *EFSA Journal*, *17*(4), 8-16.

Seca, A. M. L., & Pinto, D. C. G. A. (2018). Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. *International Journal of Molecular Sciences*, *19*(1), 6-13.

Seow, L. J., Beh, H. K., Majid, A. M. S. A., Murugaiyah, V., Ismail, N., & Asmawi, M. Z. (2011). Anti-angiogenic activity of Gynura segetum leaf extracts and its fractions. *Journal of Ethnopharmacology*, *134*(2), 221–227.

Shaaban, M. T., Ghaly, M. F., & Fahmi, S. M. (2021). Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles

against multidrug-resistant bacteria. *Journal of Basic Microbiology*, *61*(6), 557–568.

Shaban, N. S., Abdou, K. A., & Hassan, N. E.-H. Y. (2016). Impact of toxic heavy metals and pesticide residues in herbal products. *Beni-Suef University Journal of Basic and Applied Sciences*, *5*(1), 102–106.

Shafiq, N., Noreen, S., Rafiq, N., Ali, B., Parveen, S., Mahmood, A., Sajid, A., Akhtar, N., & Bilal, M. (2020). Isolation of bioactive compounds from Rumex hastatus extract and their biological evaluation and docking study as potential anti-oxidant and anti-urease agents. *Journal of Food Biochemistry*, *44*(8), 20-26.

Shah, P. A., Shah, J. V., Sanyal, M., & Shrivastav, P. S. (2017). LC–MS/MS analysis of metformin, saxagliptin and 5-hydroxy saxagliptin in human plasma and its pharmacokinetic study with a fixed-dose formulation in healthy Indian subjects. *Biomedical Chromatography*, *31*(3), 3809-3814.

Shah, P. A., & Shrivastav, P. S. (2018). Ion-pair solid phase extraction for the simultaneous separation and quantitation of metformin and canagliflozin in human plasma by LC-MS/MS. *Microchemical Journal*, *143*, 181–189.

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608.

Shang, X. F., Morris-Natschke, S. L., Yang, G. Z., Liu, Y. Q., Guo, X., Xu, X. S., Goto, M., Li, J. C., Zhang, J. Y., & Lee, K. H. (2018). Biologically active quinoline and quinazoline alkaloids part II. *Medicinal Research Reviews*, *38*(5), 1614–1660.

Shanmugam, K. R., Shanmugam, B., Subbaiah, G. V., Ravi, S., & Reddy, K. S.

(2021). Medicinal plants and bioactive compounds for diabetes management: important advances in drug discovery. *Current Pharmaceutical Design*, *27*(6), 763–774.

Shettar, A. K., Sateesh, M. K., Kaliwal, B. B., & Vedamurthy, A. B. (2017). In vitro antidiabetic activities and GC-MS phytochemical analysis of Ximenia americana extracts. *South African Journal of Botany*, *111*(2), 202–211.

Siemen, A., Kosciow, K., Schweiger, P., & Deppenmeier, U. (2018). Production of 5-ketofructose from fructose or sucrose using genetically modified Gluconobacter oxydans strains. *Applied Microbiology and Biotechnology*, *102*(4), 1699–1710.

Simón-Manso, Y., Lowenthal, M. S., Kilpatrick, L. E., Sampson, M. L., Telu, K. H., Rudnick, P. A., Mallard, W. G., Bearden, D. W., Schock, T. B., Tchekhovskoi, D. V., Blonder, N., Yan, X., Liang, Y., Zheng, Y., Wallace, W. E., Neta, P., Phinney, K. W., Remaley, A. T., & Stein, S. E. (2013). Metabolite profiling of a NIST standard reference material for human plasma (SRM 1950): GC-MS, LC-MS, NMR, and clinical laboratory analyses, libraries, and web-based resources. *Analytical Chemistry*, *85*(24), 11725–11731.

Stein, S. (2012). Mass spectral reference libraries: An ever-expanding resource for chemical identification. *Analytical Chemistry*, *84*(17), 7274–7282.

Steiner, D., Krska, R., Malachová, A., Taschl, I., & Sulyok, M. (2020). Evaluation of matrix effects and extraction efficiencies of LC-MS/MS Methods as the essential part for proper validation of multiclass contaminants in complex feed. *Journal of Agricultural and Food Chemistry*, *68*(12), 3868–3880.

Steyn, M., Couchman, L., Coombes, G., Earle, K. A., Johnston, A., & Holt, D. W.

(2018). A herbal treatment for type 2 diabetes adulterated with undisclosed drugs. *The Lancet*, *391*(10138), 2411-2423.

Subash-Babu, P., Alshatwi, A. A., & Ignacimuthu, S. (2014). Beneficial antioxidative and antiperoxidative effect of cinnamaldehyde protect streptozotocin-induced pancreatic β-cells damage in Wistar rats. *Biomolecules & Therapeutics*, *22*(1), 47-54.

Subramanian, R., Asmawi, M. Z., & Sadikun, A. (2008). In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. *Acta Biochimica Polonica*, *55*(2), 391–398.

Sudharsan, S., Saravanan, R., Shanmugam, A., Vairamani, S., Mohan, R., Menaga, S., & Ramesh, N. (2011). Isolation and Chemical characteristics of rhamnose enriched polysaccharide from G rateloupia lithophila. *Journal of Bioterrorism & Biodefense*, *2*(1), 8-19.

Sveshnikova, N., Yuan, T., Warren, J. M., & Piercey-Normore, M. D. (2019). Development and validation of a reliable LC-MS/MS method for quantitative analysis of usnic acid in Cladonia uncialis. *BMC Research Notes*, *12*(1), 1–6.

Sylvestre, M., Pichette, A., Longtin, A., Nagau, F., & Legault, J. (2006). Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. *Journal of Ethnopharmacology*, *103*(1), 99–102.

Tan, W., Zhang, Y., He, X., Xi, B., Gao, R., Mao, X., Huang, C., Zhang, H., Li, D., Liang, Q., Cui, D., & Alshawabkeh, A. N. (2016). Distribution patterns of phthalic acid esters in soil particle-size fractions determine biouptake in soil-cereal crop systems. *Scientific Reports*, *6*(1), 1–15.

Tatipamula, V. B., Ketha, A., Nallapaty, S., Kottana, H., & Koneru, S. T. (2021).

Moss Octoblepharum albidum Hedw.: Isolation, characterization, in vitro and in vivo antidiabetic activities. *Advances in Traditional Medicine. 21*(2), 351–360.

Thakor, P., Mehta, J. B., Patel, R. R., Patel, D. D., Subramanian, R. B., & Thakkar, V. R. (2016). Extraction and purification of phytol from Abutilon indicum: cytotoxic and apoptotic activity. *RSC Advances*, *6*(54), 48336–48345.

Thipanyane, M. P., Nomatshila, S. C., Musarurwa, H. T., & Oladimeji, O. (2022). The roles and challenges of traditional health practitioners in maternal health services in rural communities of Mthatha, South Africa. *International Journal of Environmental Research and Public Health, 19*(20), 13597-13602.

Thompson, L. A., & Darwish, W. S. (2019). Environmental chemical contaminants in food: Review of a global problem. *Journal of Toxicology, 2019*, 3-15.

Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews: Endocrinology*, *18*(9), 525-537.

Tong, Y. ru, Su, P., Guan, H. yu, Hu, T. yuan, Chen, J. long, Zhang, Y. feng, Zhao, Y. jun, Gao, L. hui, Zhang, X. nan, Huang, L. qi, & Gao, W. (2018). Eudesmane-type sesquiterpene diols directly synthesized by a sesquiterpene cyclase in Tripterygium wilfordii. *Biochemical Journal*, *475*(17), 2713–2725.

Tran, N., Pham, B., & Le, L. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology*, *9*(9), 1–31.

Tshering, G., Pimtong, W., Plengsuriyakarn, T., & Na-Bangchang, K. (2021). Anti-angiogenic effects of beta-eudesmol and atractylodin in developing zebrafish embryos. *Comparative Biochemistry and Physiology, 243*(3), 108980-108986.

Tugume, P., & Nyakoojo, C. (2019). Ethno-pharmacological survey of herbal

remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District, Uganda. *BMC Complementary and Alternative Medicine*, *19*(1), 9-17. 6

Türkez, H., Kübra, C., & Basak, T. (2014). Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. *Cytotechnology*, *66*(2), 597–603.

Uchiyama, N., Kikura-Hanajiri, R., Ogata, J., & Goda, Y. (2010). Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Science International*, *198*(1–3), 31–38.

United Nations News. (2023, May 19). *Chronic diseases taking 'immense and increasing toll on lives', warns WHO*. Retrieved from https://news.un.org/en/story/2023/05/1136832

Valdez, C. A., Leif, R. N., Hok, S., & Alcaraz, A. (2018). Assessing the reliability of the NIST library during routine GC-MS analyses: Structure and spectral data corroboration for 5,5-diphenyl-1,3-dioxolan-4-one during a recent OPCW proficiency test. *Journal of Mass Spectrometry : JMS*, *53*(5), 419–422.

van de Ven, H. C., Gargano, A. F. G., Van der Wal, S. J., & Schoenmakers, P. J. (2016). Switching solvent and enhancing analyte concentrations in small effluent fractions using in-column focusing. *Journal of Chromatography A*, *1427*(4), 90–95.

Verbitski, S. M., Gourdin, G. T., Ikenouye, L. M., McChesney, J. D., & Hildreth, J. (2008). Detection of Actaea racemosa Adulteration by Thin-Layer Chromatography and Combined Thin-Layer Chromatography-Bioluminescence. *Journal of AOAC International*, *91*(2), 268–275.

Viana, C., Ferreira, M., Romero, C. S., Bortoluzzi, M. R., Lima, F. O., Rolim, C. M. B., & De Carvalho, L. M. (2013). A capillary zone electrophoretic method for the determination of hypoglycemics as adulterants in herbal formulations used for the treatment of diabetes. *Analytical Methods*, *5*(8), 2126–2133.

Vicent, D., Villanueva-Penacarrillo, M. L., Malaisse-Lagae, F., Leclercq-Meyer, V., Valverde, I., & Malaisse, W. J. (1994). In vivo stimulation of insulin release by succinic acid methyl esters. *Archives Internationales de Pharmacodynamie et de Therapie*, *327*(2), 246–250.

Vieira, A. J. S. C., Gaspar, E. M., & Santos, P. M. P. (2020). Mechanisms of potential antioxidant activity of caffeine. *Radiation Physics and Chemistry*, *174*, 108968.

Vogeser, M., & Stone, J. A. (2020). A suggested standard for validation of LC-MS/MS based analytical series in diagnostic laboratories. *Clinical Mass Spectrometry, 16*(1), 25-38.

Wambalaba, F. W., Son, B., Wambalaba, A. E., Nyong'o, D., & Nyong'o, A. (2019). Prevalence and capacity of cancer diagnostics and treatment: A demand and supply survey of health-care facilities in Kenya. *Journal of the Moffitt Cancer Center, 26*(1), 6-18.

Wanandi, S. I., Limanto, A., Yunita, E., Syahrani, R. A., Louisa, M., Wibowo, A. E., & Arumsari, S. (2020). In silico and in vitro studies on the anti-cancer activity of andrographolide targeting survivin in human breast cancer stem cells. *PLOS ONE*, *15*(11), 20-27.

Wang, X., Song, Z. J., He, X., Zhang, R. Q., Zhang, C. F., Li, F., Wang, C. Z., & Yuan, C. S. (2015). Antitumor and immunomodulatory activity of genkwanin

on colorectal cancer in the APCMin/+ mice. *International Immunopharmacology*, *29*(2), 701–707.

Wang, Y., & Qian, H. (2021). Phthalates and their impacts on human health. *Healthcare*, *9*(5), 34-44.

Wang, Z., Cao, Y., Lu, Y., Zhang, F., Su, Y., & Guo, Y. (2020). Ultrasonic extraction and nebulization in real-time coupled with carbon fiber ionization mass spectrometry for rapid screening of the synthetic drugs adulterated into herbal products. *Analytica Chimica Acta*, *1136*(5), 62–71.

Wei, L. S., Wee, W., Siong, J. Y. F., & Syamsumir, D. F. (2011). Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of Peperomia pellucida leaf extract. *Acta Medica Iranica*, *49*(10), 670–674.

Wen, L., Guo, Z., Li, Q., Zhang, D., She, Z., & Vrijmoed, L. L. P. (2010). A new griseofulvin derivative from the mangrove endophytic fungus Sporothrix SP. *Chemistry of Natural Compounds*, *46*(3), 363–365.

Wen, S., Gu, D., & Zeng, H. (2018). Antitumor effects of beta-amyrin in Hep-G2 liver carcinoma cells are mediated via apoptosis induction, cell cycle disruption and activation of JNK and P38 signalling pathways. *JBUON*, *23*(4), 965–970.

WHO. (2007). *WHO Guidelines for Assessing Quality of Herbal Medicines with reference to Contaminants and Residues*. Retrieved from https://apps.who.int/iris/handle/10665/43510

WHO. (2019). *WHO Global Report on Traditional and Complementary Medicine 2019*. Retrieved from http://apps.who.int/bookorders.

WHO. (2022a). *Diabetes*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/diabetes

WHO. (2022b, February 3). *Cancer*. Fact Sheet. Retrieved from https://www.who.int/news-room/fact-sheets/detail/cancer

WHO. (2023). *Noncommunicable diseases*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

Wisitpongpun, P., Suphrom, N., Potup, P., Nuengchamnong, N., Calder, P. C., & Usuwanthim, K. (2020). In vitro bioassay-guided identification of anticancer properties from Moringa oleifera Lam. leaf against the MDA-MB-231 cell line. *Pharmaceuticals*, *13*(12), 464-472.

Wohlfarth, A., Scheidweiler, K. B., Chen, X., Liu, H. F., & Huestis, M. A. (2013). Qualitative confirmation of 9 synthetic cannabinoids and 20 metabolites in human urine using LC–MS/MS and library search. *Analytical Chemistry*, *85*(7), 3730–3738.

Wu, C. Y. C., Couto e Silva, A., Citadin, C. T., Clemons, G. A., Acosta, C. H., Knox, B. A., Grames, M. S., Rodgers, K. M., Lee, R. H. C., & Lin, H. W. (2021). Palmitic acid methyl ester inhibits cardiac arrest-induced neuroinflammation and mitochondrial dysfunction. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *165*(11), 227-234.

Wulandari, M., Urraca, J. L., Descalzo, A. B., Amran, M. B., & Moreno-Bondi, M. C. (2015). Molecularly imprinted polymers for cleanup and selective extraction of curcuminoids in medicinal herbal extracts. *Analytical and Bioanalytical Chemistry*, *407*(3), 803–812.

Xu, J. (2014). Bioactive natural products derived from mangrove-associated microbes. *RSC Advances*, *5*(2), 841–892.

Xu, Y. M., McLaughlin, S. P., & Gunatilaka, A. A. L. (2007). Sorbifolivaltrates A-D,

diene valepotriates from Valeriana sorbifolia(1). *Journal of Natural Products*, *70*(12), 2045–2048.

Yang, W.-S., Lee, S. R., Jeong, Y. J., Park, D. W., Cho, Y. M., Joo, H. M., Kim, I., Seu, Y.-B., Sohn, E.-H., & Kang, S. C. (2016). Antiallergic activity of ethanol extracts of arctium lappa l. undried roots and its active compound, oleamide, in regulating FcεRI-Mediated and MAPK signaling in RBL-2H3 cells. *Journal of Agricultural and Food Chemistry*, *64*(18), 3564–3573.

Yang, Y., Ju, Z., Yang, Y., Zhang, Y., Yang, L., & Wang, Z. (2021). Phytochemical analysis of Panax species: A review. *Journal of Ginseng Research*, *45*(1), 1–21.

Yossa, N., Patel, J., Macarisin, D., Millner, P., Murphy, C., Bauchan, G., & Lo, Y. M. (2014). Antibacterial activity of cinnamaldehyde and sporan against Escherichia coli O157:H7 and Salmonella. *Journal of Food Processing and Preservation*, *38*(3), 749–757.

Zaccai, M., Yarmolinsky, L., Khalfin, B., Budovsky, A., Gorelick, J., Dahan, A., & Ben-Shabat, S. (2020). Medicinal properties of Lilium candidum L. and its phytochemicals. *Plants*, *9*(8), 959-963.

Zeid, A. H. S. A., Haggag, M. Y., Saleh, M. M., & Mohamed, R. S. (2000). Isolation and identification of the biologically active secondary metabolites from Trichoderma harzianum Rifai and Trichoderma hamatum (Bon.) Bain. *Bulletin of the National Research Centre (Cairo)*, *25*(3), 207–222.

Zhang, A., Xue, C., & Fong, H. (2011). Integration of herbal medicine into evidence-based clinical practice. *Herbal Medicine: Biomolecular and Clinical Aspects*, *10*(3), 453–464.

Zhang, J., Wider, B., Shang, H., Li, X., & Ernst, E. (2012). Quality of herbal

medicines: Challenges and solutions. *Complementary Therapies in Medicine*, *20*(1–2), 100–106.

Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, *13*(1), 20-27.

Zhang, Y., Xue, Y., Li, G., Yuan, H., & Luo, T. (2016). Enantioselective synthesis of Iboga alkaloids and vinblastine via rearrangements of quaternary ammoniums. *Chemical Science*, *7*(8), 5530–5536.

Zhang, Y., Zhang, B., Yu, J., Yuan, M., Ji, J., Lu, H., Ma, Y., & Guo, Y. (2021). Functional biochar for efficient residue treatment of sulfonylurea herbicides by weak molecular interaction. *Biochar*, *3*(4), 545–556.

Zhao, J., Deng, J. W., Chen, Y. W., & Li, S. P. (2013). Advanced phytochemical analysis of herbal tea in China. *Journal of Chromatography A*, *1313*(6), 2–23.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., & Chen, T. (2013). In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *Journal of Agricultural and Food Chemistry*, *61*(44), 10604–10611.

Zhong, Y., Sun, C., Xiong, J., & Shi, Y. (2017). Simultaneous determination of eight adulterants in weight management supplements and herbs by HPLC–DAD and LC–MS/MS. *Journal of Liquid Chromatography & Related Technologies*, *40*(12), 640–648.

Zhou, J., Chan, L., & Zhou, S. (2012). Trigonelline: A plant alkaloid with therapeutic potential for diabetes and central nervous system disease. *Current Medicinal Chemistry*, *19*(21), 3523–3531.

APPENDICES

Appendix I: Questionnaire

Sample collector name……...Date……..

Part A

Name of the herbal medicine: ……. Sample code: …….

Outlet:

…….

County: ……. Town: ……..

Formulation:……...….

Treatment indication:…….……

Part B

How many medicinal plants have been used to make the herbal medicine?

Is the herbal medicine local? If not, which country is it from?

How should I prepare the drug?

How should I take the drug?

How much should I take and how often?

Is the medicine 100% natural?

Can I use the herbal medicine with convectional drugs or do I need to stop taking my current antidiabetic or anticancer medications?

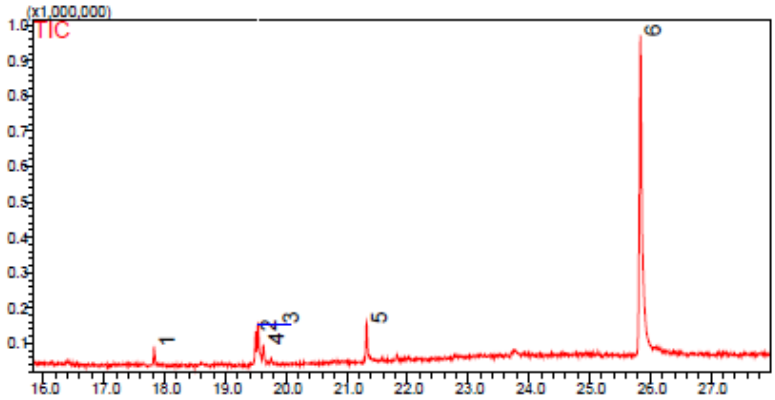
Anything else I need to know?

Appendix II: Detailed Sample Information

COUNTY COLLECTED	SAMPLE CODE	FORMULATION	OUTLET	DISEASE	COUNTRY OF ORIGIN	METHOD OF PREPARATION	MODE OF ADMINISTRATION	CONTRAINDICATIONS	INGREDIENTS USED
Uasin Gishu	EIC21P	Powder	Hawker	Cancer	Local	Hot infusion	Ingestion	Taken together with conventional drugs	Mixed plant
Uasin Gishu	EID20P	Powder	Hawker	Diabetes	Local	Decoction	Ingestion	Taken together with conventional drugs	Single plant
Uasin Gishu	EIC26P	Powder	Herbalist	Cancer	Local	Decoction	Ingestion	Taken together with conventional drugs	Mixed plant
Uasin Gishu	EID28P	Powder	Herbal clinic	Diabetes	Local	Hot infusion	Ingestion	Taken together with conventional drugs	Mixed plant
Uasin Gishu	EIC27P	Powder	Herbal clinic	Cancer	Local	Hot infusion	Ingestion	Taken together with conventional drugs	Mixed plant
Nairobi	MD43P	Powder	Hawker	Diabetes	Local	Hot infusion	Ingestion	Stop convectional drugs	Mixed plant
Nairobi	RD32P	Powder	Chandarana	Diabetes	Local	Hot infusion	Ingestion	Taken together with conventional drugs	Single plant
Nairobi	GC82P	Powder	Herbal clinic	Cancer	Local	Hot infusion	Ingestion	Taken together with conventional drugs	Single plant
Nairobi	GC81P	Powder	Hawker	Cancer	Local	Hot infusion	Ingestion	Taken together with conventional drugs	Mixed plant
Nairobi	XD72P	Powder	Herbalist	Diabetes	Local	Decoction	Ingestion	Stop convectional drugs	Mixed plant
Nairobi	C13	Powder	Vendors	Cancer	Local	Cold infusion	Ingestion	Stop convectional drugs	Mixed plant
Nairobi	D17	Capsules	Nutritional store	Diabetes	Imported	Decoction	Ingestion	Taken together with conventional drugs	Mixed plant
Nairobi	D18	Capsules	Nutritional store	Diabetes	Imported	Decoction	Ingestion	Taken together with conventional drugs	Mixed plant
Nairobi	D14	Powder	Hawker	Diabetes	Imported	Decoction	Ingestion	Stop convectional drugs	Mixed plant
Nairobi	C09	Powder	Herbal clinic	Cancer	Local	-	Ingestion	-	-
Nairobi	D12	Powder	Herbal clinic	Diabetes	Imported	Hot infusion	ingestion	-	Mixed plant
Uasin Gishu	EIC35P	powder	Herbalist	Cancer	-	Hot infusion	ingestion	-	-
Uasin Gishu	ELD/D/77P	powder	Herbalist	Diabetes	-	Hot infusion	ingestion	-	-
Uasin Gishu	ELD/C/64P	powder	herbal clinic	Cancer	Local	hot infusion	ingestion	-	-
Uasin Gishu	ELD/D/75P	powder	Herbalist	Diabetes	-	hot infusion	ingestion	-	-
Uasin Gishu	ELD/D/76P	powder	Herbal clinic	Diabetes	-	-	ingestion	-	-
Uasin Gishu	JD15P	powder	Hawker	Diabetes	Imported	-	ingestion	-	-
Nairobi	MD53P	powder	herbal clinic	Diabetes	Local	-	ingestion	-	-
Uasin Gishu	ELD47P	Powder	Herbalist	Diabetes	Local	Concusion	Inhalation	Taken together with conventional drugs	Mixed plant

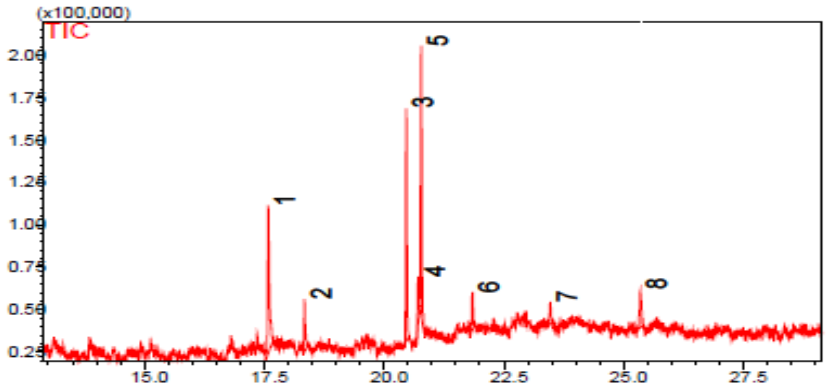
Appendix III: TICs of Selected Extracts of Sampled Herbal Medicines Using GC-MS

MD53P (acetonitrile extract)



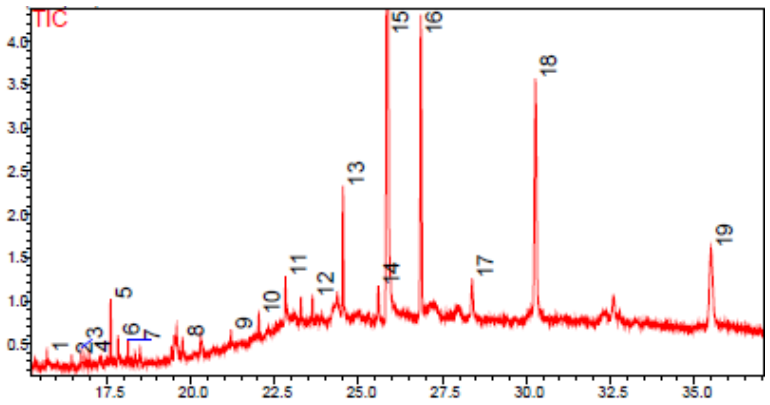
1 – Palmitic acid, methyl ester; 2 - Methyl 10-trans,12-cis-octadecadienoate; 3 - 9-Octadecenoic acid (Z)-, methyl ester; 4 – Phytol; 5 - Methyl 12-hydroxy-9-octadecenoate; 6 - 13-Docosenamide, (Z)-.

D12 (acetonitrile extract)



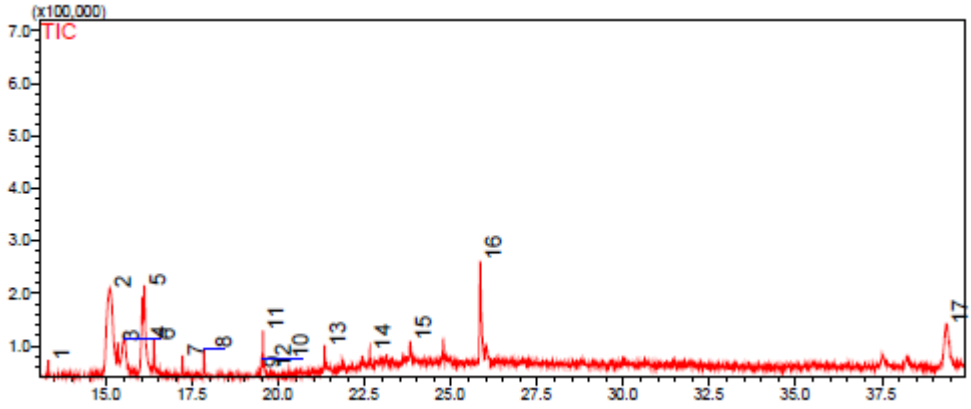
1 – Cinnamaldehyde, (E)-; 2 – Coumarin; 3 - 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one; 4 – Phytol; 5 - Caffeine; 6 - Octadecanoic acid, 3-oxo-, ethyl ester; 7 - Olean-12-en-28-al; 8 - Diisooctyl phthalate.

MD43P (hexane extract)



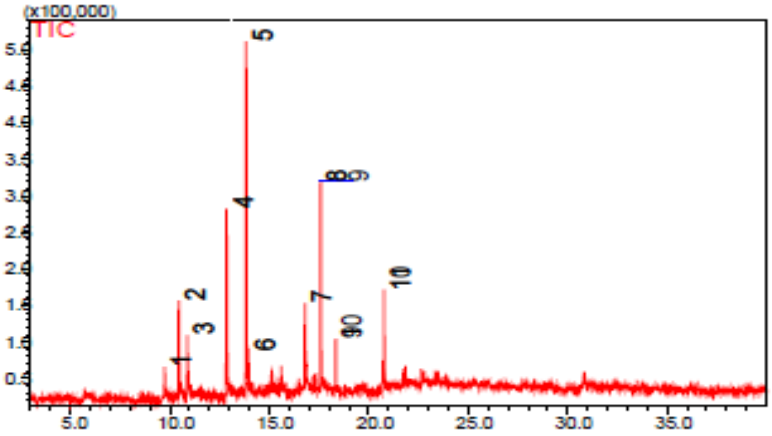
1 – Tridecanoic acid, 3-methyl-, methyl ester; 2 – Pentadecane; 3 - Isopropyl myristate; 4 – 2-Pentadecanone, 6,10,14-trimethyl-; 5 - 1-Naphthalenepropanol, .alpha.-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2-methylene-, [1S-[1.alpha.(R*),4a.beta.,8a.alpha.]]-; 6 - Hexadecanoic acid, methyl ester; 7 - m-Camphorene; 8 - Methyl stearate; 9 - Eicosane, 10-methyl-; 10 - 2-methyloctacosane; 11 – Tetratriacontane; 12 - 2-methyloctacosane; 13 – Pentatriacontane; 14 – Hexatriacontane; 15 - 13-Docosenamide, (Z)-; 16 – Tritetracontane; 17 – Squalene; 18 – Hentriacontane; 19 – Tetratetracontane.

GC81P (acetonitrile extract)



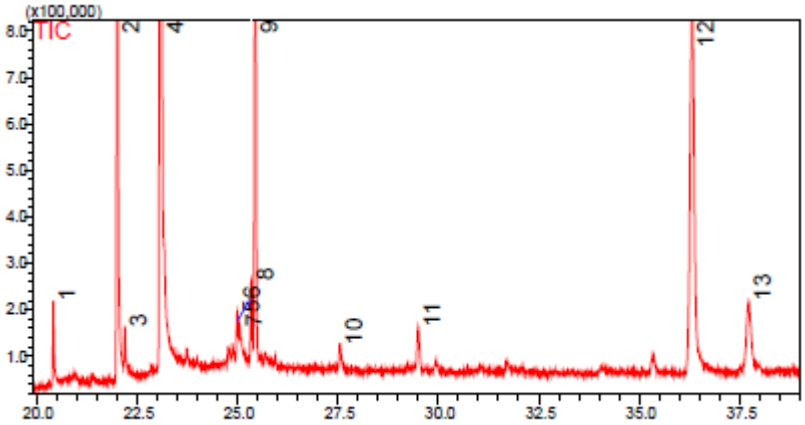
1 – 2,4-Di-tert-butylphenol; 2 – 3-O-Methyl-d-glucose; 3 - Eudesm-4(14)-en-11-ol; 4 – 3-Methylmannoside; 5 - Methyl 3,4-ethylidene-.alpha.-D-galactopyranoside; 6 - Lanceol, cis; 7 - (1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol; 8 - Hexadecanoic acid, methyl ester; 9 - E-15-Heptadecenal; 10 - 9,12-Octadecadienoic acid, methyl ester; 11 – 11-Octadecenoic acid, methyl ester, (Z)-; 12 - Heptadecanoic acid, 9-methyl-, methyl ester; 13 - Methyl ricinoleate; 14 – Triethylene glycol di(2-ethylhexoate); 15 - Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate; 16 – 13-Docosenamide, (Z)-; 17 – beta.-Amyrin.

C09 (acetonitrile extract)



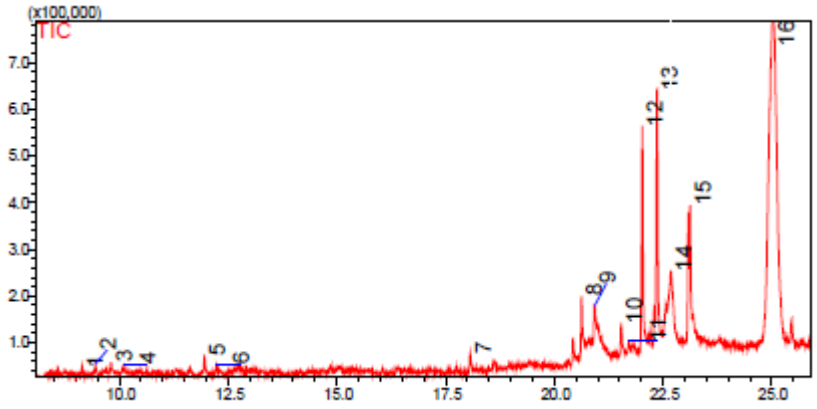
1 – 3-Phenylpropanol; 2 – Cinnamaldehyde, (E)-; 3 - Cinnamic alcohol; 4 – Trans-o-Coumaric acid; 5 - (Z)-2-methoxycinnamaldehyde; 6 - 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl; 7 - 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one; 8 - Caffeine; 9 - Dibutyl phthalate; 10 - 4,4'-((p-Phenylene)diisopropylidene)diphenol.

D17 (acetonitrile extract)



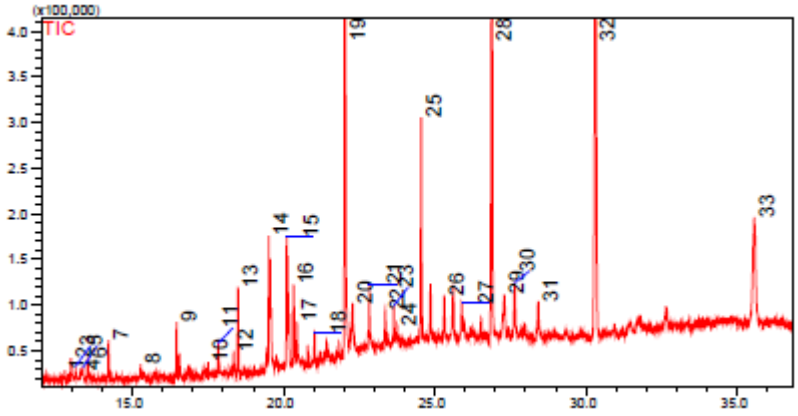
1 – 3-Phenylpropanol; 2 – Nicofuranose; 3 - 2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol; 4 – Hexadecanoic acid, methyl ester; 5 - 3,7-Cyclodecadiene-1-methanol, .alpha.,.alpha.,4,8-tetramethyl-, [s-(Z,Z)]; 6 - Hexadecanoic acid; 7 - 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one; 8 - 9-Octadecenoic acid, methyl ester, (E)-; 9 - Methyl stearate; 10 - Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-; 11 - 9-octadecanamide, (Z)-; 12 - Diisooctyl phthalate; 13 - N-(p-Methoxybenzylidene)-p-nitroaniline.

JD15P (acetonitrile extract)



1 – Terpineol; 2 – (R)-(+)-.beta.-Citronellol; 3 - Geraniol; 4 – Citral; 5 - cis-Methyl isoeugenol; 6 - Anthranilic acid, N-methyl-, methyl ester; 7 - 4-Methoxy-6-methylcoumarin; 8 - 9-Hexadecenoic acid, (Z)-; 9 - Methyl stearate; 10 - 6,9-Dimethoxy-4-methyl-2,3-dihydrofuro(3,2-c)quinoline; 11 - 9-octadecanamide, (Z)-; 12 - 13-Docosenamide, (Z)-; 13 – Skimmianine; 14 - beta.-Amyrone; 15 - Phenol, 3-pentadecyl-; 16 - Olean-12-en-3-one.

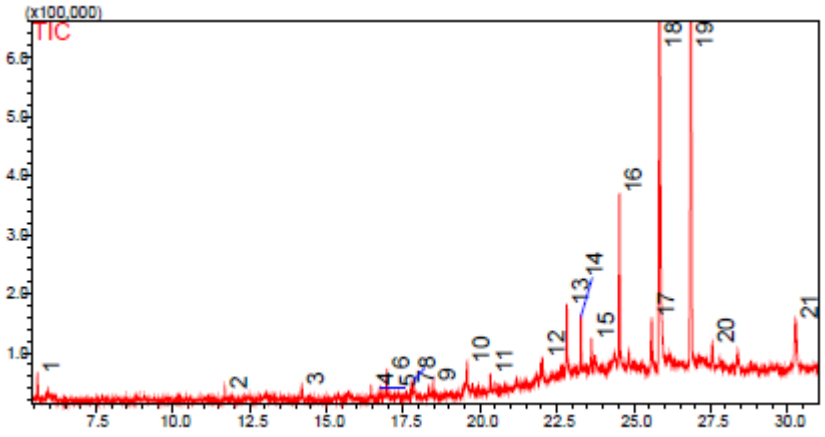
XD72P (hexane extract)



1 – Eicosane; 2 – alpha.-Curcumene; 3 - 7-epi-Sesquithujene; 4 – beta.-Bisabolene; 5 - beta.-copaene; 6 - Sesquisabinene isomer; 7 - Hexadecane; 8 - n-Eicosane; 9 - Octadecane; 10 - 2,6,10-Trimethyltridecane; 11 - Hexadecanoic acid, methyl ester;

12 - Dibutyl phthalate; 13 – Heptadecane, 2,6,10,15-tetramethyl-; 14 - Methyl 10-trans,12-cis-octadecadienoate; 15 - 9,12-Octadecadienoic acid, ethyl ester -; 16 - Undec-10-ynoic acid, tridec-2-yn-1-yl ester; 17 - Hexadecanamide; 18 - Hexadecanoic acid, methyl ester; 19 - 9-Octadecenamide, (Z); 20 – Phytol; 21 - Heneicosane; 22 - 9,12-Octadecadienoic acid, ethyl ester -; 23 – Hexacosane; 24 - Undec-10-ynoic acid, tridec-2-yn-1-yl ester; 25 – Tetracosane; 26 – 3-Methylheptacosane; 27 - Heneicosane; 28 - Squalene; 29 – 2-methyloctacosane; 30 - Hentriacontane; 31 – Tetracosane; 32 - Dotriacontane; 33 - Tritetracontane.

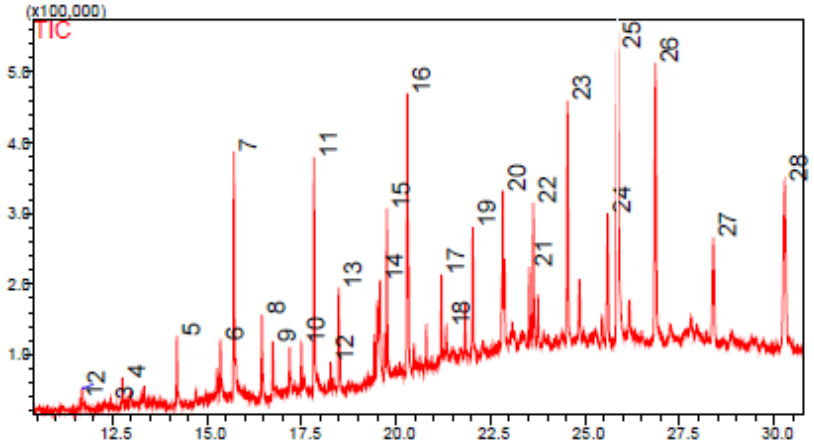
RD32P (hexane extract)



1 – Tridecanoic acid, 3-methyl-, methyl ester; 2 – Tetradecane; 3 - Hexadecane; 4 – Octadecane; 5 - i-Propyl 12-methyl-tridecanoate; 6 - 2-Pentadecanone, 6,10,14-trimethyl-; 7 - Trans-Geranylgeraniol; 8 - Hexadecanoic acid, methyl ester; 9 - Heptadecane, 2,6,10,15-tetramethyl-; 10 - Linolenic acid, methyl ester; 11 - Heneicosane; 12 – Tetracosane; 13 – 2-methyloctacosane; 14 - Diisooctyl phthalate;

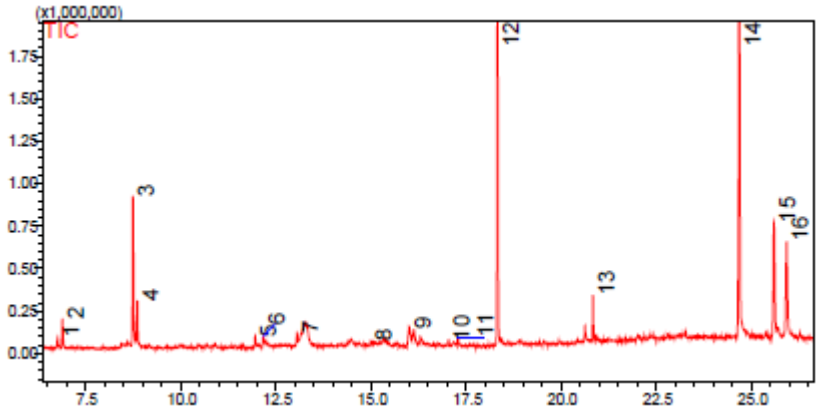
15 - Hentriacontane; 16 - Hexatriacontane; 17 - Dotriacontane; 18 - 13-Docosenamide, (Z)-; 19 – Tritetracontane; 20 – Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-; 21 – Tetratetracontane.

GC82P (hexane extract)



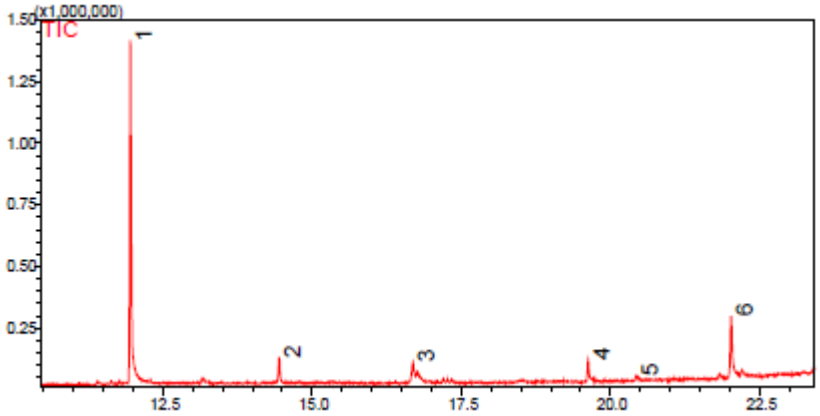
1 – Copaene; 2 – Tetradecane; 3 - 2,6,10-Trimethyltridecane; 4 – beta.-Bisabolene; 5 - Pentadecane; 6 - Hexadecane; 7 - Methyl tetradecanoate; 8 - Heptadecane; 9 - i-Propyl 12-methyl-tridecanoate; 10 - Eicosane; 11 - Hexadecanoic acid, methyl ester; 12 - Oxacycloheptadecan-2-one; 13 – Heneicosane; 14 - Linolenic acid, methyl ester; 15 - Methyl isostearate; 16 - (Z)-18-Octadec-9-enolide; 17 - Ricinoleic acid; 18 - Tetracosane; 20 – cis-10-Nonadecenoic acid; 21 - 2-methyloctacosane; 22 - Tetratriacontane; 23 – Hexatriacoctane; 24 - Tritetracoctane; 25 – Squalene; 26 – Pentatricontane; 27 - Heneicosane; 28 – Tetratetracontane.

EIC26P (acetonitrile extract)



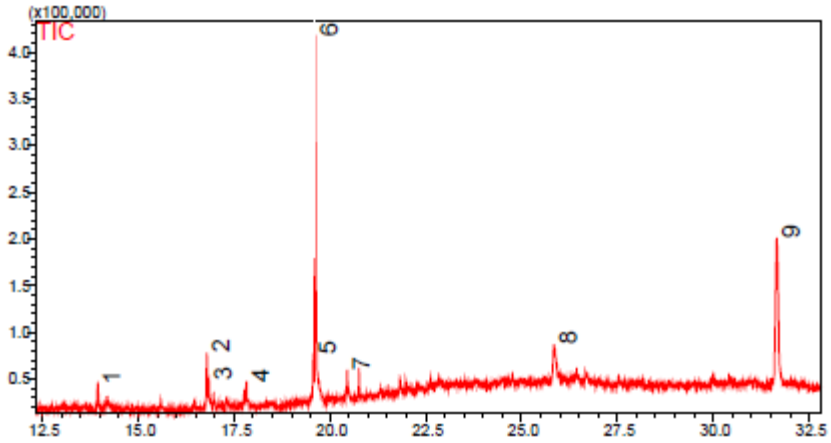
1 – alpha.-Toluenol; 2 – Pantoyl lactone; 3 - 2-acetoxyacetophenone; 4 – Menthol; 5 - cis-Methyl isoeugenol; 6 - Vanillin; 7 - 2-Tridecanone; 8 - gamma.-Eudesmol; 9 - 4-O-Methylmannose; 10 - 14,17-Octadecadienoic acid, methyl ester; 11 - Eudesm-4(14)-en-11-ol; 12 - (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one; 13 – Tributyl acetylcitrate; 14 - Columbin; 15 Matairesinol; 16 – Pinoresinol.

ELD/D/77P (acetonitrile extract)



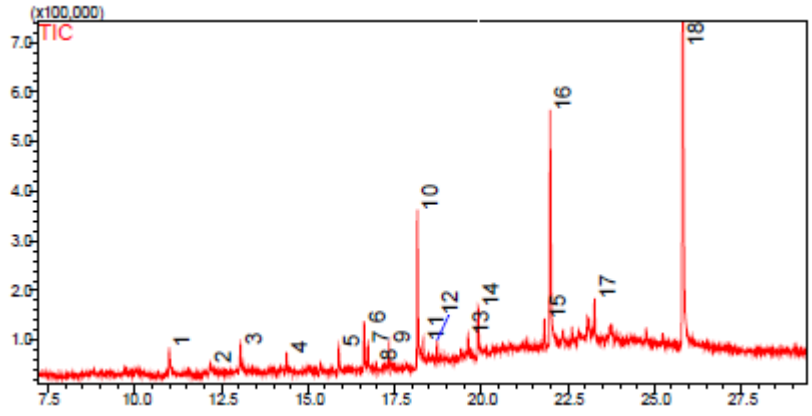
1 – cis-Methyl isoeugenol; 2 – Caryophyllene oxide; 3 - Cinnamaldehyde, 3,4-dimethoxy-; 4 – Phytol; 5 - Hexadecanamide; 6 - 9-Octadecenamide, (Z)-.

ELD/C/64P (hexane extract)



1 – 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-; 2 – 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one; 3 - Neophytadiene; 4 – Palmitic acid, methyl ester; 5 - Linolenic acid, methyl ester; 6 - Phytol; 7 - 2-Tridecanone; 8 - 3-Docosenamide, (Z)-; 9 - (+)-.gamma.-Tocopherol, O-methyl-.

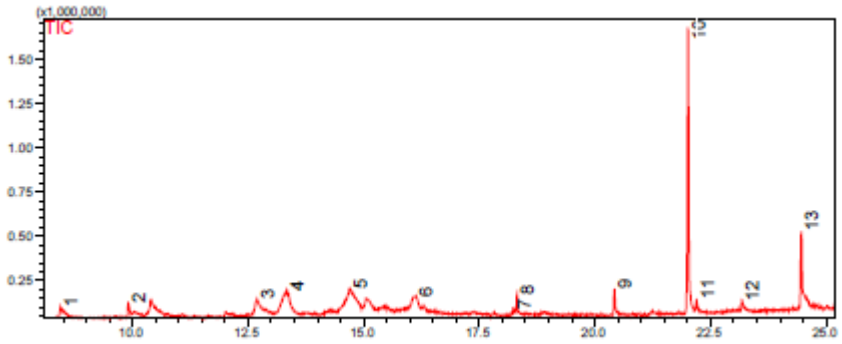
ELD47P (acetonitrile extract)



1 – 4,4-Dimethyl-1,3-oxazolidin-2-one; 2 – Vanillin; 3 - 2-Tridecanone; 4 – Diethyl Phthalate; 5 - (2E,4E)-N-Isobutylocta-2,4-dienamide; 6 - Ascabin; 7 - Isopropyl myristate; 8 - 6,10,14-Trimethyl-2-pentadecanone; 9 - Diisobutyl phthalate; 10 - (E,E)-N-Isobutyl-2,4-decadienamide; 11 - Dibutyl phthalate; 12 - Isopropyl

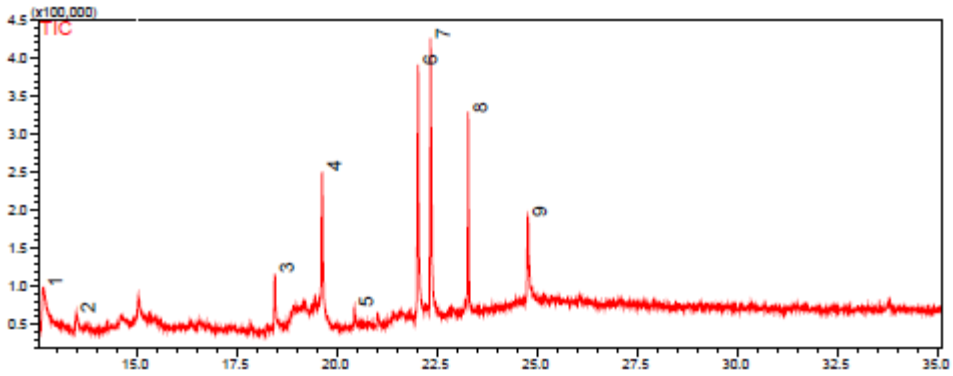
palmitate; 13 – Phytol; 14 - Ketone, 7-methoxy-2-benzofuranyl methyl; 15 - 4,8,12,16-Tetramethylheptadecan-4-olide; 16 - 3,4-Methylenedioxybenzylidene acetone; 17 - Diisooctyl phthalate; 18 - 13-Docosenamide, (Z)-.

EIC27P (methanol extract)



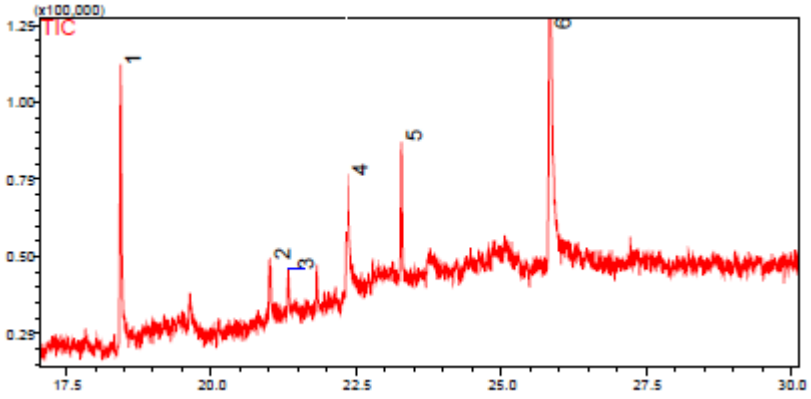
1 – 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one; 2 – 3-Acetoxy-3-hydroxypropionic acid, methyl ester; 3 - Isobutylglycerol, nitro-; 4 – 2H-Pyran-2-methanol, 3,6-dihydro-3-hydroxy-6-(1-methylethoxy)-; 5 - 3-Deoxy-d-mannonic acid; 6 - 3-O-Methyl-d-glucose; 7 - Palmitic acid; 8 - (4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one; 9 - Hexadecanamide; 10 - 9-Octadecenamide, (Z)-; 11 - Octadecanamide; 12 - Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester; 13 – Columbin.

EIC21P (methanol extract)



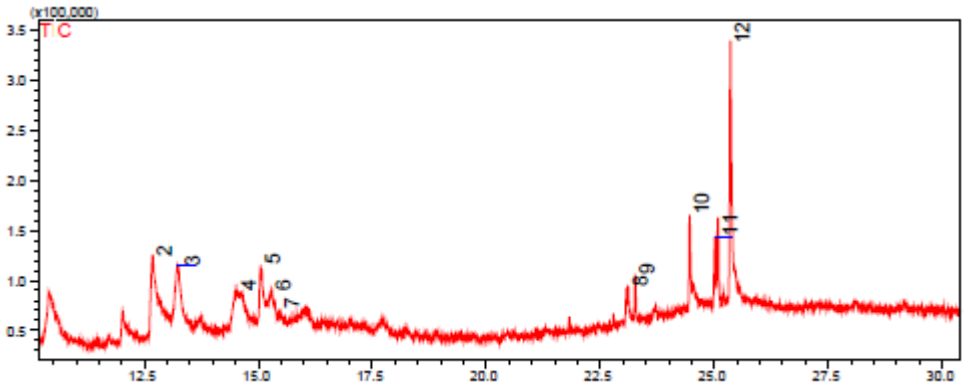
1 – n-Propyl heptyl ether; 2 – D-Allose, methyl ester; 3 - 2-propenoic acid, 2-cyano-3-[4-(diethylamino)phenyl]-, ethyl ester; 4 – 1-(6-Hydroxy-2-isopropenyl-1-benzofuran-7-yl)ethanone; 5 - Octadecanamide; 6 - 9-Octadecenamide, (Z)-; 7 - 9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-; 8 - Diisooctyl phthalate; 9 - Anthraquinone, 1,8-dihydroxy-3-methyl-.

ELD20P (methanol extract)



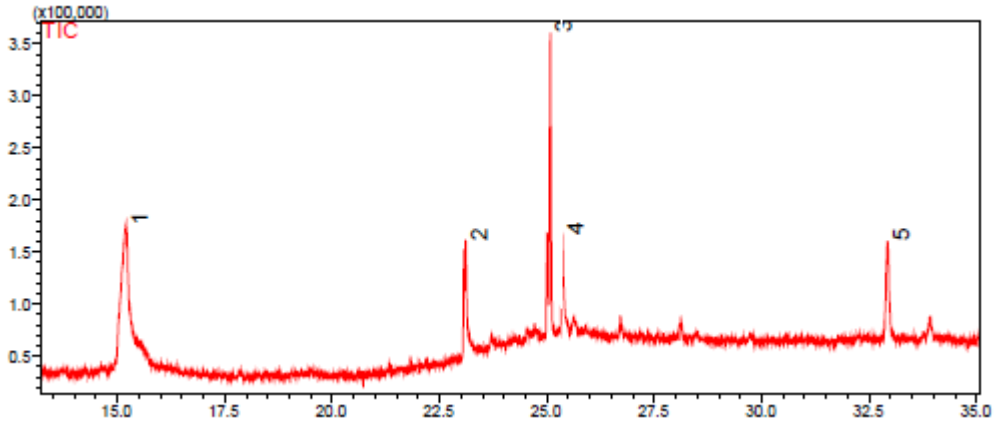
1 – 2-propenoic acid, 2-cyano-3-[4-(diethylamino)phenyl]-, ethyl ester; 2 – Xanthen-9-one, 1-hydroxy-3,5,8-trimethoxy-; 3 - 9-Octadecenoic acid, 12-hydroxy-, methyl ester, (Z)-; 4 – 1,8-Dihydroxy-3-methyl-anthraquinone; 5 - Diisooctyl phthalate; 6 - 13-Docosenamide, (Z)-.

ELD/D/76P (methanol extract)



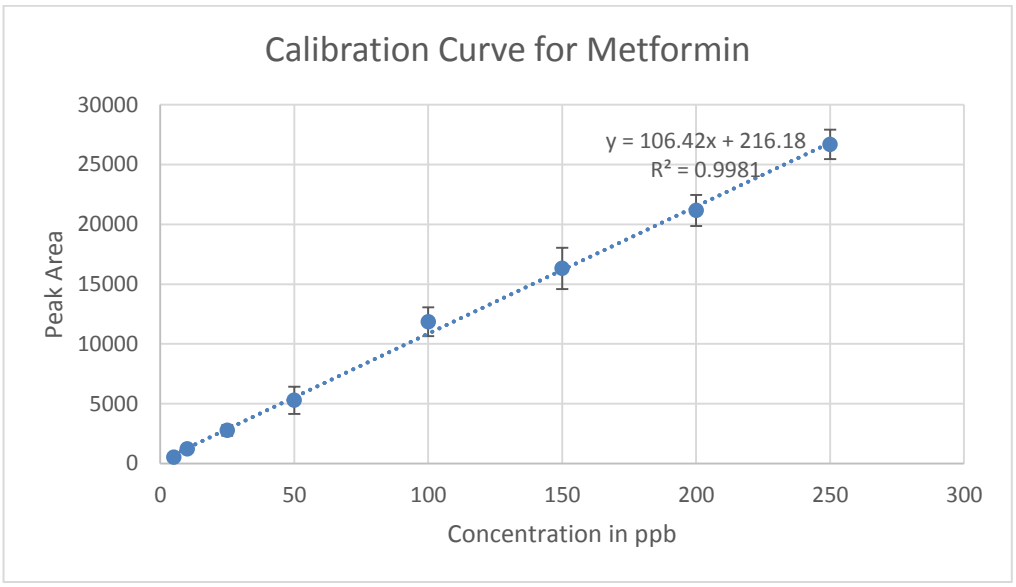
1 – 3-Acetoxy-3-hydroxypropionic acid, methyl ester; 2 – Isobutylglycerol, nitro-; 3 - Isopropyl 2,3-dideoxyhex-2-enopyranoside; 4 – Inositol, 1-deoxy- (1,2,3,4,5-Cyclohexanepentol); 5 - 1,2,3,5-Cyclohexanetetrol; 6 - 3-O-Methyl-d-glucose; 7 - 2-Butenoic acid, 4-hydroxy-, methyl ester; 8 - Phenol, 3-pentadecyl-; 9 - Diisooctyl phthalate; 10 - Columbin, (Z)-; 11 - Ibogamine; 12 - 13-Docosenamide, (Z)-.

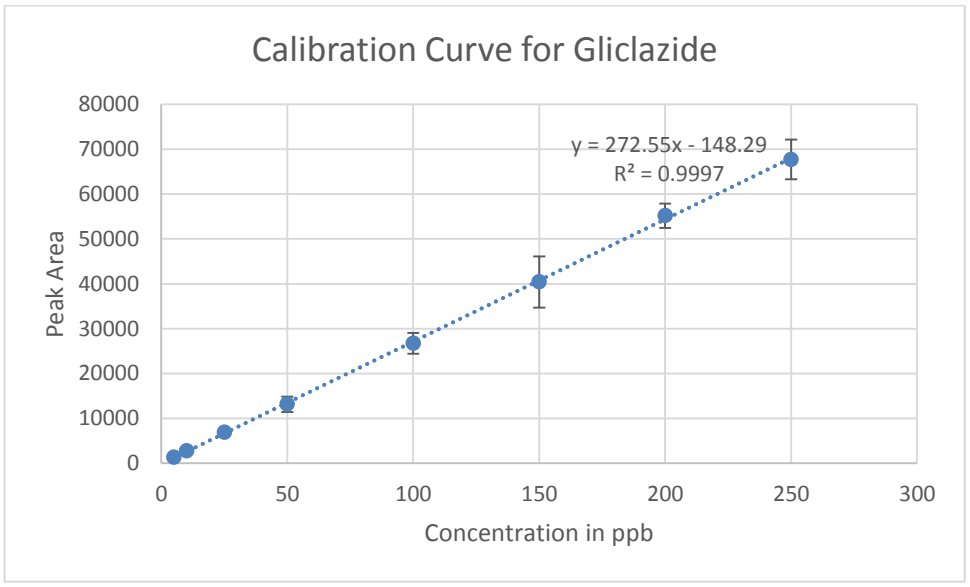
ELD/D/75P (methanol extract)

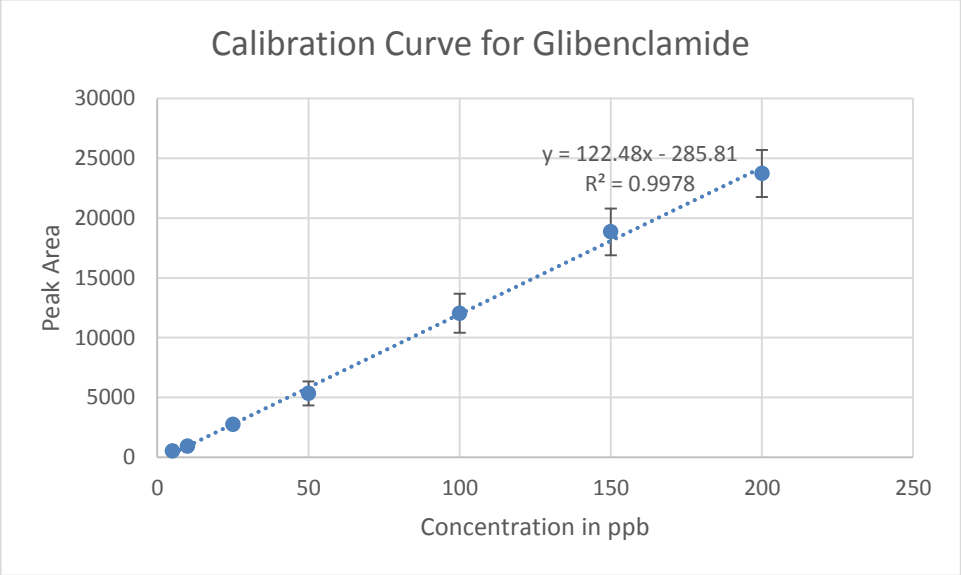


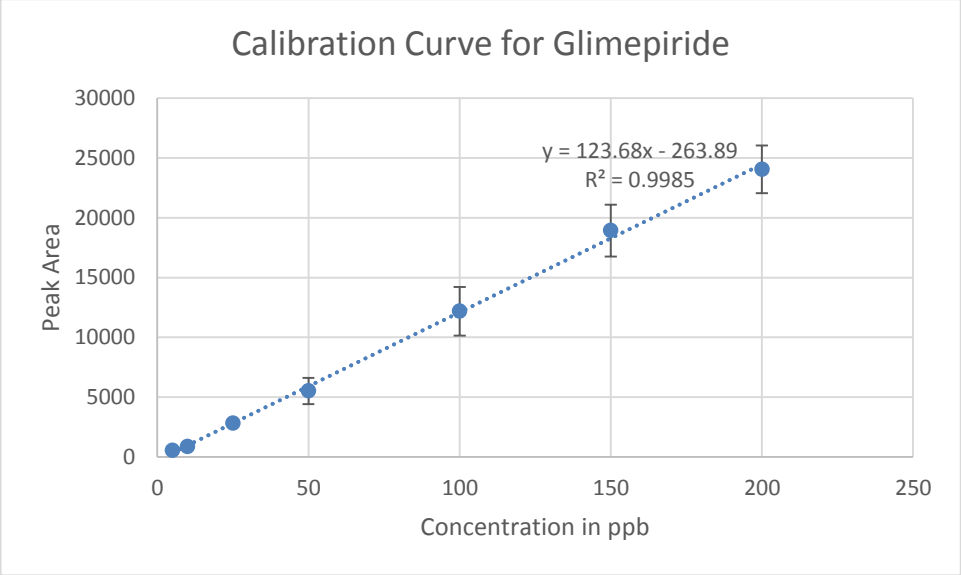
1 – 3-O-Methyl-d-glucose; 2 – (Z)-3-(pentadec-8-en-1-yl)phenol; 3 – Pseudokopsinine; 4 – 13-Docosenamide, (Z)-; 5 - Lanosterol.

Appendix IV: Calibration Curves of Selected Antidiabetic Pharmaceuticals Using LC-MS/MS






Calibration Curve for Glibenclamide
y = 122.48x - 285.81
R² = 0.9978
Peak Area
Concentration in ppb
30000
25000
20000
15000
10000
5000
0
0
50
100
150
200
250


Calibration Curve for Glimepiride
y = 123.68x - 263.89
R² = 0.9985
Peak Area
Concentration in ppb
30000
25000
20000
15000
10000
5000
0
0
50
100
150
200
250