EVALUATION OF ANTI-DIABETIC ACTIVITIES AND PROFILES OF SELECTED ERITREAN MEDICINAL PLANTS

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Evaluation of Anti-Diabetic Activities and Profiles of Selected Eritrean Medicinal Plants

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DECLARATION

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

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DEDICATION

I dedicate this work to my late father **SIUM DEMOZ**; his unwavering faith in me has been a source of constant inspiration and to my beloved wife **SABA** and our Children **FEVEN**, **YONATHAN**, **ELIANA** and **HIYAB'EL,** who continually loved, encouraged and prayed for me to complete this research work.

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ABBREVIATIONS AND ACRONYMS

ADA	American Diabetic Association
ANOVA	One-way analysis of variance
AOTT	Acute Oral Toxicity Test
β-Cell	Any of the insuling producing cells in the islets of Langerhans
CC	Column chromatography
ChemSpider	A chemical structure database with over 50 million structures
Chromasolv	High purity solvents used for HPLC and LC/MS
CRMs	Certified reference materials
DC	Diabetic control
DM	Diabetes mellitus
DMSO	Dimethyl sulphoxide
DNSA	Dinitro salicylic acid
EI	Electron impact
EIT	Eritrea Institute of Technology
ESI	Electrospray Ionization
FAAS	Flame Atomic Absorption Spectroscopy
FBGL	Fasting blood glucose level
FC	Flash chromatography
FT-IR	Fourier Transformer Infrared Spectroscopy
GC	Gas chromatography
GC-MS	Gas Chromatography and Mass Spectrometry
GDM	Gestational diabetes mellitus
HbA1C	Average levels of blood glucose over the past 3 months
HPLC	High Performance Liquid Chromatography
IC_{50}	Inhibitor concentration at which 50% inhibition of the enzyme activity occurs
ICP-MS	Inductively coupled plasma mass spectrometer
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IDF	International Diabetes Federation

In vitro	Experiment performed or taking place in a test tube, culture dish, or elsewhere outside a living organism
In vivo	Experment performed or taking place in a living organism
ISO	International organisation for standardisation
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LC	Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
METLIN	Metabolite network
MIC	Minimum inhibition concentration
NCCLS	National Committee for Clinical Laboratory Standards
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
OCC	open-column chromatography
OECD	Organization for Economic Cooperation and Development
OGTT	Oral Glucose Tolerance Test
PNPG	p-Nitrophenyl-α-D-glucopyranoside
RDA	Retro Diel-Alder
RDA	Recommended dietary allowance
R_f	Retention factor
SEM	Standard error mean
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TIC	Thin Layer Chromatography
TLC	Total ion chromatogram
UL	Tolerable upper levels
UHPLC	Ultra-high-performance liquid chromatography
UV-VIS	Ultraviolet Visible Spectrosopy
VLC	Vacuum-liquid chromatography
WHO	World Health Organization

ABSTRACT

Diabetes mellitus has become one of the largest health emergencies of the 21st century. The increase in resistance of conventional drugs, the side effects associated with the prolonged use of insulin and other hypoglycemic drugs, the unaffordable cost of buying drugs has currently brought renewed interest in plant based medicines for diabetes. Herbal drugs are cheap, readily available, safe and effective and culturally acceptable and thus WHO has recommended the investigations for alternative hypoglycemic agents from plants. The aim of the present study was to evaluate the anti-diabetic activities and profile of bioactive compounds present in the selected Eritrean medicinal plants. Based on the ethnomedicinal surveys conducted in Central and Southern Zones of Eritrea, 42 different anti-diabetic plants were identified. Preliminary phytochemical screening of the plants confirmed the presence of flavonoids, alkaloids, phenols, coumarins, steroids and others. The anti-diabetic profile of the crude methanol extracts and fractions of *Aloe camperi*, *Meriandra dianthera* and a Polyherb were initially screened using *in vitro* assays and elicited dose dependent inhibitory activities against α-glucosidase and α-amylase; comparable to Acarbose. Besides, the anti-diabetic activities of the extracts, using the *in vivo* model, showed significant decrease in the fasting blood glucose level (FBGL) by the end of the experimental day; the FBGL was significantly brought down ($P < 0.001$) by all the extracts at the end of the experimental day relative to the control. Moreover, the acute oral toxicity tests were estimated and the LD_{50} of the crude extracts was greater than 2000 mg/kg. Statistical analyses for the *in vitro* and *in vivo* experiments were performed using One-Way ANOVA followed by Dunnett's test for multiple comparisons. The levels of trace essential elements were determined using FAAS and ICP-OES, and thus the plants contain significant levels of elements including Zn, Cr, Mn, V, Se and Mg. Identification and tentative charachterisation of bioactive phytochemicals using LC-MS, GC-MS and FT-IR displayed diverse compounds. The main bioactive compounds found in *M. dianthera* were flavonoids, essential oils and fatty acid methyl esters (FAMEs). Similarly, the main compounds found in *A. camperi* were

flavonoids, phenolic acids, coumarines and FAMEs. Moreover, alkaloids, paramount FAMEs, essential oils and steroids were identified in the Polyherb. Therefore, the selected anti-diabetic plants have displayed potential bioactivity and good profile of bioactive compounds and thus can be possible candidates for herbal drug development.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes mellitus (DM) is a complex and chronic illness characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association (ADA), 2014). DM is marked by the disturbed homeostasis of carbohydrates and lipids resulting in a high concentration of glucose in the blood. DM is one of the largest health emergencies of the 21st century and projected to be the 7th leading cause of death by 2030 (Mathers & Loncar, 2006). This serious metabolic disease has a significant effect on health, quality of life, and life expectancy of diabetic patients (Sancheti & Seo, 2009). Type 2 diabetes mellitus (T2DM) is the commonest form of diabetes, has now reached epidemic proportions in most parts of the World (International Diabetes Federation (IDF) Atlas, 2015). This could be associated with the modern-day lifestyles, characterized by unhealthy diets, lack of physical inactivity, and increasing obesity (Hurt, Kulisek, Buchanan & McClave, 2010).

There are many synthetic anti-diabetic agents currently available; oral hypoglycemic drugs and insulin injections are the conventional in the management modalities (Jung *et al*., 2006). The drugs treat DM by lowering glucose levels in the blood; with the exceptions of insulin, and few others, all are administered orally. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors (Powers, 2008). Currently, medicinal plants continue to play an important role in the management of DM, especially in developing countries (Chikezie, Ojiako & Nwufo, 2015). It is estimated that more than 1200 plant species have hypoglycemic activities (Kuete, 2013). Frequently, however, it is mandatory to provide scientific proof in order to justify the use of these plants or their active components (Kayarohanam & Kavimani, 2015).

The presence of alkaloids, glycosides, flavonoids, saponins, etc. with potent anti-diabetic activities have been reported (Marles & Farnsworth, 1995). Moreover, *in vivo and in vitro* experimental models have been used to screen the anti-diabetic potential of different medicinal plants that display several effects besides lowering of the blood glucose (Rees & Alcolado, 2005; Phoboo, Shetty & ElObeid, 2015). In view of the lack of parallel studies of toxicity studies, the *in vivo and in vitro* experiments are considered screening steps in the search for drugs for the treatment of DM (Frode & Medeiros, 2008). Figure 1.1 describes some of the plant derived anti-diabetic compounds.

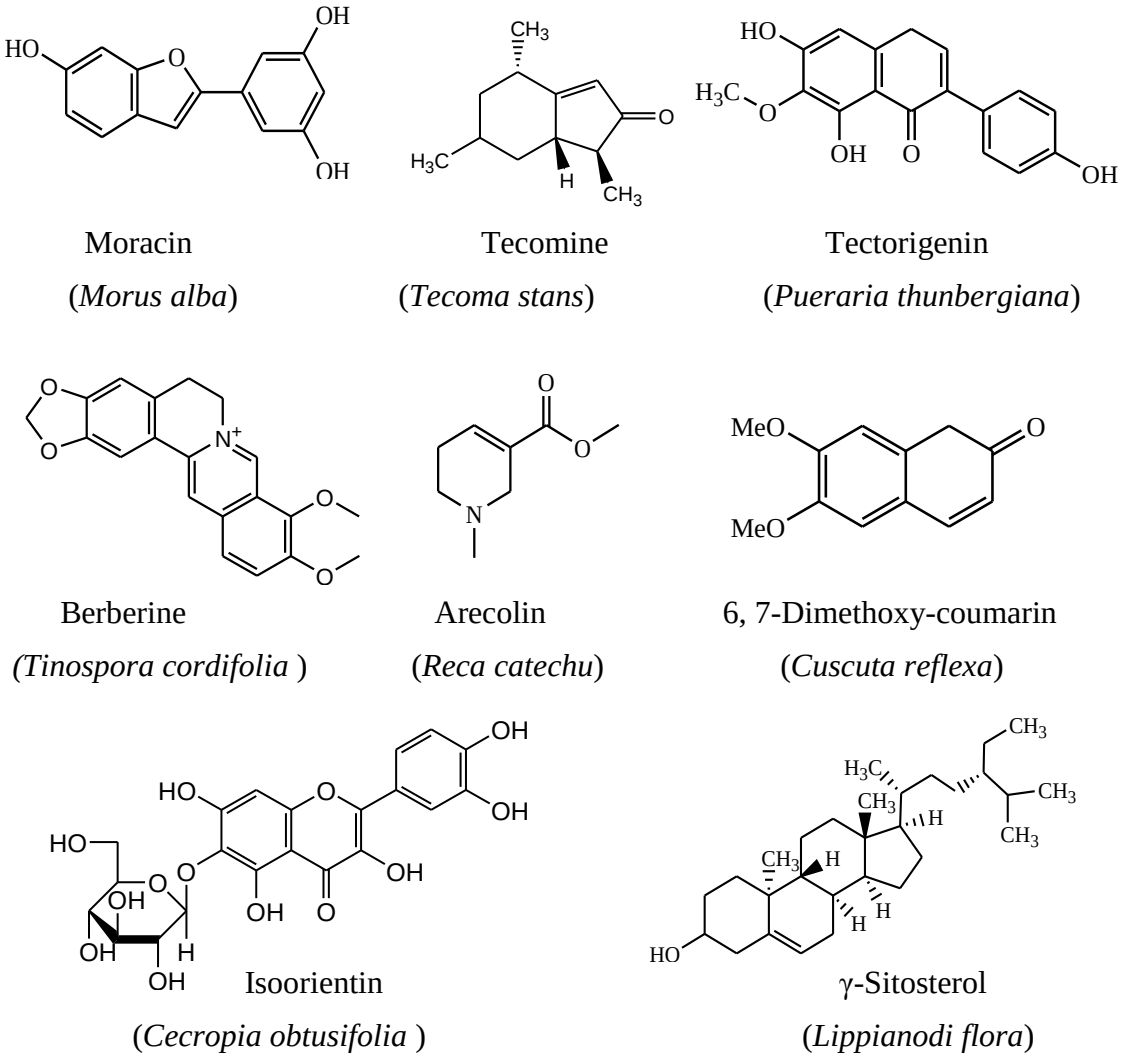


Figure 1.1: Plant derived anti-diabetic compounds

An ethnomedicinal survey is today recognized as the most viable method of identifying new medicinal plants with bioactive constituents for various ailments (Khafagi & Dewedar, 2000). The method is reported to show a greater percentage yield of bioactive useful medicinal compounds over other methods of random selection and screening. So far, several surveys and investigations have been conducted and many plants have been documented and shown positive activity for DM (Shinwaikar, Rajendran & Dinesh, 2004).

Aerial part of Aloe camperi *Aerial Part of Meriandra dianthera*

Plate 1.1: Photos of *Aloe camperi* and *Meriandra dianthera*

In this study, an ethnomedicinal survey was conducted to identify and document medicinal plants traditionally used in Central and Southern Zones of Eritrea for the treatment of DM. Thus, the plants involved in this research were selected based on the ethnomedicinal survey and literature review. The plants include *Aloe camperi, Meriandra dianthera* and a Polyherb prepared from the seeds of *Lepidium sativum, Brassica nigra and Nigella sativa.* The anti-diabetic activities and phytochemical studies related to the *Aloe camperi* and *Meriandra dianthera* are reported for the first time in this research. The plants shown in Plate 1.1 and Plate 1.2 were collected from Eritrea and brought to Kenya for bioactivity and phytochemical investigations.

Brassica nigra

Nigella sativa

Lepidium sativum

Plate 1.2: Seeds of the plants constituting of the Polyherb

1.2 Statement of the Problem

The incidence of diabetes especially T2DM is rapidly growing in the World. There is scientific consensus that in recent years the condition of diabetes has grown dramatically into a global pandemic of alarming proportions (WHO, 2016). According to the IDF Atlas 2015, estimates suggest that about 415 million adults worldwide have diabetes and were expected to rise to 642 million or more by 2040. Diabetes is an important cause of prolonged ill health and premature mortality, and

claims more lives per year than HIV-AIDS with nearly 1 death every 10 seconds (Ahmed, 2002). Approximately 5.0 million people aged between 20 and 79 years died from diabetes in 2015 and it accounted for 14.5% of global all-cause mortality among people in this age group (IDF Atlas, 2015). Diabetes mellitus is no longer a disease of predominantly rich nations and thus most markedly developing countries are at the forefront of this epidemic (WHO, 2016). It is estimated that total global health spending on diabetes accounted for USD 673 to 1,197 billion in 2015. Patients living with diabetes may need 2 to 3 three times the health-care resources compared to people without diabetes (IDF Atlas, 2015).

Moreover, the prescribed mixed medications, hypoglycemic drugs and insulin therapy, for the treatment of diabetes have been reported to have adverse effects (Halimi *et al*., 2008). The continuous use of certain synthetic drugs has been reported to worsen heart disease, increase body weight, induce hypoglycemia and others diseases. (Hollander, 2007; Lau & Teoh, 2015). Even though medicinal plants have been used as alternative therapy in the treatment of diabetes for centuries (Pandey *et al*., 2011); their scientific evaluation has not constituted a substantial area of front-line pharmacological research in diabetes. Only a limited number of these plant species have been studied and validated for their hypoglycemic activities (Mentreddy, 2007). In Eritrea, the indigenous knowledge of medicinal plants has been eroded, and no comprehensive data exists on the number and types of medicinal plants used for treatment of chronic diseases like diabetes. Research in herbal drug development and standardization is in its preliminary stage and there is no systematic and concerted approaches to these activities, for want of expertize sophisticated equipment and high-cost of chemicals (Senai, 2010).

1.3 Justification

Currently, there is growing interest in herbal remedies for diabetes mellitus due to the side effects associated with the oral hypoglycemic agents for the treatment of the disease and the higher cost of conventional drugs (Rao *et al*., 2010). Herbal drugs are prescribed due to their good effectiveness, fewer side effects in clinical

experience, relatively low costs and easier accessibility (Chawla *et al*., 2013). Moreover, herbal medicines are demanded due to culturally linked traditions and social acceptability in using them. Even though, several plant extracts have been claimed to have hypoglycemic activity but the toxicities of those extracts, active principles and mechanisms of action are generally not well known (Yeh, Eisenberg, Kaptchuk & Phillips, 2003). Therefore, there should be some mechanism to embrace the use of medicinal plants into the modern system so as to share knowledge from both traditional and modern medicine and as a means of searching drugs (Abdullahi, 2011).

To fight the global diabetes pandemic, an accessible cost-effective easily-compliant intervention that has high clinical efficacy and that is free of adverse side effects is mandatory. Therefore, this research is expected to identify and document medicinal plants used in the treatment of diabetes and contribute the knowledge and clarification on the efficacy and toxicity of some of the commonly used plants in the treatment of diabetes in Eritrea. Moreover, profiling the levels of trace elements and the bioactive compounds in the plants would support the use of the plants. The results of this research can be used in the development of herbal drugs that could be used as an alternative in the public health practices of the society.

1.4 Hypothesis

- The selected Eritrean medicinal plants do not have anti-diabetic activities.
- The plants do not constitute bioactive phytochemicals.

1.5 Objectives

1.5.1 General Objective

To evaluate the anti-diabetic activities and study the phytochemical constituents present in the selected Eritrean medicinal plants.

1.5.2 Specific Objectives

i. To carry out an ethnomedicinal survey and document the anti-diabetic plants traditionally used in in Central and Southern Zones of Eritrea.
ii. To determine the phytochemical constituents present in the crude extracts of the plants of interest.
iii. To determine the composition of trace elements associated with the anti-diabetic activity of the medicinal plants.
iv. To evaluate the toxicity and anti-diabetic efficacy of the selected plants using *in vitro* and *in vivo* bioassays.
v. To identify and characterize the bioactive secondary metabolites using different spectroscopic techniques.

CHAPTER TWO

LITERATURE REVIEW

2.1 Types of Diabetes Mellitus

The three common types of diabetes are type 1, type 2 and gestational diabetes which are briefly described below:

2.1.1 Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes mellitus, previously known as insulin dependent diabetes or juvenile onset diabetes, is an autoimmune disease characterized by deficient insulin production (ADA, 2009). T1DM mainly occurs in children and young adults and represents about 10% of all diabetes. People with type 1 diabetes are always dependent on insulin injections for survival and there is yet no proven widely available therapy to prevent or cure Type 1 diabetes. The cause of T1DM is not known and it is not preventable with current knowledge (National Institute for Health and Care Excellence (NICE), 2015).

2.1.2 Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes mellitus, previously called non-insulin dependent or mature onset diabetes, is characterised by insufficient synthesis of insulin and its secretion, secondary to insulin resistance (ADA, 2009). It is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes mellitus and the incidence and prevalence of T2DM are found to increase with age (González, Johansson, Wallander & Rodríguez, 2009). T2DM is largely the result of excess body weight and physical inactivity and is usually treated with tablets but may also require insulin injections. Some of the typical symptoms of the disease include increased thirst, excess urine, weight loss, and a clearly raised plasma glucose level. T1DM

and T2DM have some characteristic difference and thus comparisons on their clinical characteristics are shown in Table 2.1.

2.1.3 Gestational Diabetes Mellitus (*GDM*)

Gestational diabetes mellitus is glucose intolerance with onset or first recognition during pregnancy; the blood glucose values are above normal but below those diagnostic of diabetes (WHO, 2013). It is a heterogeneous pathogenic condition affecting about 2–5 % of all pregnant women during pregnancy (Shaat & Groop, 2007; Pasquel *et al*., 2009). Undiagnosed or inadequately treated GDM can lead to larger than normal babies and higher rates of maternal and infant deaths and fetal abnormalities. Women with GDM and their children are also at increased risk of type 2 diabetes later in life. Gestational diabetes is diagnosed through prenatal screening, rather than through reported symptoms (Leinonen, Hiilesmaa, Kaaja & Teramo, 2001).

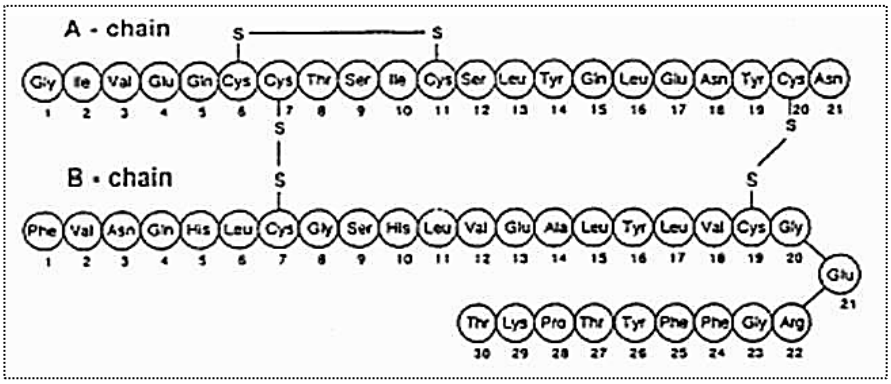
Table 2.1: Characteristics of T1DM and T2DM

Characteristics	Type 1	Type 2
Age	Childhood	Pubertal
Onset	Acute; severe	Mild-severe; often insidious
Insulin secretion	Very low	Variable
Insulin sensitivity	Normal	Decreased
Insulin dependence	Permanent	Temporary; may occur later
Genetics	Polygenic	Polygenic
Proportion of those with diabetes	About 10%	About 90%
Association: obesity	No	Strong
Autoimmune etiology	Yes	No

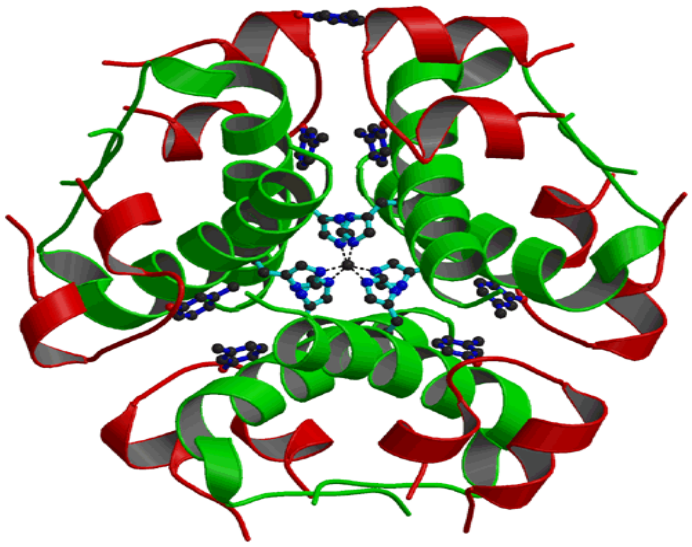
2.2 The Human Insulin

Insulin is a peptide protein hormone produced by the β-cells of the pancreas, which constitute 70% of pancreatic endocrine cells. Pancreatic β-cells are the only endocrine cells known to produce insulin. Insulin binds with and activates 80% of cells and it regulates the metabolism of glucose, fat, and protein in the body (Tengholm, 2012). It acts on all cells in the body to increase the uptake of glucose

from the blood into the cells and increases glucose transport into liver, skeletal muscle, adipose tissue, the heart and even the uterus (Wilcox, 2005). The lack or ineffectiveness, of insulin in a person with diabetes means that glucose remains circulating in the blood. Over time, the resulting high levels of glucose in the blood cause damage to many tissues in the body leading to the development of disabling and life-threatening health complications (Dimitriadis *et al*., 2011; IDF Atlas, 2015). The structure of human insulin is shown in Figure 2.1.



A. Primary Structure



B. Quaternary structure

Figure 2.1: The primary and quaternary structures of human insulin

As shown in Figure 1.4 (A), insulin is composed of two chains of amino acids: the A- chain has 21 amino acids and B- chain has 30 amino acids. The two chains are linked with each other by two cysteine disulphide bonds between CYS A7 and CYS B7 and second bond between CYS A 20 and CYS B19. There is an additional intra-chain disulphide bond connecting cysteine between A 6 and A 11 (Kansra & Sircar, 2000).

2.3 Diagnostic Criteria of Diabetes

Clinically the diabetic state is characterised by the sustained elevation of blood glucose concentration. The fasting blood glucose level (FBGL) should be used in routine screening for diabetes as well as epidemiological studies. As recommended by the ADA and WHO (WHO, 2006; ADA, 2015), the preferred method to diagnose diabetes is based on measuring the glucose levels in the blood at the different situations detailed below:

i. Random plasma glucose $\geq$ 200 mg/ dL (11.1 mmol/ L)
ii. Fasting plasma glucose $\geq$ 126 mg/ dL (7 mmol/ L)
iii. Oral glucose tolerance test (measure of plasma glucose levels 2 hour after a 75 gm oral glucose load) > 200 mg/ dL (11.1 mmol/ L)
iv. HbA_{1C} (average levels of blood glucose over the past 3 months) $\geq$ 6.5 %.

2.4 Pathogenesis of T2DM

Although the exact causes are still not known, there are multiple risk factors that could be responsible for the development of T2DM (WHO, 2016). T2DM can be triggered by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors (Cerf, 2013). The development of T2DM is clearly associated with a family history of diabetes and the genetic abnormalities reported so far, all combined, explain about 30% of the genetic factors for diabetes (Kaku, 2010). Table 2.2 describes some of the modifiable and non-modifiable risk factors for T2DM.

2.5 Pathophysiology of T2DM

In the human body a number of systems and pathways function in synchrony to bring about and maintain a healthy physiological state. At the core of these processes lies the ability of the organism to maintain a constant stable state or homeostasis. An aberration of the homeostasis leads to the development of an injury or a pathological state in various organs (Kaul *et al*., 2012).

Table 2.2: Risk factors of T2DM (Aksu, Pala & Aksu, 2006)

Non-modifiable risk factors	Modifiable risk factors
- family history of diabetes; genetic susceptibility in β-cells - ethnicity (being a member of a high-risk population) - age and gender - past history of gestational diabetes	- obesity (excess body weight) - metabolic syndrome: hypertension, decreased HDL cholesterol, increased triglyceride - lack of proper physical activity - dietary factors (poor nutrition) - other factors including dysfunctional sleep, depression and smoking

The pathophysiology of T2DM is characterized by two main pathological defects; impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance (Holt, 2004; Ozougwu, Obimba, Belonwu & Unakalamba, 2013). Impaired insulin secretion is a decrease in glucose responsiveness, which is observed before the clinical onset of disease. The impairment of insulin action in major target organs such as liver and muscles is a common pathophysiological feature of type 2 diabetes. Insulin resistance develops and expands prior to disease onset (Carrera Boada & Martínez-Moreno, 2013). When the β-cells become dysfunctional, impaired glucose tolerance and T2DM ensue because relative insulin insufficiency leads to a reduced uptake of glucose by target tissues and to an increased hepatic production of glucose (Scheen, 2003).

2.6 Complications of T2DM

People with diabetes are at higher risk of developing a number of disabling and life threatening health problems than people without diabetes (WHO, 2016). Diabetes not only kills, but is a major cause of adult blindness, kidney failure, gangrene, neuropathy and other complications. Thus, these complications have been major causes of disability, reduced quality of life and premature death (Booth, Roberts & Laye, 2012). Some of the complications are described in Section 2.6.1 to 2.6.4.

2.6.1 Diabetes Retinopathy

Diabetes is a leading cause of blindness and persistently high levels of blood glucose are the main cause of retinopathy (Hammes, Lemmen & Bertram, 2014). Retinopathy affects blood vessel formation in the retina of the eye, can lead to problems in vision like reduced vision, and potential blindness (American Academy of Ophthalmology (AAO), 2008).

2.6.2 Diabetes Nephropathy

Nephropathy is caused by damage to small blood vessels, which can cause the kidneys to be less efficient, or to fail altogether (Reutens, Prentice & Atkins, 2008). This complication on the kidneys can lead to drastic changes in the kidney tissue, loss of progressively larger amounts of protein in the urine, and gradually leading to chronic kidney disease requiring dialysis (Chan & Tang, 2015).

2.6.3 Diabetic Neuropathy

Diabetic neuropathy is the complication of diabetes affecting the nervous system, most commonly causing numbness and pain in the feet (Hinder *et al*., 2012). Diabetic neuropathy is a vascular disease effecting circulation of blood in the legs, contributing to the risk of diabetes-related foot problems (such as diabetic foot ulcers) that are difficult to treat and occasionally require limb amputation (Tesfaye *et al*., 2010).

2.6.4 Cardiovascular Diseases

Diabetes is usually associated with cardiovascular diseases which are the leading cause of death for people with diabetes. About two out of three people with diabetes die of heart disease or stroke (Bloom *et al*., 2011; Stirban & Tschoepe, 2008). The cardiovascular diseases that accompany diabetes include angina, heart attack and stroke. High blood pressure, high cholesterol, high blood glucose and other risk factors contribute to the increased risk of cardiovascular complications (ADA, 2015)

2.7 Epidemiology of T2DM

2.7.1 Global

T2DM has become an observably global public health problem. It has several new epidemiological characteristics; like the developed countries, it has also become a serious issue at an alarming rate in developing countries (Wu, Ding, Tanaka & Zhang, 2014). The prevalence of diabetes has been steadily increasing for the past 3 decades and is growing most rapidly in low- and middle-income countries (IDF Atlas, 2015). Percentage of adult diabetic population was 8.8% in 2015 and it is estimated that 10.4% of the World's population aged between 20 and 79 years will be diagnosed with diabetes. Table 2.3 describes the global prevalence in the year 2015 and a future projection of T2DM. Moreover, the estimated number of people with diabetes Worldwide and per region in adults is shown in Figure 2.2.

Table 2.3: IDF diabetes atlas global estimates of T2DM, 2015 and 2040

Diabetes in the World (20-79 years)	**2015**	**2040**
Total World population	7.3 billion	9.0 billion
Adult population	4.72 billion	6.16 billion
Child population (0-14 years)	1.92 billion	-
Global prevalence	8.8%	10.4%
Undiagnosed cases (millions)	193 million	-
Number of people with diabetes	415 million	642 million
Number of deaths due to diabetes	5.0 million	-
Total health expenditure (in USD)	673 billion	802 billion
Number of live births affected	20.9 million	-

NB: *Table extracted from IDF DIABETES ATLAS, Seventh Ed., 2015

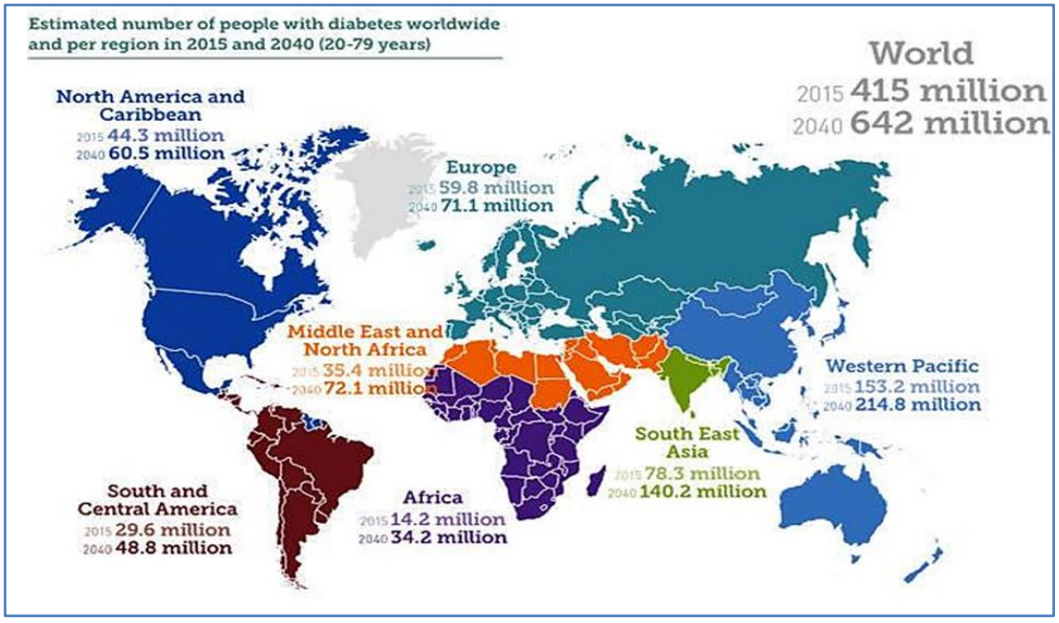


Figure 2.2: Estimated number adults with diabetes worldwide (IDF Atlas, 2015)

2.7.2 Sub-Saharan Africa

Africa is experiencing a rapid epidemiological transition with the burden of non-communicable diseases especially diabetes that will overwhelm the health care systems which are already overburdened by HIV/AIDS, tuberculosis (TB) and malaria (Agyei-Mensah & Aikins, 2010). The rapid uncontrolled urbanization and major changes in lifestyle could be driving this epidemic (Hu, 2011). Diabetes has been rising significantly across sub-Saharan African countries and has been posing a substantial public health and socio economic burden (Renzaho, 2015; Jean, 2010).

In 2015, 14.2 million adults in the African region of WHO were estimated to be living with diabetes mellitus. IDF estimates that more than two thirds of people with diabetes in Africa are undiagnosed and more than 321,100 deaths in the Africa region could be attributed to diabetes (IDF Atlas, 2015). Of all of WHO's regions, the African region is expected to have the largest proportional increase (90.5%) in the number of adult diabetics by 2030 (Whiting, Guariguata, Weil & Shaw, 2011). In sub-Saharan Africa, where resources are often lacking and governments may not prioritise screening for diabetes, the average proportion of people with diabetes who are undiagnosed is 66.7% (Mbanya *et al*., 2010).

2.7.3 The Situation of Diabetes in Eritrea

In Eritrea, the prevalence of DM in adults was estimated at 74, 200 cases (about 3.6 % of the population) and the number of deaths in adults due to diabetes was 1,194 in 2015. The number is expected to rise largely because there is still a large number, above 50%, of undiagnosed patients (IDF Atlas, 2015). Even though, there hasn't been recent published data on the prevalence of diabetes in Eritrea, mortality data from hospitals and health centers show that, diabetes mellitus, is one among the 10 leading causes of deaths in recent years (Usman *et al*., 2006; Seyum *et al*., 2010).

2.8 Management of T2DM

The main aim in the management of diabetes is to maintain blood glucose levels as near to normal as possible, while avoiding hypoglycemia (Inzucchi *et al*., 2015; Kaul *et al*., 2012). The major components of the treatment of T2DM using pharmacological and non-pharmacological interventions are described under Sections 2.8.1 and 2.8.3.

2.8.1 Non-Pharmacological Interventions

For those who are diagnosed with diabetes, a series of cost-effective interventions can improve their outcomes. Good glycemic control can be considered as the cornerstone management in the prevention of diabetic complications (WHO, 2016). Lifestyle modifications are the foundations in the management of DM and include the prescription of healthy diet, regular exercise, the management of stress, and avoidance of tobacco (Tuomilehto, 2009; Khan, 2012). Eating healthily is of fundamental importance as part of diabetes healthcare behaviour and has beneficial effects on weight, metabolic control and general well-being (Chan & Woo, 2010). People with T2DM diabetes can be prescribed dietary choices for achieving weight loss that may also improve glycemic control. Options include simple caloric restriction, reducing fat intake, consumption of carbohydrates with low rather than high glycemic index, and restricting the total amount of dietary carbohydrate (ADA, 2004). Moreover, physical activity promotes weight reduction and improves insulin

sensitivity, thus improve glucose uptake by increasing insulin sensitivity and lowering body adiposity (Knowler, Barrett-Connor & Fowler, 2002). Physical inactivity and poor physical fitness have been associated with increased mortality among persons with established T2DM (Wei *et al*., 2000).

2.8.2 Pharmacological Intervention

The main pharmacological interventions for diabetes management include oral hypoglycemic and insulin therapies.

2.8.2.1 Oral Hypoglycemic Therapy

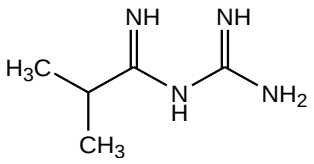
Oral hypoglycemic therapies are considered only after a regimen of dietary treatment combined with exercise has failed to achieve the therapy targets set (Garcia-Perez *et al*., 2013). Currently available oral anti-diabetic agents include sulfonylureas, biguanides, α- glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation (Luan & Feingllos, 2001; Krentz & Bailey, 2005). Examples of oral hypoglycemic drugs, their mechanism of action and some adverse effects are summarized in Table 2.4 and the structures of some of the oral hypoglycemic agents are shown Figure 2.3.

2.8.2.2 Insulin Therapy

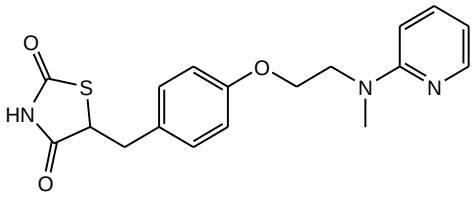
Insulin therapy is indicated in the treatment of T2DM for initial therapy of severe hyperglycemia, after failure of oral agents or during perioperative or other acute hyperglycemic states (Irons & Minze, 2014). The need for insulin depends upon the balance between insulin secretion and insulin resistance; patients with T2DM require insulin as their β- cell function declines over time (Cerf, 2013). Weight gain and hypoglycemia are common side effects of insulin therapy. Vigorous insulin treatment may also carry an increased risk of atherogenesis (Pandey *et al*., 2011).

Table 2.4: Oral hypoglycemic agents and their mode of actions

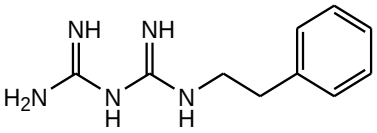
Drug class	Drug name	Physiological action	Some adverse effects
Biguanides	Metformin Phenformin	reduce gluconeogenesis in the liver (insulin sensitizers)	diarrhea, abdominal cramps, nausea and increased vomiting
Sulfonylureas (2^{nd} generation)	Glimepiride Glipizide Glibenclamide	increase insulin section by pancreatic β-cells (insulin secretagogues)	hypoglycemia,weight gain, increased risk of cardiovascular death
Meglitinides	Repaglinide Nateglinide	increase insulin secretion by pancreatic β- cells (insulin secretagogues)	increased risk for infection, headache
Thiozolidinediones	Pioglitazone Rosiglitazone	increase glucose uptake by skeletal muscles (insulin sensitizers)	increased cardiovascular risk, oedema, weight gain
Alpha glucosidase inhibitors	Acarbose Migitol	Inhibit carbohydrate absorption in the small intestine	flatulence, diarrhoea, pneumatosis



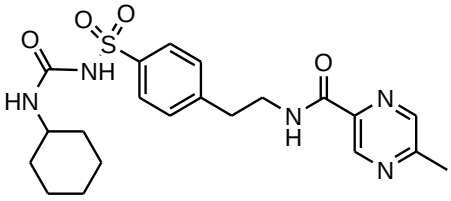
Metformin



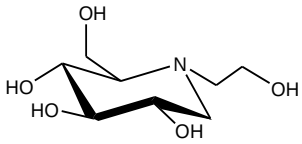
Rosiglitazone



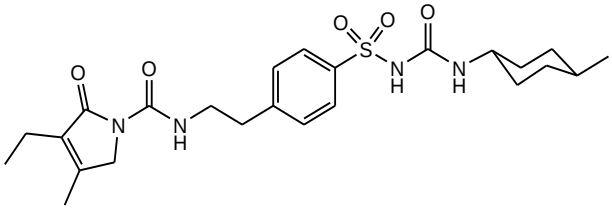
Phenphormine



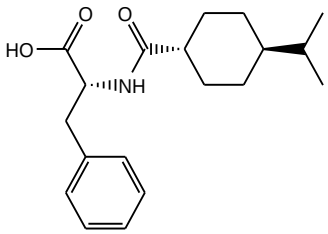
Glipizide



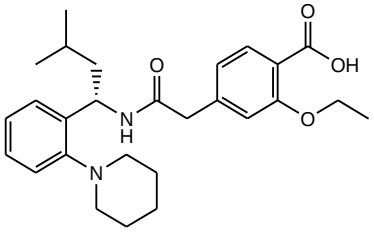
Migitol



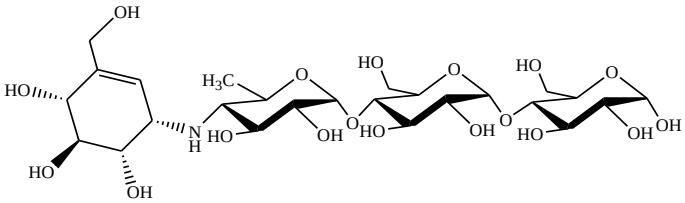
Glimepiride



Nateglinide



Repaglinide



Acarbose

Figure 2.3: Chemical structures of some oral anti-diabetic drugs

2.9 Ethnobotany in Medicinal Plants Research

Popular knowledge of plants used by humans is based on thousands of years' experience. By "trial and error", people learnt how to recognize and use plants, including those with a magic-religious function (Camejo-Rodrigues, Ascensão, Bonet, & Vallès, 2003). Ethnomedicinal studies have become increasingly valuable in the development of health care and conservation programs in different parts of the world (Ososki *et al*., 2002). The term ethnobotany was first coined by an American botanist John Harshburger, in 1896, in an attempt to study the plants used by the primitive and aboriginal people. Since then, it has been defined as the traditional knowledge of indigenous communities, about surrounding plant diversity and how various people make use of indigenous plants found in their localities. Ethnobotany involves the study of how communities of a particular region make use of indigenous plants in the region for food, clothing and medicine (Aiyeloja & Bello, 2006). Ethnobotany focuses on documenting, analysing and using of indigenous knowledge, beliefs and practices related to plant resources (Aumeeruddy-Thomas & Shengji, 2003). Research by Hostettmann *et al*. (2000) showed that the knowledge on the use of medicinal plants is enormous but if this traditional knowledge is not rapidly researched and recorded, indications are that it will be lost with succeeding generations.

2.10 Medicinal Plants for Diabetes Management

2.10.1 Introduction

Alternative systems of medicine based on plant extracts have thrived through the ages and are still practiced by a large population for the management of diabetes (Rizvi, Matteucci & Atukeren, 2013). Ethnomedicinal surveys indicate that more than 1,200 plants have been used in traditional medicine systems following claims of their hypoglycemic properties (Dey, Attele & Yuan, 2002). A large number of the plants have proved their efficacy as hyperglycemic (Chikezie *et al*., 2015). Recent scientific investigation has confirmed the efficacy of many anti-diabetic plant preparations some of which are very effective and relatively non-toxic (Piero

et al., 2011). The hypoglycemic effects of these plants or their preparation have been evaluated and confirmed using *in vitro* assays and animal models (Phoboo *et al.*, 2015); some have also been evaluated in humans (Jayawardena, De-Alwis, Hettigoda & Fernando, 2005). Most of these plants contain glycosides, alkaloids, terpenoids, flavonoids, polysaccharides, and saponins, which are frequently implicated of having anti-diabetic effect (Jung *et al*., 2006). The WHO expert committee on diabetes recommended that medicinal herbs be further investigated as they are frequently considered to be less toxic and free from side effects. Therefore, a search for safe and more effective agents has continued to be an important area of active research (Halberstein, 2005).

2.10.2 Mechanism of Action of Anti-Diabetic Plants

The anti-diabetic activity of medicinal plant depends upon a variety of mechanisms. Medicinal plants with blood lowering effect have the ability to modulate one or more of the pathways that regulate insulin resistance, β-cell function, GLP-1 homeostasis, and glucose (re) absorption (Chang *et al*., 2013).

Some of the proposed mechanisms of action of anti-diabetic plants include:

i. Stimulation of insulin secretion, inhibition of insulin degradative processes and reduction of insulin resistance (Pulok *et al*, 2006).
ii. Providing certain essential elements like calcium, zinc, magnesium, manganese and copper for the β - cells (Dwivedi & Daspaul, 2013).
iii. Increasing the size and number of cells in the islets of langerhans, regenerating and/or repairing pancreatic β - cells and protecting the destruction of the β- cells (Oh, 2015).
iv. Prevention of the pathological conversion of starch to glucose and stimulation of glycogenesis and hepatic glycolysis (Chawla *et al*., 2013).
v. Inhibition of α-amylase and α–glucosidase enzymes (Kazeem, Adamson & Ogunwande, 2013).
vi. Preventing oxidative stress in pancreatic β- cell dysfunction (Hosseini, Shafiee-Nick, & Ghorbani, 2015).

2.10.3 Commonly Used Anti-Diabetic Plants

Many studies have confirmed the benefits of medicinal plants with hypoglycemic effects in the management of diabetes mellitus. The plant families most studied for their hypoglycemic effects include: Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, Euphorbiaceae and Araliaceae (Bnouham *et al*., 2006). Although there is no herbal substitute for insulin, some herbs may help adjust blood sugar levels or manage other diabetic symptoms (Crutchfield, 2003). Some of the commonly used anti-diabetic plants and their proposed mode of actions are listed below.

i. Bitter melon (*Momordica charantia*) is commonly used in the traditional medicine for its glucose-lowering effects (Sridhar *et al*., 2008).
ii. Fenugreek (*Trigonella foenum-graecum*) has been shown in some studies to reduce blood insulin and glucose levels while also lowering cholesterol (Jelodar *et al*., 2005).
iii. Aloe vera (*Aloe barbadensis*) contains glucomannan, a hydro-soluble fiber which has a glucose-lowering effect (Surjushe, Vasani & Saple, 2008).
iv. Neem (*Azadirachta indica*) increased insulin sensitization, insulin secretion, and β- cell regeneration (Hosseini, Shafiee-Nick, & Ghorbani, 2015).
v. Garden cress (*Lepidium sativum)* inhibits renal glucose reabsorption (Eddouks & Maghrani, 2008).
vi. Black cumin (*Nigella sativa*) inhibits hepatic gluconeogenesis (Abbasnezhad, Hayatdavoudi, Niazmand & Mahmoudabady, 2015).
vii. Black mustard (*Brassica nigra*) lowers blood glucose by unclear mechanisms (Anand *et al*., 2007).
viii. Gurmar (*Gymnema sylvestre*) increases utilization of glucose or by direct stimulation of glucose uptake through hepatic metabolism, increased insulin release from pancreatic β- cells (Patel, Prasad, Kumar & Hemalatha, 2012).
ix. Onion (*Allium cepa*) and garlic (*Allium sativum*) inhibit insulin breakdown and thus lower blood sugar (Lucy *et al*., 2002).
x. Winter cherry (*Withania somnifera*) regenerates pancreatic β-cells thereby preventing diabetes induction (Andallu & Radhika, 2000).

2.10.4 Limitations and Challenges of Herbal Medical Practices

Herbal medications are the most commonly used alternative therapy for blood sugar control. However, their safety and efficacy need to be further evaluated by well-designed, controlled clinical studies (Dey, Attele & Yuan, 2002). The number of reports of patients experiencing negative health consequences caused by the use of herbal medicines has increased in recent years (Ekor, 2013). The following are some of the common limitations of traditional herbal treatments for diabetes and other diseases.

i. All herbal drugs are not safe; some may be poisonous or may cause allergenic reactions (Bent, 2008).
ii. Unlike the modern medicines, designed to work at molecular level of physiology, many herbal drugs take a long time to deliver the results (Devi, Jain & Valli, 2010).
iii. Self-treatment with herbal drugs may consist of many risk factors, over dosage and inappropriate use of the herb (Phua, Zosel & Heard, 2009).
iv. Consumption of herbal drugs without correct identification of the plant i.e. the inadvertent use of the wrong plant species or wrong part of plant may cause harm (Ekor, 2013).
v. There is some contamination with undeclared toxic and/or hazardous substances, caused by environmental factors (WHO, 2007).
vi. Lack of scientific data in support of the medicinal activity claimed and their safety and efficacy assumed (Moreira *et al*., 2014).
vii. Lack of safe and standardization procedures in herbal preparations (Bent, 2008).

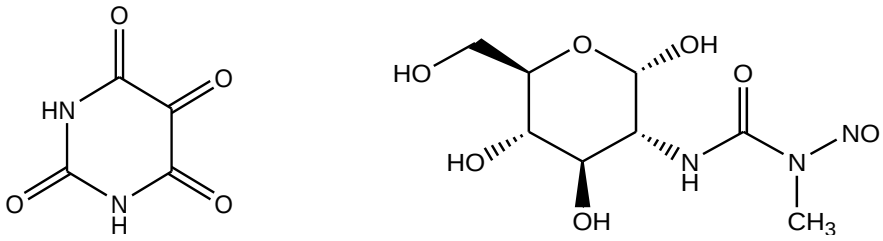
The most criticized aspect of utilizing herbal medicines is the lack of standardization. The quality of herbal medicine and the profile of the constituents in the final product have implication in efficacy and safety (Bent, 2008). Due to the complex nature and inherent variability of the constituents of plant based drugs, it is difficult to establish quality control parameters but modern analytical technique are

expected to help in circumventing this problem (Kunle, Egharevba & Ahmadu, 2012).

2.11 *In Vivo* Anti-Diabetic Assays

Experimental diabetes mellitus has been induced in laboratory animals by several methods. The induction can be made by pharmacologic, surgical or genetic manipulations in several animal species (Frode & Medeiros, 2008). The second method for creating diabetes in animals is injecting drugs such as Alloxan or Streptozotocin (STZ) (Akbarzadeh *et al*., 2007; Ojiako, Chikezie & Ogbuji, 2016). Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals (Rees & Alcolado, 2005). Alloxan (31 %) and STZ (69 %) are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease (Etuk, 2010; Bajpai, Rather & Nam, 2016). Both drugs exert their diabetogenic action when they are administered parenterally (intravenously, intraperitoneally or subcutaneously). The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status (Lenzen, 2008).

Alloxan, a pyrimidine derivative shown, is a very unstable chemical compound (Rohilla & Ali, 2012) with a molecular shape resembling glucose (Figure 2.4). It has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase and it causes a state of insulin-dependent diabetes through its ability to induce reactive oxygen species formation, resulting in the selective necrosis of β-cells (Mir & Darzi, 2009). Streptozotocin (Figure 2.4) inhibits insulin secretion and causes a state of insulin-dependent diabetes mellitus. Both effects can be attributed to its specific chemical properties, namely its alkylating potency (Lenzen, 2008).



Alloxan Streptozotocin

Figure 2.4: Chemical structures of Alloxan and Streptozotocin

2.12 *In Vitro* Anti-Diabetic Assays

In vitro tests can play a very important role in the evaluation of anti-diabetic activity of drugs as initial screening tools where the screening of large number of potential therapeutic candidates may be necessary (Thorat, Patil, Limaye & Kadam, 2012). Some of the commonly used methods *in vitro* anti-diabetic screenings include cytotoxicity study in L6 rat skeletal muscle cells, glucose uptake activity in L6 rat skeletal muscle cells and inhibition of carbohydrate-metabolizing enzymes pancreatic α-amylase and intestinal α-glucosidase activities (Ramachandran, Rajasekaran & Adhirajan, 2013). The methods employed in this research are the enzyme inhibition approaches. These methods target at lowering the corresponding post-prandial blood glucose values by impeding the breakdown and intestinal absorption of glucose through the inhibition of the enzymes and the control of which is therefore, an important aspect in treatment of diabetes (Franco, Rigden, Melo & Grossi-de-sa, 2002; Pinto *et al*., 2009). α-Amylase is a key enzyme in digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally to glucose. Degradation of this dietary starch proceeds rapidly and leads to elevated post-prandial hyperglycemia (Tarling *et al*. 2008). Similarly, the α-glucosidase inhibitors are considered to inhibit the degradation of disaccharides to monosaccharides, thereby maintaining the normal blood sugar level (Goldstein & Muller-Wieland, 2013).

2.13 Trace Elements and Diabetes

2.13.1 Introduction

The human physiology requires a number of minerals in order to maintain a proper health because macro- and microelements influence biochemical processes in life (Ajasa *et al*., 2004). A number of studies have been conducted and published related to the macro and micro-nutrient contents of medicinal plants. It has been thought that medicinal plants contain elements with remarkable importance for human metabolism, disease prevention and healing (Obianjunwa, Adeleke, Adebajo & Omubuwajo, 2004).

Trace element studies play an important role in pharmaceuticals and clinical studies; quantitative analysis of different trace elements is important for determining the efficacy and toxicity of medicinal plants (Rajua *et al*., 2006; Arceusz, Radecka & Wesolowski, 2010). Some researchers have shown that some minerals and trace elements beneficially affect the complications of DM (Guerrero-Romero & Rodriguez-Moran, 2009; Purnima & Kazi, 2015). It is widely believed that some trace elements, such as Zn, Cr, V, Mg, Mn, and Se serve as cofactors of antioxidative enzymes and play an important role in protecting the insulin secreting pancreatic β-cells, which are sensitive to free radical damage (Ngugi *et al*., 2012, Candilish, 2000). It has also been reported that the imbalance of some essential metals might adversely affect pancreatic islet and cause development of diabetes (Chen *et al*., 2009) and thus some trace elements have been recommended as dietary supplement to alleviate the impaired insulin metabolism in diabetic patients (Li *et al*., 2013; Clark *et al*., 2014). These elements are important for the normal functioning of the body but they can be harmful and toxic when their concentration exceeds the recommended limit (Khan, Ali & Tullah, 2008).

The most widely and commonly used techniques of elemental analysis, providing acceptable levels of precision and accuracy, include inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometer (ICP-MS) and flame atomic absorption spectroscopy (FAAS) (Rury,

2016). A brief summary of the bioactivity related to the trace elements studied in this research, including Zn, Cr, V, Mn, Mg and Se are summarized in Sections 2.13.2 to 2.13.7.

2.13.2 Zinc (Zn)

Zinc is an essential mineral that is required to maintain the normal structure, function, and proliferation of cells and is involved in many biochemical reactions. It plays a crucial role in the storage and secretion of insulin, which subsequently increases the uptake of glucose (Rungby, 2010). Supplementation of zinc to diabetic rats has mediated up-regulation of cardiac metallothionein as a potent anti-oxidant and thus prevented the development of diabetic cardiomyopathy (Tang *et al*., 2008). Zinc supplementation is also effective for preventing or ameliorating diabetes in several rodent models of Type 1 and Type 2 diabetes (Taylor, 2005). Moreover, it may aggravate the insulin resistance in Type 2 diabetes and thus cause complications (Hashemipour *et al*., 2009). Other data have also suggested that the defect in zinc absorption is associated with hyperglycemia or diabetes (Singh, 2014).

2.13.3 Chromium (Cr)

Chromium is a crucial trace element with many sites of action and has a vital biological activity which is necessary in glucose homeostasis (Balk *et al*., 2007). It regulates insulin and blood glucose levels by stimulating insulin signalling pathway and metabolism and thus it may improve insulin sensitivity. Modulation of lipid metabolism by chromium in peripheral tissues may represent an additional novel mechanism of action (Qiao *et al*., 2009; Cefalu *et al*., 2010). Deficiency of chromium or its biological active form has been implicated in the pathogenesis of some forms of glucose intolerance, reversible insulin resistance and diabetes (Cefalu & Hu, 2004). Moreover, its deficiency has also been held responsible for vascular complications associated with diabetes mellitus (Rabinowitz, Levin & Gonicket, 1980).

2.13.4 Vanadium (V)

Vanadium has been known for long to possess anti-diabetic properties (Thompson & Orvig, 2006). It affects various aspects of carbohydrate metabolism including glucose transport, glycolysis and glucose oxidation and glycogen synthesis (Wiernsperger & Rapin, 2010). The vanadium acts primarily as an insulin mimetic agent, although enhanced insulin activity and increased insulin sensitivity have also been noted (Cam, Brownsey & McNeill, 2000). Besides, it stimulates glucose uptake without affecting endogenous levels (García-Vicente *et al*., 2007). Vanadium has been shown to improve sensitivity to insulin in both Type 1 and Type 2 diabetes and also to lower cholesterol levels and blood pressure (Cusi *et al*., 2001).

2.13.5 Manganese (Mn)

Manganese acts as a cofactor in several enzymes including those involved in bone marrow production and metabolism of carbohydrates, proteins and fats (Orbea el. al., 2002). It has been reported that manganese involved in normal immune functions, regulation of blood sugar and cellular energy, and the defence mechanisms against free radicals (Aschner & Aschner, 2005). It activates certain enzymes that play important roles in the metabolism of carbohydrates, amino acids, and cholesterol. Manganese is also required for normal insulin synthesis, its secretion and an alteration in its metabolism has been implicated in diabetes development (Kazi *et al*., 2008). Manganese deficiency can result in impaired glucose tolerance, altered carbohydrate and lipid metabolism, impaired insulin secretion and skeletal abnormalities (Nicoloff, Mutaftchiev, Strashimov & Petrov, 2004).

2.13.6 Selenium (Se)

Selenium is involved in the complex system of protection against oxidative stress through selenium-dependent glutathione peroxidases and other selenoproteins (Burk, 2002). Due to its anti-oxidant properties, selenium might be preventing the development of diabetes and prevents the development of complications in diabetic

patients (Pallauf *et al*., 2008). In addition, selenate, an inorganic form of selenium, mimics insulin activity in experimental models (Mueller & Pallauf, 2006). In humans and experimental studies, selenium deficiency has been suggested to result in increased risk of various pathologies including cardiovascular diseases (Tanguy, Grauzam, de Leiris & Boucher, 2012). Selenium can inhibit high glucose- and high insulin-induced expression of adhesion molecules and thus it may be considered as a potential preventive intervention for diabetes-accelerated atherosclerosis (Zheng *et al*., 2007).

2.13.7 Magnesium (Mg)

Magnesium is a cofactor of various enzymes in carbohydrate oxidation and plays an important role in glucose transporting mechanism of the cell membrane. It is also involved in insulin secretion, binding and activity (Lopez-Ridaura *et al*., 2004). Magnesium has a vital role in the phosphorylation reactions of glucose and its metabolism and it may influence the release and activity of the hormones that helps control blood glucose levels (Chaudhary, Sharma & Bansal, 2010). Magnesium deficiencies occur frequently in diabetics and thus oral magnesium supplementation has been found to improve insulin sensitivity even in overweight, nondiabetic subjects with normal magnesium levels (Mooren *et al*., 2011). It was demonstrated that magnesium deficiency might lead to a decrease in insulin mediated glucose uptake and has been associated with the development of insulin resistance (Viktorinova, Toserova, Krisko & Durackova, 2009).

2.14 Flavonoids and Diabetes

2.14.1 Common Flavonoids Found in Plants

Flavonoids constitute the largest group of natural polyphenolic molecules of plant origin which function as phytochemicals (Corradini *et al*., 2011; Pinent *et al*., 2008). There are more than 6000 known flavonoids and are widely distributed in many frequently consumed beverages and food products of plant origin such as fruit, vegetables, wine, tea and cocoa (Ross & Kasum, 2002; Vinayagam & Xu, 2015).

Flavonoids are bioactive phenols with low molecular weight and play a major role in the cell synthesis and they are known for their anti-oxidant, anti-inflammatory, anti-viral, anti-carcinogenic and anti-diabetic properties (Nijveldt *et al.*, 2001; Batra & Sharma, 2013; Tapas, Sakarkar & Kakde, 2008). Flavonoids are categorized, according to their chemical structures, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. All flavonoids share the basic C6- C3- C6 structural skeleton (Figure 2.5), consisting of two aromatic C6 rings (A and B) and a heterocyclic ring (C) that contains one oxygen atom.

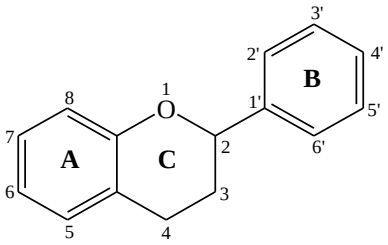


Figure 2.5: Structure of flavonoid backbone

Although they are sometimes found as their aglycones, flavonoids most commonly occur in plant materials as flavonoid O-glycosides, in which one or more hydroxyl groups of the aglycones are bound to a sugar, forming an acid-labile glycosidic O-C bond (Tsao, 2010). Flavonoids are usually isolated as mixtures from several plants and thus the isolation and identification of each compound is often difficult, due to similarities of their structures and polarities (Tsimogiannis, Samiotaki, Panayotou G. & Oreopoulou, 2007).

2.14.2 Bioactivity of Flavonoids

The main anti-diabetic activity and related anti-oxidant activities of flavonoids are discussed below.

2.14.2.1 Anti-Oxidant Properties of Flavonoids

Flavonoids can exert their anti-oxidant activity by various mechanisms, e.g., by scavenging or quenching of free radicals, donating of hydrogen, chelating of metal ions, or inhibiting enzymatic systems responsible for free radical generation (Firuzi *et al*., 2005; Bischoff, 2008). The anti-oxidant effects of flavonoids are enhanced by the number and position of hydroxyl groups in the molecule, the catechol structure (O-dihydroxy group in the B ring), presence of unsaturation (C2 - C3 double bond) and 4- oxo function (keto double bond at position 4 of the C ring) in the C- ring (Heim, Tagliaferro & Bobliya, 2002). The catechol structure possess electron donating properties and are capable of readily donating electron to stabilize radical species, the 4-oxo in association with the C2- C3 double bond increases the radical scavenging activity by delocalizing electrons from B ring, the 3- OH moiety of the C-ring generates an extremely active scavenger. Moreover, the 5- OH and 7- OH groups may also add scavenging potential in certain cases (Amic *et al*., 2007). Flavonoids may be capable of binding the transition metal ions, such as copper and iron, which play a role in glycoxidation, thus preventing metal-catalysed formation of hydroxyl radicals or related species from H_2O_2 (Treml & Smejkal, 2016).

2.14.2.2 Anti-Diabetic Properties of Flavonoids

Flavonoids are among the classes of compounds that have received the most attention with regard to their anti-diabetic properties (Soumyanath, 2006). Numerous studies have been carried out to explore the potential role of flavonoids and thus the anti-diabetic activities of flavonoids have been reported (Kesari *et al*., 2006; Qi *et al*., 2010). The role flavonoids are quite important in fighting with the complications of diabetes mellitus than any other method of treatment. They are capable of improving, stabilizing and long sustaining the insulin secretion, human islets and pancreatic cell respectively (Abdulrahman & Hasan, 2013). Regulation of the postprandial glucose by inhibiting starch digestion, delaying the gastric emptying rate and reducing active transport of glucose across intestinal brush border membrane is one of the mechanisms by which flavonoids in

diet can reduce the risk of T2DM (Heilbronn, Smith & Ravussin, 2004). The general mechanism of action of flavonoids is outlined in Figure 2.6.

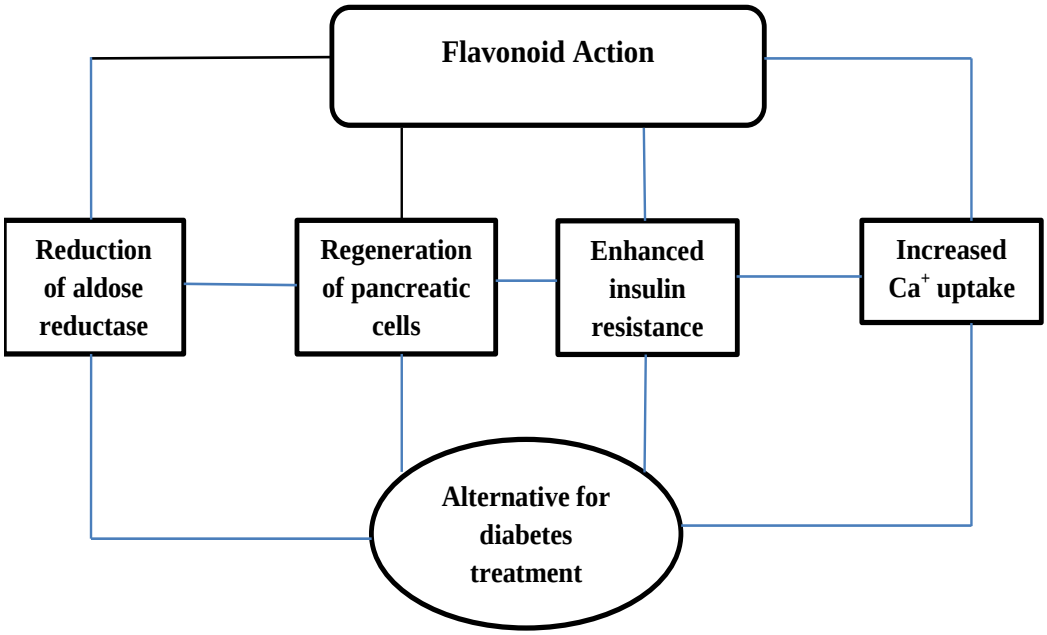


Figure 2.6: Flavonoids function in diabetes mellitus

2.14.3 Common Fragmentation Patterns of Flavonoids

In flavones and flavonols, the most important retro diel-alder (RDA) fragmentations are the 0/2, the 0/4, the 1/3. While the 0/4 pathway appears to be exclusive of flavones, where it leads to the $^{0,4}B^{+}$ ion, which loses water to form the $^{0,4}B^{+}$ - H_2O, the $^{0,2}A^{+}$ is an exclusive of flavonols; also, the $^{1,3}B^{+}$ ion appears to be exclusive of flavones. In common, flavonoids from both classes give rise to the product ions $^{1,3}A^{+}$, and $^{0,2}B^{+}$ (Cuyckens & Claeys, 2005). In particular, losses of one and two C_2H_2O moieties from the precursor ion, involving all the rings, and the formation of $^{1,3}A^{+}$ - C_2H_2O ion, are the most useful (Justino, Borges & Florencio, 2009). Moreover, fragments resulting from fission of the C- rings are specific of each subgroup and revealed the substitution pattern of A- and B- rings. The plausible fragmentation patterns of flavones, flavonols and glycosylated flavonols are shown in Figure 2.7.

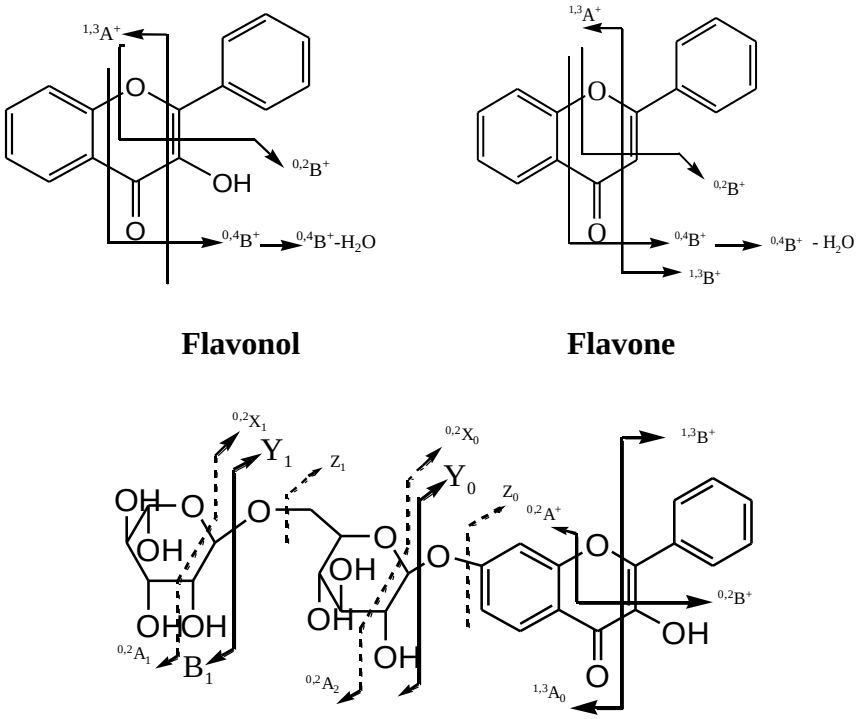


Figure 2.7: Common fragmentation patterns of flavonoids

2.14.4 Common Adducts in the Postive Ion Mode in Electrospray Ionization (ESI)

There are some common background ions observed during the analysis of compounds of interest (Keller, Sui, Youngc & Whittal, 2008). The peaks observes in the spectrum may not be from the compound of interest, they can arise from impurities in the sample (residual solvent, or phthalate plasticisers) or may be present in the spectrometer (contamination of the instrument from the analysis of previous samples) and thus results in the formation of certain adducts (Varghese *et al*., 2012). Table 2.5 describes the common adducts observed in spectra of a positive ion mode electrospray ionizations (Methodology for Accurate Mass Measurement of Small Molecules, 2006).

Table 2.5: The common molecular ion adducts often observed in ESI mass spectra

Adduct ion formed		m/z of Ion	Cause
Lithium	$[M + Li]^+$	$[M+1+6]^+$	Lithium salts
Ammonia	$[M + NH_4]^+$	$[M+1+17]^+$	Ammonia/NH_4OH
Water	$[M +H_30]^+$	$[M+1+18]^+$	Water/acids
Sodium	$[M + Na]^+$	$[M+1+23]^+$	Sodium salts
Methanol	$[M + CH_3OH]^+$	$[M+1+32]^+$	Methanol in solvent
Potassium	$[M + K]^+$	$[M+1+39]^+$	Potassium salts
Acetonitrile	$[M + CH_3CN]^+$	[M+1+41]+	Acetonitrile in solvent
Acetic acid	$[M + CH_3CO_2H]^+$	$[M+1+60]^+$	Acetic acid

2.14.4 Spectral Data Sources Used for Analysis

The main challenges in metabolite analysis include chromatographic data processing and metabolite characterization. The challenge has prompted the development of METLIN (METabolite LINk), a metabolite database that incorporates MS data from multiple sources, including high-accuracy FTMS mass measurements and tandem MS data (Smith *et al*., 2005). ChemSpider is a free chemical structure database providing fast text and structure search access to over 58 million structures from hundreds of data sources. Moreover, a number of mass spectral and metabolite data repositories have previously been created. One such example is the NIST database, a heavily used resource library for electron ionization (EI) mass spectrometry data on over 100,000 compounds (Wagner, Sefkow & Kopka, 2003).

2.15 Common Extraction Techniques

Plant metabolites often occur as complex mixtures of many substances of a wide range of polarity and hydrophobicity. The most important groups of substances in plant material are: low polar (waxes, terpenoids), semi-polar (lipids, phenolic compounds), low-polar (alkaloids), and high-polar (polar glycosides, polar alkaloids, saccharides, peptides, proteins) (Romanik, Gilgenast, Przyjazny & Kamiński, 2007). Therefore, extraction of bioactive compounds from plant materials can be conducted by various classical techniques. Most of these techniques are based on the extracting

power of different solvents in use and the application of heat and/or mixing. In order to obtain bioactive compounds from plants, as indicated in Table 2.6, the existing classical techniques are Soxhlet extraction, maceration and hydrodistillation (Azmir *et al*., 2013)

Table 2.6: The common methods of plant extractions

Parameters	Extraction Techniques		
	Soxhlet	**Sonification**	**Maceration**
Common Solvents	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water	Methanol, ethanol, or mixture of alcohol and water
Temperature (°C)	Depending on solvent used	Can be heated	Room temperature
Pressure applied	Not applicable	Not applicable	Not applicable
Time required	3 - 18 hr	1 hr	3 - 4 days
Volume of solvent (ml)	150 – 200	50 – 100	Depending on the sample size

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari *et al*., 2011). Table 2.7 shows the common solvents employed for the extraction of different metabolites of interest.

Table 2.7: Comparison of the common solvents used for extraction

Water	**Ethanol**	**Methanol**	**Chloroform**	**Ether**	**Acetone**
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarines	
Saponins	Flavonols	Tannins		Fatty acids	
Terpenoids	Terpenoids	Terpenoids			
Polypeptides	Polypeptides	Polypeptides			
Lectins	Lectins	Lectins			
		Lactones			
		Flavones			
		Polyphenols			

2.16 Analytical Tools for Separation and Characterisation

The development of authentic analytical methods which can reliably profile the phytochemical composition includes quantitative analysis of marker/bioactive compounds and other major constituents (Khanvilkar, Ayare & Kadam, 2016). When investigating the complete phytochemical profile of a given plant species, the separation and purification of plant constituents are mainly carried out by one or more chromatographic techniques (Harborn, 1984). A number of different separation techniques such as thin-layer chromatography (TLC), open-column chromatography (OCC), flash chromatography (FC) and vacuum liquid chromatography (VLC) and HPLC are the most popular techniques for natural product isolation (Boligon & Athayde, 2014). TLC fingerprinting is of key importance for herbal drugs made up of essential oils, resins and complex mixtures of constituents (Vaidya & Devasagayam, 2007). TLC gives a quick answer as to how many components are in a mixture and is also used to support the identity of a compound given the retention factor (R_f) of a known compound (Sasidharan *et al*., 2011).

When FC and OCC are used as first fractionation steps of crude plant extracts, solvent systems of increasing polarity are often employed. VLC is a popular method for fractionation of crude extracts due to its ease of use and high sample capacity. Eluted fractions are usually analysed by TLC for their composition. VLC and FC are mainly used for rapid fractionation of crude extracts or coarsely purified fractions (Bucar, Wubea & Schmidb, 2013). HPLC is the preferred method for quantitative analysis of more complex mixtures. It is a versatile, robust, and widely used technique for the isolation of natural products. It can identify, quantify and purify the individual components of a mixture (Martin & Guiochon, 2005). HPLC has been coupled to various sophisticated detectors such as UV photodiode array detectors (LC-UV-DAD), mass spectrometers (LC-MS) or more recently to nuclear magnetic resonance instruments (LC-NMR) (Patel *et al*., 2000).

UV-VIS spectrometry, MS, GC, and LC used alone, or hyphenated systems such as GC/MS, LC/MS, and MS/MS, and NMR are sophisticated techniques that provide a chemical fingerprint as to the nature of chemicals or impurities present in the crude plants, fractions or isolated compounds (Kareru, Keriko, Gachanja & Kenji, 2008). Moreover, FT-IR has proven to be a valuable tool for identification of functional groups present in an unknown mixture of extracts (Eberhardt, Li, Shupe & Hse, 2007; Hazra *et al*., 2007).

2.17 Botanical, Ethnomedicinal and Chemical Profile of the Plants

2.17.1 *Aloe camperi*

Aloe camperi Schweinfurth (Aloeceae) is a species of aloe native to Eritrea and Ethiopia. It is so far not known anywhere else. The specific epithet "camperi" was, according to Schweinfurth, given in honour of "an esteemed friend Manfredo Camperio, who did so much for the Italian Colony of Eritrea". The species was described in 1894 based on the type material collected near Ghindae in Eritrea. *Aloe camperi* is distinguished from the related species of the clavate perianth which is 18 - 22 mm long. It grows abundantly on rocky slopes and sandy alluvial plains along the Eastern escarpment between 550 and 2700 m above sea level. The main flowering period of the plant is from March to May (Sebsebe & Inger; 2010; Weber, 2013). In folk medicine, *Aloe camperi* has been used in treatment of malaria, wound, eye inflammation, skin and gastrointestinal problems. Recently the hyaluronidase inhibition property of the plant was reported (Gebrelibanos *et al*., 2014). There is no other report on the bioactivity and phytochemical study of this plant.

2.17.2 *Meriandra dianthera*

Meriandra dianthera (Roth) Briq. is a species of plants in the Lamiaceae family; it is native to Eastern Africa, the Arabian Peninsula, and India. *Meriandra dianthera* is a fragrant shrub 50 cm to 2 m with dense branches, found in open bush vegetation sometimes cultivated, 1800 - 3000 m. Most parts covered with short grey hairs give a white appearance. The leaves are very aromatic, long oval, 3.5 - 7.0 cm, the blade narrows at the base into winged stalk less than 2 cm, midrib clear below, leaf edge

finely round-tooth, the tip more or less pointed; both surfaces hairy, more dense below (Bein *et al*., 1996; The Global Biodiversity Information Facility, 2013). There are no detailed information about the bioactivity and chemistry of *Meriandra*. However, recently, ethnomedicinal information from Southern Tigray, Ethiopia reported that the leaves of *Meriandra dianthera* are used for hypertension and diarrhoea controls (Solomon, Balcha & Mirutse, 2015).

2.17.3 *Brassica nigra*

Brassica nigra (L.) Koch. belonging to the Brassicaceae family is well known since the Greek civilization for its medicinal proprieties (Hippocrates 480 BC). This species is widespread in the Mediterranean basin and in some central Asian and Middle East areas and is cited as "mustard" in the New Testament for its fast growing habit. It is an erect, simple, mostly branched, annual herb, 50 to 60 cm tall with lower leaves distinctly stalked, lyrate-pinnatisect, largest and ovate middle leaves shorter-petioled and upper leaves oblong-linear mostly entire and all very bright green. The fruit are long erect pods, seeds globose, 1mm. diameter, obscurely brown and black near the hilum, delicately alveolate (Kole & Borlaug, 2011). The reported activities of *Brassica nigra* (Linn) seeds include insulinotropic effect, anti-microbial and anti-hyperglycemic effect (Anand *et al*., 2009; Obi, Nwanebu, Ndubuisi& Orji, 2009). The seeds of *Brassica nigra* contain alkaloid, sinapine, myrosin, sinigrin, inosite, albumins, gums and colouring matters (Kirtikar & Basu, 1984; Anand *et al*., 2007).

2.17.4 *Lepidium sativum*

Lepidium sativum (L), commonly known as garden cress, is an annual herb, belonging to the Brassicaceae family. It is an annual erect herbaceous plant, growing up to 30 cm. It is a fast-growing plant and well-known culinary herb and the leaves are widely used as a garnish and are consumed raw in salads. The seeds are small, oval-shaped, pointed and triangular at one end, smooth, about 2 - 3 mm long, 1 - 1.5 mm wide, reddish brown, a furrow present on both surfaces extending up to two thirds downward. *Lepidium sativum* is reported to exhibit antih-ypertensive, diuretic,

analgesic, hypoglycemic, anti-diarrheal, anti-asthmatic and other activities (Maghrani, Zeggwagh, Michel, & Eddouks, 2005; Paranjape & Mehta, 2006; Patel, Kulkarni, Undale & Bhosale, 2009). The seeds contain volatile essential aromatic oils, flavonoids, isothiocynates glycoside and alkaloids (Maier, Gundlach & Zenk, 1998).

2.17.5 *Nigella sativa*

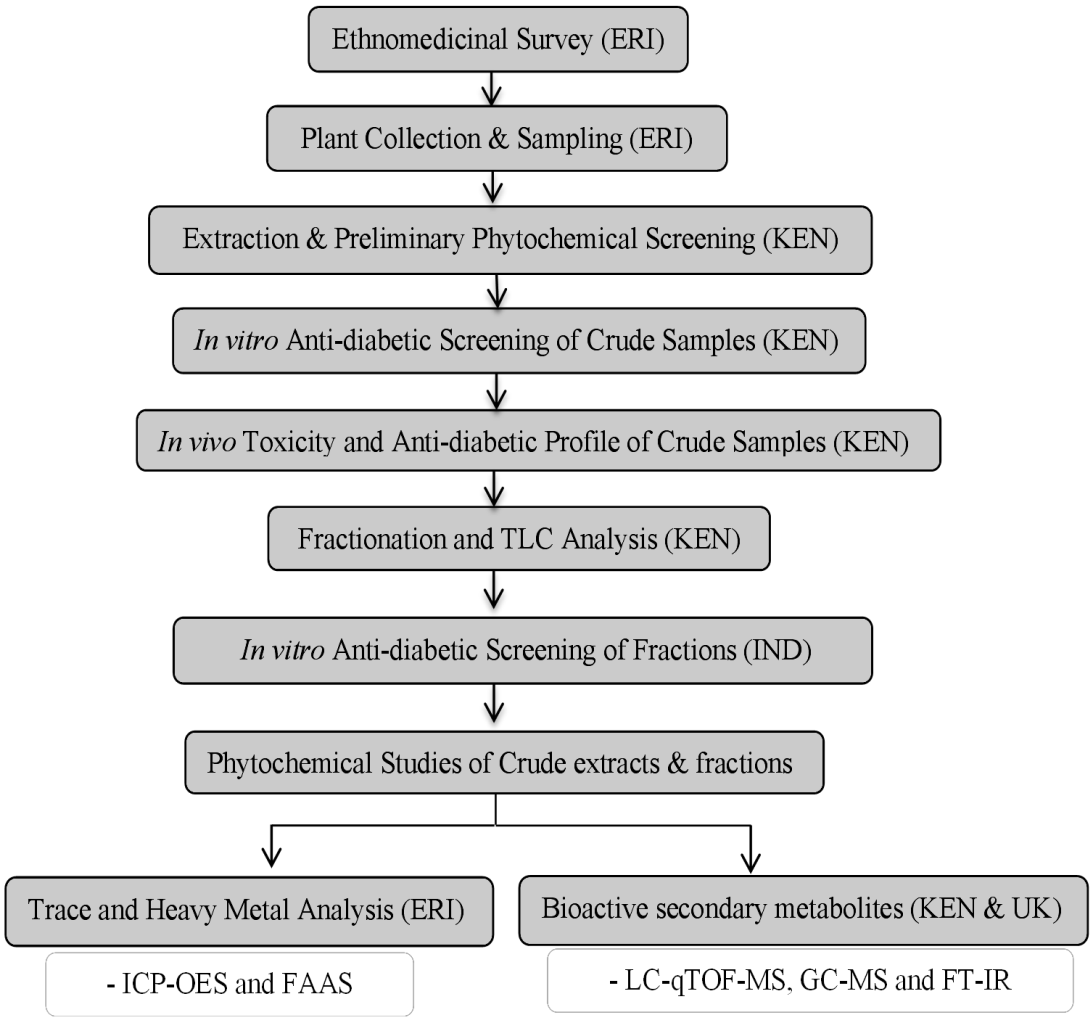
Nigella sativa L (Family: Ranunculaceae) is an annual flowering plant growing to a height of 20-30 cm. The fruits are large and inflated capsule composed of 3-7 follicles each containing numerous seeds. The seeds are tiny (1-2 mm) and black in colour and have been known as black seed or black cumin. The flowers are delicate. The seeds are used as a spice and native to Southwest Asia. The seeds have been used for medicinal purposes for centuries in Asia, the Middle East, and Africa (Ali & Blunden, 2003). *Nigella sativa* has been traditionally used as a natural remedy for a number of ailments that include asthma, chest congestion, hypertension, diabetes, inflammation, cough, bronchitis and others (Khan *et al*., 2003; Salih, Siphi & Donmez, 2009). It was reported that the main bioactive constituents of the *Nigela sativa* are p–cymene, thymoquinone and thymol (Moretti, Antuono, Filippo & Elementi, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The research involved various methods associated with the systematic identification of the plants, extraction of bioactive components, separation of components, anti-diabetic bioassays and phytochemical investigations. The research activities were mostly done in Eritrea (ERI) and Kenya (KEN). The general outline of the overall research work is represented as shown in Figure 3.1.



NB: ERI-Eritrea, KEN-Kenya, IND -India, UK-United Kingdom (Southampton)

Figure 3.1: The general outline of the overall research work

3.2 Study Area of the Ethnomedicinal Survey

The regions/zones of Eritrea are the primary geographical divisions through which Eritrea is administered. Six in total, they include the Maekel (Central), Anseba, Gash-Barka, Debub (Southern), Northern Red Sea and Southern Red Sea zones. The current ethnomedicinal surveys were conducted were in the Central and Southern Zones. The Central Zone is the smallest region in the country and contains the major city and national capital, Asmara; the area of Central Zone lies between 15º10'-15º35' N latitude and 38º41'-39º30' E longitude with an average elevation of 2,343 meters. The Southern Zone lies along a portion of the national border with Ethiopia 14º25'-15º10' N latitude and 38º15'-39º-45' E longitude and it has an elevation of 1915 m above sea level. Figure 3.2 shows the map of Eritrea and the Zones where the ethnomedicinal survey and plant collection are made.

Figure 3.2: Map of Eritrea with labelled Central and Northern Zones

3.3 Collection of Ethnomedicinal Data

The ethnomedicinal surveys were carried out from November 2013 to April 2014, in selected localities of Central and Southern Zones, by interviewing 66 informants using a semi-structured questionnaire (Appendix 1). The informants were mainly herbalists and diabetic patients. A brief profile of the informants in summarized in Appendix 2. Prior to the use of the questionnaire, conversations with the informants were held with the assistance of community administrators in order to explain the objectives of the study and to building trust with the objectives of documenting and preserving the indigenous knowledge. The data collected includes local names of the plants, the plant parts used, methods of herbal preparation and the manner of administration. Moreover, some profile of the informants was recorded and the data was entered into excel data sheets for analysis.

3.4 Collection of Plant Samples

Based on the frequency of their wider application and some literature data, 11 plants were initially selected for a preliminary phytochemical screening. The majority of the plants of interest were collected during the field trips and some from market areas guided by the herbalists. The plants subjected for the preliminary phytochemical screening include *Acacia nilotica, Aloe camperi, Azadirachta indica, Balanites aegyptiaca, Brassica nigra, Eucalyptus globulus, Meriandra dianthera, Lepidium sativum, Nigella sativa, Trachyspermum ammi* and *Trigonella foenum-graecum*. The plants are widely used in folk medicine for different purposes and are believed to have anti-diabetic activities (Mussie, Kareru, Keriko & Berhane, 2015).

The fresh leaves of *Acacia nilotica* and *Azadirachta indica* were collected from Hagaz area (15°42'26.8"N 38°16'5.2"E) and the fresh leaves of *Eucalyptus globulus* were collected from Adi-abieto, around Asmara (15°20′N 38°56′ E). The fresh leaves of *Aloe camperi* and *Meriandra dianthera* were collected from Adi-hawisha ($15^{o}14'$N $38^{o}58'$E). The seeds of *Brassica nigra, Lepidium sativum, Nigella sativa, Trachyspermum ammi* and *Trigonella foenum-graecum* were collected from a herbal shop in Asmara, Eritrea. The plants were initially identified by their vernacular

names and later validated by a plant taxonomist of Eritrea Institute of Technology (EIT), Prof. Ghebrehiwet Medhanie. Based on the results of the preliminary phytochemical screening, ethnomedicinal uses of the plants and exhaustive literature review, three extracts were selected for comprehensive profiling of their activities and phytochemical constituents throughout the research work. The plants were *Aloe camperi, Meriandra dianthera* and a Polyherb (prepared from equal proportion of the seeds of *Lepidium sativum, Brassica nigra* and *Nigella sativa*).

3.5 Chemicals and Reagents

Analytical grade n-hexane, methanol, petroleum ether, ethyl acetate, dimethyl sulfoxide, chloroform, 65% nitric acid, 30% hydrogen peroxide, 32% hydrochloric acid, 98% sulphuric acid (H_2SO_4), formic acid, ultrapure-deionised water (18 Ω), LC−MS grade methanol, LC-grade ultrapure water, LC-grade acetonitrile, LC-grade dichloromethane, rutin, camphor, alloxan monohydrate, α-amylase enzyme, α-glucosidase enzyme, maltose and starch were purchased from Sigma-Aldrich Company. Metformin, potassium bromide, sodium sulphate, acarbose, dinitro salicylic acid (DNS), glucose kit reagent and Tris buffer were obtained from Cipla Pharmaceuticals, Kenya.

Whatman No. 1 filter papers, extraction thimbles, TLC Silica gel 60 F254 (20 × 20 cm) plates (Merck KGaA, Germany), micro tips, sample vials, silica gel 60 (0.063 - 0.2000 mm; Merck KGaA Germany), Glucometer (On Call Plus, ACON Laboratories, Inc., USA), diagnostic Kit, glucometer strips were purchased from Doxpan Venture, Nairobi, Kenya. The glassware for the elemental analysis was soaked in 3M HNO_3 for the whole night, washed and rinsed with deionized water to minimize the chances of interferences and all the chemical analysis were conducted under extractor hood and a digital IR Vortex Mixer (S/N296058 made in Italy) was used for mixing of the solutions.

3.6 Extraction and Sample Preparation

The leaves and seeds of the plants were washed with distilled water and dried under shade at room temperature separately. The dried plants were powdered using an electric blender and filtered using 35 mesh size (200 mm) sieve. The powdered plants were stored in sealed plastic containers until further use. The two main types of extraction methods used are described in Section 3.6.1 and Section 3.6.2 (Handa, Khanuja, Longo & Rakesh, 2008; Bandar *et al*., 2013).

3.6.1 Maceration

For the preliminary phytochemical screening, three different extracts were prepared for each plant by maceration using hexane, methanol and water. The hexane and methanol extracts were prepared as follows: A portion (10 g) of the powdered plants was macerated into the solvent (100 ml) for 7 days with occasional stirring. The aqueous extracts were prepared by soaking the powdered plants (10 g) in distilled water (100 mL) for 48 h in conical flasks. The solvent and aqueous extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at about 40 ^{o}C using a rotary evaporator (Buchi Rotavapor R-200, Tokyo).

The extracts for the bioactivity and structural studies are prepared as follows: Each powdered sample (100 g) was defatted three times using light petroleum (60-80 ^{o}C) and then the dried marcs were macerated in methanol (1000 ml) for five days with occasional stirring. The methanol extracts were evaporated *in vacuo* at 50 ^{o}C and the concentrated extracts were stored in air tight containers at 4 ^{o}C until further use. The extraction afforded 20.82, 28.84 and 21.14 g from the *Aloe camperi, Meriandra dianthera* and the Polyherb, respectively. This method was employed frequently when large amount of extracts were needed.

3.6.2 Soxhlet Extraction

Soxhlet extraction was used mainly when small amount of sample for instrumental analysis were required. During the extraction, each powdered plant sample (5 g at a time) was placed in a thimble and extracted successively initially with

hexane (3x) and then with methanol (3x) at a volume of 250 mL solvent for a single extraction using Soxhlet extractor for about 8 h. The extracts were concentrated under reduced pressure and the crude methanol extracts were preserved at 4 ˚C in airtight bottle containers until further use.

3.6.3 Sample Digestion for Elemental Analysis

The plants of interest during the elemental analysis were *Aloe camperi, Meriandra dianthera* and the Polyherb. *Lepidium sativum, Nigella sativa* and *Brassica nigra,* the constituents of the Polyherb, were investigated separately. The finely powdered plants were initially heated on a heating block in an oven for complete dryness. Each sample (0.5 g) was weighed accurately using a 4 decimal places analytical balance and placed in a digestive tube. The samples were initially digested with concentrated HNO_3 (5 ml) at 175 ^oC for 40 min and then at 150 ^oC for 90 min. After the mixture was cooled, 1 ml HNO_3 and 0.5 ml H_2SO_4 were added and the mixture was heated at 175 ^oC for 60 min. The mixture was allowed to cool and H_2O_2 (2 ml) was added dropwise and heated at 140 ^oC for 10 min to remove any remaining NO_2 that might interfere in the measurement. The resulting mixture was transferred to a calibrated flask and HCl (8 ml) was added and the entire filtrate was diluted suitably with ultrapure deionized water to 25 ml. Samples were immediately analysed following the digestion (Abou-Arab & Abou Donia, 2000; Maiga *et al*., 2005).

3.7 Preliminary Phytochemical Analysis

The solvent and aqueous extracts were subjected to preliminary phytochemical studies in order to screen the presence of secondary metabolites including alkaloids, phenols, flavonoids, terpenoids, coumarines, steroids, saponins, tannins, glycosides and others by using standard protocols (Trease & Evans, 1989; Harbone, 1998). The detailed experiments used for the preliminary phytochemical analysis are summarized in Sections 3.7.1 to 3.7.12.

3.7.1 Test for Alkaloids

i. Wagner's test: A fraction (about 2 ml) of each extract was treated with 3 - 5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of a reddish brown precipitate (or colouration).

ii. Mayer's test: A fraction (1 ml) of each extract was acidified and treated with a few drops of Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids.

iii. Dragendroff's test: A fraction (1 ml) of each extract was acidified and treated to a few drops of Dragendroff's reagent (solution of potassium bismuth iodide) and gave reddish brown precipitate

3.7.2 Test for Glycosides

i. Keller Kelliani's test: Each extract (5 ml) was treated with glacial acetic acid (2 ml) in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with concentrated sulphuric acid (1 ml). A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

ii. Legal's test: Each extract (1 ml) was dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation of a pink to red color showed the presence of glycosides.

3.7.3 Test for Flavonoids

i. Alkaline reagent test: An extract (2 ml) was treated with a few drops of 20% sodium hydroxide solution; the formation of an intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

ii. Shinoda's test: An alcoholic extract (1 ml) was treated with magnesium foil and concentrated hydrochloric acid; formation of intense cherry red color indicated the presence of flavonones or orange red color indicated the presence flavonols.

3.7.4 Test for Phenols

i. Ferric chloride test: A fraction of the extracts (2 ml) was treated with aqueous 5% ferric chloride and observed for formation of a deep blue or black colour.
ii. Lead acetate test: A quantity of the extracts (1 ml) was mixed with basic lead acetate solution. Formation of white precipitates indicated the presence of tannins.

3.7.5 Tests for Steroids

i. Salkowski Tests: A chloroform solution of the extract (1 ml) was shaken with concentrated sulphuric acid and on standing yielded a red color.
ii. Lieberman Burchardt tests: A chloroform solution of the extract (1 ml) with few a drops of acetic anhydride and concentrated sulphuric acid (1 ml) from the sides gave a reddish ring at the junction of 2 layers.

3.7.6 Test for Saponins

Foam test: Water (6 ml) was added to an extract (2 ml) in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirmed the presence of saponins.

3.7.7 Test for Sterols

Liebermann-Burchard test: An extract (1 ml) was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of a dark pink or red color.

3.7.8 Test for Tannins

Braymer's test: An extract (2 ml) was treated with 10% alcoholic ferric chloride solution and observed for a formation of blue or greenish colour solution.

3.7.9 Test for Terpenoids

Salkowki's test: Chloroform (1 ml) was added to each extract (2 ml) followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

3.7.10 Tests for Coumarins

A moistened extract (0.5 g) was taken in a test tube. The test tube was covered with a filter paper treated with 1 N NaOH solution. The test tube was placed for few min in boiling water and then the filter paper was removed and examined under the UV light for a yellow fluorescence indicated the presence of coumarins.

3.7.11 Tests for Carbohydrates

i. Fehling's test: Equal quantities (1 ml) of Fehling solution A and B were added to the extract (1 ml) and upon heating formation of a brick red precipitate indicated the presence of sugars.
ii. Benedict's test: A Benedict's reagent (5 ml) was added to the extract solution (1 ml) and boiled for 2 min and then cooled. Formation of a red precipitate showed the presence of sugars.

3.7.12 Test for Protein and Amino Acids

i. Biuret test: 40% Sodium hydroxide solution and 1% copper sulphate solution (2 drops) was mixed until a blue color produced, and then the extract (1 ml) was added. Formation of a pinkish or purple violet color indicated the presence of proteins.
ii. Ninhydrin test: Freshly prepared (2 drops) ninhydrin reagent (0.1% solution in n-butanol) was added to the extract (1 ml) and heated. Development of a blue color revealed the presence of proteins, peptides or amino acids.

3.8 *In Vitro* and *In Vivo* Anti-Diabetic Studies

The crude extracts and fractions were screened for their anti-diabetic activities using in *vitro* and *in vivo* assays. Moreover, the toxicity of the crude plants was investigated using *in vivo* model.

3.8.1 *In Vitro* Anti-Diabetic Screening

The *in vitro* screening of the crude extracts and fractions were done using the α-amylase and α-glucosidase inhibition assays as per reported standard procedures (Rao *et al*., 2010; Subramanian, Asmawi & Sadikun, 2008). The plant extracts for both assays were initially prepared as described in Section 3.6.

3.8.1.1 α-Amylase Inhibition Activity

Different concentrations (25 - 800 µg/ml) of the plant samples and standard acarbose were prepared in dimethyl sulfoxide from1 mg/ml stock solution. Each sample and standard (500 µl) were added to a 0.5 mg/ ml α-amylase solution (500 µl) and incubated for 10 min at room temperature. Then 1.0% starch solution (500 µl) was added and incubated for another 10 min. After that the colouring reagent, dinitrosalicylic acid (DNS, 1 ml) was added to the reaction mixture and heated in a boiling water bath for 5 min. After cooling, it was diluted with 10 ml of distilled water. The absorbance was measured at 540 nm against the blank reagent. The percentage enzyme inhibition activity of the extracts/standard was calculated using the following formula:

$$\% \text{ Inhibition } = \frac{A_{CO} - A_t}{A_{CO}} \times 100$$

Where, A_{CO} is absorbance of the control and A_t is absorbance of the test samples. Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with the vehicle (25 µl dimethylsulfoxide and distilled water). For blank incubation, in order to allow for the absorbance produced by the extract, the enzyme solution was replaced by the buffer solution and the

absorbance was recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme.

3.8.1.2 α-Glucosidase Inhibition Activity

From 1 mg/ml stock solution different concentrations (25 - 800 µg/ml) of the plant samples were prepared in 5% dimethyl sulfoxide. Each sample and standard (500 µl) were added to a 1 U/ml of α- glucosidase (500 µl) and then incubated for 5 min at room temperature. Then 37 mM maltose solution (500 µl) was added and incubated for 30 min. After that the glucose kit reagent (1 ml) was added to the reaction mixture and kept aside for 15 min. Tris buffer (1 ml) was then be added to the mixture. The absorbance was measured at 505 nm against the blank reagent. The α-glucosidase inhibition was expressed as a percentage of inhibition. IC_{50} and the percentage inhibition were calculated in the same way as described in Section 3.7.1.1.

3.8.2 *In Vivo* Anti-Diabetic Sreening

3.8.2.1 Experimental Animals

Wistar albino rats (150 – 170 g) of 8 - 10 weeks of either sex were obtained from Kabete Veterinary Farm, Nairobi, Kenya (Plate 3.1). All the experiments were conducted in the Animal House of Jomo Kenyatta University of Agriculture and Technology (JKUAT). After randomized grouping and before initiation of the experiment, animals were housed, for two weeks, in ventilated polypropylene cages under standard laboratory conditions of temperature and humidity to acclimatize. During this period, the rats were fed on standard laboratory diet, in the form of rat pellets from Unga Feeds Limited, Nairobi, Kenya, and water was allowed *ad libitum*. Twelve light-dark cycles was also allowed in the entire period of the experiment (Animal Research Review Panel, 2007). The experimental protocols were approved by the Institutional Animal Ethics Committee of JKUAT (JKU/2/4/IPC/013).

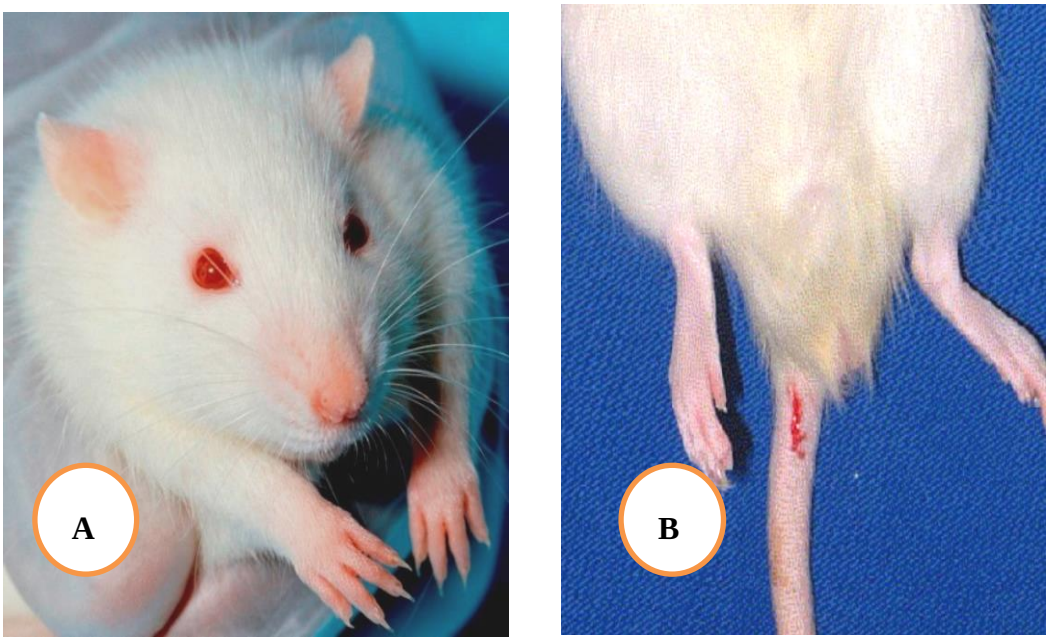


Plate 3.1: Typical Wistar albino rat (A) and incision of tail vein for blood reading (B)

3.8.2.2 Acute Oral Toxicity Test (AOTT)

An acute oral toxicity study was performed as per the protocols of the Organization for Economic Cooperation and Development (OECD) guidelines 425 (OECD, 2001). Nulliparous female animals were fasted overnight prior to dosing. The fasting body weight of each animal was determined and the dose was calculated accordingly. The crude methanol extracts (2000 mg/Kg) of *A. camperi*, *M. dianthera* and the Polyherb were administered in a single dose by gavage. The rats were then kept under strict observation for physical and behavioural changes for 24 h, with special attention during the first 4 h. Following the results from the first rat, other four rats were recruited and fasted overnight and administered a single dose of 2000 mg/kg and were observed in the same manner. These observations continued for a further 14 days for any signs of overt toxicity. Hence, 1/ 10^{th} (200 mg/kg) and 1/ 5^{th} (400 mg/kg) of this dose were adopted for further anti-hyperglycemic studies.

3.8.2.3 Oral Glucose Tolerance Test (OGTT)

OGTT was assessed, according to standard methods, to the overnight fasting rats (Ayala *et al*., 2010; USQ Animal Ethics Committee, 2014). The Wistar albino rats were divided into 8 groups of 6 rats each. The aim was to study the effect of plant extracts on changes in the blood glucose level in normal rats. The three plant extracts and standard drug (metformin) were administered as shown in Table 3.1. Thereafter, following 30 min post extracts and standard drug administration, glucose solution (40% aqueous solution) at 2 g/kg per body weight was loaded orally to all the groups. Blood was obtained from the tail vein using a sterile needle and the changes in blood glucose levels were measured, using a glucometer and recorded after 30 min of treatment (considered as 0 min) and after 30, 60 and 120 min of glucose loading.

Table 3.1: Grouping of animals for OGTT and anti-hyperglycemic studies

Group label	Description
Normal control	normal control received normal saline (0.9 % NaCl)
Positive control	administered 5 mg/kg body of metformin
AC200	administered 200 mg/kg body weight of *Aloe*
AC400	administered 400 mg/kg body weight of *Aloe*
MD200	administered 200 mg/kg body weight of *Meriandra*
MD400	administered 400 mg/kg body weight of *Meriandra*
PH200	administered 200 mg/kg body weight of Polyherb
PH400	administered 400 mg/kg body weight of Polyherb
DC*	diabetic control received 150 mg/kg Alloxan

DC*–diabetic control (Alloxanized) used for the anti-hyperglycemic studies only

3.8.2.4 Anti-Hyperglycemic Activity

A single dose of Alloxan at 150 mg/kg (5% solution) was prepared using normal saline (0.9 % NaCl) at room temperature and immediately administered intraperitoneally to the rats. Animals were then kept for the next 24 h on 10 % glucose to prevent hypoglycemia. Diabetes was confirmed after 72 h of Alloxan injection, the blood samples were taken from the tail vein and plasma glucose levels were estimated with the help of a Glucometer using the strip method. The rats with

FBGL higher than 200 mg/dl were included in the study (Erhirhie *et al*., 2013; Sharma & Garg, 2009). The plant extracts were administered orally for 3 weeks using gavage. Starting from the 1st day (3rd day of Alloxan injection) of extract administration to diabetic rats, FBGL was measured in the 4th, 7th, 14th and 21st days. As indicated in Table 3.1, the experiment was carried on 9 groups of each six rats and the animals were grouped in a similar way as OGTT experiment except that there was a diabetic control group.

3.8.2.6 Assessment of Body Weight

Once the rats were confirmed as diabetic after 72 h, body weights were measured pre-treatment (after 3 days of diabetes induction) and end of the treatment (after 21 days of treatment) by using a digital weighing balance. The relationship in body weight of each group versus the activity of the extracts was thus investigated.

3.9 TLC Analysis and Chromatographic Separation

The components of the crude plant extracts were initially screened using TLC using different solvent combinations. Moreover, after fractionation of the crude samples, the fractions were analysed using TLC.

3.9.1 TLC Analysis

TLC plates were used for the screening. Different proportions mobile phases (hexane: ethyl acetate, hexane: ethyl acetate: chloroform, hexane: ethyl acetate: methanol, chloroform: ethyl acetate: methanol: water and chloroform: methanol) were employed in order to select the best solvent combination for the column work. The samples were applied as separate spots to the TLC plates about 1.0 cm from the edge using micro tips. All TLC separations were performed at room temperature. After sample application the plates were placed vertically into a solvent vapour saturated TLC chamber. The spotting line was about 0.5 cm from the developing solution. After the mobile phase had moved about 80% from the spotting line, the plates were removed from the developing chamber. The sheets were dried in hot air oven for 4 - 5 min and observed using a UV-VIS before and then after colorization

were developed by passing iodine fumes over the dried sheets. R_f values of some fractions were determined and used for comparison purposes.

3.9.2 Chromatographic Separation

The crude plant extracts (20.82, 28.84 & 21.14 g of *A. camperi*, *M. dianthera* and the Polyherb) were subjected to column chromatography. In each case, slurry of silica gel (150 g) was prepared using hexane and poured down carefully into a column. A varying solvent combination of increasing polarity was used as the mobile phase. Each sample was prepared in a ceramic mortar by adsorbing 8.0 g of the extract to 20 g of silica gel 60 (0.063 - 0.2000 mm; Merck KGaA Germany) in methanol and dried on a hot plate. The dry powder was allowed to cool and then gently layered on top of the column. Elution of the extract was done with solvent systems of gradually increasing polarity using hexane, ethyl acetate and methanol. The following ratios of solvent combinations were sequentially used in the elution process; Hexane: ethyl acetate 100:0, 80:20, 60:40, 40:60 and 20: 80, 100:0 and further with ethyl acetate: methanol 100:0, 80:20, 60:40, 40: 60, 20: 80 and 0:100. The eluted fractions were collected in aliquots of 50 ml vials.

3.10 Instrumental Analysis

3.10.1 Instrumentation

Different instruments were employed in the overall research work and the LC-MS and GC-MS used for the analysis of the crude extracts and fractions were different. A dual viewing ICP-OES (Perkin Elmer Optima 8300, made in Singapore) coupled to an ultrasonic nebulizer CETAC 6000AT+ (CETAC, Omaha, NE, USA) and FAAS (Agilent 240FS, made in Australia) were employed for the analysis of the trace and other elements. A Waters ACQUITY UPLC (ultra-performance liquid chromatography) I-class system (Waters Corporation, Milford, MA, USA) fitted with Waters ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm; Waters Corporation, Dublin, Ireland) and 7890A GC/MS gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) linked to a 5975C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA) were used for the

identification and characterisation of compounds in the crude extracts. Furthermore, the components of the fractions were analysed using UPLC-MS (MaXis (Bruker Daltonics, Bremen, Germany) fitted with Waters UPLC BEH C18 (50 mm x 2.1 mm 1.7 µm) column and equipped with a Time of Flight (TOF) analyser, and a Thermo (Hemel Hempstead, UK) Trace GC-MS (J & W Innowax 60 m x 0.25 mm 0.5 µm thickness polar column) equipped with a single quadrupole analyser. The infrared (IR) spectra of the crude methanol and hexane extracts were obtained from a Fourier Transformer Infrared (FT-IR 8400, Shimadzu).

3.10.2 Operating Conditions of the Instruments

The operating conditions of the instruments are described below. Table 3.2 and Table 3.3 represent the operating conditions of the instruments involved in the elemental analysis. Similarly Table 3.4 and Table 3.5 furnish the working conditions of the LC-MS used for the analysis of crude samples and GC-MS used for fractions respectively. Furthermore, the working condition of the GC-MS used for the crude samples and LC-MS used for the fractions are described in Section 3.10.2.1 and Section 3.10.2.2 respectively.

Table 3.2: The operating parameters of the ICP-OES

Condition	Setting
Power	1.3 Kw
Plasma gas glow	15 L/min
Auxiliary gas flow	1.5 L/min
Spray chamber type	Glass cyclonic (single-pass)
Torch	Standard one piece quartz axial
Nebulizer type	Sea Spray
Nebulizer flow	0.7 L/min
Pump speed	2-4 rpm
Total sample usage	2 ml
Replicate read time	5 s
Number of replicates	2
Sample undertake delay time	15 s
Stabilization time	40 s
Rinse time	20 s
Fast pump	Off
Background correction	Fitted

Table 3.3: The operating conditions of the FAAS

	Element matrix			
Parameter	**Cu**	**Pb**	**Zn**	**Fe**
Wavelength	324.8 nm	283.3 nm	213.9 nm	372.0 nm
Slit Width	0.5 nm	0.5 nm	0.5 nm	0.2 nm
Gain	60 %	29 %	33 %	55 %
Lamp Current	4.0 mA	10.0 mA	5.0 mA	5.0 mA
Flame Type	air/acetylene	air/acetylene	air/acetylene	air/acetylene
Air Flow	13.50 L/min	13.50 L/min	13.50 L/min	14.90 L/min
Acetylene Flow	2.20 L/min	2.20 L/min	2.50 L/min	2.20 L/min

3.10.2.1 Instrumental Condition of LC-MS Used for Crude Analysis

The UPLC-Qtof-MS was operated at 40 °C and an auto sampler tray cooled to 15 °C was used for injection of samples. The mobile phase consisted of two components: solvent A: LC-grade ultra-pure water with 0.1% formic acid and solvent B: LC-grade acetonitrile with 0.1% formic acid. The following gradient was used: 0-0.2 min., 10% B; 0.2-3 min., 10 - 60% B; 3- 5 min., 60 - 80% B; 6- 8 min., 80% B; 8- 9 min., 100% B; 9- 10 min., 100% B; 10- 10.5 min. 100- 10% B; 10.5- 12 min. 10% B. The injection volume was 0.5 µL and the flow rate held constant at 0.4 ml min^{-1}. The working conditions of the LC-MS are shown in Table 3.4.

Table 3.4: The working conditions of the UPLC-Qtof-MS used for crude analysis

Parameter	**Set value**
Ionization interface	ESI
Mass scanning (positive-ion mode)	50-1,200 m/z
Scan time	1s
Capillary voltage	3.0 kV
Cone voltage	70 V
Desolvation temperature	380°C
Desolvation gas flow	400 L/h
Extract voltage	5 V
RF voltage	0.5 V
Source temperature	110°C
High-energy collision energy ramp	25−45 eV
Lock mass spray reference	leucine encephalin at 10 s intervals
Data collection	centroid mode

3.10.2.2 Instrumental Condition of GC-MS Used for Crude Analysis

The GC/MS used for the analysis of crude samples has the following working conditions: inlet temperature 270°C, transfer line temperature of 280°C, and column oven temperature programmed from 35 to 285 °C with the initial temperature maintained for 5 min then 10°C min^{-1} to 280°C, held at this temperature for 20.4 min. Helium at a flow rate of 1.25 ml min^{-1} served as the carrier gas. The mass selective detector was maintained at ion source temperature of 230°C and a quadrupole temperature of 180°C. Electron impact (EI) mass spectra were obtained at the acceleration energy of 70 eV. A 1.0 µl aliquot of sample was injected in the split less mode using an auto sampler 7683 (Agilent Technologies, Inc., Beijing, China). Fragment ions were analyzed over 40-550 *m/z* mass range in the full scan mode. The filament delay time was set at 3.3 min.

Table 3.5: Instrumental conditions of the Thermo Trace GC-MS

Oven Method	Right SSL Method	Right Carrier Method
Initial temp (^{o}C): 60	Base temperature: On	Mode: constant flow
Initial time (min): 8.00	Base temperature (C): 240	Initial value: On
Number of ramps: 1	Mode: Split less	Initial value (ml/min): 1.00
Rate #1 (deg/min): 15.0	Split flow: Off	Initial Time: 1.00
Final temp #1 (^{o}C): 240	Split flow (ml/min):10	Gas saver: On
Hold time #1 (min): 16.00	Split less time (min): 1.00	Gas saver flow (ml/min): 20
Post run temperature: Off	Surge pressure: Off	Gas saver time: 2.00
	Surge pressure (kPa): 3.00	Vacuum compensation: On
	Surge duration (min): 0.00	
	Constant purge: On	
	Stop purge at: (min): 0.00	

3.10.2.3 Instrumental Condition of HPLC-MS Used for Analysis of Fractions

Electrospray ionization (ESI) was used as the interface and was operated in positive selected ion monitoring (SIM) mode. The probe temperature was set at 500 °C and needle voltage was set at 2000 V. The cone voltage was set at 50 V for all SIM scans with a span of 0.3 amu for each SIM.

3.10.3 Standards and Calibrations

3.10.3.1 Standards and Calibration for the ICP-OES and FAAS

Different custom-grade multi-element standard solutions of PerkinElmer Pure were employed for the calibration and in-house certified reference materials (CRMs) for biological materials, developed using the "ISO Guide 80", were used for comparison purposes (ISO Guide 80, 2014). Calibration curves for each element were constructed in triplicate using six different concentrations, giving regression coefficient (r^2) values which ranged from 0.9957 to 0.9996. An internal-standard stock solution of 100 mg/L Lutetium (Lu) was prepared from single-element stock solutions (Appendix 7 & Appendix 8).

3.10.3.2 Standards for the LC-MS and GC-MS

Serial dilutions of the authentic standards rutin (1.8 - 181 ng/µl) was analyzed by LC-Qtof-MS in MS^E mode to generate linear calibration curves (peak area vs. concentration) (Figure 3.3) with the following linear equation: *y = 5578.4x - 39094* (R^2 = 0.9960) which served as the basis for the external quantification of the flavonoids and other compounds present in the extracts. Similarly, serial dilutions of authentic standard (camphor; 99 %, Gillingham, Dorset, England) (1 ng/µl, 35 ng/µl, 70 ng/µl, 140 ng/µl and 280 ng/µl) were prepared and analyzed by GC-MS. The linear regression of the standard camphor (y = 203482x – 451578; R^2 = 0.9997) is shown in Figure 3.4.

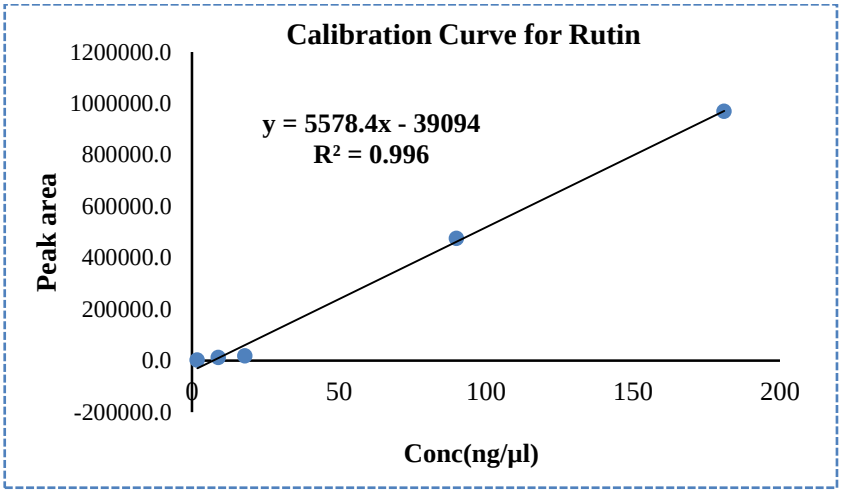


Figure 3.3: Linear calibration curve of rutin

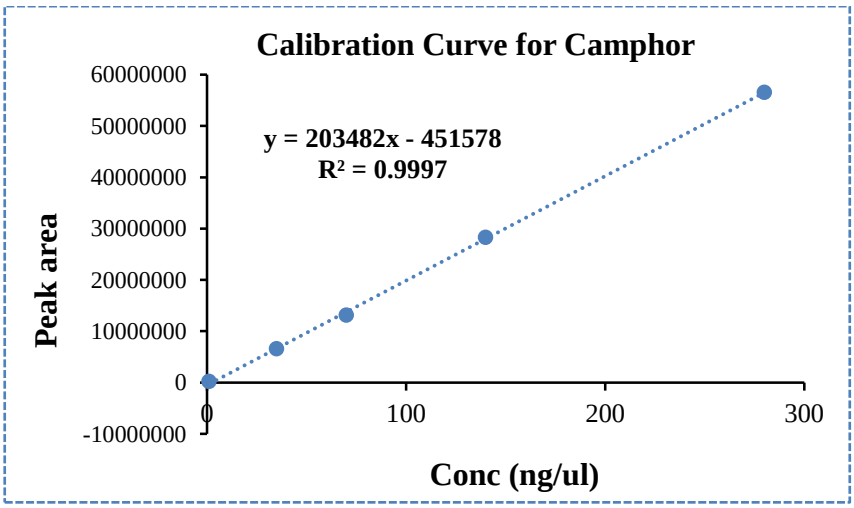


Figure 3.4: Linear calibration curve of camphor

3.10.4 Sample Analysis Using the Instruments

3.10.4.1 ICP-OES and FAAS

For the ICP-OES and FAAS, the dilute filtrate solutions of the digested plant samples were transferred into test tubes and about 20 ml of the resulting solution was then fed in to the air-acetylene FAAS. Suitable hollow cathode lamps were used to measure the absorbance of the elements at their resonance wavelengths.

3.10.4.2 FT-IR

The infrared (IR) spectra of the crude methanol and hexane extracts were obtained from the FT-IR. Each sample (5 mg) was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR-spectrometer was operated at spectral range 4000 - 500 cm^{-1}.

3.10.4.3 LC-MS and GC-MS

The dried crude methanol extracts were placed in test tubes and dissolved in 3 mL of LC−MS grade CHROMASOLV methanol (95:5 MeOH: H_2O; Sigma-Aldrich, St. Louis, MO), and centrifuged at 14,000 rpm for 5 min to pellet the dried extracts at the bottom of the tube, after which 0.5 μL of each sample was automatically injected into LC−QtoF−MS. Similarly, for the GC-MS, each sample (1 mg) was weighed and dissolved in dichloromethane (sigma Aldrich gc-grade) and the samples were vortexed for 30 s and sonicated in an ultra-bath for 15 min before being centrifuged at 14,000 rpm for 5 min. The supernatant was passed through Na_2SO_4 to remove moisture before analysis.

3.11 Data Analysis of the Results

The ethnomedicinal data extracted from the questionnaires were first stored in Microsoft Excel data sheet and then analysed using IBM SPSS 21. The *in vitro* experiments and the elemental analysis were performed in triplicate and the results were recorded as the mean ± SEM. Statistical difference between the control (Acarbose) and treatment groups for the *in vitro* screening was determined using one-way analysis of variance (ANOVA) followed by Tukey's test for *post hoc* analysis. The IC_{50} values were determined by linear regression plots (Appendix 3 & Appendix 4) with varying concentrations of the plant extracts against percentage inhibition. Similarly, for the *in vivo* results, a two tailed paired t-test was used first to determine any significant difference between baseline and intervention. Further statistical analysis was then performed using One-Way ANOVA followed by

Dunnett's test for multiple comparisons. A value of $P < 0.05$ was considered to be statistically significant.

During the elemental analysis, a window 7 compatible S/W with the instruments provided by Perkin Elmer was used to process the spectral data for calculating sample concentrations by comparing light intensities measured at various wavelengths for standard solutions with intensities from the sample solutions. For the LC-MS analysis, MassLynx version 4.1 SCN 712 (Waters Corporation, Maple Street, MA) was used for data acquisition and processing. The elemental composition was generated for every analyte. Potential assignments were calculated using mono-isotopic masses with a tolerance of 10 ppm deviation and both odd- and even-electron states possible. The number and types of expected atoms was set as follows: carbon ≤ 100; hydrogen ≤ 100; oxygen ≤ 50; nitrogen ≤ 6; sulfur ≤ 6. Thus, the empirical formula generated was used to predict the tentative structures which were proposed based on online sources (Metlin and Chemspider), fragmentation patterns and previous work stored in the computer. Similarly, for the GC-MS analysis, the structures were proposed on the basis of their general fragmentation and using reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 05, 08. The target peaks were further identified through comparison of their mass spectra with Adams2.L, Chem ecol.L, and NIST05a.L library data.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Ethnomedicinal Survey

The ethnomedicinal survey was conducted in Central and Southern Zones of Eritrea. The two Zones were chosen on their ease of access and greater number of traditional healers reported from previous surveys (Senai, 2010). The 66 informants, from the two Zones, with different sex and age, shared relevant information. As described in Figure 4.1, most of the informants were aged above 50, and thus the indigenous knowledge should be collected before the current generation passes away; lest the information is in danger of extinction. The ethnomedicinal data was analysed and thus presented a total of 42 anti-diabetic plants belonging to 24 families (Table 4.1). According to the survey data shown in Figure 4.2, the dominant families were Apiacea (14.29%), Lamiaceae (9.52%), Fabaceae (9.52%) and Asteraceae (7.14%). The finding of the present survey is similar to a previously published report; Thorne (1981) reported that the most often used anti-diabetic plants belong to Fabaceae, Asteraceae, Lamiaceae, and Liliaceae families.

Different plant parts were employed for the preparation of the herbal preparations. The leaf (42%), stem bark (21%) and seed (14%) were the most commonly used plant parts in the herbal preparations for diabetes (Figure 4.3). Moreover, the methods of herbal preparation and administration for diabetes were documented (Table 4.2). The commonest methods of herbal preparation reported were maceration, decoction, and infusion. It was reported that plant leaves are about 20% more favorable for storing active ingredients as compared to other parts of herbal plants. Similarly, in the traditional medicinal practices decoction, soaking/maceration and percolations are broadly employed (Chan, Ngoh & Yusoff, 2012). Furthermore, apart the uses of the plants for diabetes, their application in the treatment of other ailments have been documented. The common ailments treated by the plants and their frequency of citation, by the traditional practices, were anaysed

as follows: diarrhoea (12), hypertension (12), diuretics (11), malaria (10), gastrointestinal problems (10), wound healing (9), anthelminthic (8), bronchitis (9), asthma (8) and skin problems (7) (Table 4.2). Even though the herbal medicinal practices for bacterial and fungal diseases were common among the different populations of Eritrea, the knowledge of herbs for diabetes and other cardiovascular diseases has not been prominent. Therefore, the survey has displayed an array of medicinal plants with anti-diabetic potential and other diseases. The data can be used as a reference for further studies related to the biological activities and phytochemical profile of the plants.

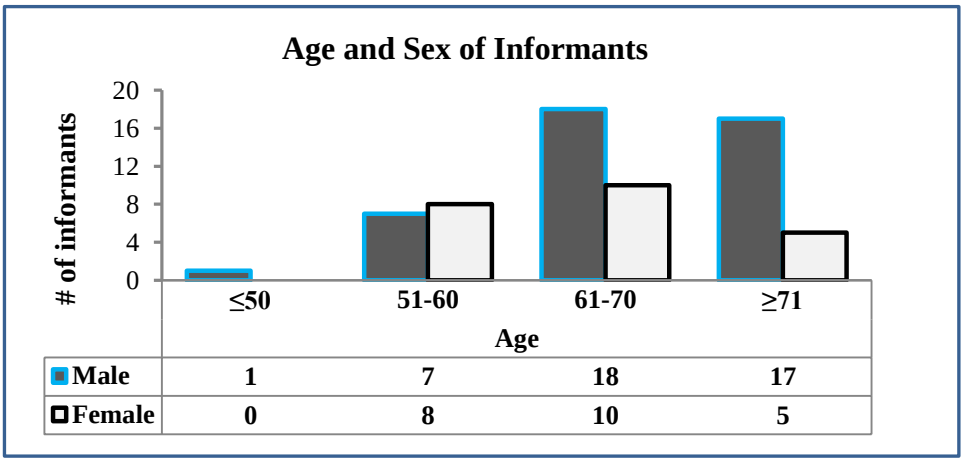


Figure 4.1: Number of informants involved in the survey vs their age

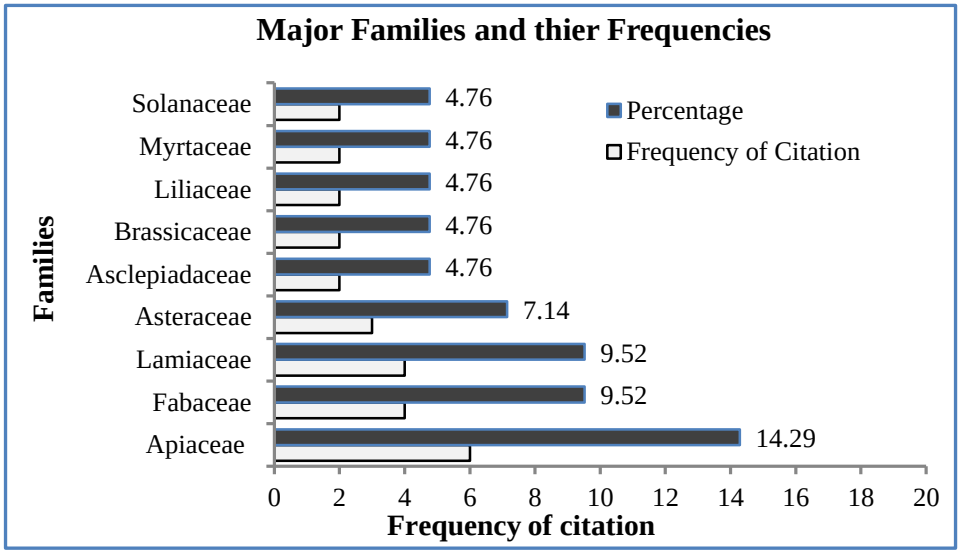


Figure 4.2: The frequency of citation of the major families

Some of the plants are predominantly used with higher frequency than the others. These include *Meriandra dianthera*, *Trigonella foenum-graceum*, *Aloe camperi*, and *Allium sativum* (Table 4.1). Most of the anti-diabetic plants, documented from the surveys, were also reported in the scientific literatures to have either direct or indirect effect on diabetes. However, some of the anti-diabetic plants like *Meriandra dianthera*, *Aloe camperi*, *Psiada panctualata*, *Steganotaenia araliaceae* and *Otostegia integrifolia* have not been reported to have anti-diabetic activities in the literature. Thus, the plants can be targeted for further studies.

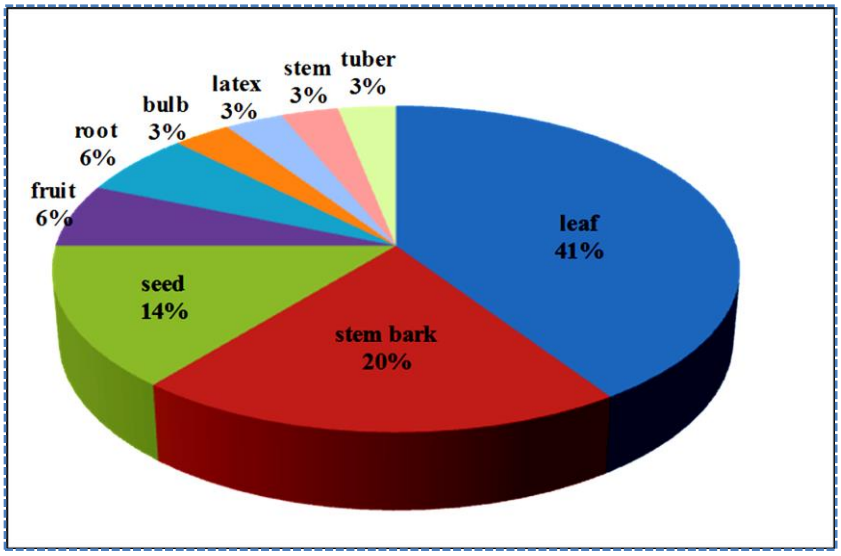


Figure 4.3: Frequency of the plant's parts used for the treatment of diabetes

Based on the analysis of the ethnomedicinal data, some polyherbal combinations were also reported for the treatment of diabetes. The two common polyherbs and their methods of preparation are described below.

i. The dried and powdered seeds of *Lepidium sativum*, *Brassica nigra* and *Nigella sativa* are mixed in equal proportion and thus macerated in water overnight, filtered and the filtrate is drunk twice a day until the patient gets better.

ii. Three teaspoons of *Nigella sativa*, a teaspoon of *Trachyspermum ammi* and seven pieces of the seeds of *Acacia nilotica* are pounded together and immersed in water for few hours, the filtrate of the mixture is drunk every morning before breakfast.

Table 4.1: List of anti-diabetic plants used in Central and Southern Zones of Eritrea

Scientific Name	Family	Vernacular Name	Common/English Name	Plant Part/s Used	Frequency Citation
Acacia nilotica (L.)	Fabaceae	Ghered	Gum Arabica	Seed, stem bark	3
Allium cepa L.	Liliaceae	Shiguerti-keyih	Onion	Bulb	6
Allium sativum L.	Liliaceae	Shiguerti-tsaeda	Garlic	Bulb	15
Aloe camperi Schweinfurth	Aloaceae	Sandai-ere	Aloe	Leaf, latex	8
Ammi visnaga (L.) Lam.	Apiaceae	E'bna	Khella/Picktooth	Leaf	2
Anethum graveolens Linn	Apiaceae	Shilan-maedo	Dill	Leaf	2
Azadirachta indica A. Juss.	Meliaceae	Neem	Neem	Leaf, stem bark	6
Balanites aegyptica (L.) Del.	Balanitaceae	Mekie	Desert Date	Leaf, fruit	4
Brassica nigra Koch.	Brassicaceae	Adri	Black Mustard	Seed	5
Calotropis procera (Ait.)	Asclepiadaceae	Ghinde'a	Sodom Apple	Stem bark, latex	3
Capparis decidua (Forssk.)	Caparidiacea	Sorob	Caper Berry	Stem bark, leaf	3
Carica papaya L.	Caricaceae	Papayo	Papaya	Leaf, seed	4
Carissa edulis (Forssk)	Apocynaceae	Agam	Num-num	Stem bark	2
Cichorium endivia L.	Asteraceae	Shikoria	Succory	Leaf	4
Clerodendrum myricoides (Hochst)	Lamiaceae	Sur-betri	Ugandense	Stem bark, leaf	2
Clutia lanceolata (Forssk)	Euphorbiaceae	Tish-belalito	Cerra Cipapau Apple	Leaf	1
Daucus carota L.	Apiaceae	Caroti	Carrot	Tuber	4
Entada abyssinica Steud. ex A.	Fabaceae	Halke	Tree entanda	Stem bark, leaf	1
Eucalyptus globulus (Labill.)	Myrtaceae	Tsaeda-kelamintos	Eucalyptus	Leaf	5
Ferula communis L.	Apiacea	Diog	Giant fennel	Seed, leaf	3
Gymnema sylvestre Roxb.	Asclepiadaceae	Shankuk	Australian Cow Plant	Leaf	2
Kigelia africana (Lam.) Benth.	Bignoniaceae	Mederba/Zelzale	Sausage Tree	Fruit	1

Continued (Table 4.1)

Scientific Name	Family	Vernacular Name	Common/English Name	Plant Part/s Used	Frequency Citation
Lepidium sativum L.	Brassicaceae	Shinfae	Garden Cress	Seed	6
Mangifera indica L.	Anacardiaceae	Mangus	Mango	Leaf, stem bark	3
Meriandra dianthera (Roth) Briq.	Lamiaceae	Nehiba/Mezeguf	-	Leaf	11
Moringa oleifera Lam.	Moringaceae	Moringa	Horseradish Tree	Leaf	4
Nigella sativa L.	Ranunculaceae	Abosoda	Black Cumin	Seed	5
Otostegia integrifolia Benth.	Lamiaceae	Ch'endog	-	Leaf	3
Plumbago zeylanica L.	Plumbaginaceae	Aftooh	Leadwort	Root, stem	3
Psiada panctualata (DC.) Vatke	Asteraceae	Tsehaiferhet	-	Leaf, root	2
Psidium guajava L.	Myrtaceae	Zeitun	Guava	Leaf	3
Rosmarinus officianilis L.	Lamiaceae	Azmarino	Rosemary	Leaf, stem	4
Solanum incanum L.	Solanaceae	Uengule	Poison Berry	Fruit	2
Steganotaenia araliacea Hochst.	Apiaceae	Mewets denagl	Carrot Tree	Leaf, seed	2
Tamarindus indica L.	Fabaceae	Humer	Tamarind Tree	Fruit	3
Terminalia brownii Fresen	Combretaceae	Weiba	Terminalia	Stem bark, leaf	2
Trachyspermum ammi (L.)	Apiaceae	Kamun/Tsakida	Bishop's Weed	Seed	5
Trigonella foenum-graecum L.	Fabaceae	Abe'ake	Fenugreek	Seed	9
Vernonia amygdalina Del.	Asteraceae	Grawa	Bitter Leaves	Leaf, stem bark	2
Withania somnifera (L.)	Solanaceae	Agol	Winter Cherry	Root, leaf	2
Zingiber officinale Roscoe	Zingiberaceae	Zingible	Ginger	Root	3
Zizyphus spina-christi (L.)	Rhamnaceae	Gaba	Christ's Thorn Jujube	Leaf	2

Table 4.2: Methods of herbal preparations for the treatment of diabetes and other diseases

Medicinal Plant	Method of Preparation and/or Administration for Diabetes Treatment	Other Ethnomedicinal Uses of the Plant
Acacia nilotica	- decoction of the seed or bark is used regularly	- malaria, cough, diarrhoea, dysentery, insect repellent
Allium cepa	- freshly cut bulb is often used	- antipyretic, gastrointestinal disorders, antiseptic, anthelminthic
Allium sativum	- fresh bulb is eaten raw or added in sauce	- asthma, antiseptic, diuretic, hypertension, expectorant,
Aloe camperi	- extract of the latex or leaf is used regularly	- skin burns, dandruff, stomach pain, hypertension, hair fall
Ammi visnaga	- leaf extract is used in the morning	- diuretic, hypotensive, hair-care, antispasmodic, asthma
Anethum graveolens	- tea of the leaves is taken twice daily	- diarrhea, eye problems, indigestion, stomachache, antispasmodic
Azardichta indica	- leaf or bark decoction used for drinking	- insect repellent, malaria, skin diseases, anthelmintic, diuretic
Balanites aegyptiaca	- leaf extract or ripe fruit is taken	- purgative, insecticidal, laxative, diarrhoea, stomach aches
Brassica nigra	- seed decoction is used regularly	- gastrointestinal disorder, stimulant, diuretic, bronchitis
Calotropis procera	- crushed bark or latex are mixed with butter	- skin diseases, anthelmintic, expectorant, wounds, diarrhoea
Capparis decidua	- infusion of stem bark or leaf is used	- tooth ache, cough, arthritis, anthelmintic, malaria, inflammation
Carica papaya	- decoction of seed or leaf is drunk 1 beaker in the morning	- amoebicide, hypertension, constipation, expel worms, laxative
Carissa edulis	- extract of stem bark is drunk regularly	- anthelmintic, inflammation, hypotensive, diuretics, headache
Cichorium endive	- cooked properly and eaten with enjera	- appetizer, febrifuge, anti-allergic
Clerodendrum myricoides	- leaf or stem bark extract is drunk regularly	- abdominal pains, snake bites, hemorrhoids, eye disease
Clutia lanceolate	- leaf extract is taken twice a day	- malaria, diarrhea, colds, gynecological problems
Daucus carota	- tuber is eaten in raw or with salad	- diuretic, inflammation, leprosy, worms troubles
Entada abyssinica	- decoction of the stem bark or leaf	- gastrointestinal problems, cold, candidiasis
Eucalyptus globulus	- leaf extract is taken 1 cup per day	- insect repellent, tuberculosis, bronchitis, malaria, skin diseases
Ferrula communis	- decoction of fresh leaf or dried seed	- antispasmodic, diarrhea, expectorant ,dermatitis
Gymnema sylvestre	- leaf extract is taken daily	- cough, inflammations, constipation, haemorrhoids, bronchitis
Kigelia Africana	- fruits are eaten	- constipation, tapeworm, dysentery, gynecological disorders

Continued (Table 4.2)

Medicinal Plant	Method of Preparation and/or Administration for Diabetes Treatment	Other Ethnomedicinal Uses of the Plant
Lepidium sativum	- the seed extract is taken before bed	- diuretic, cough, laxative, asthma, diarrhea, malaria
Mangifera indica	- decoction of leaf or stem bark is used regularly	- diuretic, diarrhea, dysentery, anemia, bronchitis, hypertension
Meriandra dianthera	- extract of the leaf is taken 1 cup daily	- hypertension, gastritis, bronchial asthma, purgative, diarrhea
Moringa oleifera	- fresh leaf juice taken every morning in empty stomach	- asthma, constipation, skin diseases, diarrhea, stomach pain, hypertension
Nigella sativa	- seeds added in bread or a spoon of powdered seeds taken orally before meal	- asthma, anthelmintic, dysentery, hypertension, gastrointestinal Problems
Otostegia integrifolia	- extract of the leaves taken in the evening	- gynecological problems, inflammation, anti-oxidant
Plumbago zeylanica	- decoction of stem or roots used trice a day	- stimulant, abortifacient, inflammation, bronchitis, itching
Psiada panctualata	- decoction of the leaves or roots is used	- cough, anti-abortion, asthma
Psidium quajava	- decoction of the leaves is used regularly	- wounds, ulcers, bronchitis, dysentery, antiseptic, antispasmodic
Rosmarinus officianilis	- a spoon of leaf or stem is added in daily food	- stomach pains, gynecological problems, bad breath, migraine
Solanum incanum	- fruits boiled in hot water or mixed with yogurt	- expectorant, stomach ache, fever, snake bite, bronchitis
Steganotaenia araliacea	- decoction of either leaf or seed mixed with milk is taken in the morning	- sore-throat, ulcer, diuretic, stomach ache, dysentery, Hypotensive
Tamarindus indica	- extract of the fruit is taken twice a day	- malaria, fever, stomach ache, wounds, purgative
Terminalia brownie	- decoction of the stem bark or leaf	- wounds, malaria, dermatitis, tuberculosis
Trachyspermum ammi	- seed powder is soaked in water or milk	- hypertension, diarrhea, asthma, anthelminthic, diuretic
Trigonella foenum-graceum	- extract of the dried and powered seed used regularly	- diuretic, antipyretic, stomachic, anemia, hypertension, gastrointestinal pains, carminative
Vernonia amygdalina	- the extract of fresh leaves in water is used	- stomach ache, skin infections, tooth ache, cough, malaria,
Withania somnifera	- decoction of the roots are used	- constipation, skin infection, rheumatism
Zingiber officinale	- tea of the rhizome frequently used	- digestive disorders, stimulant, bronchitis, throat infections
Ziziphus spina-christi	- infusion of the leaves used regularly	- diarrhea, dandruff, skin infections, bronchitis, malaria

Several plant species have been used for the prevention or management of diabetes by Native Americans, Chinese, South Americans and Asian Indians (Mentreddy, Mohamed, & Rimando, 2005). In China, it is estimated that more than 200 species of plants exhibit hypoglycemic properties and hundreds of herbal formulations have been reported to have been used for the treatment of diabetes (Jia, Gao & Tang, 2003). In Nigeria, about 115 anti-diabetic plants were screened either *in vivo* or *in vitro*. One-third of the plants have been studied for their mechanism of action, while isolation of the bioactive constituent(s) has been accomplished for twenty three plants (Ezuruike & Preto, 2014).

4.2 Preliminary Phytochemical Analysis

As shown in Table 4.3, the preliminary phytochemical analysis of the methanol and aqueous extracts established the presence of alkaloids, saponins, phenols, steroids, flavonoids, coumarines, glycosides and carbohydrates in most of the anti-diabetic plants. However, the hexane extracts did not show the characteristic metabolites of interest. During the analysis of the methanol and aqueous extracts, very intense coloration and precipitation which are characterstic of phenols and flavonoids were observed in *M. dianthera*. The chemical analysis can be used as an infererence to the possible classes of metabolites present in the plant extracts, thus systematic instrumental analysis is mandatory for the final identification and characterisation of the bioactive molecules present in the samples.

Table 4.3: Preliminary phytochemical screening of the anti-diabetic plants

Medicinal Plant	Solvent Used	Alkaloids	Saponins	Steroids	Phenols	Tannis	Flavonoids	Resins	Glycosides	Carbohydrates	Proteins	Coumarines	Terpenoids
Acacia nilotica	Methanol	+	+	+	+	+	+	+	+	+	+	-	+
	Hexane	-	+	-	-	-	-	-	-	-	+	+	-
	Aqueous	+	-	+	+	+	+	-	+	-	+	-	+
Aloe camperi	Methanol	+	+	+	+	+	-	+	+	+	+	+	+
	Hexane	-	+	+	-	-	-	-	-	-	-	+	+
	Aqueous	+	-	+	+	+	+		+	+	-	+	+
Azadirachta indica	Methanol	+	+	+	+	+	+	-	+	+	+	-	+
	Hexane	-	+	-	-	-	-	-	-	-	-	+	-
	Aqueous	+	+	+	+	+	+	+	+	+		+	+
Balanites aegyptiaca	Methanol	-	+	+	+	+	-	+	+	+	+	-	
	Hexane	-	+	-	-	-	-	-	-	-	-	-	-
	Aqueous	+	+	+		+	+	+	+	+		+	+
Brassica Nigra	Methanol	+	-	+	+	+	+	-	+	+	-	+	-
	Hexane	-	+	+	-	-	-	-	-	-	-	-	+
	Aqueous	+	-	-	-	+	+		+	-	-	+	-
Eucalyptus globulus	Methanol	+	-	+	+	+	-	+	-	+	-	+	-
	Hexane	-	+	-	-	-	-	-	-	-	-	-	-
	Aqueous	+	+	+	+	+	+		+	+	+	+	+
Lepidium Sativum	Methanol	+	+	+	+	+	+	+	+	-	+	+	+
	Hexane	-	-	-	-	-	+	-	-	-	-	-	-
	Aqueous	+	+	+	+	+	+	+	+	-	+	+	+
Meriandra dianthera	Methanol	+	+	+	+	+	+	+	+	+	+	+	+
	Hexane	-	-	+	-	-	-	-	-	-	-	+	+
	Aqueous	+	+	+	-	+	+	+	+	+	-	+	+
Nigella sativa	Methanol	+	-	+	+	+	+	+	+	+	+	-	
	Hexane	-	-	-	+	-	-	-	-	-	-	-	-
	Aqueous	+	+	+	+	-	-		+	+	+	-	-
Trachyspermum ammi	Methanol	+	+	+	+	+	+	+	+	+	+	+	+
	Hexane	-	-	-	-	-	-	-	-	-	-	-	-
	Aqueous	+	-	+	+	+	+		+	+	-	+	+
Trigonella foenum-graceum	Methanol	+	+	+	+	+	+	-	+	+	+	-	
	Hexane	-	-	-	+	-	-	-	-	-	-	-	-
	Aqueous	+	+	+	+	+	+		+	+	+	-	+

NB: '- & +' - represent absence and presence of the metabolite respectively

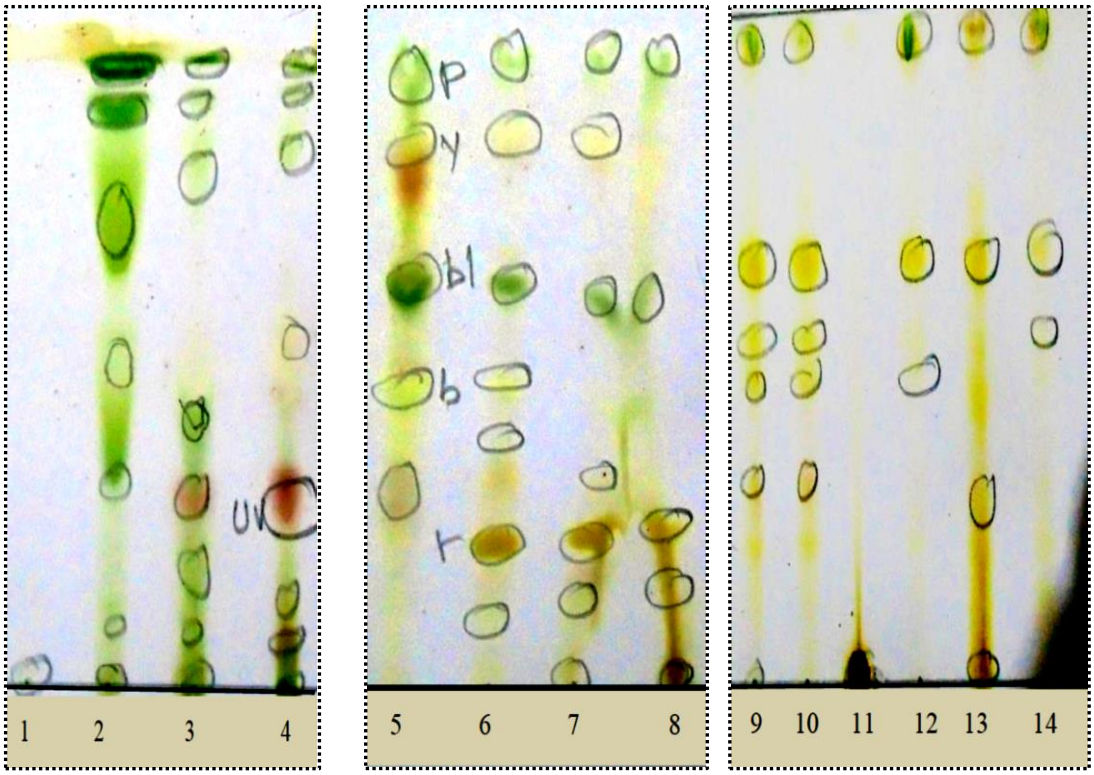
4.3 Separation of Components Using Column Chromatography

4.3.1 TLC Analysis

The crude methanol extracts were initially screened using TLC. As described in Section 3.9.1, various solvent systems were tried during the analysis. The dried TLC plates were observed using spraying agents and UV light (at 254 and 365 nm), the solvent system of hexane: methanol and hexane: ethyl acetate: methanol gave good separation of the components of the crude mixture. Thus, the gradient solvent system of hexane: ethyl acetate: methanol was chosen for column work. Some representative TLC results prepared using hexane: ethyl acetate: methanol of certain fractions of *Meriandra dianthera* are shown in Plate 4.1 and the calculated R_f values of the separated components are summarized in Table 4.4. As displayed in the TLC, some of the fractions (like 7 & 8; 9 &10) had similar separations. Similar results were observed in the fractions of the other plants and thus this solvent system was adopted in studying the components of the crude and fractions analysed. Analysis of some of the TLC plates under UV confirmed the presence of UV active compounds that may infer the presence of conjugated aromatic systems.

Table 4.4: Characteristic spots and their corresponding R_f values

Fraction	Characteristic Spots	Solvent System	Calculated R_f values
1	0 spot		-
2	6 spots	Hex: EtOAc	0.10, 0.29, 0.46, 0.67, 0.83, 0.96
3	7 spots	(8:2)	0.10, 0.17, 0.27, 0.39, 0.74, 0.83, 0.89
4	7 spots		0.10, 0.13, 0.26, 0.50, 0.76, 0.84, 0.89
5	5 spots		0.28, 0.43, 0.59, 0.78, 0.91
6	7 spots	Hex:EtOAc:MeOH	0.10, 0.19, 0.34, 0.43, 0.56, 0.78, 0.91
7	6 spots	(10:4:1)	0.10, 0.19, 0.31, 0.56, 0.40, 0.78, 0.91
8	4 spots		0.10, 0.19, 0.56, 0.92
9	5 spots		0.28, 0.46, 0.53, 0.64,
10	5 spots		0.28, 0.44, 0.53, 0.63,
11	0 spots		-
12	3 spots	Hex:EtOAc:MeOH	0.46, 0.64, 0.96
13	3 spots	(15:8:1)	0.28, 0.54, 0.64, 0.97
14	3 spots		0.54, 0.64, 0.97



Key: uv- uv active, r- red, b-blue, y-yellow, P- purple

Plate 4.1: TLC analysis of certain fractions of *Meriandra dianthera*

4.3.2 Fractionation of Crude Extracts

The components of the crude extracts were fractionated using both open-column chromatography (CC) and vacuum-liquid chromatography (VLC). An example of the collected fractions, using the gradient solvent system of hexane: ethyl acetate: methanol, from *M. dianthera* is described in Table 4.5. The similar fractions, among the large number of fractions present in the vials, were pooled together using TLC analysis. Due to the large number of fractions, the similar fractions of *M. dianthera* and the other plants were eventually combined together into seven fractions each. The fractions were then screened for their anti-diabetic activities using the *in vitro* assays and structural studies using GC-MS and LC-MS.

Table 4.5: The fractions collected during the column work of *M. dianthera*

Sample	Fractions	Percentage of solvent	Volume (ml) of solvent used
1	1-10	0% EtOAc / Hex	500
2	11-20	20 % EtOAc / Hex	500
3	21-30	40 % EtOAc / Hex	500
4	31-40	60 % EtOAc / Hex	500
5	41-50	80 % EtOAc / Hex	500
6	51-60	100 % EtOAc / Hex	500
7	61-70	20 % MeOH /EtOAc	500
8	71-80	40 % MeOH /EtOAc	500
9	81-90	60 % MeOH /EtOAc	500
10	91-100	40 % MeOH /EtOAc	500
11	101-110	80 % MeOH//EtOAc	500
12	111-120	100 % MeOH	500

NB: EtOAc– Ethyl acetate**, MeOH–** methanol, Hex-Hexane

4.4 Anti-Diabetic Effects of the Plants

4.4.1 *In Vitro* Results of Crude Extracts

The results of the *in vitro* anti-diabetic activities of the plants are displayed in Table 4.6 and Table 4.7. The IC_{50} wasccalculated using linear and non-linear regression regression analyses and the associated equations are furnished in Appendix 3 and Appendix 4. All the extracts and Acarbose (positive control) elicited dose dependent α-amylase and α-glucosidase inhibition activities. A maximum of 87.8, 63.5, 58.7 and 55.3 % inhibition of α-glucosidase activity was observed at 0.800 mg/ml concentration of the crude extracts from *M. dianthera*, *A. camperi*, Polyherb and Acarbose, respectively *M. dianthera* showed the highest α-glucosidase inhibitory (IC_{50}: 0.074 ± 0.032 mg/mL) effect at highest concentration tested relative to *A. camperi*, Polyherb and Acarbose (IC_{50}: 0.37 ± 0.052, 0.56 ± 0.024 and 0.55 ± 147 0.029), respectively. This indicates that the extracts are very potent α-glucosidase inhibitors in comparison with acarbose. Acarbose is a known α-amylase and α-glucosidase inhibitor, currently used in anti-diabetic therapy for reducing postprandial increase in blood glucose levels. As shown in Figure 4.4, *A. camperi* and the Polyherb displayed lower α-amylase inhibitory activities (IC_{50}: 1.72 ± 0.06 and 2.57 ± 0.07 mg/mL, respectively) compared to Acarbose and *M. dianthera* (IC_{50}: 0.31 ± 0.01 and 0.43 ± 0.02 mg/mL, respectively).

Table 4.6: *In vitro* α-glucosidase inhibitory activities of the crude extracts

Test sample	% Inhibition Concentration (µg/ml)						IC_{50} (µg/ml)
	25	50	100	200	400	800	
M. dianthera	14.6 ± 0.41	28.74 ± 0.16	63.07 ± 0.24	76.84 ± 0.26	82.42 ± 0.38	88.01 ± 0.49	**74.13 ± 0.32**
A. camperi	2.61 ± 0.34	10.80 ± 0.73	21.96 ± 0.87	38.61 ± 0.27	54.51 ± 0.26	63.54 ± 0.66	**371.30 ± 0.52**
Polyherb	11.41 ± 0.37	20.32 ± 0.28	23.55 ± 0.07	35.42 ± 0.12	38.46 ± 0.19	58.71 ± 0.40	**562.30 ± 0.24**
Acarbose	10.71 ± 0.19	15.49 ± 0.19	21.10 ± 0.40	32.50 ± 0.38	45.89 ± 0.38	55.26 ± 0.19	**549.54 ± 0.29**

NB: Results reported as mean ± SD

Table 4.7: *In vitro* α-amylase inhibitory activities of the crude extracts

Test sample	% Inhibition Concentration (µg/ml)						IC_{50} (mg/ml)
	25	50	100	200	400	800	
M. dianthera	18.2 ± 1.40	23.65 ± 1.42	27.33 ± 1.30	28.05 ± 1.07	38.87 ± 0.28	78.30 ± 3.07	**0.43 ± 0.02**
A. camperi	5.03 ± 0.04	5.55 ± 0.45	6.32 ± 0.21	7.01 ± 0.07	7.87 ± 0.27	15.87 ± 0.44	**1.72 ± 0.06**
Polyherb	1.68 ± 0.26	2.41 ± 0.18	2.72 ± 0.11	8.51 ± 0.10	9.24 ± 0.25	16.42 ± 0.19	**2.57 ± 0.07**
Acarbose	26.66 ± 0.42	29.50 ± 0.42	41.23 ± 0.41	43.02 ± 0.49	56.19 ± 0.57	82.92 ± 1.45	**0.31 ± 0.01**

NB: Results reported as mean ± SD

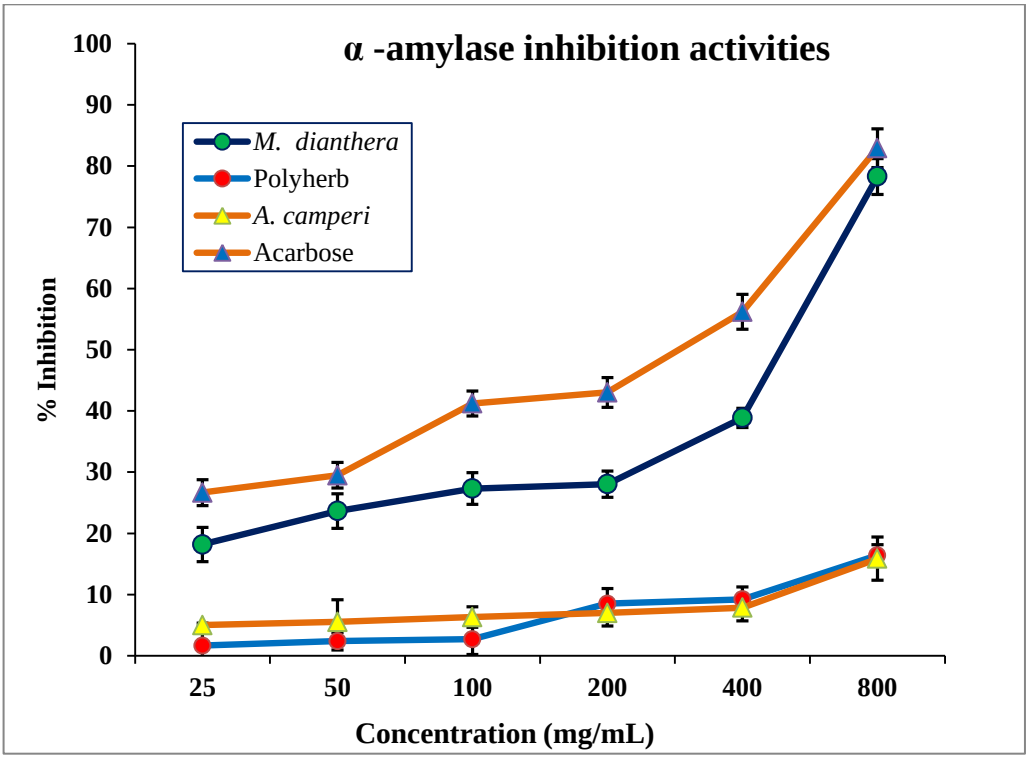


Figure 4.4: α-Amylase inhibitory activities of the crude extracts

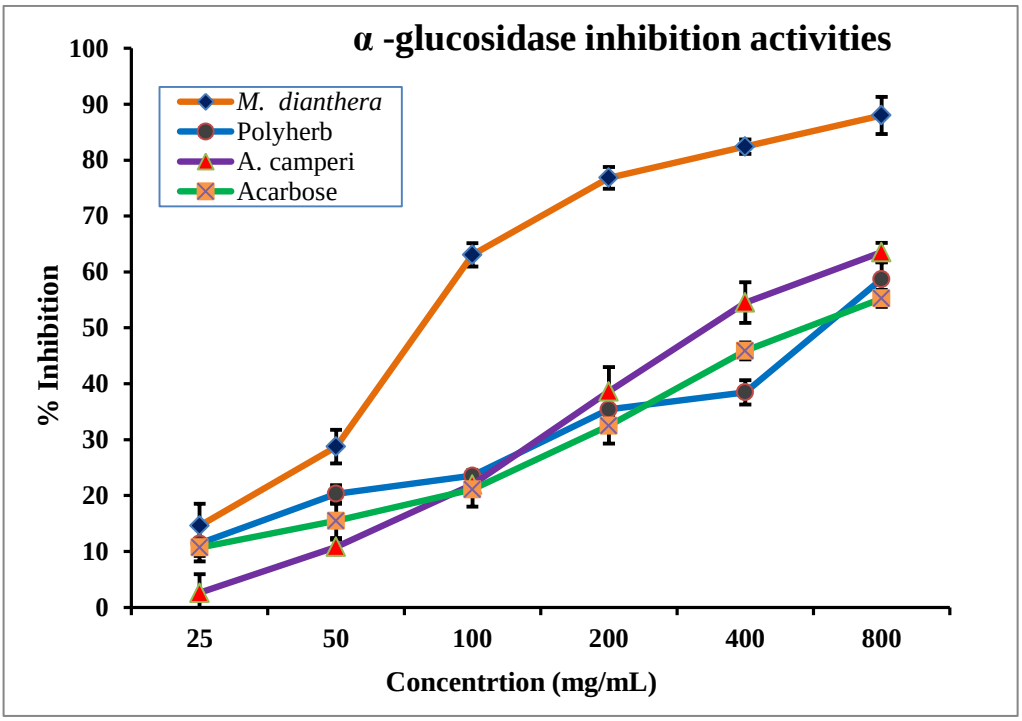
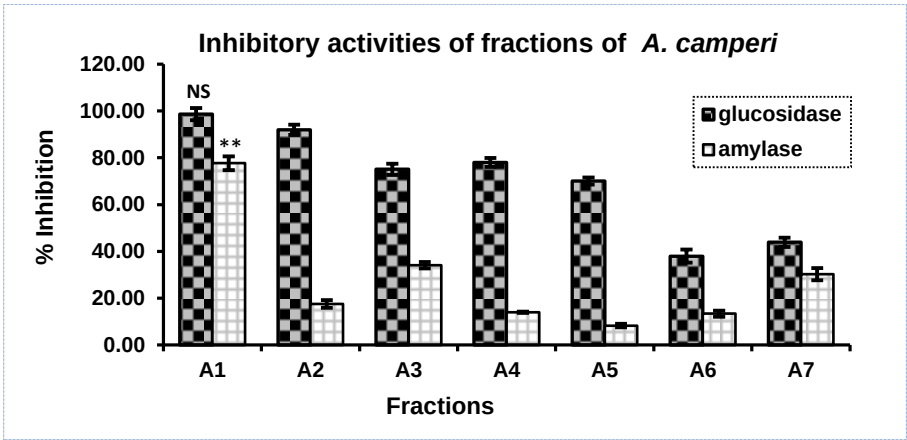


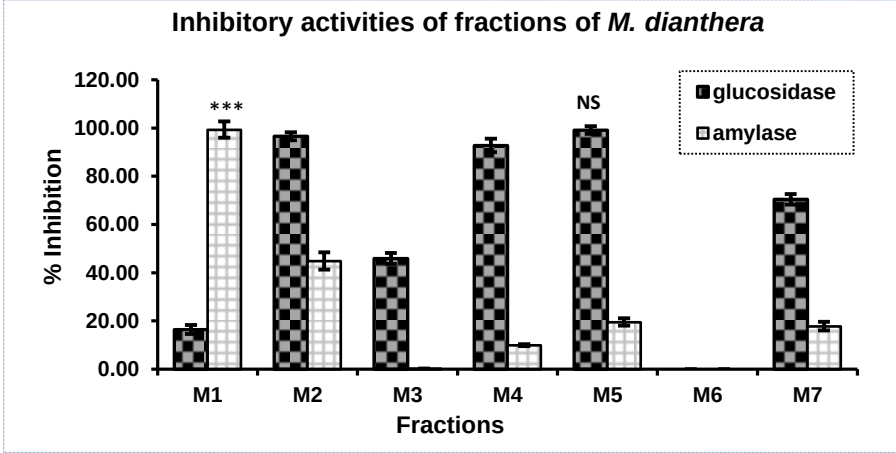
Figure 4.5: α-Glucosidase inhibitory activities of the crude extracts

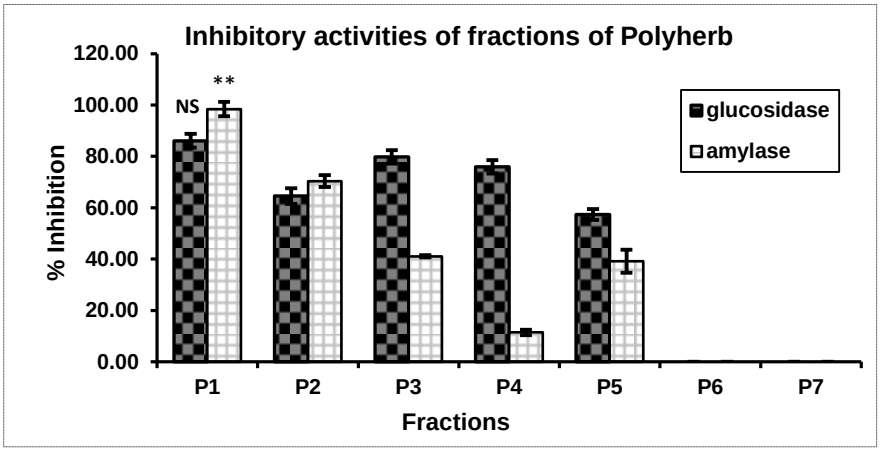
For the α-amylase inhibition activities, even at lower concentrations (25 mg/ml), there was a significant difference ($P < 0.01$) among the values generated for all the extracts (Figure 4.4). Moreover, there was a very significant difference ($P < 0.001$) among the percentage inhibition of the groups at higher concentration (800 mg/ml). Similarly, in the α-glucosidase inhibition activities (Figure 4.5), except for the polyherb and standard, there was significant difference ($P < 0.05$) at various ranges of concentrations (25 - 800 µg/ml) for the *A. camperi* and *M. dianthera*. From the results, it is evident that the methanolic extracts of the plants had potential α-amylase and α-glucosidase inhibitory activities comparable to the commercial drug acarbose. The enzyme inhibitory activities have been adopted as reliable techniques of evaluating the anti-diabetic potential of different medicinal plants and thus inhibition of the action of the digesting enzymes prolong overall carbohydrate digestion time by delaying its breakdown, causing a decrease in the rate of glucose absorption and consequently blunting the post prandial rise in plasma glucose (Powers & David, 2011).

4.4.2 *In Vitro* Results of Fractions

The *in vitro* anti-diabetic activities of the fractions, of the three plants of interest, are described in Figure 4.6. Most of the fractions displayed characteristic inhibition activities. The non-polar fractions of the *A. camperi* (A1, A2), *M. dianthera* (M2) and Polyherb (P1) and the moderately polar fractions of Meriandra (M4, M5) and Polyherb (P3, P4) elicited relatively higher α-glucosidase inhibitory activities. However, the most polar fractions of *M. dianthera* (M6, P6) and Polyherb (P7) showed no inhibitory activities against α-glucosidases. Similarly, the non-polar fractions labeled as A1, M1, M2, P1 and P2 displayed higher α-amylase inhibition activities as compared to the polar fractions. The moderately polar fraction labeled as M3 and the most polar fractions labeled as M6, P6, and P7 showed no inhibitory activities against α-amylase. The fractions with characteristic good α-glucosidase and α-amylase inhibitory activities were subjected to GC-MS and LC-MS in order to identify and characterize the bioactive metabolites responsible for the reported activities.







Remarks: A1-A7: fractions from *A. camperi*, **M1-M7:** fractions from *M. dianthera* and **P1-P7:** fractions from Polyherb; *** for $P < 0.001$, ** for $P < 0.01$ and ^{NS} for not-significant (Statistical significance compare to the other fractions); Results reported as mean ± SD (n=3)

Figure 4.6: Comparison of the enzyme inhibitory activities of the fractions

4.4.3 *In Vivo* Results of Crude Extracts

Efficacy and toxicity of the crude extracts were investigated using *in vivo* models.

4.4.3.1 Acute Oral Toxicity Test Results

During the toxicity tests, the albino rats were safe up to a maximum dose of 2000 mg/kg per body weight for all the three extracts. There were some temporary changes in the normal behavioural pattern of the rats administered especially with *A. camperi* and *M. dianthera*. However, there were no signs and symptoms of acute toxicity and mortality when fed with all the extracts. The results confirmed that the three plant extracts have LD_{50} value greater than the test dose (2000 mg/kg per body weight) because at higher dose (5000 mg/kg) the rats were not able to survive. Recent reported results show that the LD_{50} values of the leaves of *Hibiscus rosa-sinensis* and *Java tea* were estimated to be greater than 2000 mg/kg and 5000 mg/kg respectively (Nath & Yadav, 2015; Pariyani *et al*., 2015).

4.4.3.2 Oral Glucose Tolerance Test (OGTT)

The OGTT results of the crude extracts are summarized in Table 4.8. Administration of *A. camperi* (AC), *M. dianthera* (MD) and Polyherb (PH) at doses of 200 and 400 mg/kg body weight to the glucose loaded rats showed significant reduction in blood glucose levels (BGL). Post to the extracts' and standard drug administration (0 min), there was no significant difference in BGL among all the groups ($P > 0.05$). All groups, however, showed significant increase ($P < 0.05$) in BGL 30 min following glucose loading, confirming the induction of hyperglycemia (Figure 4.7). Compared to the normal control (NC) all groups, except PH200, showed significant difference (PC and MD400 with $P < 0.001$, AC400 and MD200 with $P < 0.01$, AC200 and PH400 with $P < 0.05$) in BGL 30 min following glucose loading. OGTT is commonly used to monitor how blood glucose homeostasis is maintained following glucose overload. This test can be applied for the diagnosis of pre-diabetes and diabetes. This test is complementary to glycemia monitoring for

diabetes care and could be necessary to detect more subtle changes during the development of insulin resistance (DeFronzo & Abdul-Ghani, 2011).

Table 4.8: Effect of the crude extracts on OGTT

Group	Blood glucose level (in mg/dl)			
	0 min	30 min	60 min	120 min
NC	98.28 ± 2.70	117.77 ± 1.71^{a2}	114.52 ± 1.49	112.21 ± 1.70
PC	97.36 ± 1.51	105.49 ± 1.48^{a1}	99.04 ± 1.32^{c3}	85.67 ± 1.20^{b3,c3}
AC200	98.54 ± 1.68	110.40 ± 1.73^{a2}	105.10 ± 1.25^{c2}	98.52 ± 1.56^{b2,c3}
AC400	97.32 ± 1.43	107.57 ± 1.75^{a1}	103.24 ± 1.62^{c3}	93.68 ± 1.33^{b2,c3}
MD200	97.04 ± 1.22	108.07 ± 1.73^{a2}	102.64 ± 1.84^{c3}	92.70 ± 1.90^{b3,c3}
MD400	97.92 ± 1.67	105.65 ± 1.97^{a1}	98.55 ± 1.15^{c3}	86.21 ± 1.96^{b3,c3}
PH200	98.28 ± 2.32	111.25 ± 1.52^{a2}	106.48 ± 1.44^{c2}	102.29 ± 1.79^{b1,c2}
PH400	98.64 ± 2.57	109.71 ± 1.96^{a1}	103.64 ± 2.20^{c3}	99.56 ± 1.71^{b1,c3}

All data are expressed as mean ± SEM; n=6 in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. NC-normal control, PC-positive control, $^{1}P < 0.05$, $^{2}P < 0.01$ and $^{3}P < 0.001$, a-compared to 0 min, b-compared to 30 min, c-compared to NC

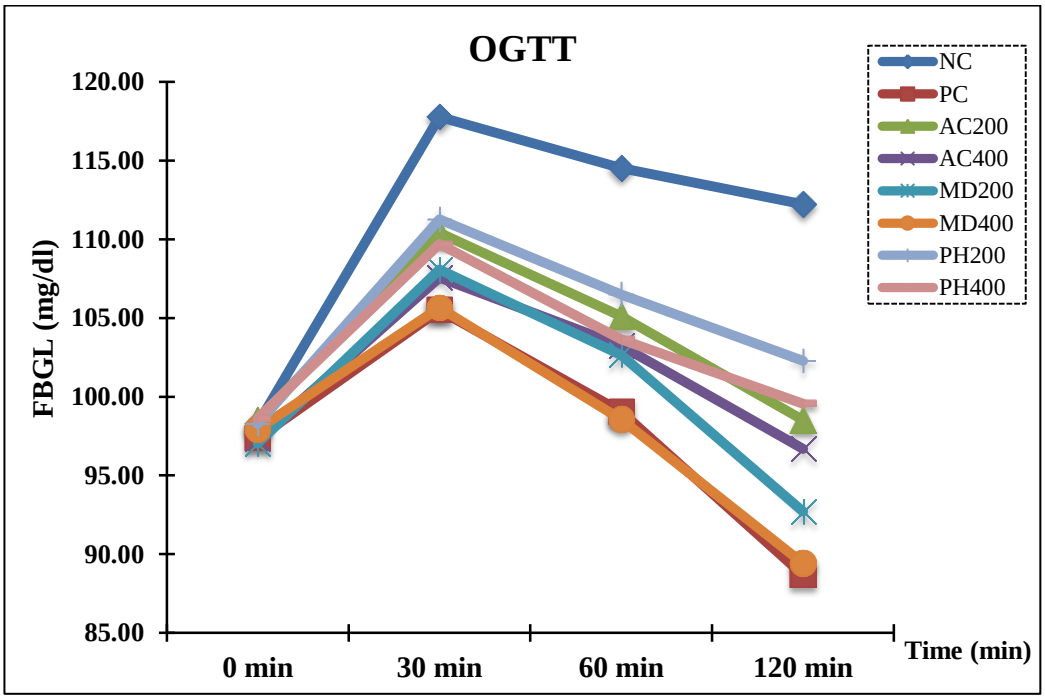


Figure 4.7: Oral glucose tolerance effect of the extracts

On the other hand, in the inter-group analysis, hyperglycemia with glucose challenge was significantly ($P < 0.001$) brought down with PC, AC400, MD200, MD400, and PH400 at 60 min relative to the negative control. AC200 and PH200 also showed significant ($P < 0.01$) decrease in BGL at 60 min. At 120 min, a significant difference (mostly $P < 0.001$) was achieved from the groups compared to the negative control. Moreover, at 120 min a significant difference (mostly with $P < 0.01$) was observed compared to the peak hyperglycemia (BGL at 30 min).

4.4.3.3 Anti-Hyperglycemic Effect

As shown in Table 4.9 and Figure 4.8, a very significant ($P < 0.001$) reduction in blood glucose level was observed at the 7^{th} day of the extracts administration of AC400, MD200, MD400 and metformin. Similarly, significant reduction in BGL was observed with AC200 ($P < 0.01$), PH400 ($P < 0.01$) and PH200 ($P < 0.05$) relative to the diabetic control. A very significant ($P < 0.001$) reduction in the BGL was recorded by all the extracts at the end of the 2^{nd} week treatment, which remained persistent up to the 3^{rd} week. The decline in blood glucose level is generally used as the main directory for anti-hyperglycemic effect of drugs. Thus, treatment with methanol extract has proved to be highly effective in causing significant anti-hyperglycemic response in the experimentally diabetic rats.

Comparatively, each group showed significant reduction ($P < 0.05$) in BGL as compared to the first day extract administration (0 day). Moreover, comparing the 21^{st} day to the 7^{th} day, all the extracts showed a very significant ($P < 0.01$) reduction in BGL. By the end of the 21^{st} day, the reduction in BGL of AC200, AC400, MD200, MD400, PH200, and PH400 was 35.05, 37.29, 37.74, 42.10, 31.53, and 33.63% respectively. Comparing the reduction brought about by the plant extracts to metformin (46.84 %), it is evident that the reduction in BGL is quite significant. After 21 days of treatment, the maximum reduction in BGL was observed in the group treated with *M. dianthera* at a concentration of 400 mg (MD400) as compared to the other treatment groups. FBG level was estimated before diabetic induction and after extracts administration. All the three extracts (at doses of 200 and 400

mg/kg per body weight) and standard drug produced significant reduction in the blood glucose level with maximum reduction being achieved with the dose 400 mg/kg for all the extracts showing dose dependent activity. Similar results on the anti-hyperglycemic activities of other medicinal were reported recently. Treatment with prolonged dose of exudates of *Aloe vera* leaves showed hypoglycemic effect in alloxanized diabetic rats and the methanolic extract of *Aegle marmelos* was found to reduce blood sugar in alloxan induced diabetic rats; reduction in blood sugar was seen from the 6^{th} day after continuous administration. Moreover, the root extract of *Anthocephalus indicus* (at 500 mg/ kg body weight) for 21 days in alloxan induced diabetic rats resulted in significant decrease in the levels of blood glucose (Attanayake, Jayatilaka, Pathirana, & Mudduwa, 2013; Prakash *et al*., 2015).

Table 4.9: Anti-hyperglycemic effect of the crude extracts

Group	Blood glucose level (in mg/dl)			
	0 day	**7 day**	**14 day**	**21 day**
NC	88.2 ± 3.55	91.08 ± 3.40	90.72 ± 3.25	92.52 ± 4.21
DC	236.76 ± 3.84	238.08 ± 2.72	237.80 ± 2.54	239.28 ± 2.84
PC	233.80 ± 2.61	169.92 ± 4.78^{c3,a3}	141.12 ± 4.05^{c3}	124.28 ± 3.61^{c3,b3}
AC200	231.80 ± 2.93	210.44 ± 3.98^{c2,a1}	180.10 ± 3.28^{c3}	144.32 ± 3.58^{c3,b3}
AC400	233.16 ± 2.36	205.16 ± 3.52^{c3,a2}	175.19 ± 2.56^{c3}	135.12 ± 2.91^{c3,b3}
MD200	230.00 ± 3.37	210.44 ± 3.98^{c3,a2}	180.10 ± 3.28^{c3}	144.32 ± 3.58^{c3,b3}
MD400	233.16 ± 2.36	205.16 ± 3.52^{c3,a2}	175.19 ± 2.56^{c3}	135.12 ± 2.91^{c3,b3}
PH200	234.28 ± 2.60	222.56 ± 2.96^{c1,a1}	199.88 ± 3.74^{c3}	160.40 ± 4.03^{c3,b3}
PH400	232.44 ± 2.89	218.20 ± 1.82^{c2,a2}	192.33 ± 2.75^{c3}	154.28 ± 3.53^{c3,b3}

NB: All data are expressed as mean ± SEM; n=6 in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. NC-normal control, DC-diabetic control, PC-positive control, $^{1}P < 0.05$, $^{2}P < 0.01$ and $^{3}P < 0.001$, a-compared to 0 day, b-compared to 7 day, c-compared to DC

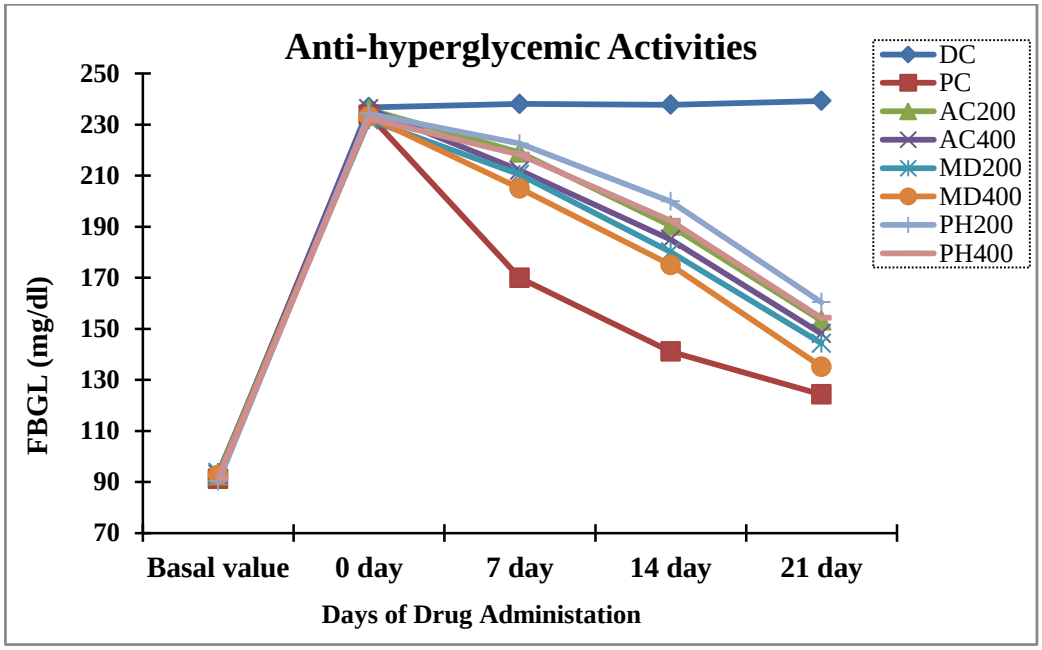


Figure 4.8: Anti-hyperglycemic effects of the extracts

4.4.3.4 Assessment of Body Weight

Table 4.10 represents the effects of methanol extracts of *A. camperi*, *M. dianthera* and the Polyherb on the changes in the body weight of normal control and treated diabetic rats. Statistical analysis by One-Way ANOVA revealed that there was no significant difference among the groups during the estimation of initial body weight ($P > 0.05$). However, a steady decrease in the body weight was observed in the diabetic control ($P < 0.001$) by the end of the 3rd week of Alloxan treatment.

Administration of metformin to the diabetic rats resulted in increase of the body weight compared to diabetic control rats which suggests that metformin treatment has positive effect on maintaining body weight. However, diabetic rats treated with plant extracts at doses of 200 and 400 mg/kg per body weight showed decrease in body weight but it was significant improvement as compared to the body weight of the diabetic control group. Moreover, the methanol extracts of *A. camperi*, *M. dianthera* and Polyherb showed significant change ($P < 0.05$) in body weight compared to the normal control group. Diabetes is characterized by weight loss and thus was observed in this study. Initially, Alloxan administration brought about

marked reduction in body weight of rats. However, the decrease in body weight of the rats was improved by the treatment of the herbal extracts. The extracts with highest activities showed better improvement in body weight compared to those with lowest activities.

Table 4.10: Comparison of body weight of the normal and treated diabetic rats

Treatment Group	Initial body Weight (in grams)	Final body Weight (in grams)	Change in body Weight (%)
NC	163.35 ± 3.13	169.6 ± 2.97	6.25
DC	160.18 ± 3.27	130.05 ± 3.21***	30.13
PC	161.06 ± 2.41	163.90 ± 2.49 ^{NS}	2.84
AC200	160.73 ± 3.39	148.01 ± 2.78 **	12.72
AC400	161.56 ± 3.07	150.23 ± 3.14 **	11.32
MD200	162.43 ± 2.85	152.06 ± 2.99 *	10.38
MD400	161.57 ± 3.10	155.54 ± 3.14 *	6.04
PH200	161.89 ± 2.75	136.86 ± 3.12 **	25.03
PH400	162.70 ± 3.48	141.70 ± 3.21 **	21.00

NB: All data are expressed as mean ± SEM; n = 6 in each group. Statistical significant test for Comparison was done by two tailed paired t-test. NC-normal control, DC-diabetic control, PC-positive control, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the initial body weight

4.5 Trace Elements and Heavy Metals Analysis

Before the analysis of the elements, the accuracy of the methods was verified using in-house certified reference materials (CRMs) digested using dry ashing. As exhibited in Figure 4.9, the calculated relative errors were as follows: Mg (0.36), Ba (0.58), Ca (1.56), K (0.26), Na (1.11), Al (0.17), Sr (0.61), Zn (0.44), Cr (- 1.65), V (1.89), Mn (- 0.27), Se (0.06), Fe (0.28), Cu (0.28), Li (1.96) and Co (7.69). Except for Cr and Mn, all the elements demonstrated positive relative errors and the deviations from the mean values were small. There was no significant difference in the measured and certified values. Therefore, the calculated relative errors revealed high accuracy of the method, suggesting that this method can be used for routine analysis of trace and heavy metals in herbal products.

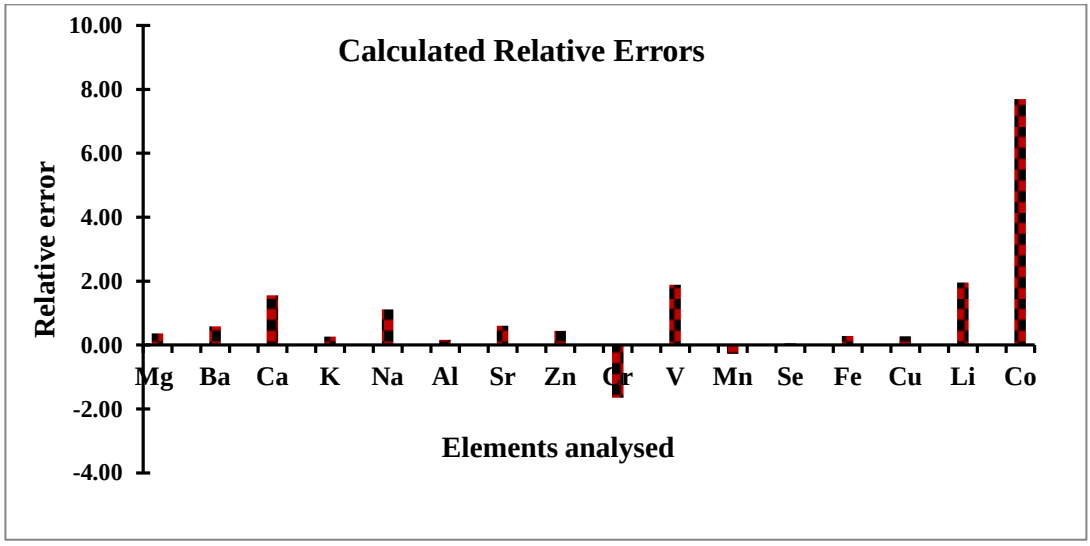


Figure 4.9: Relative errors based on comparison of certified and measured values

The concentrations of the elements analysed using the ICP-OES and FAAS are furnished in Table 4.11 and Table 4.12. A total of 16 elements were analysed and special emphasis was given to the levels of the essential elements associated with diabetes including Zn, Cr, Se, Mg, Mn, V and Mg. In this study, a substantial quantity of zinc was determined in all the plants. Maximum amount (in ppm, dry weight) of zinc was found in the seed samples of *N. sativa* (52.23), followed by *B. nigra* (37.90) and the lowest in *A. camperi* (23.25). Based on Tukey's multiple comparisons test, there was significant difference ($P < 0.01$) in the levels of zinc among the plants. Chromium was found in the range of 1.18 to 1.86 ppm, where the highest concentration was detected in *M. dianthera*, while the lowest value in *N. sativa*. There was no significant difference in the levels of chromium among the plants. The concentration (in ppm) of vanadium in the medicinal plants ranged from 1.05 in *L. sativum* to 9.38 in *M. dianthera*. The levels of vanadium were significantly different ($P < 0.01$) among the plants.

Based on the results portrayed in Table 4.12, the lowest concentration of manganese was observed in *L. sativum* and amounted 18.51 ppm, while the highest level (82.03 ppm) was in *M. dianthera*. The level of manganese, among the plants, was significantly different ($P < 0.001$). Investigation of the level of selenium revealed

that the element exists in a very low concertation in all the plants. The range of Se was 25.47 up to 72.64 ppb whereby the highest level of the element was observed in *N. sativa*. The values of selenium were not statistically different among the investigated plants. The level of magnesium was detected in substantial quantity in all the plants. It ranged from 10.8 ppm in *A. camperi* to 25.05 ppm in *N. sativa*. The level of magnesium was relatively variable and thus was statistically different ($P < 0.01$).

M. dianthera showed the highest level of the major elements including Ca, Al and Li. Special mention should be made of Ca and Al, which have an anomalous values (in ppm) of 7341.51 and 2810.91, respectively. These values are extremely higher compared to the values measured in the other plants ($P < 0.001$). Similarly, as shown in Table 4.11, *A. camperi* displayed the highest levels (in ppm) of Mg, K and Sr (10811, 10646 and 169, respectively) compared to the other plants ($P < 0.01$). However, the plant showed lowest levels of Zn, V and Se compared to the other plants (Figure 4.10). There are no reported levels of trace and major elements related to *M. dianthera* and *A. camperi*. The highest levels of Cu and Se were observed in the seeds of *N. sativa* (36.21 ppm and 72.64 ppb, respectively). Low concentrations of Co (0.19 – 1.48 ppm) and Li (0.18 – 0.86 ppm) were observed in all the plant samples. Besides, the concentrations of metals such as lead (Pb), cadmium (Cd) and arsenic (As) present in the leaves and seeds of the plants were not quantifiable. It is known that excess concentration of these elements is toxic and thus can disrupt the glucose uptake and alter the related molecular mechanism in glucose regulation (Khan, Ali and Tullah, 2008). Table 4.11 and Table 4.12 also display the recommended dietary allowance (RDA) and tolerable upper levels (UL) (EFSA, 2006; WHO, 2006; Dhonukshe-Rutten, 2012), of most of the elements and thus it can be deduced that the existing profile of the trace and other elements, based on WHO 2008 and WHO 2011 reports, were within the permissible limits.

Table 4.11: Concentration (ppm or mg.kg^{-1}, dry weight) of macro elements determined using ICP-OES

Samples	Mg	Ba	Ca	K	Na	Al	Sr
N. sativa	2505.52 ± 11.43	36.21 ± 1.08	478.35 ± 5.99	2496.26 ± 11.43	21.84 ± 0.22	126.71 ± 4.73	92.97 ± 0.81
L. sativum	2516.46 ± 13.62	31.77 ± 4.99	497.27 ± 4.41	2464.39 ± 13.62	18.51 ± 0.32	131.90 ± 1.67	15.50 ± 0.68
B. nigra	3460.00 ± 9.12	32.97 ± 3.30	1406.30 ± 3.04	3388.48 ± 9.12	36.31 ± 0.98	446.42 ± 5.16	53.89 ± 1.60
M. dianthera	5263.06 ± 9.49	28.61 ± 3.51	7341.51 ± 4.05 [a]	5219.54 ± 9.49	82.03 ± 1.10 [a]	2810.91 ± 7.08 [a]	82.12 ± 0.80
A. camperi	10811.66 ± 13.13 [b]	18.41 ± 1.00	545.32 ± 7.96	10655.91 ± 9.13 [a]	25.78 ± 0.38	196.79 ± 4.13	169.51 ± 2.95 [a]
RDA	280 - 350 mg	1.1 mg	1000 mg	3.5 g	1.5 g	-	-
UL	350 mg	-	2500 mg	3000 mg	2300 mg	-	-

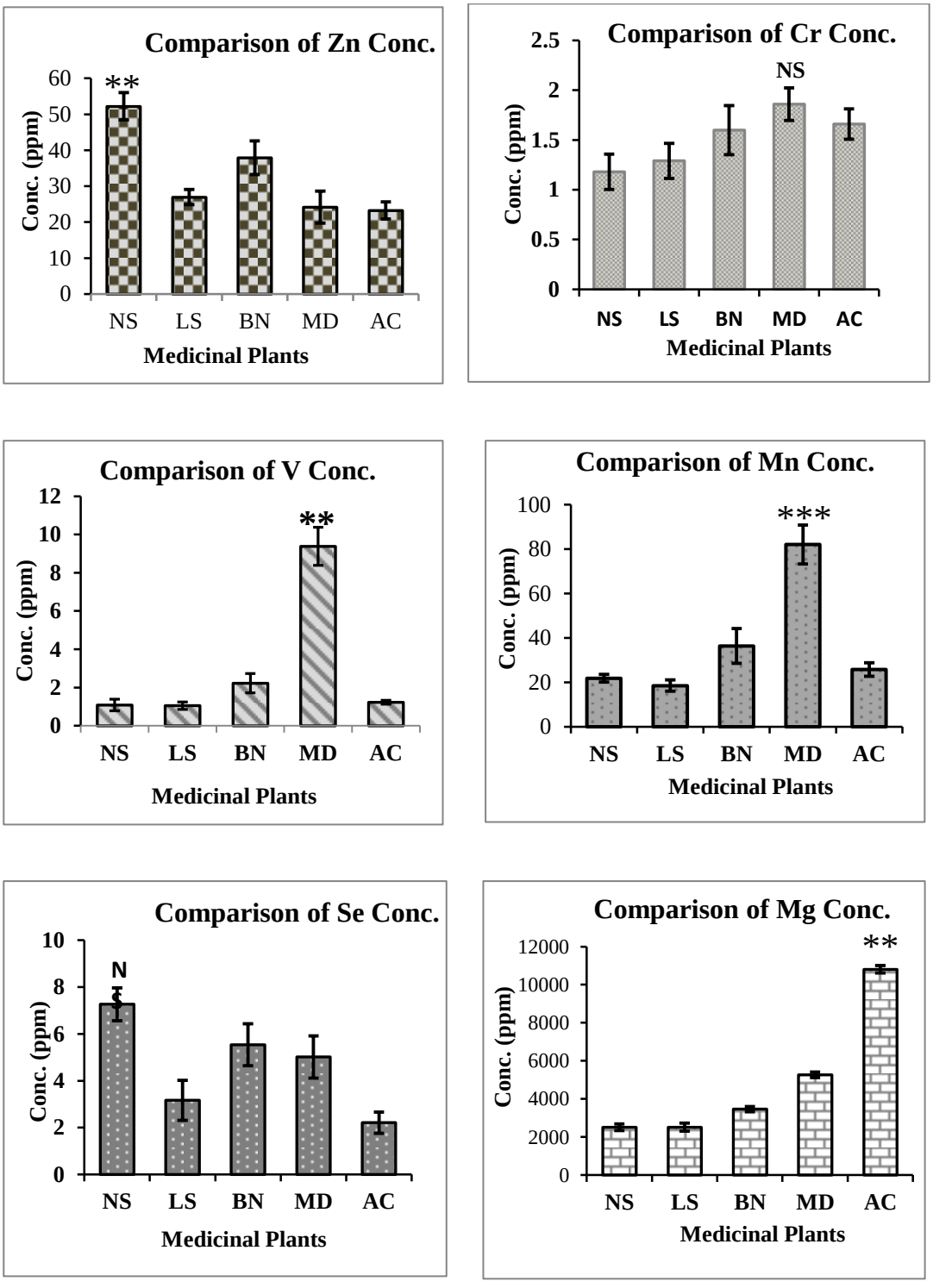
NS - *Nigella sativa*, LS - *Lepidium sativum*, BN - *Brassica nigra*, AC -*Aloe camperi* and MD - *Meriandra dianthera*
RDA - Recommended daily dietary allowance per day for adults, UL –Tolerable upper intake level per day for adults

Concentration values are expressed as mean ± SD (n = 3), [a]P < 0.001, [b]P < 0.01, [c]P < 0.05 (Statistical significance compared to all the other plants)

Table 4.12: Concentration (ppm or mg.kg^{-1}, dry weight) of trace elements determined using ICP- OES and FAAS

Samples	Zn	Cr	V	Mn	Se [#]	Fe	Cu	Li	Co
N. sativa	52.23 ± 0.95 [b]	1.18 ± 0.04	1.08 ± 0.03	21.84 ± 0.22	72.64± 2.98[NS]	478.35 ± 5.99	36.21 ± 1.08[NS]	0.52 ± 0.05	0.28 ± 0.02
L. sativum	26.94 ± 1.53	1.29 ± 0.05	1.05 ± 0.02	18.51 ± 0.32	33.64 ± 5.47	497.27 ± 4.41	31.77 ± 4.99	0.23 ± 0.02	0.19 ± 0.03
B. nigra	37.90 ± 1.84	1.60 ± 0.04	2.23 ± 0.05	36.31 ± 0.98	58.73 ± 3.92	847.64 ± 3.09	32.97 ± 3.30	0.18 ± 0.06	0.29 ± 0.01
M. dianthera	24.17 ± 1.10	1.86 ± 0.03[NS]	9.38 ± 0.10[b]	82.03 ± 1.10[a]	46.81 ± 5.96	1241.05 ± 3.17[a]	28.61 ± 3.51	0.86 ± 0.10 [c]	1.48 ± 0.08[a]
A. camperi	23.25 ± 0.63	1.66 ± 0.01	1.23 ± 0.01	25.78 ± 0.38	25.47 ± 3.66	545.32 ± 7.96	18.41 ± 1.00	0.39 ± 0.05	0.25 ± 0.04
RDA	7 - 9 mg	25 - 35 µg	10 - 20 µg	3 mg	50 - 60 mcg	9 -15 mg	900 µg	-	-
UL	25 mg	1000 mcg	1.8 mg	11 mg	300 mcg	25 mg	5 mg	-	-

NS- *Nigella sativa*, LS- *Lepidium sativum*, BN- *Brassica nigra*, AC- *Aloe camperi* and MD- *Meriandra dianthera*
RDA - Recommended daily dietary allowance per day for adults, UL –Tolerable upper intake level per day for adults
Concentration values are expressed as mean ± SD (n = 3), [#] Conc of Se expressed as ppb (µg.kg^{-1})
[a]P < 0.001, [b]P < 0.01, [c]P < 0.05, [NS]-not significant (Statistical significance compared to all the other plants)



NB: NS-*Nigella sativa*, LS-*Lepidium sativum*, BN-*Brassica nigra*, AC-*Aloe camperi* and MD-*Meriandra dianthera* and *** ($P < 0.001$) and ** ($P < 0.01$)

Figure 4.10: Comparison of the levels of Zn, Cr, V, Mg, Mn and Se

The results found in the present work were compared with the values previously reported and thus analysis of *N. sativa* seeds from Turkey were found to have 0.12, 117.32, 41.42, 30.26, 28.56 and 2.55 ppm of Co, Fe, Zn, Cu, Mn and Cr, respectively (Vatansev *et al*., 2013). Apart the levels of Fe and Co, all the values found in this study were similar to the levels of the elements reported from Turkey. Moreover, Shomar (2012) reported the levels of Cr, Cu, Fe, Mn, Al, Ba, Mg, and K in *N. sativa* from Egypt as 4.2, 14.5, 114, 47.4, 99.2, 14.0, 2356 and 9900 ppm, respectively. Current results presented elevated levels of Cu, Fe and Al compared to the values reported from Egypt. On contrary, the levels of Mn, Cr, Ba and K reported from Egypt were higher than the values determined in this paper.

Similarly, analysis of the levels (in ppm, dry weight) of *B. nigra* seeds from India using AAS (Rathee, Hooda, Sushila & Kumar, 2015) gave lower levels of Cu (5.50) and Fe (184.0) compared to the current report of the elements (32.97 and 847.64 respectively). However, the levels of Zn and Mn were very similar in both reports. Plants have the ability to uptake metals as nutrient from the soil and its environment which are so essential for their physiological and biochemical growth and thus the difference in the levels of the trace elements from different countries could be due to the variation in in soil types, agricultural and industrial activities, and local growing conditions such as differences in water, plant interactions and weather (Kabata-Pendias, 2011; Anal, 2014).

4.6 FT-IR Analysis

During the analysis of the IR spectra, the absorption bands in the group frequency region (4000- 1250 cm^{-1}) were given emphasis and thus the characteristic of the main functional groups (OH, NH_2, C=O, C-H, etc.) were observed in this range. Moreover, various relevant peaks were observed in the fingerprint region (< 1250 cm^{-1}). The most intense calculated bands of the IR spectra (Figure 4.11 to Figure 4.16) and the corresponding assignments were summarized in Table 4.13 and Table 4.14. Examination of the IR spectra showed a broad and very strong O–H stretch peak between 3450- 3300 cm^{-1} and was the most common characteristic peak of the methanol and hexane extracts. This could infer the presence of alcohols, phenols and flavonoid in those plants.

Characteristic peaks in the range of 1680 - 1740 cm^{-1} of carbonyls were also observed in the methanol extracts of *A. camperi* (1737.7 cm^{-1}, weak) and *M. dianthera* (1681.8 cm^{-1}, strong), and hexane extract of the Polyherb (1724.2 cm^{-1}, strong). The presence of esters might be suggested in the methanol extract of the *A. camperi* due to the presence the characteristic C-O stretching around 1028 cm^{-1} and 1261 cm^{-1}. Moreover, C-H vibrational frequencies of saturated hydrocarbons were observed in all the extracts ranging from 2950 to 2830 cm^{-1}. In the analysis of IR results, the characteristic signals of fatty acids and their esters at 725 (CH_2 rocking); 951 (-CH_2 wagging), 1097 (OCH_2-C), and 1292 (C-CO-O); 1711, 1736 (C=O esters); 2851 and 2918 cm^{-1} (CH_2 sym. and asym. str.) were observed in some of the spectra.

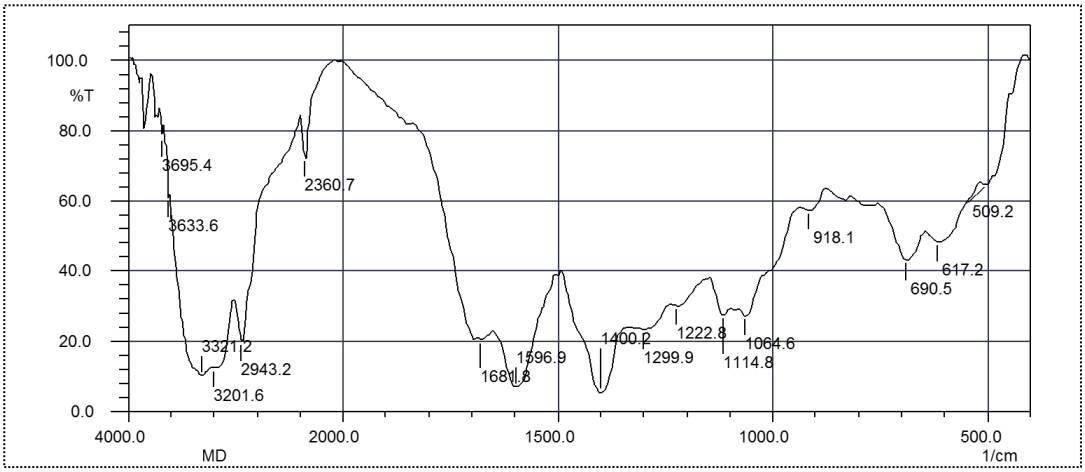


Figure 4.11: FT-IR spectrum of the crude methanol extract of *M. dianthera*

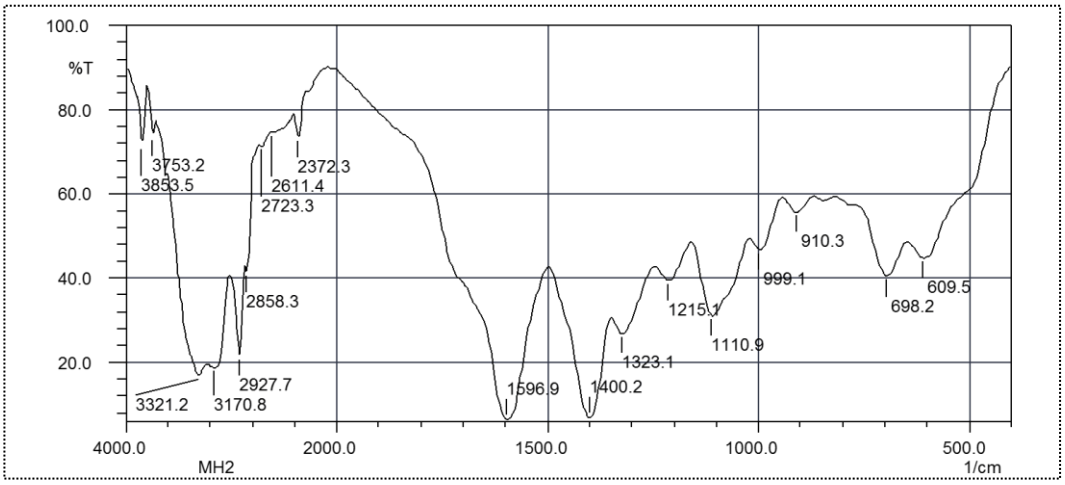


Figure 4.12: FT-IR spectrum of the crude hexane extract of *M. dianthera*

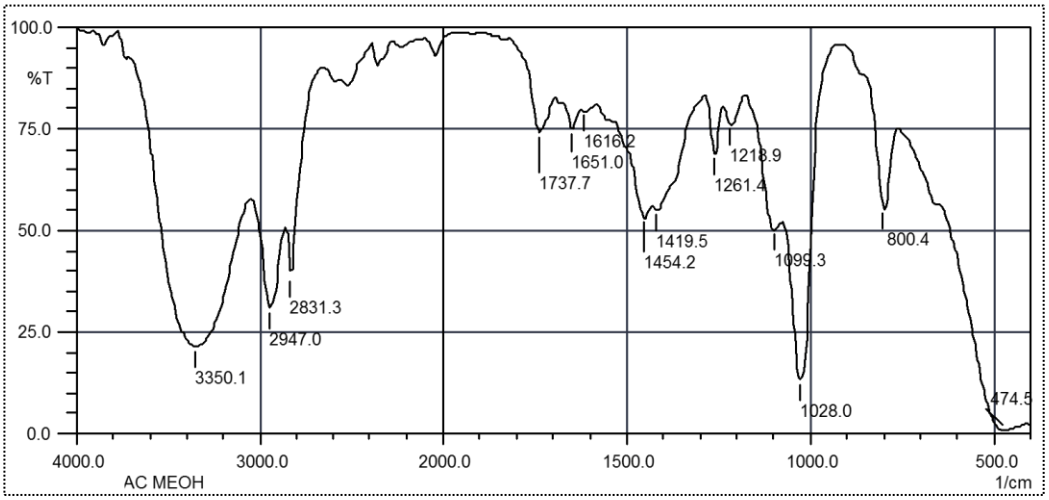


Figure 4.13: FT-IR spectrum of the crude methanol extract of *A. camperi*

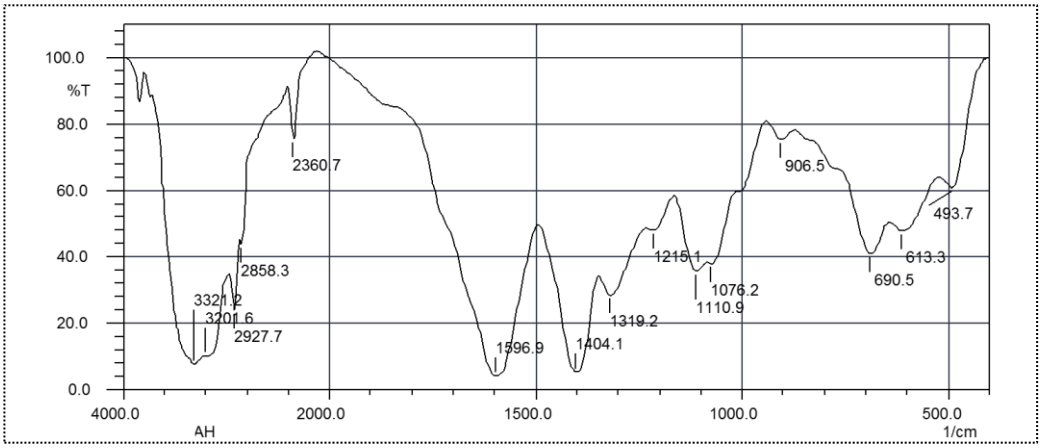


Figure 4.14: FT-IR spectrum of the crude hexane extract of *A. camperi*

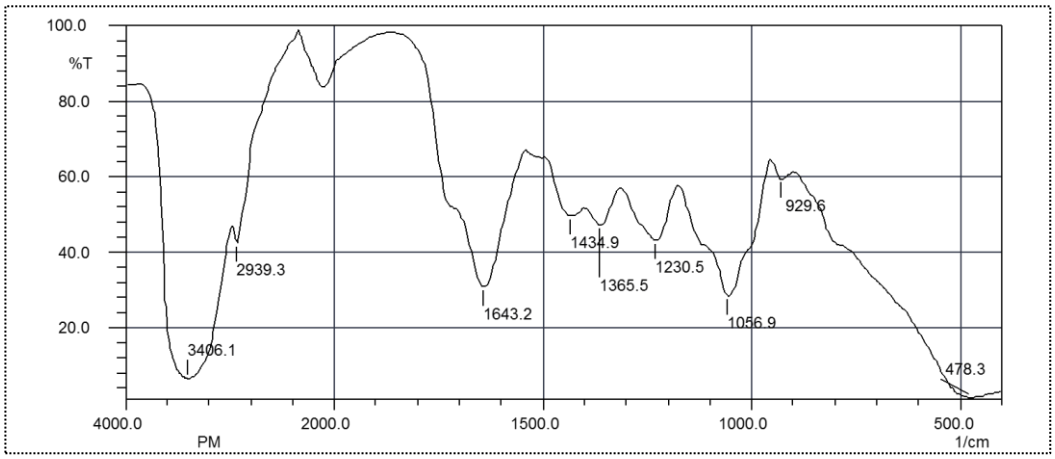


Figure 4.15: FT-IR spectrum of the crude methanol extract of Polyherb

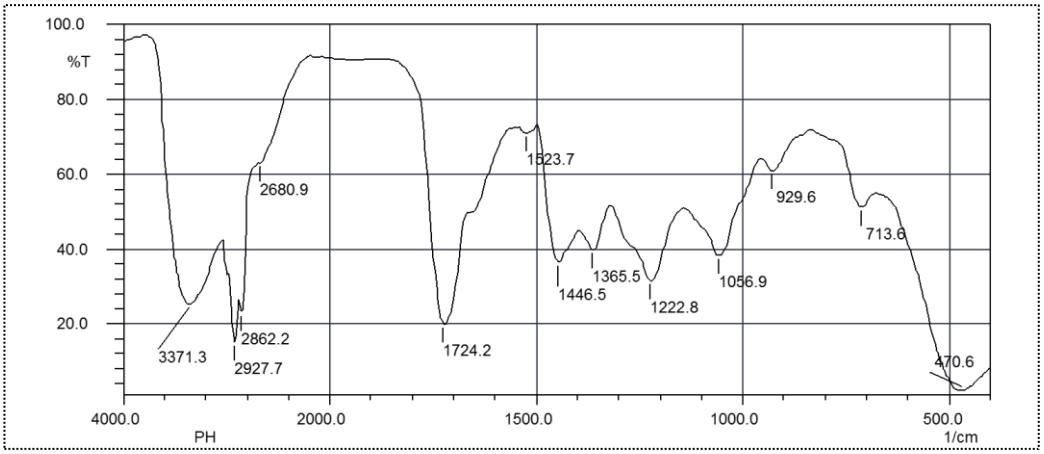


Figure 4.16: FT-IR spectrum of the crude hexane extract of Polyherb

Table 4.13: IR modes and their corresponding assignments of methanol extracts

AM		MM		PM		Corresponding assignments
υ (cm^{-1})	I	υ (cm^{-1})	I	υ (cm^{-1})	I	
3350.1	vs	3321.2	vs	3406.1	vs	stretching vibration of O–H groups
		3201.6	vs			hydrogen bonded (alcohols and hydroxyls)
2947.0	s	2943.2	vs	2939.3	m	sp^3 C–H stretching (hydrocarbon skeleton)
2831.3	s					
		2360.7	w	-	-	
1737.7	w	-	-	-	-	C=O stretch (could be ester)
-	-	1681.8	s			Quinone or conjugated ketone
1651.0	w	-	-	-	-	C=C stretching vibration
-	-	-	-	1643.2	s	bending vibration in N-H
1616.2	w	1596.9	vs	-	-	Benzene (C=C benzene)
1454.2	m					
1419.5	m	1400.2	vs	1434.1	m	C–C stretching of phenyl groups
		1299.9	s	1365.5	m	
1261.4	w	1222.8	m	1230.5	m	bending of O–H groups and/or C-O
1218.9	w	1114.8	m			stretching in phenols / alcohols
1099.3	m	1064.6	m	1056.9	s	C–O stretching (due to hydroxyl)
1028.0	s					C–O stretching (due to esters)
800.4	m	918.1	w	926.6	w	C–H bend or OH (oop)
		690.5	m			
-	-	617.2	m	-	-	C–H bend/ -CH_2 rocking

NB: υ: frequency, I: intensity, vs: very strong, s: strong, m: medium, w: weak; AM, MM and PM stand for the methanol extracts of the *Aloe, Meriandra* and Polyherb respectively

Table 4.14: IR modes and their corresponding assignments of hexane extracts

AH		MH		PH		Corresponding assignments
υ (cm^{-1})	I	υ (cm^{-1})	I	υ (cm^{-1})	I	
3321.2	vs	3321.2	vs	3371.3	Vs	O–H stretch, hydrogen bonded
3201.6	vs	3170.9	vs			
2927.7	s	2927.7	vs	2927.7	Vs	C–H stretch
2858.3	s	2858.3	s	2862.2	vs	
-	-	-	-	2680.9	M	-$R_2NH_2^+$, R_3NH^+, =NH^+-
2360.7	m	2372.3	w	-	-	amino acids /acetals
-	-	-	-	1724.2	Vs	C=O bond (phenolic esters)
1596.9	vs	1596.9	vs	1523.7*	W	aromatic C=C stretch and/or
1404.1	vs	1400.2	vs	1446.5*	m	ester skeleton ($RCOO^-$)
1319.2	s	1323.1	s	1365.5	M	C–C stretching of phenyl groups
1215.1	m	1215.1	m	1222.8	m	C–O stretch (due to hydroxyl)
1110.9	m	1110.9	m	1056.9	m	
1076.2	m	999.1	w			
906.5	m	910.3	w	929.6	W	- OH (oop)
690.5, 613.3	m	698.2, 609.5	m	713.6	M	C–H bend/ -CH_2 rocking

NB: AH, MH and PH stand for the hexane extracts of the *Aloe, Meriandra* and Polyherb respectively

4.7 Identification and Characterisation of Compounds

Both LC-MS and GC-MS are the most popular hyphenated techniques in use today for identification and characterisation of natural products (Wildon & Brinkman, 2003). Thus, these techniques were employed in the study of the major phytochemicals present in the plants of interest.

4.7.1 LC-MS Analysis of Crude Extracts

In the present work, the metabolites from the methanolic extracts of the plants were identified and tentatively characterized by a non-targeted analytical approach using ultra-high-performance liquid chromatography (UHPLC) coupled to QTOF-MS (Prasain, Wang & Barnes, 2004; Li *et al*., 2015). The total ion chromatogram (TIC) of the mass spectrometry was in the positive ionization mode.

4.7.1.1 LC-MS Results of *Meriandra dianthera*

The analysis of the TIC identified different bioactive compounds with different concentrations (ng/µl) in *M. dianthera*. The quantification was done by using rutin as a standard. Figure 4.17 displayed different characteristic peaks representing various compounds in the plant. Using the online search (Metlin and Chemspider), about 27 known compounds and 1 unknown were identified and tentatively characterised (Table 4.15) in *M. dianthera*. Flavonoids were the most dominant compounds among the other phenolic acids, terpenoids, alkaloids and a carbohydrate. Some of the structures of of the compounds found in *M. dianthera* are shown in Figure 4.18.

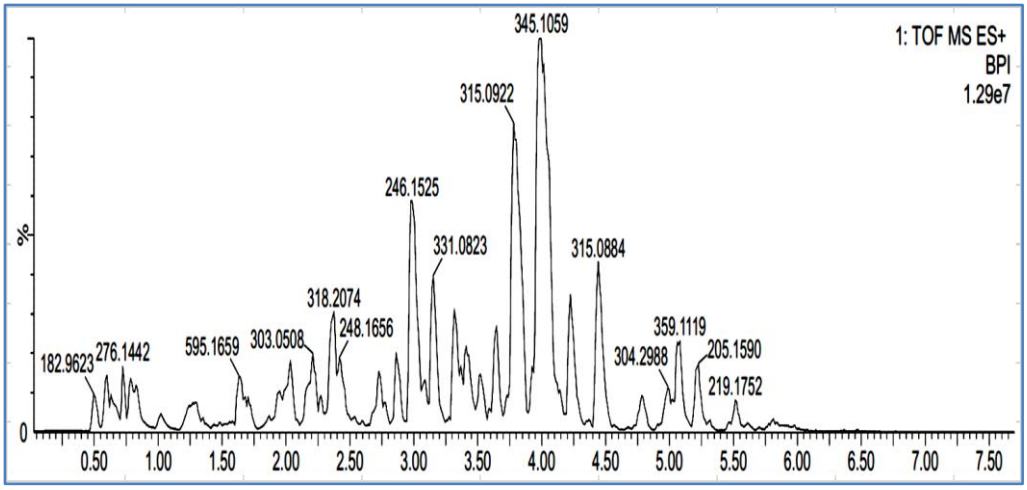


Figure 4.17: TIC for crude methanol extract of *M. dianthera* obtained from UPLC

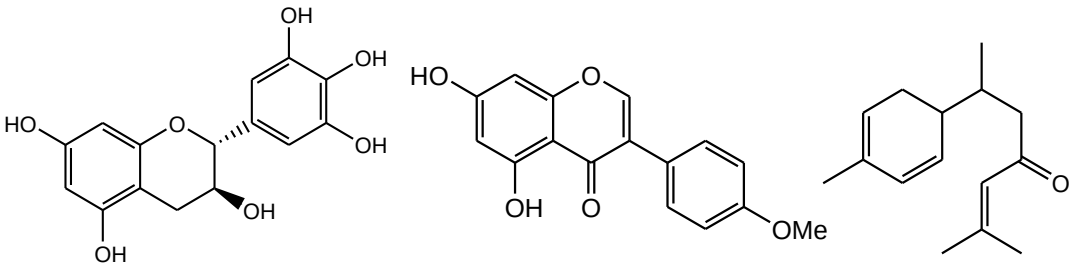
Table 4.15: List of compounds identified using LC-MS in *M. dianthera*

RT (min)	Compound Name	[M+H]+	Peak area	Conc (ng/µl)
0.51	3,4-Dihydroxycinnamic acid	182.96	174471.30	38.28
0.59	Belladonnine	543.13	186083.00	40.37
0.64	6-Oxoalstophylline	381.08	1245.80	7.23
0.73	2-Amino-5,9,12,15,17-octadeca-pentaen-3-ol	276.14	16589.10	9.98
1.03	Quercetin 7-(6"-tiglylglucoside)	547.13	1457.80	7.27
1.28	Indoleacrylic acid	188.07	100388.60	25.00
1.64	Kaempferol 3-O-rhamnosyl-glucoside	595.17	15896.30	9.86
1.71	Riboflavine 2',3',4',5'-tetrabutanoate	657.31	14789.20	9.66
1.95	Quercetin 4'-O-glucoside	465.10	13258.30	9.38
2.03	Myricetin	319.21	12369.20	9.23
2.21	Quercetin	303.05	201811.90	43.19
2.37	Luteolin	287.06	402977.50	79.25
2.72	Ellagic acid acetyl-xyloside	477.14	112693.20	27.21
2.88	Pendulin	507.15	112894.90	27.25
3.01	Quercetin 3-O-acetyl-rhamnoside	491.29	311564.00	62.86
3.17	5,7,4'-Trihydroxy-6,3'-dimethoxyflavone	331.08	190830.80	41.22
3.32	Apigenin	271.06	165897.20	36.75
3.42	Gallocatechin	307.13	132547.20	30.77
3.52	Ligstroside-aglycone	361.09	142598.20	32.57
3.81	Cirsimartin	315.09	136897.10	31.55

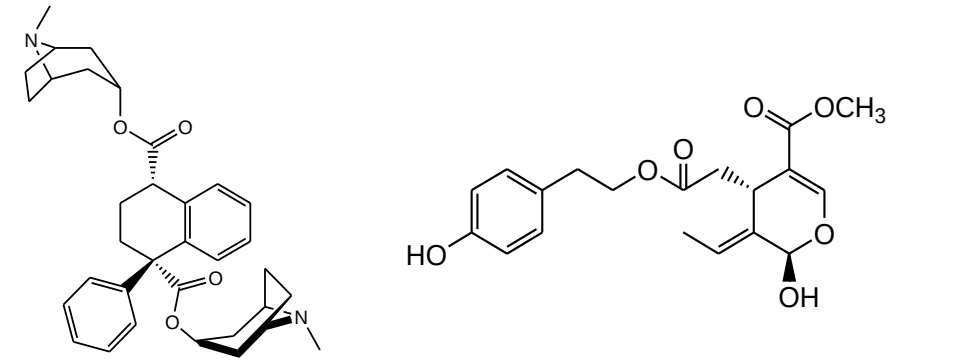
Table 4.15 (Continued)

RT (min)	Compound Name	[M+H]+	Peak area	Conc (ng/µl)
4.00	Cirsilineol	345.11	1106565.50	205.37
4.24	5,7-Dihydroxy-4'-methoxyisoflavone	285.08	13265.30	9.39
4.78	Kaempferol 3,7,4'-trimethyl ether	329.10	71045.10	19.74
4.99	Aestivine	304.30	6325.30	8.14
5.07	Gardenin B	359.12	281004.60	57.38
5.23	Ledol	205.16	130215.40	30.35
5.52	α-Turmerone	219.18	114589.30	27.55

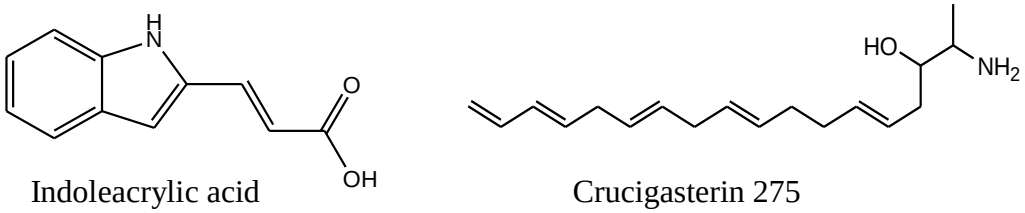
The structure of the flavonoids found in *M. dianthera*, except gallocatechin and 5, 7-dihydroxy-4'-methoxyisoflavone, with their $[M+H]^+$ values are shown in Table 4.16 and Table 4.17. The flavonoids identified in *M. dianthera* belong to different subgroups i.e. flavonols, flavones, flavanol and isoflavone. Some of the flavonols were O-glycosylated and the flavones were mostly methoxylated. The LC conditions permitted a good separation of these compounds and were optimized for further separations of crude plant extracts containing aglycones and glycosylated flavonoid derivatives. Moreover, the reported anti-diabetic and related anti-oxidant activities of the flavonoids and other compounds are also furnished in Table 4.18. Compounds including myricetin, quercetin, ellagic acid acetyl-xyloside, apigenin cirsilineol and α-Turmerone were reported to have anti-diabetic activities (Table 4.18). Many flavonoids were demonstrated to act on biological targets involved in Type 2 diabetes mellitus such as: α-glycosidase, glucose cotransporter or aldose reductase (Nicolle, Souard, Faure & Boumendje, 2011). Similarly, naturally occurring flavonoids such as myricetin, quercetin and apigenin have been proposed as effective supplements for management and prevention of diabetes and its long-term complications based on in vitro and animal models (Vinayagam & Xu, 2015)



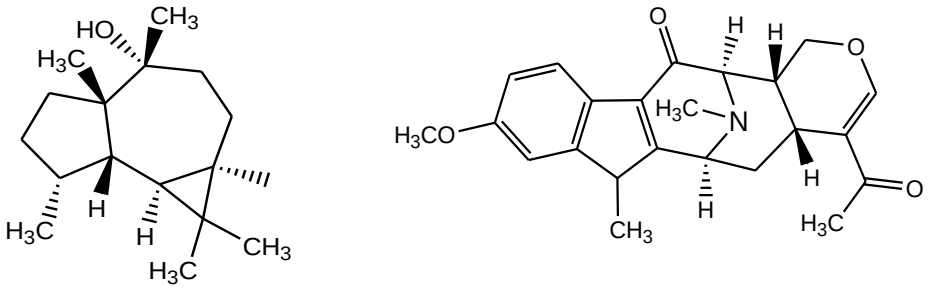
Gallocatechin 5 ,7-Dihydroxy-4'-methoxyisoflavone α-turmerene



Belladonnine Ligstroside-aglycone



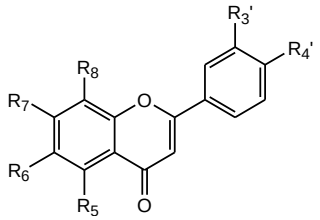
Indoleacrylic acid Crucigasterin 275



Ledol 6-Oxoalstophylline

Figure 4.18: Compounds identified in *M. dianthera* using LC-MS analysis

Flavone type



Flavonol type

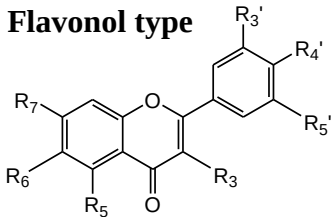


Table 4.16: Flavone aglycones identified in *M. dianthera*

Compounds	Position of the group in the flavone skeleton						
	$[M+H]^+$	R_5	R_6	R_7	R_8	R_3'	R_4'
Luteolin	287.06	OH	OH	OH	H	OH	H
Apigenin	271.06	OH	OH	H	H	OH	H
Cirsilineol	345.11	OH	OMe	OMe	H	OH	OMe
Cirsimaritin	315.09	OH	OMe	H	H	OH	OMe
Gardenin B	359.12	OH	OMe	H	OMe	OMe	OMe
5,7,4'-Trihydroxy-6,3'-dimethoxyflavone	331.08	OH	OH	OMe	H	OH	OMe

Table 4.17: Flavonol glycones and glycosides identified in *M. dianthera*

Compounds	Position of the group in the flavonol skeleton							
	$[M+H]^+$	R_3	R_5	R_6	R_7	R_3'	R_4'	R_5'
Quercetin	303.05	OH	OH	H	OH	OH	OH	H
Myricetin	319.21	OH	OH	H	OH	OH	OH	OH
Pendulin		OMe	OH	OMe	OMe	H	OH	H
Kaempferol 3,7,4'-trimethyl ether	329.10	OMe	OH	H	OMe	H	OMe	H
Quercetin 4'-O- glucoside	465.10	OH	OH	H	OH	OH	O-glucose	H
Quercetin 7-O-(6"-tiglylglucoside)	547.13	OH	OH	H	7-O-(6"-tiglyl glucose)	OH	OH	H
Quercetin 3-O-acetyl-rhamnoside	491.29	O- acetyl-rhamnose	OH	H	OH	OH	OH	H
Kaempferol 3-O-rutinoside	595.17	O-rutinose	OH	H	OH	H	OH	H

Table 4.18: Reported bioactivities of the compounds found in *M. dianthera*

Compound Name	Class of Compound	Bioactivity reported	References
3,4-Dihydroxycinnamic acid	Hydroxycinnamic acid	- anti-oxidant	Abraham, 2003
Quercetin 7-(6''-tiglylglucoside)	Flavonol	- anti-oxidant	Murota & Terao, 2003
Kaempferol 3-O-rhamnosyl-glucoside	Flavonol glycoside	- anti-oxidant	Calderón-Montaño *et al*, 2011
Riboflavine 2',3',4',5'-tetrabutanoate	Flavin	- anti-hyperlipidaemic	Collins, 2006
Spiraeoside	Flavonol	- anti-oxidant	Gary *et al* 1996
Myricetin	Flavonol	- anti-oxidant - anti-diabetic	Ross & Kasum, 2002; Li & Ding, 2012
Quercetin	Flavonol	- anti-oxidant - anti-diabetic	Manach, 1998; Vessal, Hemmati &Vasei, 2003
Luteolin	Flavonol	- anti-oxidant	Seelinger, Merfort & Schempp, 2008
Ellagic acid acetyl-xyloside	Hydroxybenzoic acid	- anti-oxidant -α-glucosidase inhibitor	Zafrilla *et al*, 2001 Tabopda *et al*, 2009
Pendulin	Flavone	- anti-oxidant	Heo *et al*, 2001
Quercetin 3-O-acetyl-rhamnoside	Flavonol	- anti-oxidant	Agnihotri *et al*, 2008
Jaceosidin	Flavone	- anti-oxidant	Kim *et al*, 2008
Apigenin	Flavonol	- anti-oxidant - anti-hyperglycemic	Jeyabal *et al*., 2005 Panda & Kar, 2007
(+)-Gallocatechin	Flavanol	- anti-oxidant	Someya, Yoshiki & Okubo, 2002
Ligstroside-aglycone	Tyrosol	- anti-oxidant	Obied *et al*., 2007
Cirsimaritin	Flavones	- anti-oxidant	Ibañez *et al*., 2003
Cirsilineol	Flavone	- anti-oxidant - anti-diabetic	Linn. Kelm *et al* 2000; Polya, 2003
Kaempferol 3,7,4'-trimethyl ether	Flavonol	- anti-oxidant	Yang *et al*., 2009
α-Turmerone	Sesquiterpenoid	- anti-diabetic	Lekshmi *et al*., 2012

4.7.1.2 LC-MS Results of *Aloe camperi*

The TIC of the *A. camperi* displayed an array of peaks which based on the online search afforded 20 known and 1 unknown compounds (Figure 4.19). Different classes of compounds including flavonoids, phenolic acids, coumarins, unsaturated carboxylic acids and aliphatic alcohols were identified and tentatively characterized (Table 4.19). Similar to *M. dianthera*, flavonoids were the most dominant compounds in the *A. camperi*. The flavonoids found in *A. camperi*, except cirsilineol, were different to those found in *M. dianthera*. The flavonoids belong to the different classes of flavonoids including flavanol, flavone, chalcon, isoflavonoid and anthocyanidin. It was reported that some of the flavonoids rutin, isoorientin and phloretin 2'-O-xylosyl-glucoside were reported to have anti-diabetic property. The detailed information of the flavonoids along with their $[M+H]^+$ values and their reported bioactivities are described in Table 4.20. Some of the structures of the compounds identified in *A. camperi* are shown in Figure 4.20.

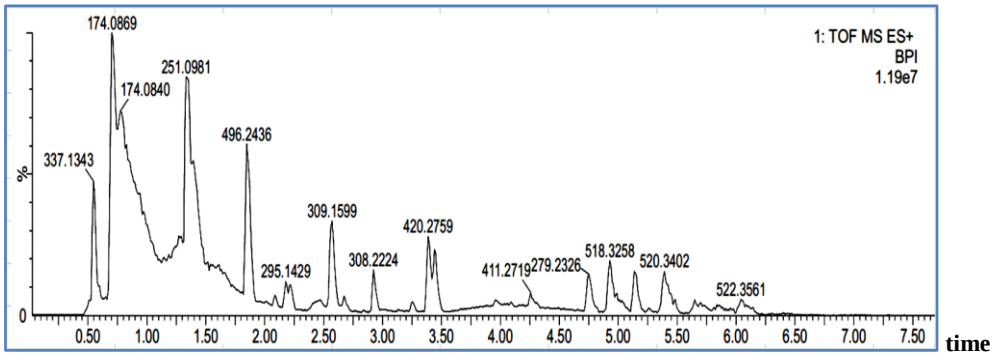


Figure 4.19: TIC from LC-MS of crude extract of *A. camperi*

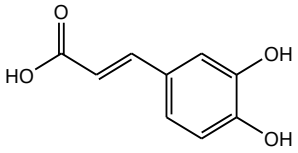
Table 4.19: Non-flavonoid compounds identified using LC-MS in *A. camperi*

RT(min)	Compound Name	$[M+H]^+$	Peak area	Conc (ng/µl)
0.51	3,4-Dihydroxycinnamic acid	182.96	73277.20	20.14
1.42	Lariciresinol-sesquilignan	557.19	71745.10	19.87
1.56	3E,5E-tridecadienoic acid	211.16	114933.30	27.61
2.79	7-Ethoxycoumarin	191.07	1459.60	7.27
2.93	p-Coumaric acid ethyl ester	193.05	1785.10	7.33
3.39	4-Hydroxybenzoic acid 4-O-glucoside	301.07	10092.30	8.82
3.95	(Z,Z,Z)-9,12,15-Octadecatrien-1-ol	265.08	198509.60	42.59

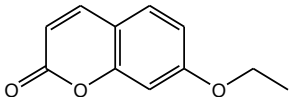
Table 4.20: Flavonoids found in *A. camperi* and their reported bioactivities

Compound Name	$[M+H]^+$	Peak area	Conc (ng/µl)	Class of Compound	Reported Bioactivities	References
Procyanidin trimer C1	867.24	72366.30	19.98	Flavanol	-	
Rutin	611.16	142706.40	32.59	Flavonol	- anti-oxidant	Choi *et al*., 2009
					-, anti-diabetic	Niture, Ansari & Naik, 2014
Isoorientin	449.11	121503.20	28.79	Flavone	- hypoglycemic	Aragão *et al*., 2010
Rotenone	395.14	512682.30	98.91	Isoflavone	-	
Phloretin 2'-O-xylosyl-glucoside	590.23	185960.10	40.34	Chalcone	- insulin mimetic	Lisa, 2014
Daidzin	417.12	12132.30	9.18	Isoflavonoid	- anti-oxidant	Xie *et al*., 1994
Malvidin 3-O-galactoside	529.13	185905.00	40.33	Anthocyanidin	-	-
Rhamnetin 3-rhamnosyl-(1->3) (4'''-acetylrhamnosyl)(1->6)-galactoside	813.25	82156.60	21.74	Flavone	-	-
Acacetin 7-rhamnosyl-(1->4'') [glucosyl-(1->6'')(6'''-acetylsophoroside)]	959.28	164324.10	36.47	Flavone	- anti-oxidant	Kim, Park & Jung, 2013
Scutellarein 6,4'-dimethyl ether 7-(3'''-acetylrutinoside)	665.17	164423.20	36.48	Flavone	-	-
Butein	273.11	184228.30	40.03	Chalcone	- anti-oxidant	Jayasinghe *et al*., 2004
Cirsilineol	345.10	42714.60	14.67	Flavone	-	-
Catechin 3-O-glucose	454.29	9861.30	8.78	Flavanol	- anti-oxidant	Gali *et al*., 1994

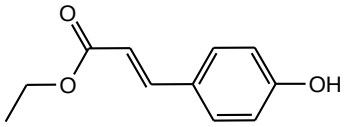
Many *Aloe* species have been studied for their phytochemical constituents and related bioactivities. Recently 26 bioactive compounds were identified in the ethanolic extract of *Aloe vera* and include hexadecanoic acid, octadecanoic acid, tricosane, 1-octadecanol, sitosterol and stigmasterol as the main constituents of the plant (Bowankar *et al*., 2013). Moreover, twenty-two compounds including hydroxyquinones, flavonoids, coumarins, triterpenes, and organic acids were obtained from *Aloe hijazensis* of Saudi Arabia (Abd-Alla *et al*., 2009). Some of the previously reported compounds, including the flavonoids and fatty acid methyl ester are similar to those identified in this report.



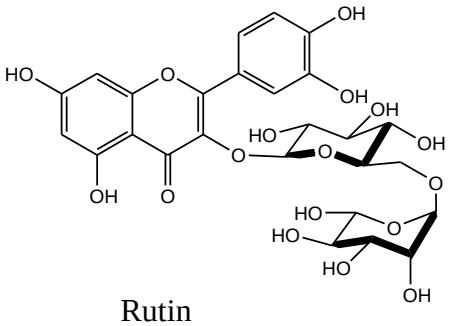
Caffeic acid



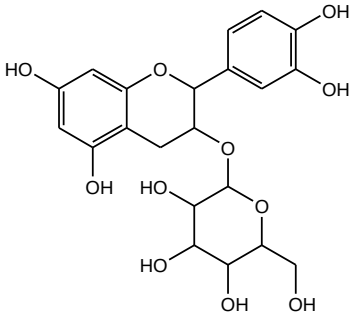
7-Ethoxycoumarin



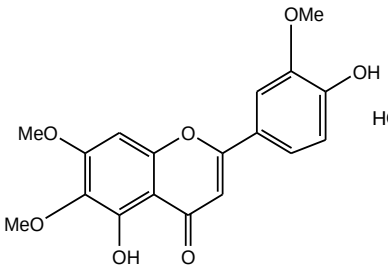
p- Coumaric acid ethyl ester



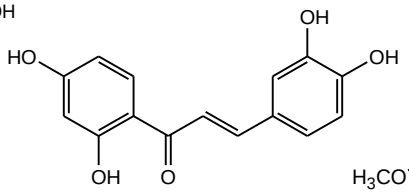
Rutin



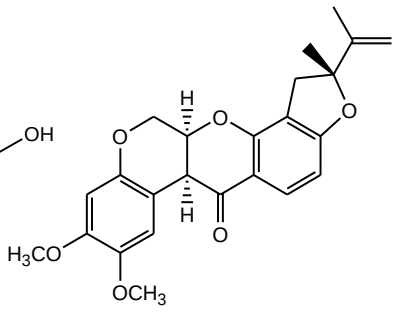
Catechin 3-O-glucose



Cirsilineol



Butein



Rotenone

Figure 4.20: Some of the compounds identified in *A. camperi* using LC-MS

4.7.1.3 LC-MS Results of the Polyherb

As shown in Figure 4.21, the TIC of the Polyherb also shows numerous peaks which were analysed to give about 20 compounds containing of 4 unknowns. The most prominent compounds were alkaloids. Moreover, aromatic acids, steroids, carbohydrates and a diterpenoid were among the compounds (Table 4.21). The alkaloids belong to ephedrine, isoquinoline, acridone and diterpenoid alkaloids. Based on literature survey, none of the alkaloids were reported to have anti-diabetic or anti-oxidant activities. However, some of the non-alkaloid compounds found in the plant including bisdemethoxycurcumin, dihydrotetrabenazine glucuronide and sterol 3-β-D-glucoside were reported to have anti-diabetic activities (Kuroda *et al*., 2005; Goland *et al*., 2009; Ivorra, Payá & Villar, 1990).

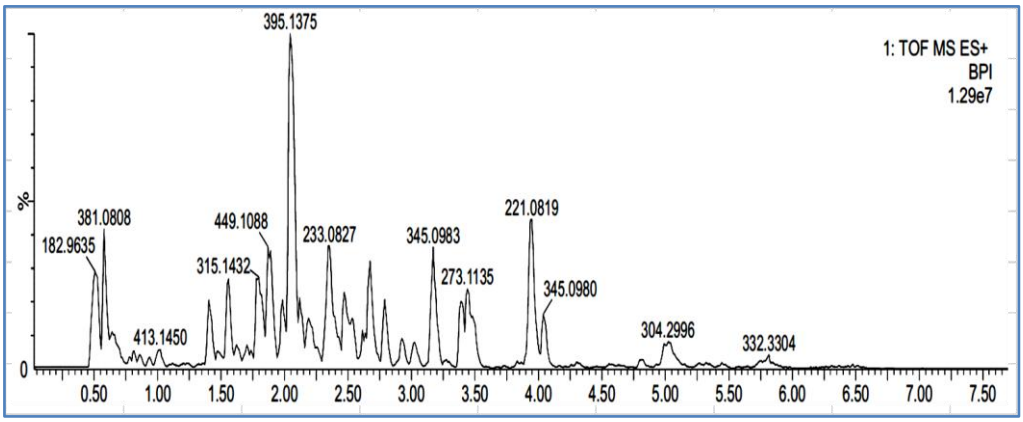


Figure 4.21: TIC for crude methanol extract of the Polyherb

Table 4.21: List of compounds identified using LC-MS in the Polyherb

RT (min)	Compound Name	$[M+H]^+$	Peak area	Conc (ng/µl)
0.55	6",6"-Dimethylpyrano[2",3":6',5']-2'-hydroxy-4,4'-dimethoxychalcone	367.14	123692.10	29.18
0.72	Rosmanol	347.15	263071.80	54.17
0.78	DL-Norephedrine	174.08	1816214.90	332.59
1.33	Fluperlapine	310.17	962480.90	179.55
1.86	Dihydrotetrabenazine glucuronide	496.24	450132.90	87.70
2.18	Tubulosine	475.26	143654.10	32.76
2.22	Spiramide	458.22	246560.60	51.21
2.56	Bisdemethoxycurcumin	309.16	256060.30	52.91
2.68	N-Tricosanoyl anthranilic acid	474.25	60155.60	17.79

Table 4.21(Continued)

RT (min)	Compound Name	$[M+H]^+$	Peak area	Conc (ng/µl)
2.92	Des-N-methyl acronycine	308.22	70766.20	19.69
3.25	Rutin	611.16	50144.70	16.00
3.40	Dolaconine	420.28	252945.00	52.35
3.97	6'-O-D-Xylopyranosyl alangiside	638.40	15489.20	9.78
4.26	Sterol 3-beta-D-glucoside	411.27	101668.70	25.23
4.75	Goyaglycoside c	662.42	201554.00	43.14
5.15	(25S)-5β-spirostan-3β-yl β-D-glucoside	601.38	163263.90	36.28

4.7.2 GC-MS Results of the Crude Extracts

GC-MS has been demonstrated to be a valuable analytical tool for the analysis of mainly non-polar components and volatile natural products, e.g., mono- and sesquiterpenes (Delazar, Reid & Sarker, 2004). The GC-MS of the crude extracts were analysed and thus are reported below. The quantification of the compounds was based on the standard (camphor).

4.7.2.1 GC-MS Results of *Meriandra dianthera*

As shown in Figure 4.22, the TIC of the complex mixture of *M. dianthera* displayed numerous compounds. Based on analysis, about 45 compounds are presented in Table 4.22 and the main compounds include octadecanoic acid (4.20%), caparratriene (4.30%), phytol acetate (4.92%), n-hexadecanoic acid (6.36%), linolenic acid methyl ester (8.14%) and camphor (9.08%). The mixture comprises of 26 essential oils (28.6% of the total composition). The main components of the essential oil of the plant include camphor (31.7%), oplopanone (13.45%), phytol (6.68%), ledol (6.12%), isolongifolen-5-one (5.40%), borneol (4.97%), palustrol (4.36%), verbenol (3.57%) and β-ocimene (3.19%). The percentage of the compounds was calculated based on the total essential oil composition of the plant. Some of the essential oils found in *M. dianthera* were reported to have anti-diabetic activities (Berraaouan & Bnouham, 2013; Ezuruike *et al.*, 2014). Moreover, coumarins, a steroid and methoxy flavonol were some of the identified compounds among the other. Based on literature review, the fatty acid methyl esters and

essential oils were also responsible to reported bioactivity of the plant. Examples of some of the compounds found in *M. dianthera* are shown in Figure 4.23.

In *Meriandra benghalensis*, plant of the same family to *Meriandra dianthera*, from Yemen, 12 essential oils were identified. The most abundant constituents were camphor (43.6 %), 1, 8-cineole (10.7 %), α-eudesmol (5.8 %), caryophyllene oxide (5.8 %), camphene (5.3 %) and borneol (3.4 %). Both plants share common essential oils and camphor was reported as the most prominent compound in both plants (Ali *et al*., 2012).

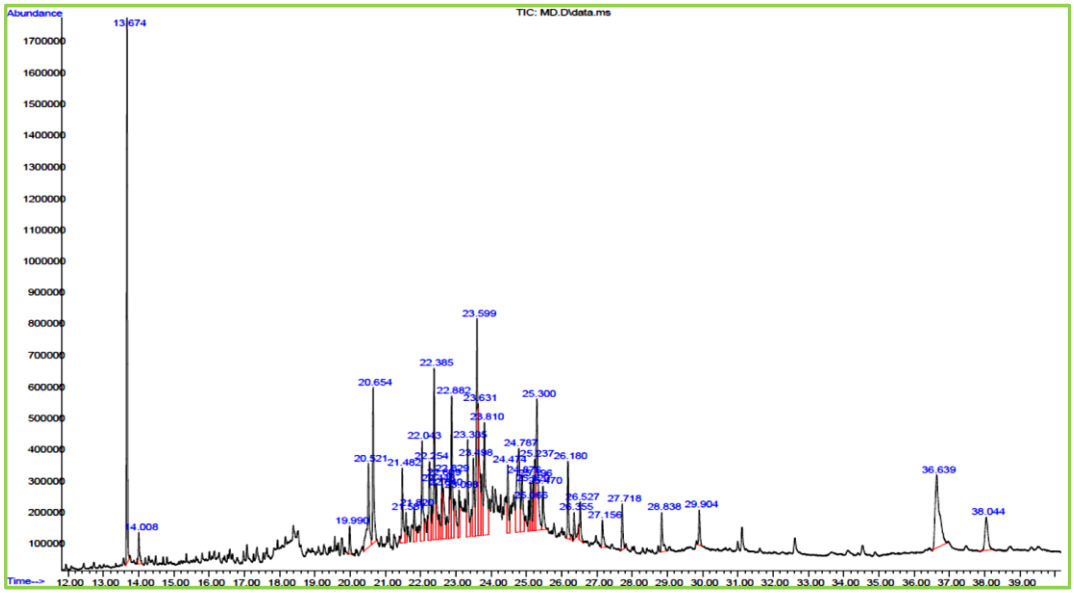


Figure 4.22: TIC of the methanol extract of *M.dianthera*

Table 4.22: Compounds identified in *M. dianthera* using GC-MS analysis

RT (min)	Library/ID	Area (Ab*s)	Conc (ng/µl)
10.01	Camphene	1199113	8.1
10.78	Phenol	357680	4.0
11.41	α-Terpinene	416958	4.3
11.56	o-Cymene	654105	5.4
11.63	Limonene	349262	3.9
11.69	Eucalyptol	637188	5.4
12.65	Camphenilone	406480	4.2

Table 4.22 (Continued)

RT (min)	Library/ID	Area (Ab*s)	Conc (ng/µl)
12.73	p-Cymenene	1038936	7.3
13.58	*trans*-Pinocarveol	1644490	10.3
13.68	Camphor	31039547	154.8
13.97	Pinocarvone	852320	6.4
14.01	Borneol	4871487	26.2
14.38	α-Terpineol	1679383	10.5
14.49	Myrtenol	1934169	11.7
14.70	Verbenone	1525506	9.7
14.80	Trans-Carveol	1593066	10.0
15.35	Piperitone	1732752	10.7
15.81	β-Ocimene	3128039	17.6
16.01	Diosphenol	2590590	15.0
16.53	Limonene glycol	1481879	9.5
17.07	Verbenol	3497902	19.4
19.45	7-Amino-4-methyl-coumarin	2398075	14.0
19.57	Palustrol	4271411	23.2
19.99	Ledol	5993230	31.7
21.40	Isolongifolen-5-one	5290106	28.2
21.48	Oplopanone	13171678	67.0
21.75	4-(1,1-Dimethylpropyl)-phenol	6947878	36.4
22.39	Phytol, acetate	16831869	84.9
22.75	4'-Phenoxyacetophenone	6997148	36.6
22.83	(Z)- 11-Tetradecyn-1-ol acetate	8534491	44.2
23.26	Pentadecanoic acid, 14-methyl-, methyl ester	8021424	41.6
23.33	Caparratriene	14721296	74.6
23.60	Palmitic acid	21749752	109.1
23.95	But-2-enoic acid, 2-methyl-, (7,8-dihydro-8,8-dimethyl-6H-pyrano[3,2]coumarin-7-yl) ester	7364329	38.4
24.39	7-Methoxy-6-(3-methyl-2-butenyl)-coumarin	6273945	33.1
25.07	Phytol	6537267	34.3
25.30	Linolenic acid	27823106	139.0
25.47	Stearic acid	14362571	72.8
26.18	Apocyanin	12844172	65.3
26.49	2,4,4-Trimethyl-3-(3-methylbuta-1,3-dienyl) cyclohexanone	5897978	31.2
30.87	Squalene	2342515	13.7
34.79	α-Tocopherol	1172284	8.0
36.49	3, 6, 8-Trimethoxy-5-hydroxy- flavonol	1495958	9.6
37.91	γ-Sitosterol	1153960	7.9

4.7.2.2 GC-MS Results of *Aloe camperi*

Analysis of the TIC of the GC-MS (Figure 4.24) of *A. camperi* afforded about 21 compounds (Table 4.23). The main compounds in the methanol extract of the *A. camperi* were linolenic acid methyl ester (5.85 %), phytol (6.44 %), palmitic acid (11.54 %), linolenic acid (15.41 %) and γ-sitosterol (26.40 %). The result showed the presence of appreciable quantity of free fatty acids (FFAs) and fatty acid methyl esters (FAMEs). FFAs and FAMEs are reported to have anti-diabetic activities and thus the reported bioactivities of FFAs and FAMEs are summarized under Section 4.18.

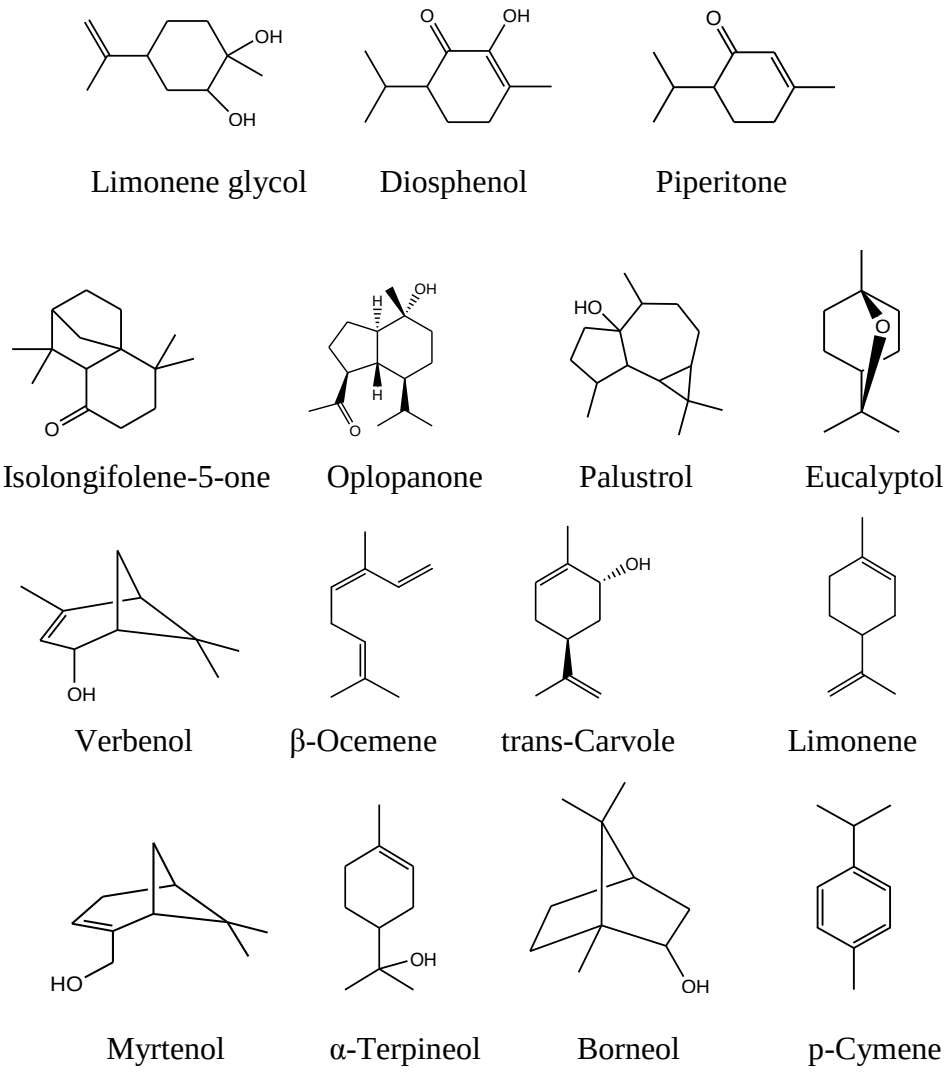


Figure 4.23: Some compounds identified in *M. dianthera* using GC-MS

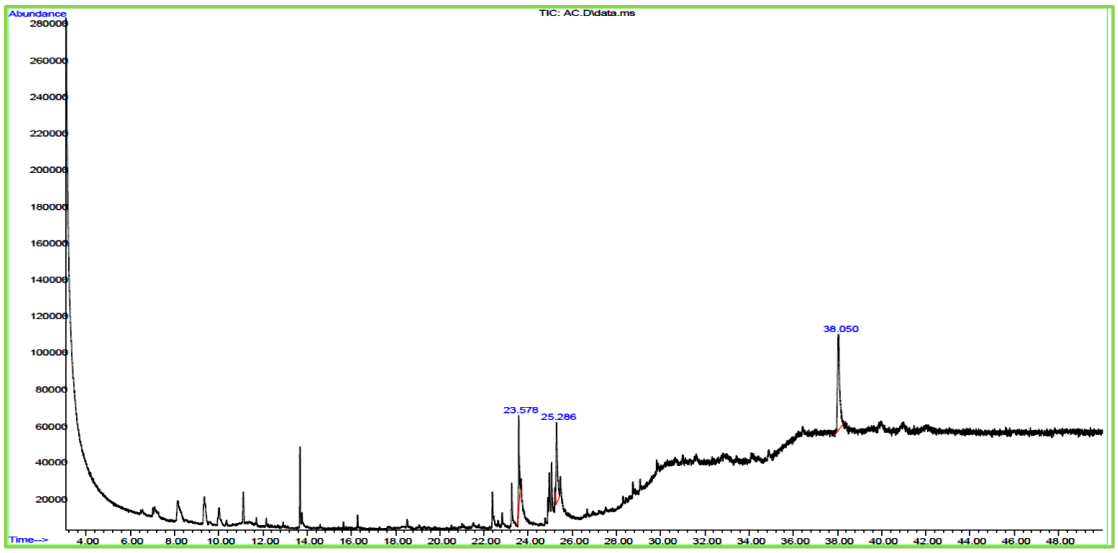
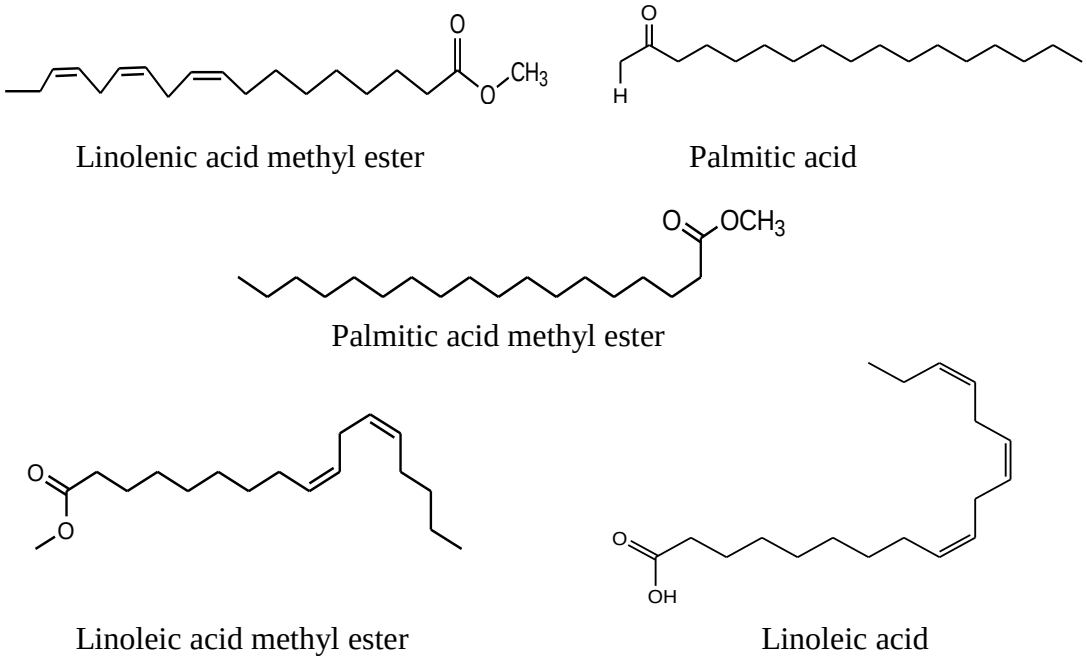


Figure 4.24: TIC of the methanol extract of *A. camperi*

Some structures of of FFAs and FAMEs found in *A. camperi* are represented in Figure 4.20.



Linolenic acid methyl ester

Palmitic acid

Palmitic acid methyl ester

Linoleic acid methyl ester

Linoleic acid

Figure 4.25: Fatty acids and fatty acid methyl esters identified in *A. camperi*

Table 4.23: Compounds identified in *A. camperi* using GC-MS

RT (min)	Compound name from Library	Area (Ab*s)	Conc (ng/µl)
8.15	4-Methyl-2-hexanol	778209	6.0
9.35	3-Methyl-2-butenal	1042324	7.3
10.01	Oxalic acid, monoamide, N-cycloheptyl pentyl ester	527182	4.8
11.65	4-(1-Methylethenyl)-1-cyclohexene-1-methanol	49169	2.5
12.25	Oxalic acid, isohexyl pentyl ester	47763	2.5
12.92	Oxalic acid, 2-ethylhexyl isohexyl ester	80000	2.6
13.68	Camphor	811597	6.2
15.64	2,3,5-Trimethyl-decane	70861	2.6
15.93	(2-Methyl-1-propenyl)-hydrazine,	27159	2.4
22.39	11,13-Dimethyl-12-tetradecen-1-ol acetate	509402	4.7
22.83	Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester	348059	3.9
23.26	Methyl hexadecanoate	907375	6.7
23.58	Palmitic acid	2265402	13.4
24.77	n-Pentadecanol	206300	3.2
24.90	Linoleic acid, methyl ester	390935	4.1
24.96	Linolenic acid, methyl ester	1148481	7.9
25.07	Phytol	1262874	8.4
25.17	16-Methyl- heptadecanoic acid methyl ester	291147	3.7
25.22	Linoleic Acid	514117	4.7
25.29	Linolenic acid	3024419	17.1
38.03	γ-Sitosterol	5180397	27.7

4.7.2.3 GC-MS Results of Polyherb

Analysis of the TIC of the polyherb showed a spectrum of compounds in the range of retention time 9 up to 38 min (Figure 4.26). The Polyherb had three constituent plants and thus the GC-MS result showed a large number of compounds and were presented in Table 4.24 and Table 4.25. The main parts of the mixture were FFAs and FAMEs. The main compounds include octadecanoic acid (4.35%), methyl hexadecanoate (4.63%), (E)-9-octadecenoic acid methyl ester (7.88%), n-hexadecanoic acid (10.42%), methyl linoleate (12.37%) and linoleic acid (40.35%). The FFAs and FAMEs comprise of about 89% of the overall composition of the extract and unlike *M. dianthera*, the essential oil composition of the Polyherb was only 1% of the overall components of the mixture. Steroids including

cholesterol, (3α,5α, 6β)-3',6-dihydro-cyclopropa [5,6] cholestan-3-ol, stigmasterol and γ-sitosterol were identified at very low concentration. Some steroids like sterol 3β-D-glucoside are reported to have anti-diabetic activities (Ivorra *et al*., 1990).

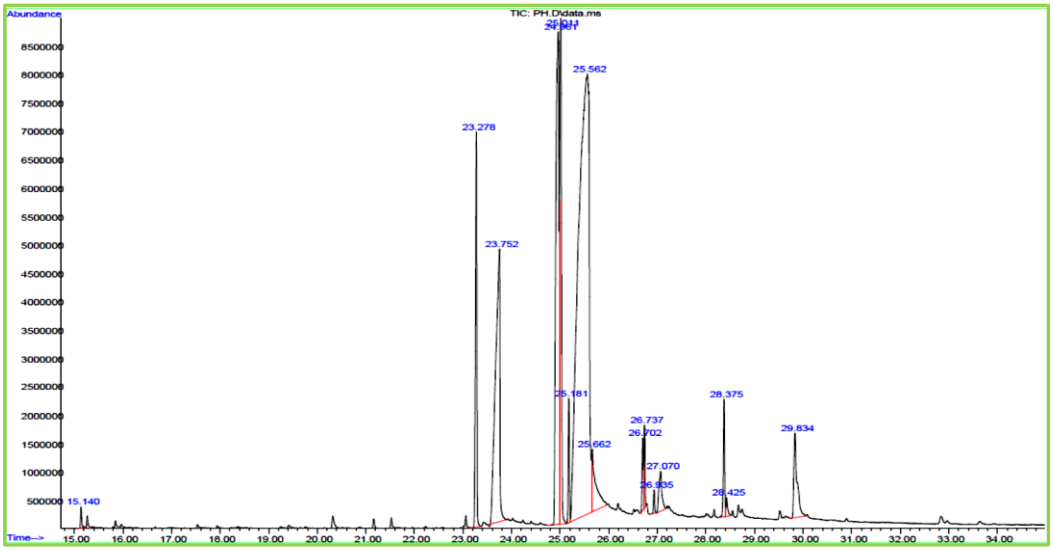


Figure 4.26: TIC of the methanol extract of the Polyherb

Table 4.24: Essential oils identified in the Polyherb using GC-MS

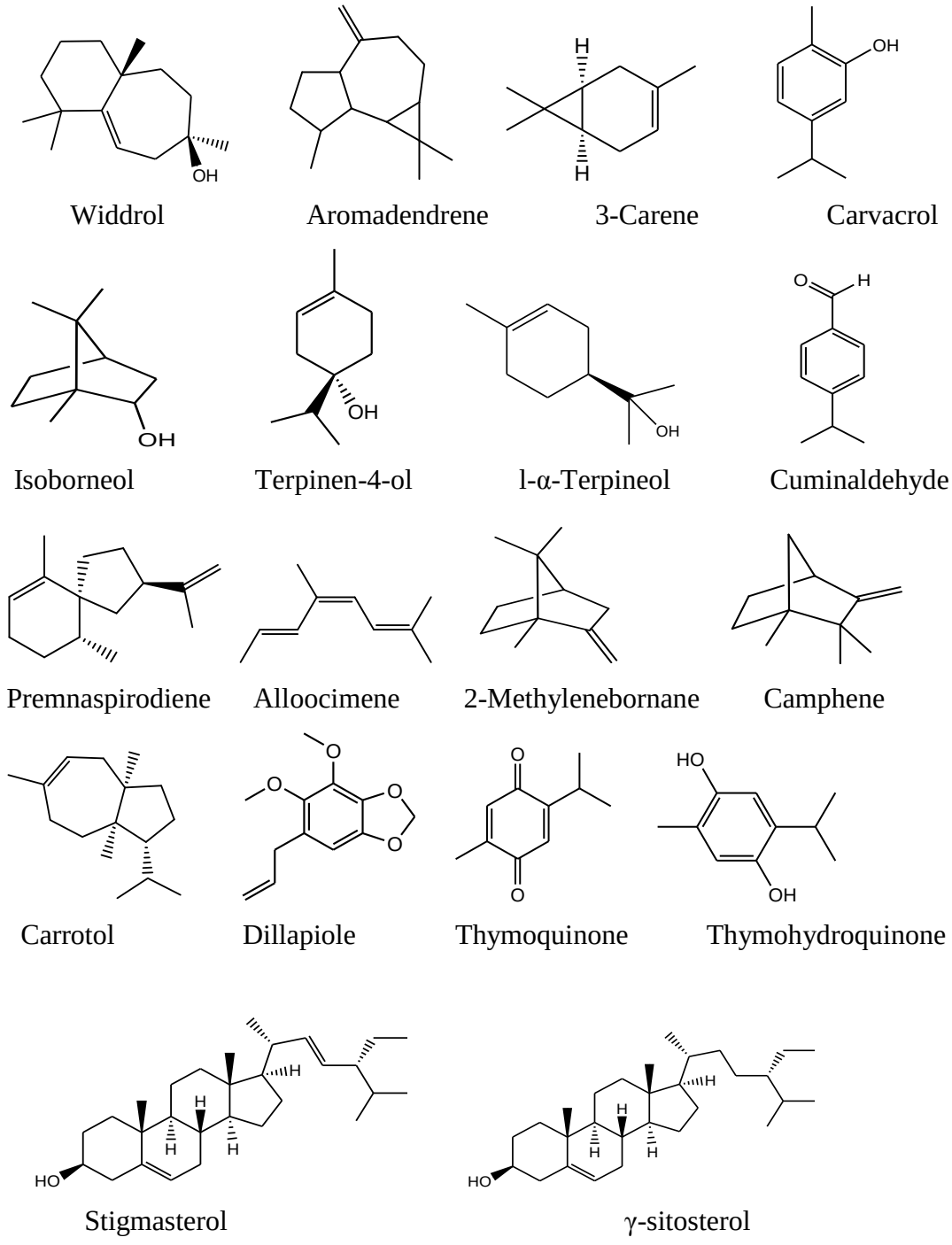
RT (min)	Library/ID	Area (Ab*s)	Conc (ng/µl)
11.65	(+)-3-Carene	194554	3.18
12.73	*p*-Cymenene	81801	2.62
13.68	Camphor	1691933	10.53
13.89	Benzoic acid	454835	4.45
14.02	Isoborneol	524317	4.80
14.18	Terpinen-4-ol	1029977	7.28
14.38	L-alpha-Terpineol	497377	4.66
15.14	Cumin aldehyde	6197811	32.68
15.27	Thymoquinone	5306931	28.30
15.85	*p*-Cymen-7-ol	2934476	16.64
15.97	Carvacrol	1943123	11.77
16.67	Camphene	599062	5.16
17.54	Longifolene	2148890	12.78
17.95	Aromadendrene	1257395	8.40
18.23	Primnaspirodiene	373000	4.05
19.25	Thymohydroquinone	1308262	8.65
19.41	cis-Stilbene	2288237	13.46
19.99	Carotol	97509	2.70
20.14	Dill apiole	299284	3.69

Table 4.25: Fatty acids and fatty acid methyl esters identified in the Polyherb using GC-MS

RT (min)	Library/ID	Area (Ab*s)	Conc (ng/µl)
10.73	Heptanoic acid	876769	6.53
18.85	Tetradecanoic acid, 12-methyl-, methyl ester	175247	3.08
19.15	Nonanedioic acid, dimethyl ester	108139	2.75
21.16	Methyl tetradecanoate	3569452	19.76
21.53	Tetradecanoic acid	4374308	23.72
22.23	Pentadecanoic acid, methyl ester	955321	6.91
22.57	Pentadecanoic acid	749248	5.90
23.06	Methyl hexadec-9-enoate	4532268	24.49
23.28	Methyl hexadecanoate	143082969	705.39
23.43	Palmitoleic acid	7193106	37.57
23.76	Palmitic acid	321751582	1583.45
23.92	Palmitic acid, ethyl ester	8682909	44.89
24.02	(Z)- 9-Octadecenoic acid methyl ester	8345701	43.23
24.23	Heptadecanoic acid, methyl ester	7118211	37.20
24.59	Heptadecanoic acid	9129071	47.08
24.96	Methyl linoleate	381913578	1879.11
25.01	(E)-9-Octadecenoic acid, methyl ester	243508408	1198.93
25.18	Methyl octadecanoate	39018311	193.97
25.56	Linoleic acid	1.246E+09	6124.38
25.66	Octadecanoic acid	134184763	661.66
26.52	Linoleic acid, methyl ester	11248455	57.50
26.70	11,13-Eicosadienoic acid, methyl ester	33987381	169.25
26.74	cis-11-Eicosenoic acid, methyl ester	35193355	175.17
26.94	Eicosanoic acid, methyl ester	26925049	134.54
27.24	Eicosanoic acid	24021123	120.27
28.38	(Z)-13-Docosenoic acid, methyl ester	48985711	242.96
28.55	Docosanoic acid, methyl ester	13173749	66.96
28.67	Erucic acid	17871694	90.05
30.08	Tetracosanoic acid, methyl ester	19355202	97.34

Some of the essential oils and other compounds analysed using GC-MS in the methanolic extracts of the polyherb are shown in Figure 4.27. The essential oils present in the polyherb were mostly different from those found in *M. dianthera*. The Polyherb contains three different plants and the powdered seed of *N. sativa* was oily and thus the plant was expected to contribute good number of essential oils. Forty-eight compounds have been identified in the essential oil from *N. sativa* seeds from Poland (Wajs, Bonikowski & Kalemba, 2008). A number of fatty acids were also reported in *N. sativa* which could be responsible to the expected bioactivity of the

polyherbal extract (Nickavar *et al*., 2003). Data generated from different sources demonstrated the anti-diabetic effect of essential oils (Irshaid, Mansi & Aburjai, 2010; Bakkalia, Averbeck, Averbeck, & Idaomar, 2008)



Widdrol Aromadendrene 3-Carene Carvacrol

Isoborneol Terpinen-4-ol l-α-Terpineol Cuminaldehyde

Premnaspirodiene Alloocimene 2-Methylenebornane Camphene

Carrotol Dillapiole Thymoquinone Thymohydroquinone

Stigmasterol γ-sitosterol

Figure 4.27: Structure of compounds analysed using GC-MS in the Polyherb

4.7.3 Analysis of the Fragmentation Patterns of Some of the Compounds

Study of the fragmentation patterns of the spectra of the compounds obtained from the LC-MS and GC-MS were necessary in order to justify the reasonable structures of some of the compounds. Representative spectra of the compounds are described in Figure 4.28 up to Figure 4.41. Flavonoids were the main compounds in *M. dianthera and A. camperi* and thus the spectra of the flavonoids were carefully analysed according to the common fragmentation pattern described under Section 2.14.3. As shown in Figure 4.28, the spectrum of 5, 4'-dihydroxy, 3, 6, 8-trimethoxyflavone was characterized by the loss of 15 u, resulting in a $[M\text{-}CH_3]^+$. Therefore, the characteristic peaks at m/z 328.9, 314.9, and 301.0 indicated the loss of methyl group. In particular, loss of one and two C_2H_2O moieties from the precursor ion, involved all the rings and the formation of $^{1,3}A^+ - C_2H_2O$ ion, were the most useful. Thus the characteristic peaks at m/z = 301.0 and 257.9 could be due to the loss of C_2H_2O moiety. The common fragmentation pattern of flavones at $^{0,2}B^+$ and $^{0,4}B^+$ with characteristic peaks of m/z 121 and 153 respectively were observed as reported before (Justensen, 2001; Justino *et al*., 2009).

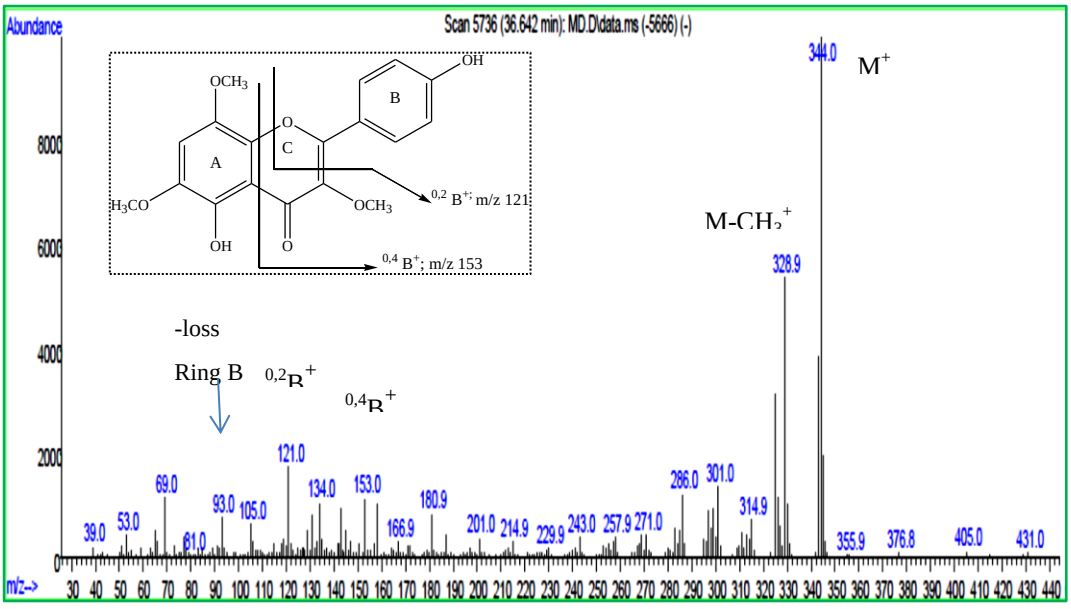


Figure 4.28: Fragmentation pattern of 5,4'-dihydroxy- 3,6,8-trimethoxyflavone

In the analysis of the spectra of quercetin 4'-glucoside, the fragment from the aglycone moiety was observed; cleavage at the glycosidic O-linkages (the sugar linked involves C-O bond) with a concomitant H-rearrangement lead to the elimination of monosaccharide residue (loss of 162 a.m.u.) and thus the base peak observed in Figure 4.29 was due to the loss of the sugar moiety. In the case of luteolin 6-C-glucoside (Figure 4.30) the sugar was directly linked to the flavonoid moiety via C-C bond (C-glycosides). Thus, a prominent peak due to the loss of 120 Da $[M+H- 120]^+$ was observed and was published as a diagnostic ion for C-glycosides (Cuyckens & Claeys, 2004).

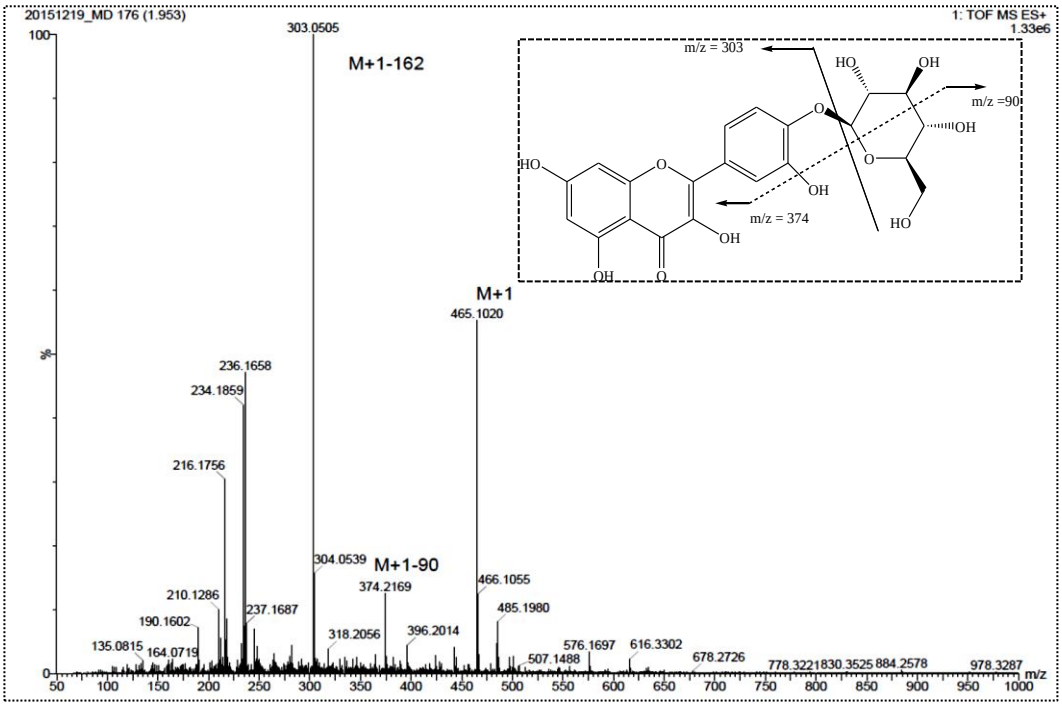


Figure 4.29: Spectrum and fragmentation pattern of quercetin 4'-glucoside

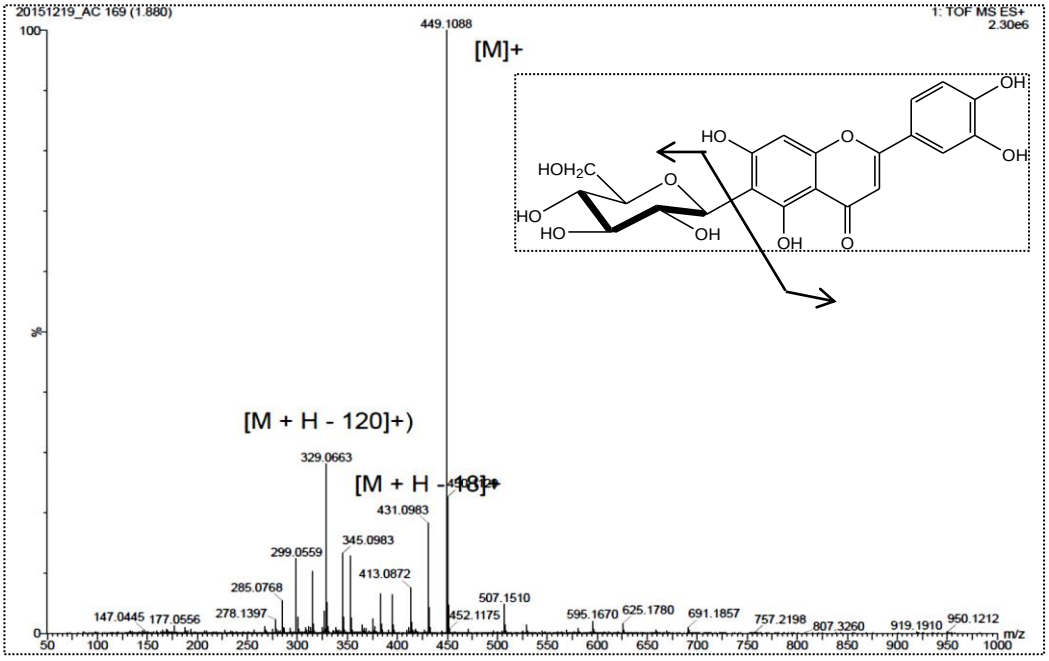


Figure 4.30: Spectrum and fragmentation pattern of luteolin 6-C-glucoside

Fatty acids and fatty acid methyl esters were some of the main constituents, especially in the Polyherb and *M. dianthera*, observed during the the GC-MS and LC-MS spectal analysis. Most of the compounds displayed similar fragmentation pattern due to the presence of the long hydrocarbon chain and similar functional groups. Examples of the fragmentation patterns of FFAs and FAMEs are shown from Figure 4.31 upto Figure 4.34. In linolenic acid (9,12,15-octadecatrienoic acid), the characteristic peaks with m/z = 79, 95, 108 and 236 can be deduced by the proposed fragmentation pattern shown in Figure 4.31 (A). Moreover, the prominent peaks with m/z = 55, 135, 149, 182, and 222 can be explained by the fragmentation pattern shown in Figure 4.31 (B).

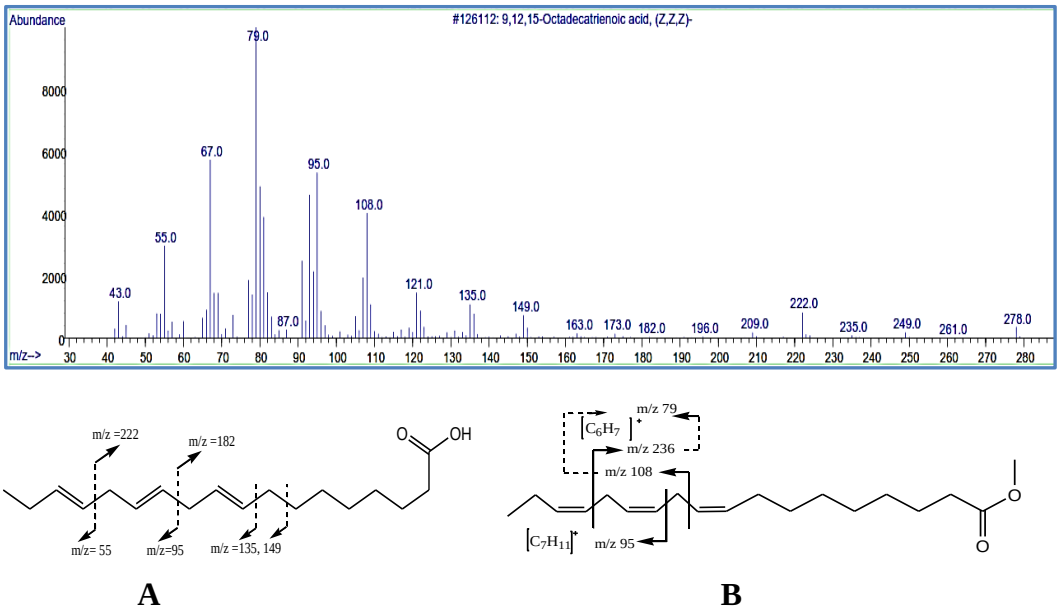


Figure 4.31: Spectrum and fragmentation pattern of linolenic acid

Similarly, in the analysis of the spectrum of palmitic acid (Figure 4.32), the molecular ion peak (m/z = 256) was small but detectable. The loss of hydroxyl radical ($OH^{\cdot}$) lead to the weak peak with m/z = 239 $([M-17]^{+})$ and fragmentation of the C-CO bond also lead to m/z 43 and m/z = 213 $([M - 43]^{+})$. Cleavage of the γ bond (relative to CO) lead to one of the prominent peaks due to $+CH_2CH_2COOH$ (m/z = 73). The characteristic peak at m/z = 60 was due to McLafferty rearrangement (Figure 4.34) and resulted in the loss of acetic acid (CH_3COOH). The other visible peaks in the spectrum (m/z 115, 129, 143, 157, 171, 185, 241...) were characteristics of long-chain aliphatic acids (Pretsch, Buhlmann & Affolter, 2000).

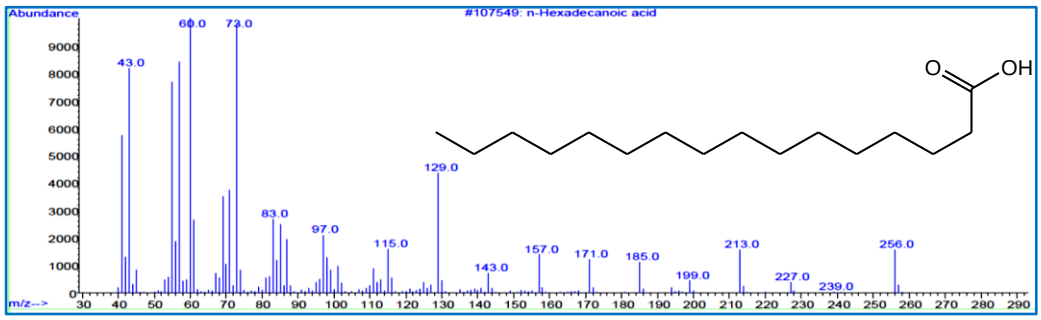


Figure 4.32: Mass spectrum of palmitic acid

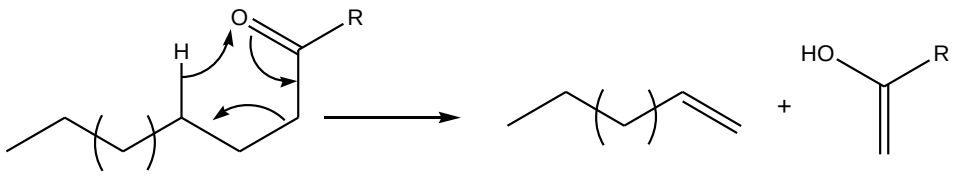


Figure 4.33: Common McLafferty rearrangement of carbonyl arrangements

The mass spectrum of most saturated FAMEs exhibited the presence of the corresponding $[M]^+$, $[M–31]^+$ and $[M–43]^+$, as well as intensive peaks at m/z 74, 87 and 143, which are characteristics of saturated FAMEs (Gangadhar, Tanushri, Shraddha & Ganesh, 2015). The spectrum along with the proposed fragmentation analysis is shown in Figure 4.34.

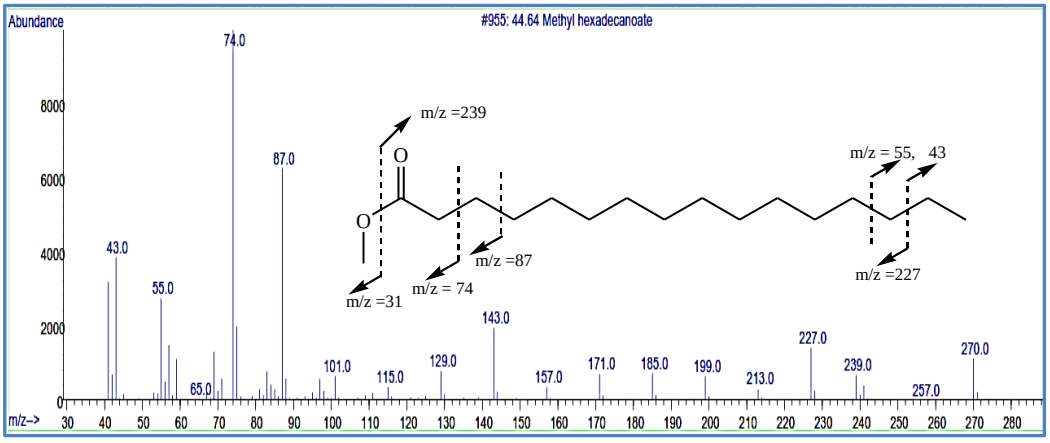


Figure 4.34: Mass spectrum of methyl hexadecanoate

In the case of steroids, the spectrum and a proposed fragmentation pattern of β-sitosterol are displayed in Figure 4.35 and Figure 4.36, respectively. The peaks with m/z 396 and 381 were due to the loss of water from the molecular ion (m/z = 414) and followed by a loss of methyl group from the molecular ion. The characteristic ion peak at m/z 273 was in a close agreement with the loss of side chain caused by the fission of C17 - C20 bond. This was further fragmented producing water and a structure with a signal at m/z 255 which on successive dealkylation would yield ions at m/z 186, 173, 159, 145, 132, 119, 105, 92, 81, 57 and 43 (Zhang et. al. 2005).

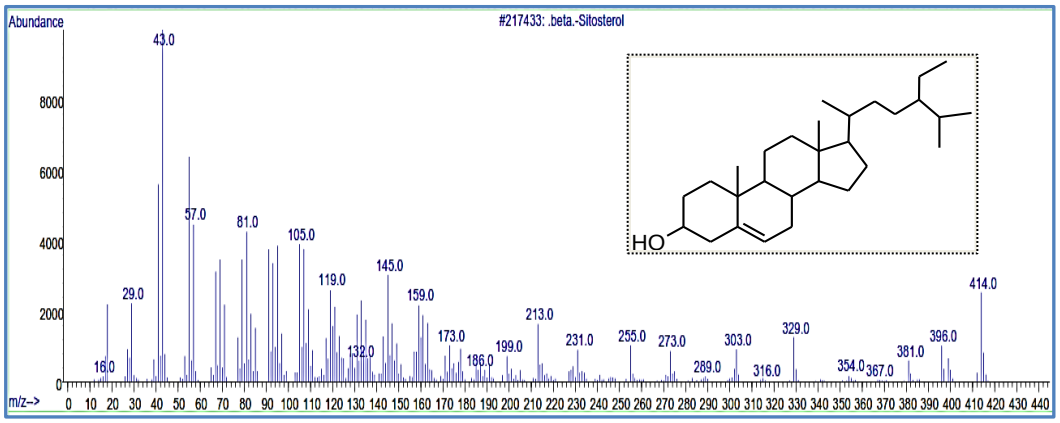


Figure 4.35: Mass spectrum of β-sitosterol

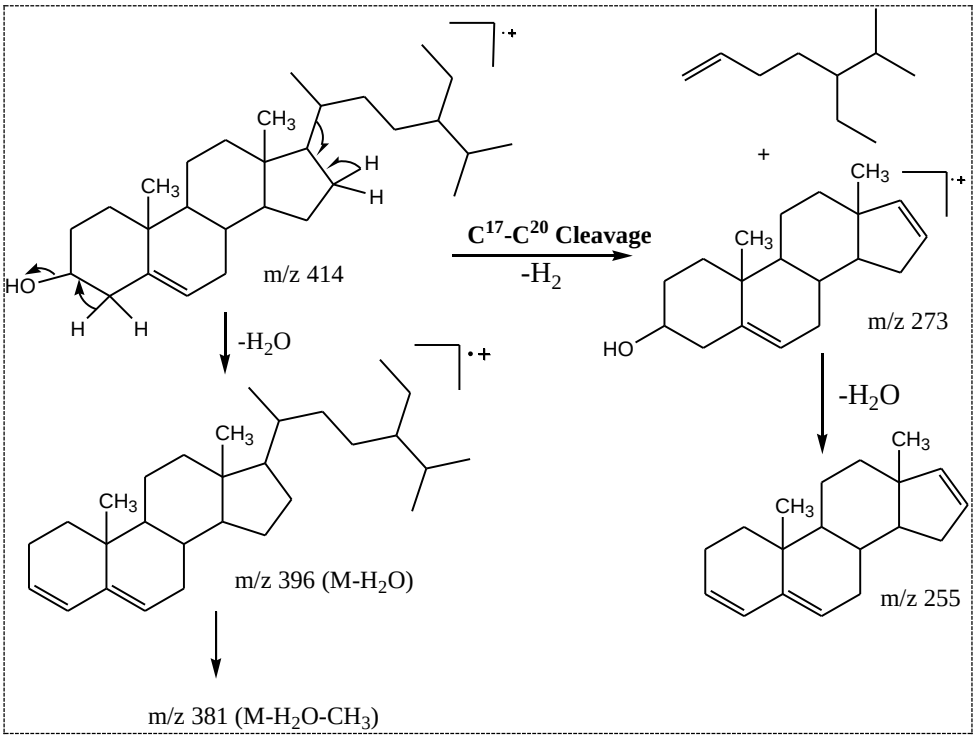


Figure 4.36: Fragmentation pattern of β-sitosterol

Additional structural analysis of the fragmentation patterns of other types of compounds including aldehydes, ketones, aromatic alcohols and phenolic compounds, found in the plants, are described below.

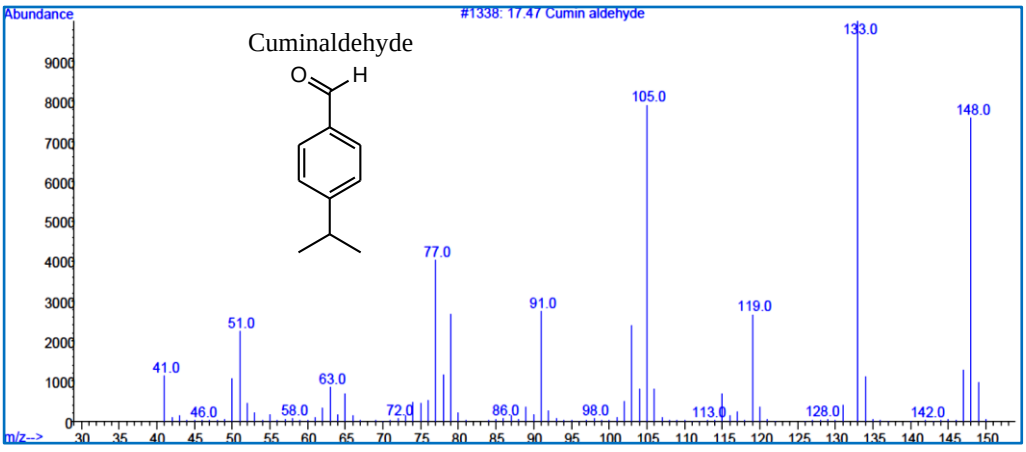


Figure 4.37: Mass spectrum of cumminaldehyde

Like all aromatic aldehydes, the mass spectrum of cumminaldehyde (Figure 4.37) was characterized by the loss of $H^{\cdot}$ to yield the corresponding benzoyl ion, [M-1; m/z = 147]$^{+}$, followed by decarbonylation to a phenyl ion, [M-1-28; m/Z = 119]$^{+}$, of lower intensity. The peaks at m/z = 41, 51, 63, 77, 91 were typical of an aromatic arrangement. The prominent peak with m/z =133 was obtained from the loss of a single methyl branch and decarbonylation [M-CH_3-COH]$^{+}$.

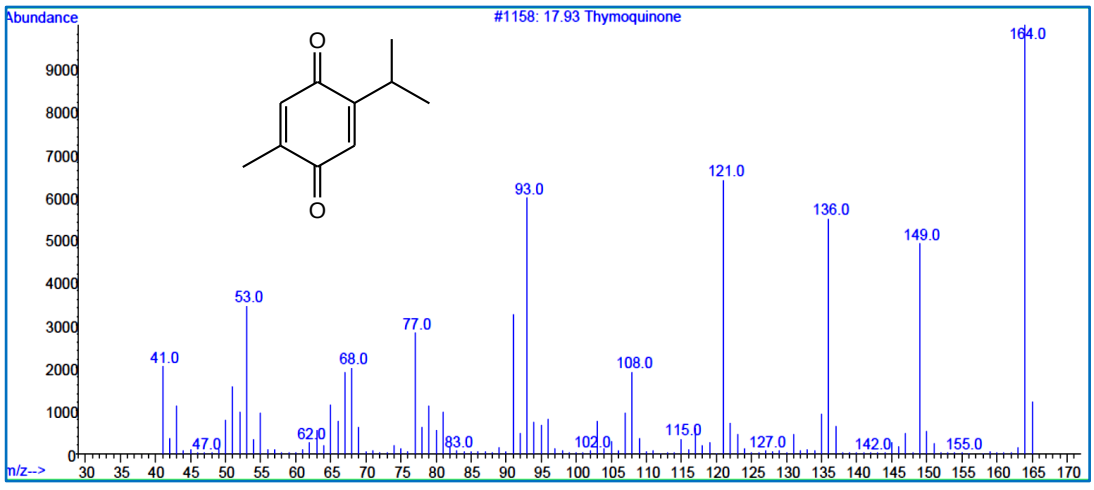


Figure 4.38: Mass spectrum of thymoquinone

In thymoquinone, the abundant molecular ion peak and predominant intensive peaks around the molecular ion region showed the resonance stabilized arrangement of thymoquinone (Figure 4.38). A characteristic loss of CO [M - CO]$^{+}$ gave m/z 136.

The prominent peaks with m/z of 121, 136 and 149 were due to the loss of the alkyl (-CH_3, -C_2H_6, -C_3H_9 and - C_4H_{10} respectively) substituents. Moreover, the aromatic hydrocarbon fragmentation gave corresponding *m/z* values of 53, 68 and 77.

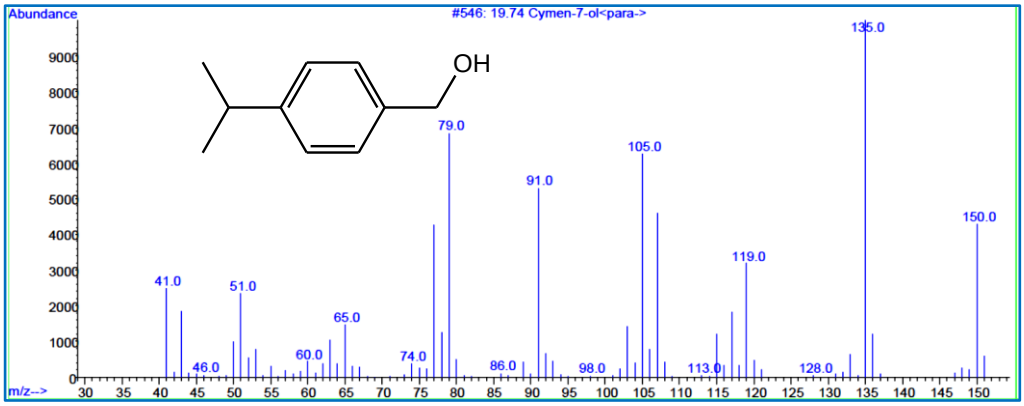


Figure 4.39: Mass spectrum of p-cymen-7-ol

In the spectrum of p-cymen-7-ol (Figure 4.39), the base peak (m/z = 135) appeared due to the loss of CH_3^+ from the parent ion. As shown in the Figure 4.40, the main route of the fragmentation pattern of the aromatic alcohol was the loss of $H^{\cdot}$ and consecutive elimination of CO gave a protonated benzene molecule, which further lost H_2. The second fragmentation path was due to the elimination of $OH^{\cdot}$ and thus formed the tropylium cation (m/z =91) (Pretsch, Buhlmann & Affolter, 2000).

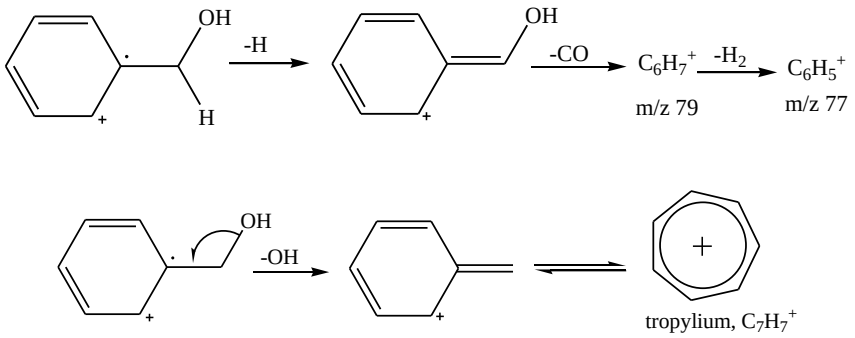


Figure 4.40: Common fragmentation pattern of benzyl alcohols

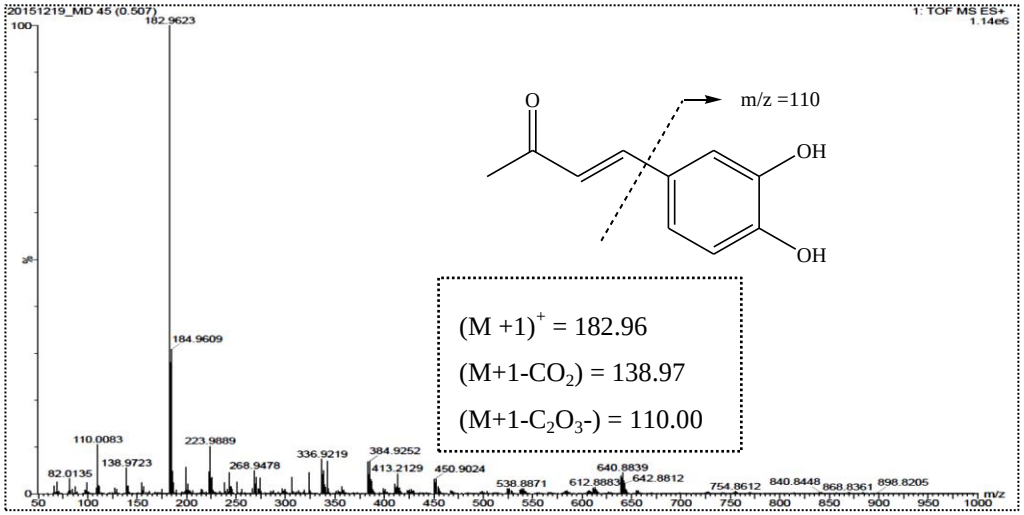


Figure 4.41: Spectrum of 3, 4-dihydroxycinnamic acid

In most ESI system adducts are usually observed as background peaks in the spectra. During the analysis of the spectra of many compounds, additional background peaks were observed as a result of the common adducts described in Section 2.14.4. The presence of adducts was observed in the spectrum of 3, 4- dihydroxycinnamic acid (Figure 4.41). The spectrum displayed characteristic peaks above the molecular ion peak (M+1 ion of m/z 182.96). The possible adducts in the spectrum were observed at m/z = 223.98, 268.94 and 336.92 and these could be due to acetonitrile (+ 46), DMSO (+ 85) and DBU (+ 253) respectively.

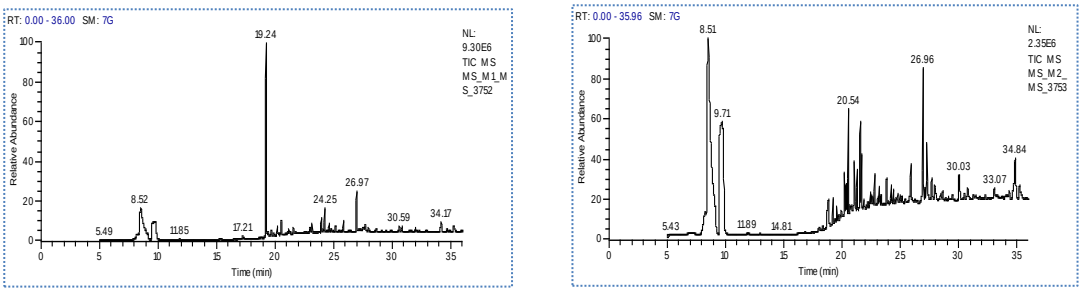
4.7.4 GC-MS and LC-MS Analysis of Bioactive Fractions

The most active fractions described under Section 4.4.2 were analysed using GC-MS and LC-MS. The phytochemical profiles related to some of the bioactive fractions are described below.

4.7.4.1 Non-Polar Fractions

The most active non-polar fraction were analysed using GC-MS and various compounds were identified. The first 2 non-polar fractions of *M. dianthera* (Section 4.4.2 described as M1 and M2) were pooled together and analysed and thus the GC-MS analysis (Figure 42) showed various essential oils including camphor, terpineol, caryophyllene oxide, ledol, isolongifolen-5-one, borneol, spathulenol,

p-menth-1-en-8-ol and thymol. Some of the essential oils characterised were reported to have anti-diabetic activities (Akolade, Usman, Okereke, & Muhammad, 2014; Nguyen, Um & Kim, 2011). Moreover, bioactive FAMEs like palmitic acid methyl ester, linolenic acid methyl ester and other bicyclic compounds were characterized in the bioactive non-polar fractions of *M. dianthera* and some of the FAMEs were reported to have anti-diabetic related activities (Nguyen, Um & Kim, 2011; Berraaouan, Abid & Bnouham, 2013). Therefore, the associated bioactivities of the non- polar fractions of the *M. dianthera* a can be attributed to the presence of the bioactive essentials oils and FAMEs represented in Table 4.26.



Fraction 1 **Fraction 2**

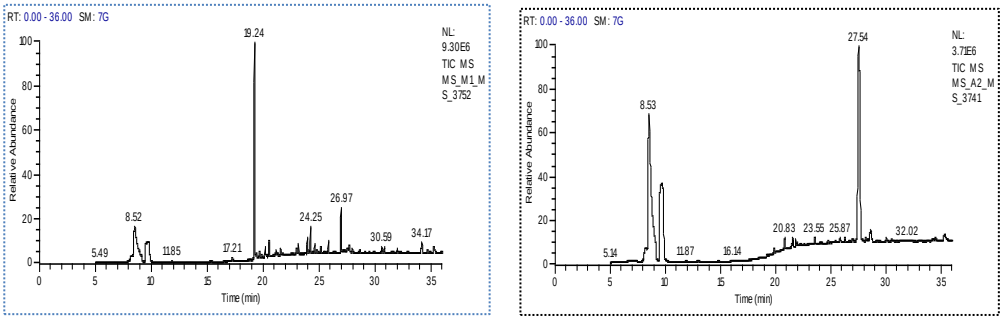
Figure 4.42: TIC from GC-MS analysis of the non-polar fractions of *M. dianthera*

Table 4.26: Compounds present in the bioactive non-polar fractions of *M. dianthera*

RT	SI	Compound	M.Wt	Fraction
19.24	916	Camphor	152	M1
19.69	919	α-Terpineol	154	M1, M2
20.19	874	Pinocarveol	152	M1
20.29	892	Verbenol	152	M2
20.54	925	Borneol	154	M1, M2
21.03	906	2-Pinen-4-one	150	M2
21.29	901	2-Pinen-10-ol	150	M2
21.66	846	Thymol	150	M2
23.95	911	Caryophyllene oxide	220	M1
24.25	914	Ledol	222	M1
24.57	832	Isolongifolene	218	M1
24.63	887	6,10,14-trimethyl-2-Pentadecanone	268	M1
25.16	842	Spathulenol	220	M1
25.85	922	Palmitic acid methyl ester	270	M1
26.97	904	β-Eudesmol	222	M1, M2
34.16	925	Linolenic acid methyl ester	292	M1

NB: RT- retention time & SI - Similarity index
M1 and M2 represent fraction 1 and fraction 2 of *M. dianthera* respectively

Similarly, the most active non-polar fractions of *A. camperi are* comprised of certain bioactive compounds. The main compounds identified were benzoic acid derivatives including 2-hydroxy-benzoic acid ethyl ester and 2-hydroxy-benzoic acid phenyl methyl ester (Figure 4.43 and Table 4.27). Benzoic acid and its derivatives were reported to have anti-oxidant and anti-diabetic properties (Ezuruike & Prieto, 2014). Other bioatctive compounds found in the bioactive non-polar fraction of *A. camperi* were 1-hexadecanol and 10-undecenoic acid methyl ester.



Fraction 1 **Fraction 2**

Figure 4.43: TIC of the non-polar fraction of *A. camperi* found using GC-MS

Table 4.27: Compounds present in the bioactive non-polar fractions of *A. camperi*

RT	SI	Compound	M.Wt	Fraction
19.84	920	Palmityl alcohol	242	A1, A2
20.83	944	10-Undecenoic acid, methyl ester	198	A2
21.54	900	1-Heptadecene	238	A1, A2
21.78	925	Benzoic acid, 2-hydroxy-, ethyl ester	166	A1, A2
23.20	854	Benzene, (1-ethyldecyl)-	246	A1
23.53	886	1-Nonadecene	266	A1, A2
25.84	886	Palmitic acid, methyl ester	270	A1
26.24	865	1-Heneicosyl formate	340	A1
26.90	881	2,4-Di-tert-butylphenol	206	A1
26.90	881	Phenol, 2,4-bis(1,1-dimethylethyl)-	206	A2
27.54	854	Benzoic acid, 2-hydroxy-, phenyl methyl ester	228	A2
29.98	876	Benzyl ether	198	A1, A2

NB: RT – retention time & SI - Similarity index
A1 and A2 represent fraction 1 and fraction 2 of *A. camperi* respectively

Mostly FFAs and FAMEs were determined as the most active non-polar fractions of the Polyherb. The main list of the bioactive molecules in the Polyherb include 11-eicosenoic acid methyl ester, longifolene, benzyl nitrile, methyl tetradecanoate, palmitic acid methyl ester, methyl stearate, 9-octadecenoic acid methyl ester and linoleic acid methyl ester (Appendix 6). In this study, GC-MS has proved a better separation and characterisation of the fatty acids and their associated esters; similar findings were reported before (Destaillats & Cruz-Hernandez, 2007).

4.7.4.1 Polar Fractions

Polar compounds, phenols and flavonoids, containing a number of hydroxyl groups, were not detected using the GC-MS analysis. Therefore, the bioactive constituents of one of the most active polar fractions of *M. dianthera* was analysed using LC-MS. The TIC from the LC-MS analysis of the fractions displayed a spectrum with 8 characteristic peaks (Figure 4.44) and the peaks were analysed using online search (METLIN) and thus characteristic bioactive compounds including three flavonoids and one phenolic acid were identified. The compounds were 5, 7- dihydroxy-4'-methoxyisoflavone (Biochanin A), 5, 7, 4'-trihydroxy-6, 3'-dimethoxyflavone, 4-hydroxybenzoic acid 4- O-glucoside and apigenin. The mass spectra of the compounds are shown in Figure 4.45. It is easily recognized that, except for apigenin, the base peaks were the molecular ion peaks of the compounds. The base peak of apigenin shows the presence of an adduct at m/z > 413. Based on literature survey, the compounds were reported to have either direct or indirect anti-diabetic effects (Ezuruike & Prieto, 2014; Khadem & Marles, 2010). The same fraction was analysed using GC-MS and did not show any characteristic compound. Therefore, the observed anti-diabetic activity of the fraction is mainly due to the compounds identified above using LC-MS.

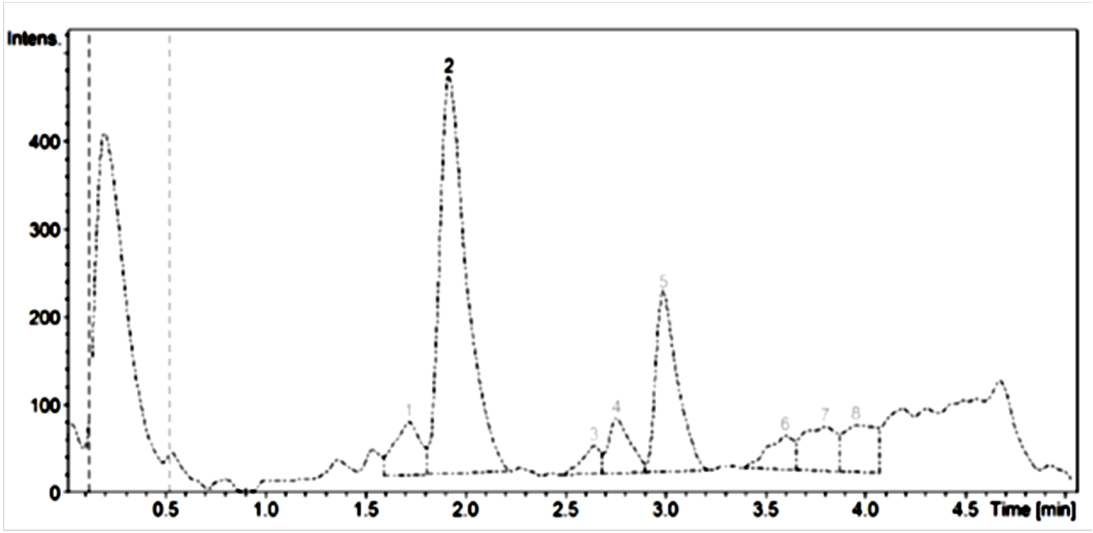
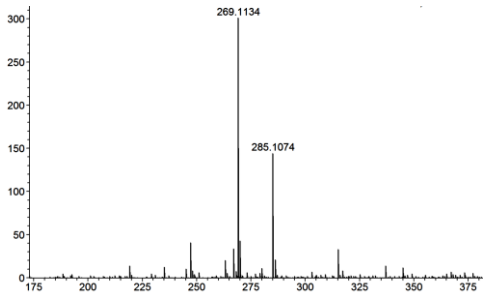
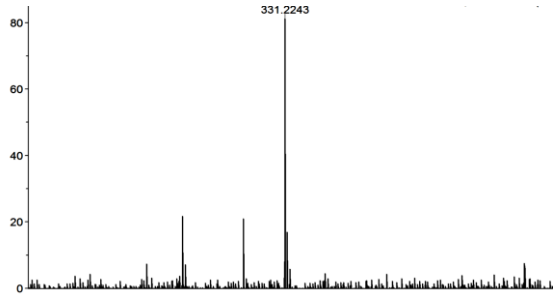


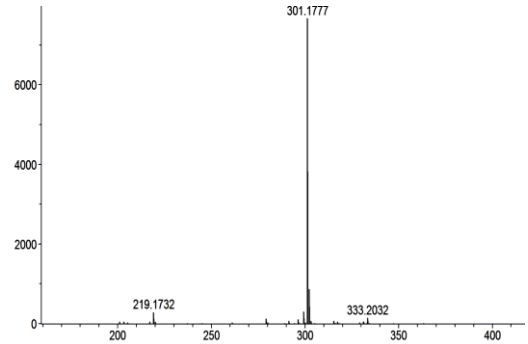
Figure 4.44: TIC of the most active polar fraction of *M. dianthera* using LC-MS



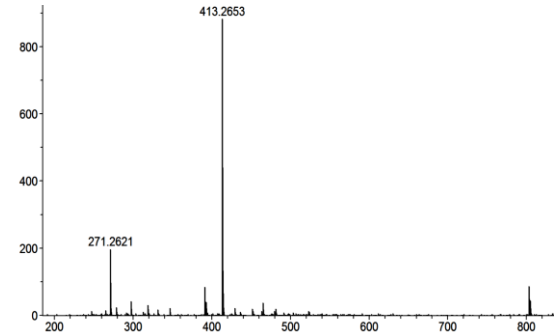
5,7-Dihydroxy-4'-methoxyisoflavone



5,7,4'-Trihydroxy-6,3'-dimethoxyflavone



4-Hydroxybenzoic acid 4-O-glucoside



Apigenin

Figure 4.45: Spectra from the LC-MS analysis of the polar fraction of *M. dianthera*

4.8 Fatty Acids and Fatty Acid Methyl Esters and thier Biological Activities

In food nutrition assessment, fatty acids have gained significance in the diagnosis of various diseases and pharmacology due to biological importance (Cherif, Frikha, Gargouri & Miled, 2008). In lowering risks of inflammation, heart diseases and for immunity enhancement, saturated fatty acids either monosaturated or polysaturated have been used (Yaqoob, 2002). FAMEs are known to have anti-bacterial, antifungal and anti-diabetic properties (Nguyen, Um, & Kim, 2011). Some of the FAMEs including palmitic acid, stearic acid methyl esters, oleic acid, linoleic acid, linoleinic acid methy esters are considered to be closely related with type 2 diabetes mellitus (T2DM) and serve as signaling molecules in insulin secretion (Tan *et al*., 2010). The simplest fatty acid like linoleic acid (C18:2) and linolenic acid (C18:3) have been reported to protect against cardiovascular and in flammatory diseases, though linolenic acid has greater health benefits. Conjugated fatty acids including linoleic acid lower insulin resistance which may help prevent adult-onset of diabetes (Aluko, 2012).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study has revealed the use of medicinal plants for diabetes control as they play a major role in the primary health care of the people of the Southern and Central Zones of Eritrea. A total of 42 plants traditionally used for diabetes were identified and documented. Furthermore, the detailed ethnomedicinal values of the plants, dosage and methods of preparation were reported.

The *in vitro* and *in vivo* assays of *Aloe camperi*, *Meriandra dianthera* and a Polyherb elicited dose dependent anti-diabetic activities. The acute oral toxicity study of *A. camperi*, *M. dianthera* and Polyherb established that the extracts administered to the Wistar Albino rats were safe with the limit dose of 2000 mg/kg per body weight. A safe dose can be estimated for developing herbal drugs using proper calculations from animal to human. Both the *in vitro* and *in vivo* studies established the anti-diabetic potential of the plants. All plants, especially *M. dianthera,* displayed good results comparable to the standard drugs.

The trace metal analysis displayed the presence of substantial levels of essential elements including Zn, Cr, V, Mg, Mn, and Se in the plants. These elements play vital roles in blood glucose reduction and thereby aiding in the management of diabetes. Moreover, analysis of heavy and toxic metals (such as Fe, Cu, Ni, Co, As, Cd and Pb) were found in the permissible limit. The reported bioactivity of the plants could partly be due to the essential elements present in the plant.

Identification and tentative characterisation of the active compounds using hyphenated techniques, LC-MS, GC-MS and FT-IR, produced detailed structural information in the crude extracts and fractions. The plants displayed a spectrum of bioactive molecules including flavonoids, fatty acids, fatty acid methyl esters, essential oils, benzoic acid derivatives, steroids, and some alkaloids. The main

bioactive compounds which could be responsible for the reported activity of *M. dianthera* were the flavonoids, essential oils and fatty acid methyl esters. Similarly, flavonoids, fatty acid methyl esters and alkaloids were the main bioactive constituents for *A. camperi* and the Polyherb. Therefore, the selected anti-diabetic plants have displayed potential bioactivity and good profile of bioactive compounds. The current report justified the ethnomedicinal uses of the plants for diabetes and thus the plants can be possible candidates for herbal drug development.

5.2 Recommendations

Based on the analysis of the survey data, observed anti-diabetic profile and identified phytochemicals, the following recommendations were made on the report of this work.

- Majority of the informants involved in the ethnomedicinal survey were old. As traditional medical knowledge is orally passed down via lifestyle, thus the informants are dying without transferring the indigenous knowledge of the plants to others. Therefore, it is important to exhaustively identify, document and publicise medicinal plant knowledge for diabetes and other diseases in the different Zones of Eritrea.
- This paper is one of the first reports related to the anti-diabetic profile and phytochemical constituents of Eritrean medicinal plants. Thus, the available data regarding the anti-diabetic plants are not adequate enough and the plants currently in use for the treatment of diabetes can't be endorsed for use without proper evaluation. Therefore, continuous pharmacological studies are compulsory to provide suggestion for a safe and effective use of the plants traditionally used in the treatment of diabetes.
- The results reported in this paper can be used as basis for the development of herbal drugs for diabetes. Therefore, with the proper and safe formulations, the plants can be employed as candidates for the development of potential and alternative herbal medicine. However, preliminary clinical studies are recommended for ensuring a better efficacy and safety.

- The plants included in this research are indigenous; therefore the plants should be preserved and cultivated in botanical gardens for future use. Further bioactivity studies and identification of bioactive compounds should be done to supplement this report.
- The high performance liquid chromatography/electrospray ionization with quadrupole time of flight tandem mass spectrometry (HPLC/QTOF-MS/MS) and GC-MS has proven to be powerful techniques for rapid identification of the constituents of the complex matrix of plant extracts and their application for qualitative and quantitative purposes can be highlighted for several future studies.
- Isolation of pure compounds, complete identification and structural elucidation of some of the bioactive anti-diabetic compounds would be mandatory for the develpment of drugs from the plants.

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APPENDICES

Appendix 1: Questionnaire Used for the Ethnomedicinal Survey

The English version of the questionnaire prepared for the data collection during the ethnomedicinal survey is as follows.

Date _____

1. Locality:

   District _____ Village _____

2. Name of the traditional healer _____

   Age _____ Sex _____

3. Vernacular name of the plant _____

4. Herbal preparation procedures:

   i. Plant part used _____

   ii. Fresh or dried _____

   iii. Crushed , powdered or other _____

   iv. Solvent (s) used _____

   v. Cold , warm or hot _____

   vi. Mixed with other materials (proportion):

_____

_____

   vii. Other preparations:

_____

_____

5. Dose and regimen:

_____

_____

6. Disease or symptoms treated:

_____

_____

Other plant(s) used together with the plant (proportion):

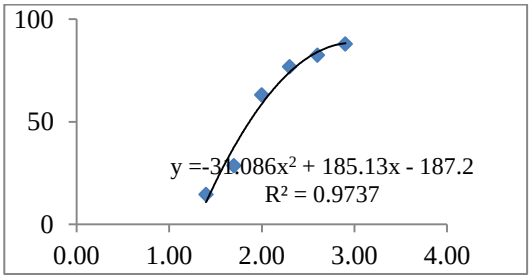
_____

Appendix 2: List of Informants Involved During the Ethnomedicinal Survey

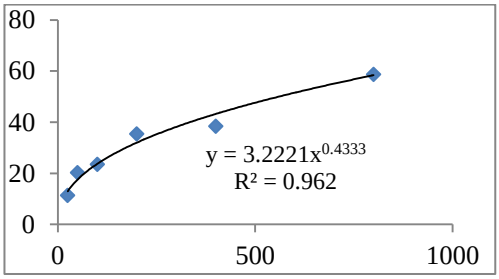
S.No	Name	Sex	Age
1	Abdelwasie	M	65
2	Abraham	M	79
3	Alganesh	F	68
4	Amna	F	62
5	Awelkier	M	61
6	Beyene A	M	64
7	Beyene B	M	83
8	Bicha	F	80
9	Ethiopia	F	61
10	Eyob	M	82
11	Ezghaharia	F	79
12	Fekre	M	60
13	Feshaye	M	69
14	Gooye	F	58
15	Habteslassie	M	63
16	Habtom	M	74
17	Hailemariam	M	55
18	Issak	M	67
19	Jaber	M	67
20	Johar	M	70
21	Johan	M	70
22	Kefle	M	69
23	Keria	F	52
24	Letay	F	72
25	Letebrhan A	F	81
26	Letebrhan B	F	65
27	Mehari	M	72
28	Meaza	F	54
29	Mebrak	F	67
30	Mehari	M	74
31	Mletu	F	63
32	Mohammed	M	90
33	Naser	M	75

S.No	Name	Sex	Age
34	Niema	F	67
35	Nuria	F	59
36	Okbamichael	M	62
37	Raqia	F	56
38	Romana	F	63
39	Romadan	M	59
40	Saedi	F	58
41	Seida	M	66
42	Semira	F	60
43	Shibeshi	M	80
44	Sieda	F	61
45	Sium	M	64
46	Sium B	M	64
47	Sulieman	M	71
48	Tecle	M	80
49	Tekea	M	60
50	Tesfay	M	61
51	Tewelde	M	54
52	Tsegai	M	72
53	Yakob	M	59
54	Yasin	M	71
55	Yohannes	M	81
56	Yoseab A	M	81
57	Yoseab B	M	59
58	Zahara	F	69
59	Zeineb	F	72
60	Zmsmm	F	60
61	Zommuy	M	75
62	Measho	M	74
63	Hadish	M	65
64	Abraham	M	66
65	Jewahi	M	50
66	Tikabo	M	70

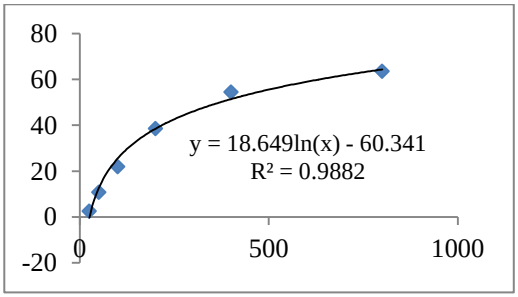
Appendix 3: Regression Analysis for the LC50 Calculations of α-Glucosidase



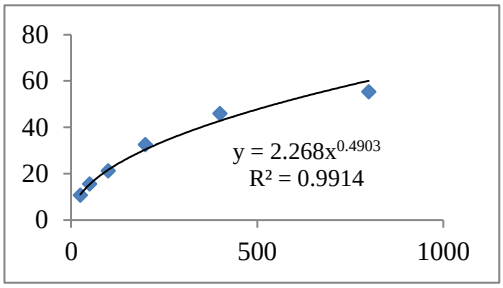
Meriandra dianthera LC_{50}= **74.13 µg/ml**



Polyherb, LC_{50} = **371.30 µg/ml**

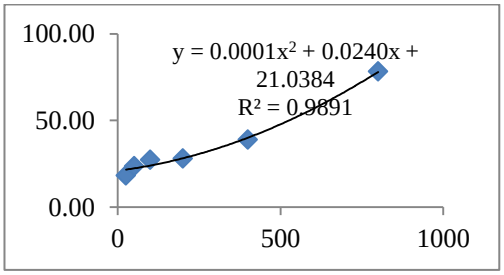


Aloe camperi, LC_{50} = **371.30 µg/ml**

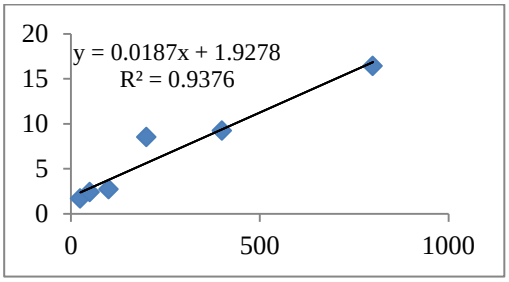


Acarbose, LC_{50} = **549.54 µg/ml**

Appendix 4: Regression Analysis for the LC50 Calculations of α- Amylase



Meriandra dianthera LC_{50}= **0.43 mg/ml**



Polyherb, LC50 = **2.57 mg/ml**

Appendix 5: Ethical Clearance Letter for the In Vivo Experiment

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

Office of the director, Production Services

(Research, Production and Extension Division)

Internal memo

To: Prof Patrick G. Kareru Date: 27th Sept 2016

From: Director Production **REF: *JKU/2/4/IPC/013***

Re: *PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTIDIABETIC POTENTIAL OF SELECTED ERITREAN MEDICINAL PLANTS JKU/2/4/IPC/013*

This is to confirm that prior to commencement of the above research, reference **JKU/2/4/IPC/013,** the proposal was subjected to peer-review and it was approved by the Ethics Sub-committee of Senate, Jomo Kenyatta University of Agriculture and Technology during the year 2013.

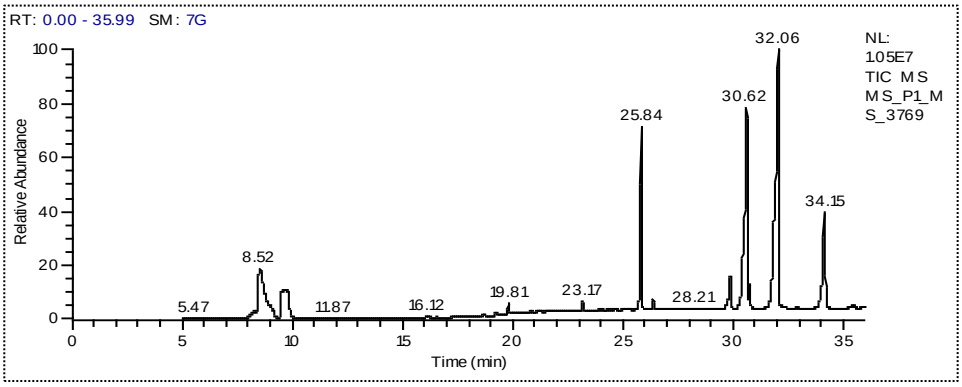
Mr. **Mussie Sium Demoz Registration Number** Sc431-4759/2013 has carried out the research in strict adherence to the ethical issues dealing with animal models.

Prof. Martin Obanda.

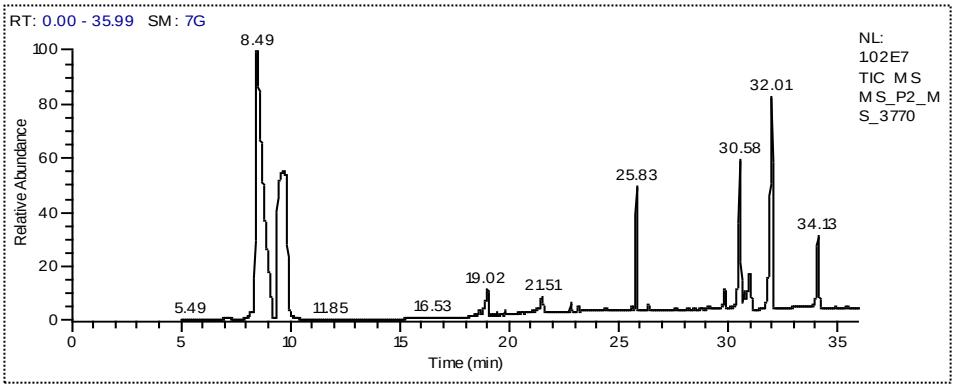
Director Production

Copy to: DVC, RPE

Appendix 6: GC-MS Analysis Data of Non-polar Fractions of the Polyherb



TIC obtained from the GC-MS analysis of Fraction 1



TIC obtained from the GC-MS analysis of Fraction 2

List of the main compounds found in the most active non-polar fraction of the Polyherb

	RT	SI	Name	M.Wt
Fraction 1 & 2	19.03	879	11-Eicosenoic acid, methyl ester	324
	19.82	836	Longifolene	204
	22.83	920	Benzyl nitrile	117
	23.17	844	Methyl tetradecanoate	242
	25.64	832	Phenol, 2-methyl-5-(1-methylethyl)-	150
	25.85	918	Palmitic acid methyl ester	270
	29.86	837	Methyl stearate	298
	30.62	893	9-Octadecenoic acid, methyl ester	296
	32.07	851	Linoleic Acid Methyl Ester	294

NB: RT – retention time & SI - Similarity index

Appendix 7. Preparation of 1000 ppm Stock AA Standards

Each standard was prepared as described and diluted into a 1 liter volumetric flask using deionized water

Aluminium
Dissolve 1.000 g of Aluminium metal in 25 ml of hydrochloric acid (S.G. 1.18) and a few drops of nitric acid (S.G. 1.42).

Barium
Dissolve 1.4380 g of barium carbonate ($BaCO_3$) in 20 ml 1M hydrochloric acid.

Calcium
Dissolve 2.4973 g of calcium carbonate ($CaCO_3$) in 25 ml of 1M hydrochloric acid. This should be added dropwise to avoid losses during the vigorous effervescence.

Chromium
Dissolve 7.6960 g of chromium nitrate ($Cr(NO_3)_3.9H_2O$) in 250 ml of deionised water.

Cobalt
Dissolve 1.000 g of cobalt metal in 50 ml of 5M hydrochloric acid.

Copper
Dissolve 1.000 g of copper metal in 50 ml of 5M nitric acid.

Iron
Dissolve 1.000 g of iron wire or granules in 20 ml of 5M hydrochloric acid

Lead
Dissolve 1.000 g of lead metal in 50 ml of 2M nitric acid.

Magnesium
Dissolve 1.000 g of magnesium metal in 50 ml of 5M hydrochloric acid.

Manganese
Dissolve 1.000 g of manganese metal in 50 ml of conc. hydrochloric acid.

Mercury
Dissolve 1.000 g of mercury metal in 20 ml of 5M nitric acid.

Potassium
Dissolve 11.9070 g of dry potassium chloride in 250 ml of deionised water.

Sodium
Dissolve 2.5420 g of sodium chloride (NaCl) in 200 ml of deionised water.

Vanadium
Dissolve 1.000g of vanadium metal in 25 ml conc.nitric acid.

Zinc
Dissolve 1.000 g of zinc metal in 30 ml of 5M hydrochloric acid.

Appendix 8: Comparison of Measured and Certified Values of the Elements

Element	Certified value (mg/kg)	Measured values (mg/kg)	% RSD *	Relative Error
Mg	2496 ± 67.00	2505 ± 11.43	0.36	9
Ba	36 ± 0.96	36.21 ± 1.08	0.58	0.21
Ca	471 ± 8.45	478.35 ± 5.99	1.56	7.35
K	2499 ± 67.08	2505.52 ± 11.43	0.26	6.52
Na	21.6 ± 0.39	21.84 ± 0.22	1.11	0.24
Al	126.5 ± 4.49	126.71 ± 4.73	0.17	0.21
Sr	92.41 ± 1.33	92.97 ± 0.81	0.61	0.56
Zn	52 ± 1.9	52.23 ± 0.95	0.44	0.23
Cr	1.21 ± 0.03	1.18 ± 0.04	2.48	- 0.03
V	1.06 ± 0.04	1.08 ± 0.03	1.89	0.02
Mn	21.9 ± 0.55	21.84 ± 0.22	0.27	- 0.06
Se #	72.6 ± 1.82	72.64 ± 2.98	0.06	0.04
Fe	477 ± 11.98	478.35 ± 5.99	0.28	1.35
Cu	36.11 ± 0.18	36.21 ± 1.08	0.28	0.10
Li	0.51 ± 0.05	0.52 ± 0.05	1.96	0.01
Co	0.26 ± 0.03	0.28 ± 0.02	7.69	0.02

Appendix 9: Custom-Grade Multi-Element Standard Solutions Employed for Calibration

Custom standards used	Targeted Element for analysis
50 ppm Custom 1 Matrix 5% HNO3 (N9307113)	- Mg
Se 5ppm	- Se
H5 - 5ppm prepared from custom 3 matrix 5% HNO_3 / 2%HF / Tr. Tart. Acid (N9307115) and custom 4 matrix 5% HNO_3 (N9307116)	- Ba, Sr, Zn, Cr, V, Mn, Cu, Li, & Co
VH50 ppm prepared from custom 1 matrix 5% HNO_3 (N9307113) and custom 2 matrix 5% HNO_3 (N9307114)	- Mg, Ca, Al, Fe, K and Na

NB: H5 (high 5) and VH50 (very high 50) are calibration levels of the elements

Appendix 10: Published Papers

European Journal of Medicinal Plants
9(2): 1-11, 2015, Article no.EJMP.18777
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

Ethnobotanical Survey and Preliminary Phytochemical Studies of Plants Traditionally Used for Diabetes in Eritrea

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[1]Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
[2]School of Pharmacy, College of Health Sciences, Asmara, Eritrea.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MSD and BGN carried out the Survey. Authors MSD, KPG and KJM drafted the manuscript and participated in the phytochemical analysis. All authors involved in the research concept, data analysis and review of the manuscript read and approved the final manuscript.

Article Information

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(2) James Adams, Pharmacology, University of Southern California, USA.
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Original Research Article

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ABSTRACT

Aims: Identify and document medicinal plants used for the treatment of diabetes mellitus. Investigate the secondary metabolites present in those plants.
Study Design: A questionnaire was employed for the survey and different chemicals and reagents were used for phytochemical screening.
Place and Duration of Study: Ethnobotanical surveys were conducted between November 2013 and April 2014 in certain localities of Central and Southern Zones of Eritrea and the phytochemical screening was conducted in September 2014 in Kenya.
Methodology: The ethnobotanical data was collected by interviewing 66 informants using a questionnaire; the majority of informants were herbalist and individuals living with diabetes. The phytochemical screening was done using wet chemical analysis.
Results: The survey showed 42 different medicinal plants used for the treatment of diabetes. The

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Journal of Diabetes Mellitus, 2015, 5, 267-276
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http://dx.doi.org/10.4236/jdm.2015.54033

Scientific Research Publishing

Evaluation of the Anti-Diabetic Potential of the Methanol Extracts of *Aloe camperi*, *Meriandra dianthera* and a Polyherb

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Received 28 August 2015; accepted 27 October 2015; published 30 October 2015

Abstract

The objective of the study was to evaluate the anti-diabetic activities of methanol extracts of *Aloe camperi* (AC), *Meriandra dianthera* (MD) and a polyherbal drug (PH) in diabetes induced Wistar albino rats. A single dose of alloxan monohydrate (150 mg/kg, i.p.) was used to induce diabetes mellitus (DM). Diabetes was confirmed by the elevated blood glucose levels determined after 72 h of induction. Animals with mean fasting blood glucose (FBG) level more than 200 mg/dl were recruited for the experiment. The herbal extracts at doses of 200 and 400 mg/kg and standard drug-metformin (5 mg/kg) were administered orally to the diabetic rats for 21 days and the FBG level was estimated on 0, 7, 14 and 21 days. The herbal extracts showed dose-dependent fall in FBG levels and the result exhibited very significant ($P < 0.001$) decreases in FBG level by the end of the experimental day as compared to the diabetic control. The highest antihyperglycemic effect was observed by MD extract at 400 mg/kg and was comparable to the standard drug. Oral glucose tolerance test (OGTT) was also conducted on normal rats and thus glucose at 2 g/kg per body weight was loaded via oral gavage to all groups 30 min after extract administration. All the groups showed significant increase ($P < 0.01$ or $P < 0.05$) in FBG level at 30 min following glucose loading. The hyperglycemia with glucose challenge was significantly brought down ($P < 0.001$) by all herbal extracts at 60 and 120 min relative to the negative control. Moreover, acute oral toxicity tests was conducted based on the protocols of OECD-425 and thus the LD_{50} of the herbal extracts was estimated to be greater than 2000 mg/kg. Statistical analysis was performed using One-Way ANOVA followed by Dunnett's test for multiple comparisons, and values of $P < 0.05$ were considered as statistically significant.

*Corresponding author.

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American Journal of Plant Sciences, 2014, 5, 3752-3760
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http://dx.doi.org/10.4236/ajps.2014.526392

Scientific Research

GC-MS Analysis of the Essential Oil and Methanol Extract of the Seeds of *Steganotaenia araliacea* Hochst

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Received 9 October 2014; revised 16 November 2014; accepted 29 November 2014

Abstract

Background: ***Steganotaenia araliacea*** **is a multipurpose plant and has wider applications in the folklore for the treatment of various ailments. Previously the presence of antileukemic lignan lactones and saponins was detected in the stem bark, root and leaf of** ***Steganotaenia araliacea.*** **Besides, the diuretic and antibacterial activities of the plant were reported. However, there has been no attempt to examine the constituents of the seeds of** ***Steganotaenia araliacea.*** **This paper reports the first such study of both the essential oil and methanol extract of the seeds of the plant. Methods: The seeds of the plant were shade dried, pounded and thus extracted using methanol. Besides, the essential oil of the seeds was collected using steam distillation. The components of the methanol extract were studied both by GC-MS and preliminary phytochemical studies; the essential oil was running on GC-MS for analysis. Results: The GC-MS analysis of the essential oil of the seeds identified the presence of** α**-linalool,** α**-pinene, m-cresol,** *p*-menth-1-en-4-ol, *p*-menth-1-en-8-ol, **myristicin and others. Besides, the methanol extract of the seeds showed the presence of falcarinol, apiol, scoparone, stigmasterol, myristicin etc. The preliminary phytochemical analysis of the methanol extract of the seeds confirms the presence of alkaloids, flavonoids, tannins, coumarines, steroids, and phenols. Conclusion: This plant contains bioactive metabolites and thus can be used as an alternative and complementary medicine in treatment of different ailments. However, further studies on the bioactivity and toxicity of the plant should be done.**

Keywords

GC-MS, ***Steganotaenia araliacea,*** **Essential Oil, Phytochemicals**

*Corresponding author.

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Research Article

Profile of Trace Elements in Selected Medicinal Plants Used for the Treatment of Diabetes in Eritrea

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Academic Editor: Kazuyuki Tobe

This study was designed to investigate the profile of certain trace elements having therapeutic properties related to diabetes mellitus. The investigated plants were *Aloe camperi*, *Meriandra dianthera*, *Lepidium sativum*, *Brassica nigra*, *and Nigella sativa*. These plants are traditionally used in the management of diabetes in Eritrea. The elemental analysis was conducted using inductively coupled plasma optical emission spectrometry (ICP-OES) and flame atomic absorption spectroscopy (FAAS) techniques. The accuracy of the methods was verified using in-house reference materials (CRMs) and no significant differences were observed between the measured and certified values. The analysis displayed variable concentrations of the different trace elements including Zn, Cr, V, Mn, and Se in the plants. Moreover, the levels of major elements, such as Mg, Ca, K, Na, and Ba, and heavy metals, such as Fe, Cu, Ni, Co, As, and Pb, were determined and found to be in the permissible limit defined by WHO. Among the plants, *Meriandra dianthera* showed the highest levels of Mn, Cr, V, and other elements and the values were significantly different ($P < 0.05$).

1. Introduction

Trace elements have been identified for a long time as potential candidates for improving metabolic disorders including diabetes [1]. It is widely believed that some trace elements, such as Zn, Cr, V, Mg, Mn, and Se serve as cofactors of antioxidative enzymes and play an important role in protecting the insulin secreting pancreatic β-cells, which are sensitive to free radical damage [2, 3]. It has also been reported that the imbalance of some essential trace elements might adversely affect pancreatic islet and cause development of diabetes [4] and thus some trace elements have been recommended as dietary supplement to alleviate the impaired insulin metabolism in diabetic patients [5, 6]. Some researchers have also shown that trace elements beneficially affect the complications of diabetes mellitus [7]. Clinical studies suggest that the body's balance of mineral trace elements is disrupted by diabetes and thus diabetic individuals are susceptible to trace element deficiency [8]. Even though trace elements are important for the normal functioning of the body, they can be harmful and toxic at high concentrations [9]. Therefore profiling the levels of these elements is mandatory in monitoring the safety of herbal preparations employed in the management of diabetes and other ailments.

The most widely and commonly used techniques of elemental analysis, providing acceptable levels of precision and accuracy, include flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) [10]. The present work focuses on analysis of the levels of trace elements and their therapeutic role in the management of diabetes from selected Eritrean medicinal plants. The investigated plants were *Aloe camperi*, *Meriandra dianthera*, *Lepidium sativum*, *Brassica nigra*, and *Nigella sativa*. These plants have been traditionally used for the treatment of diabetes and other ailments in Eritrea [11]. The profile of trace and other elements in *Aloe camperi* and