

**ANTIDIABETIC ACTIVITIES OF ETHYL ACETATE AND AQUEOUS
EXTRACTS OF *PAPPEA CAPENSIS*, *SENNA SPECTABILIS*, *MAYTENUS
OBSCURA*, *OCIMUM AMERICANUM* AND *LAUNAEA CORNUTA***

**Karau Geoffrey Muriira (MSc Biochemistry)
Reg No: I84/20608/2010**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the
Degree of Doctor of Philosophy in Medical Biochemistry of Kenyatta University**

April, 2014

DECLARATION

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

Karau, Geoffrey Muriira

Signature: _____ Date: _____
Department of Biochemistry and Biotechnology
Kenyatta University

We confirm that the work reported in this thesis was carried out by the candidate under our supervision

SUPERVISORS

Prof Eliud NM Njagi
Department of Biochemistry and Biotechnology
Kenyatta University

Signature: _____ Date: _____

Dr Alex K Machocho
Department of Chemistry
Kenyatta University

Signature: _____ Date: _____

Dr Laura N Wangai
Department of Biochemistry and Molecular Biology
Jomo Kenyatta University of Agriculture and Technology

Signature: _____ Date: _____

DEDICATION

This thesis is dedicated to my daughters; Lavender Kendi, Lindsay Kawira and Lynnelle Mueni.

ACKNOWLEDGEMENTS

I am grateful to my supervisors Prof. Eliud NM Njagi, Dr. Alex K Machocho and Dr. Laura N Wangai for their tireless effort, immense support, intelligent criticism and guidance in the entire process of designing and implementing the project. I thank Mr. James Adino of the Department of Medical Laboratory Sciences, Kenyatta University for assisting in handling the laboratory mice used in the research.

I wish to acknowledge the outstanding collaboration of Mr. Antony Njuki Maringa, Mbeere, Embu County, Mr. Nicholas Kiburi, and Mr. John Kabiro, from Meru County for helping me with the contacts of the traditional medicine men, whose contributions were invaluable in selecting specific plants used in management of hyperglycemia in their respective communities.

I wish to acknowledge the moral and financial support from my wife Betty Nkatha and our daughters Lavender Kendi, Lindsay Kawira and Lynnelle Mueni. To my parents, Mr. and Mrs. James Karau, thank you for instilling in me the urge to pursue education and supporting me all through. To my brother, Dr. Bundi Paul Karau and his wife Dr. Saumu Mueni, your moral and financial support are highly appreciated and acknowledged.

I wish to thank the National Council of Science and Technology, Kenya for funding the study through grant number NCST/5/003/2nd call PhD/102, 2011. I equally thank my employer, Kenya Bureau of Standards (KEBS) for allowing me to use their laboratory facilities.

Above all I thank God almighty for His continued love and faithfulness in enabling me go through the entire project with confidence, strength and determination.

TABLE OF CONTENT

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENTS	iv
LIST OF FIGURES	viii
LIST OF TABLES	x
ABBREVIATIONS AND ACRONYMS	xi
ABSTRACT	xii
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background information.....	1
1.2 Diabetes Mellitus.....	2
1.3 Statement of the problem	4
1.4 Research questions	5
1.5 Research hypotheses.....	5
1.6 Objectives	6
1.6.1 General objective	6
1.6.2 Specific objectives	6
1.7 Justification	7
CHAPTER TWO	8
LITERATURE REVIEW.....	8
2.1 Status of diabetes mellitus.....	8
2.2 Pathogenesis of diabetes mellitus.....	9
2.3 Classification of diabetes mellitus.....	11
2.4 Major organs affected by diabetes mellitus.....	12
2.5 Contributing factors for diabetes mellitus	15
2.6 Diagnosis of diabetes mellitus.....	16
2.7 Conventional management of diabetes mellitus.....	17
2.8 Herbal management of diabetes mellitus	22
2.9 Essential anti-diabetic mineral elements from herbs.....	23
2.10 Essential vitamins with hypoglycemic activity from herbs.....	27
2.10.1 Retinol.....	27
2.10.2 Alpha-tocopherol	28
2.10.3 Thiamine	29
2.10.4 Nicotinic acid and Nicotinamide	30
2.10.5 Ascorbic acid	31
2.11 Carotenoids from herbs	32
2.12 Safety of herbal medicine.....	34
2.13 Alloxanized mice model for diabetes mellitus	35
2.14 Biochemical markers of medicinal plant extracts toxicity	36
2.14.1 Alkaline phosphatase (ALP).....	37
2.14.2 Aspartate aminotransferase (AST).....	38
2.14.3 Alanine aminotransferase (ALT)	39
2.14.4 Creatinine kinase (CK)	39
2.14.5 Blood urea nitrogen (BUN)	40
2.15 Plants under the study.....	40
2.15.1 <i>Pappea Capensis</i>	40
2.15.2 <i>Maytenus obscura</i>	41
2.15.3 <i>Senna spectabilis</i> L	42
2.15.4 <i>Launaea cornuta</i>	43
CHAPTER THREE.....	47

MATERIALS AND METHODS	47
3.1 Study area	47
3.2 Collection of plant materials	47
3.3 Processing of plant materials	48
3.4 Experimental animals	48
3.4.1 Identification of the optimum alloxan-monohydrate dose to induce diabetes....	49
3.4.2 Induction of hyperglycemia	49
3.4.3 Experimental design	50
3.4.4 Blood sampling and blood glucose determination.....	50
3.5 <i>In vivo</i> single dose toxicity test	50
3.5.1 Determination of body weight	51
3.5.2 Determination of hematological and biochemical parameters	51
3.5.2.1 Determination of the activity of aspartate aminotransferase (AST)	52
3.5.2.2 Determination of the activity of alanine aminotransferase (ALT)	53
3.5.2.3 Determination of the activity of alkaline phosphatase (ALP)	53
3.5.2.4 Determination of the activity of creatine kinase (CK).....	54
3.5.2.5 Determination of the blood levels of urea (BUN)	54
3.5.3 Quality control (QC).....	55
3.6 Determination of phytochemicals	56
3.6.1 Qualitative screening of phytochemicals	56
3.6.2 Quantitative determination of phytochemicals	57
3.7 Determination of phytonutrients	60
3.7.1 Determination of vitamins A and E by HPLC method.....	60
3.7.2 Determination of β -carotene, β -cryptoxanthin and lycopene	63
3.7.3 Determination of ascorbic acid	64
3.7.4 Determination of thiamine and niacin	65
3.8 <i>In vitro</i> antioxidant activities of the plant extracts	66
3.8.1 Free radical scavenging activity by DPPH assay.....	66
3.8.2 Reducing power assay	67
3.8.3 Total antioxidant activity by phosphomolybdate assay	67
3.9 Proximate composition.....	68
3.9.1 Determination of the moisture content	68
3.9.2 Determination of ash content.....	68
3.9.3 Determination of crude protein content	69
3.9.4 Determination of crude fat content	70
3.9.5 Determination of crude fiber content.....	71
3.9.6 Determination of nitrogen free extract.....	72
3.9.7 Determination of the energy content	72
3.10 Chemical compounds from the ethylacetate extracts of the medicinal plants.....	72
3.11 Elemental analyses by energy dispersive X-ray fluorescence spectroscopy.....	73
3.12 Data management and statistical analysis	74
CHAPTER FOUR.....	75
RESULTS	75
4.1 Yields of ethyl acetate and aqueous crude plants extracts	75
4.2 Effects of oral administration of extracts on blood glucose levels.....	76
4.2.1 Optimum dose for induction of hypoglycemia in BALB/c mice.....	76
4.2.2 Effects of oral administration of extracts of <i>P. capensis</i>	77
4.2.3 Effects of oral administration of extracts of <i>S. spectabilis</i>	82
4.2.4 Effects of oral administration of extracts of <i>M. obscura</i>	92
4.2.5 Effects of oral administration of <i>O. americanum</i>	101
4.2.6 Effects of oral administration of <i>L. cornuta</i>	106

4.3 <i>In vivo</i> single dose toxicity of the ethyl acetate and aqueous extracts	111
4.3.1 Effect of oral administration of aqueous plants extracts on body weight and mean weekly body weight change	111
4.3.2 Effects of oral administration of the ethyl acetate and aqueous plants extracts on hematological parameters	114
4.3.3 Effect of oral administration of aqueous plants extracts on some end point biochemical parameters	119
4.3.4 Effects of oral administration of ethyl acetate stem bark and leaves extracts on end point biochemical parameters	121
4.4 Proximate composition of the plants leaves and stem barks powders	123
4.5 Phytochemical present in the plants leaves and stem barks powders.....	126
4.6.1 Free radical scavenging activity by DPPH assay of the leaves and stem bark powders	132
4.6.2 Total antioxidant activity by phosphomolybdate assay of the leaves and stem bark powders	132
4.6.3 Reducing power of the plants powders.....	133
4.6.4 Compounds of ethyl acetate stem barks and leaves of <i>P. capensis</i>	134
4.6.5 Compounds from ethyl acetate stem bark extracts of <i>S. spectabilis</i>	139
4.6.6 Compounds from ethyl acetate stem bark extracts of <i>M. obscura</i>	144
4.6.7 Compounds from ethyl acetate extracts of <i>O. americanum</i>	148
4.6.8 Compounds from <i>L. cornuta</i>	152
4.7 Mineral element composition of the plants powders.....	154
CHAPTER FIVE.....	156
DISCUSSION, CONCLUSION AND RECOMMENDATIONS	156
5.1 Discussion	156
5.2 Conclusion.....	168
5.3 Recommendations	169

LIST OF FIGURES

Figure 2.1: The chemical structure of Vitamin A.....	28
Figure 2.2: The chemical structure of Vitamin E.....	29
Figure 2.3: The chemical structure of Vitamin B1.....	30
Figure 2.4: The chemical structure of Vitamin B3.....	31
Figure 2.5: The chemical structure of Vitamin C.....	32
Figure 2.6: The chemical structure of Lycopene.....	34
Figure 2.7: The chemical structure of β -carotene.....	34
Figure 2.8: Photographs of the medicinal plants used in this study.....	46
Figure 4.1: Percentage change in blood sugar levels after oral administration of ethyl acetate leaf extracts of <i>P. capensis</i> to alloxan-induced male diabetic BALB/c mice.....	81
Figure 4.2: Percentage change in blood sugar levels after oral administration of ethyl acetate stem barks extracts of <i>P. capensis</i> to alloxan-induced male diabetic BALB/c mice	81
Figure 4.3: Percentage change in blood sugar levels after oral administration of ethyl acetate leaf extracts of <i>S. spectabilis</i> to alloxan-induced male diabetic BALB/c mice.....	86
Figure 4.4: Percentage change in blood sugar levels after oral administration of ethyl acetate stem barks extracts of <i>S. spectabilis</i> to alloxan-induced male diabetic BALB/c mice.....	86
Figure 4.5: Percentage change in blood sugar levels after oral administration of aqueous stem barks extracts of <i>S. spectabilis</i> to alloxan-induced male diabetic BALB/c mice.....	91
Figure 4.6: Percentage change in blood sugar levels after oral administration of aqueous leaf extracts of <i>S. spectabilis</i> to alloxan-induced male diabetic BALB/c mice.....	91
Figure 4.7: Percentage change in blood sugar levels after oral administration of ethyl acetate leaf extracts of <i>M. obscura</i> to alloxan-induced male diabetic BALB/c mice.....	96
Figure 4.8: Percentage change in blood sugar levels after oral administration of ethyl acetate stem barks extracts of <i>M. obscura</i> to alloxan-induced male diabetic BALB/c mice.....	96
Figure 4.9: Percentage change in blood sugar levels after oral administration of aqueous stem barks extracts of <i>M. obscura</i> to alloxan-induced male diabetic BALB/c mice.....	100
Figure 4.10: Percentage change in blood sugar levels after oral administration of aqueous leaf extracts of <i>M. obscura</i> to alloxan-induced male diabetic BALB/c mice.....	100
Figure 4.11: Percentage change in blood sugar levels after oral administration of ethyl acetate extracts of <i>O. americanum</i> to alloxan-induced male diabetic BALB/c mice.....	105
Figure 4.12: Percentage change in blood sugar levels after oral administration of aqueous extracts of <i>O. americanum</i> to alloxan-induced male diabetic BALB/c mice.....	105
Figure 4.13: Percentage change in blood sugar levels after oral administration of ethyl acetate extracts of <i>L. cornuta</i> to alloxan-induced male diabetic BALB/c mice.....	110
Figure 4.14: Percentage change in blood sugar levels after oral administration of	

aqueous extracts of <i>L. cornuta</i> to male diabetic BALB/c mice.....	110
Figure 4.15: Plots showing reducing power varying with concentration.....	134
Figure 4.16: GC-MS chromatograms of extracts of <i>P. capensis</i> stem bark.....	137
Figure 4.17: GC-MS chromatograms of extracts of <i>P. capensis</i> leaves.....	138
Figure 4.18: GC-MS chromatograms of extracts of <i>S. spectabilis</i> stem bark.....	140
Figure 4.19: GC-MS chromatograms of extracts of <i>S. spectabilis</i> leaves.....	143
Figure 4.20: GC-MS chromatograms of extracts of <i>M. obscura</i> stem bark.....	145
Figure 4.21: GC-MS chromatograms of extracts of <i>M. obscura</i> leaves.....	147
Figure 4.22: GC-MS chromatograms of ethyl acetate extracts of <i>O. americanum</i>	151
Figure 4.23: GC-MS chromatograms of ethyl acetate extracts of <i>L. cornuta</i>	153

LIST OF TABLES

Table 2.1: The medicinal plants used in the study.....	45
Table 4.1: The yields of plants extracts in mg/g of dry material.....	76
Table 4.2: Optimal ip dose of alloxan-monohydrate for induction of hypoglycemia.....	77
Table 4.3: Hypoglycemic effects of ethyl acetate leaf extracts of <i>P. capensis</i>	79
Table 4.4: Hypoglycemic effects of ethyl acetate stem barks extracts of <i>P. capensis</i>	80
Table 4.5: Hypoglycemic effects of ethyl acetate leaf extracts of <i>S. spectabilis</i>	84
Table 4.6: Hypoglycemic effects of ethyl acetate stem barks extracts of <i>S. spectabilis</i>	85
Table 4.7: Hypoglycemic effects of aqueous stem barks extracts of <i>S. spectabilis</i>	89
Table 4.8: Hypoglycemic effects of aqueous leaf extracts of <i>S. spectabilis</i>	90
Table 4.9: Hypoglycemic effects of ethyl acetate leaf extracts of <i>M. obscura</i>	94
Table 4.10: Hypoglycemic effects of ethyl acetate stem barks extracts of <i>M. obscura</i>	95
Table 4.11: Hypoglycemic effects of aqueous stem barks extracts of <i>M. obscura</i>	98
Table 4.12: Hypoglycemic effects of aqueous leaf extracts of <i>M. obscura</i>	99
Table 4.13: Hypoglycemic effects of ethyl acetate extracts of <i>O. americanum</i>	103
Table 4.14: Hypoglycemic effects of aqueous extracts of <i>O. americanum</i>	104
Table 4.15: Hypoglycemic effects of ethyl acetate extracts of <i>L. cornuta</i>	108
Table 4.16: Hypoglycemic effects of aqueous extracts of <i>L. cornuta</i>	109
Table 4.17: Effects of ethyl acetate extracts at 1 g/kg body weight daily in mice for 28 days on body weight and mean weekly body weight change.....	112
Table 4.18: Effects of aqueous extracts at 1 g/kg body weight daily in mice for 28 days on body weight and mean weekly body weight change.....	113
Table 4.19: Effects of aqueous medicinal plants extracts at 1g/kg body weight daily in mice for 28 days on hematological parameters	116
Table 4.20: Effects of aqueous medicinal plants extracts at 1 g/kg body weight daily in mice for 28 days on white blood cells count.....	117
Table 4.21: Effects of ethyl acetate medicinal plants extracts at 1 g/kg body weight daily in mice for 28 days on hematological parameters in mice.....	118
Table 4.22: Effects of ethyl acetate extracts at 1g/kg body weight daily in mice for 28 days on white blood cells count.....	119
Table 4.23: Effects of aqueous extracts at 1 g/kg body weight daily in mice for 28 days on some end-point biochemical parameters.....	120
Table 4.24: Effects of ethyl acetate extracts at 1 g/kg body weight daily in mice for 28 days on some end-point biochemical parameters.....	122
Table 4.25: Proximate compositions of the dry leaves and stem barks powders.....	125
Table 4.26: Qualitative screening of the phytochemicals in the dry powders.....	128
Table 4.27: Quantitative analyses of phytochemicals in the plants powders.....	129
Table 4.28: Phytonutrients composition of the plant powders.....	131
Table 4.29: Percentage free radical scavenging activity by DPPH assay.....	132
Table 4.30: Total antioxidant activity by phosphomolybdate assay.....	133
Table 4.31: Chemical compounds of the stem barks of <i>P. capensis</i>	135
Table 4.32: Chemical compounds of the leaves of <i>P. capensis</i>	136
Table 4.33: Chemical compounds of the stem barks extracts of <i>S. spectabilis</i>	139
Table 4.34: Chemical compounds of the leaves extracts of <i>S. spectabilis</i>	141
Table 4.35: Chemical compounds of the stem barks extracts of <i>M. obscura</i>	144
Table 4.36: Chemical compounds of the leaves extracts of <i>M. obscura</i>	146
Table 4.37: Chemical compounds of the extracts of <i>O. americanum</i>	148
Table 4.38: Chemical compounds of the extracts of <i>L. cornuta</i>	152
Table 4.39: Mineral elements composition in mg/kg dry matter.....	155

ABBREVIATIONS AND ACRONYMS

AAE:	Ascorbic acid equivalent
AAS:	Atomic absorption spectrophotometry
AI:	Adequate intake
ALP:	Alkaline phosphatases
ALT:	Alanine aminotransferases
AMP-K:	Adenine monophosphate activated protein kinase
AOAC:	Association of official analytical chemists
AST:	Aspartate amino transferases
BHT:	Butylated hydroxylated toluene
BUN:	Blood urea nitrogen
CK:	Creatinine kinases
DM:	Diabetes mellitus
DPPH:	1 1-diphenyl-2-picrylhydrazyl
EDXRF:	Energy dispersive X-ray fluorescence spectroscopy
G-6-Kinase:	Glucose 6- kinase
GC-MS:	Gas Chromatography coupled to Mass Spectrometer
GLUT:	Glucose transporter
HPLC:	High Pressure Liquid Chromatography
IDDM:	Insulin dependent diabetes mellitus
MODY:	Maturity-onset diabetes of the young
NAD:	Nicotinamide adenine dinucleotide
NCEs:	New chemical entities
NFE:	Nitrogen free extracts
NIDDM:	Non-insulin dependent diabetes mellitus
OGTT:	Oral glucose tolerance test
PPAR- γ :	Gamma peroxisome proliferator-activated receptor
PPG:	Post prandial glucose
RDA:	Recommended dietary allowance
ROS:	Reactive oxygen species
TNF- α :	Alpha tumour necrosis factor
VLDL:	Very low density lipoproteins
WHO:	World Health Organization

ABSTRACT

Pappea capensis, *Senna spectabilis*, *Maytenus obscura*, *Launaea cornuta* and *Ocimum americanum* have been used traditionally in the management of several diseases including diabetes mellitus but their efficacy and safety after long term use is not scientifically evaluated. The aim of this study was to determine *in vivo* hypoglycemic activity and safety of aqueous and ethyl acetate extracts of leaves and stem barks from plants in male BALB/c mice. The extracts were orally screened for their hypoglycemic activity in alloxan induced diabetic mice using a glucometer. The safety of these extracts was studied in mice orally administered with 1g/kg body weight daily for 28 days by monitoring the changes in body weight, hematological and biochemical parameters. Mineral composition of the plant powders were estimated using energy dispersive X-ray fluorescence system (EDXRF); vitamins were estimated using HPLC system; while the types and quantities of phytochemicals present were assessed using standard procedures. Individual volatile phytochemicals in the extracts were determined using GC-MS system. Proximate composition of the dry plants parts powers was estimated using standard procedures while the energy content was determined using the Atwater factor. Oral administration: of both the leaf and stem bark ethyl acetate and aqueous extracts of *P. capensis*, *S. spectabilis*, *M. obscura*, *L. cornuta* and *O. americanum* in doses of 50, 100 and 200 mg/kg body weight lowered blood glucose; of the three tested doses of aqueous leaf and stem bark extracts of *P. capensis* and aqueous leaves extracts of *M. obscura* could not significantly alter the blood glucose levels in alloxan induced diabetic male BALB/c mice; of the aqueous extracts of *L. cornuta* at 50 and 200 mg/kg body weight dose raised the glucose levels beyond that of the diabetic control mice. Oral administration of aqueous leaves extracts of *P. capensis*, and *O. americanum* and ethyl acetate leaves extracts of *S. spectabilis* at 1 g/kg body weight daily in mice for 28 days significantly reduced the weekly body weight gain. The same oral dose of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* altered the red blood cell count, hemoglobin levels, packed cell volume, mean cell hemoglobin concentration, mean cell volume, platelets; white blood cell count and their differential counts; altered the levels of blood urea nitrogen and the activities of alanine and aspartate aminotransferases, alkaline phosphatase and creatine kinase. The plants powders contained phenols, tannins, saponins, flavonoids, alkaloids, terpenoids, phylobatannins, cardiac glycosides, sterols, fatty acids and reducing sugars; vitamins C, E, B1, B3, retinol, β -carotene, lycopene and cryptoxanthin; minerals selenium, iron, zinc, copper, chromium, manganese, vanadium, molybdenum, cobalt and heavy metals nickel, aluminium, lead and mercury. The observed hypoglycemic activity and/or slight toxicity in the bioactive plants extracts could be associated with the phytonutrients and minerals present in these plants. This study recommends use of safe plants with antidiabetic activity as herbal remedies.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Traditional medicine, existed from ancient times, and is estimated by World Health Organization to be in use by up to 80% of the population in most of the developing countries mostly based on plants and plant materials (WHO, 2002). It is currently estimated that out of a total of an approximately 420,000 plant species that exist in nature, over 248,000 species of higher plants have been identified, and of these 12,000 plants are known to have medicinal properties. However, less than 10% of all the plants have been investigated from a phytochemical or pharmacological point of view (Ali *et al.*, 2011).

Bioprospecting refers to discovery of new or known chemical compounds from biological resources and it often draws from indigenous knowledge on the uses and characteristics of these resources. It is an effective and economical strategy for drug discovery. In spite of past success in New Chemical Entities (NCEs) generation from natural products for drug discovery and development, there has been a recession in the generation of NCEs by the pharmaceutical companies. The recession could be reversed if the discoverers embrace traditional medicines with an open mind. At the United States of America department of natural products, the mélange of NCE discovery and traditional medicines for the drug development is being pursued by adopting models for proper standardization of traditional medicines and assessment of biodiversity (Newman *et al.*, 2003). The journey to drug discovery and development starts from ethnomedicine evaluation and cultivation of important crops for investigating natural product chemistry, profiling of materials and constituents, preclinical evaluations and scale ups using both isolation and synthetic strategies and innovation products.

The pharmaceutical companies base their discovery of new drugs on New Chemical Entities (NCEs). Expectation of low toxicity for humans from plants used traditionally would be no hyperbole, as these plants have been thoroughly tested owing to their use since ages. Medicinal plants have an advantage over conventional drugs because they have been tested for their long term use by humans in various folkloric usages and are embedded in rich community fabric which accords them wide acceptance. Hence a low human toxicity of any bioactive compounds based on ancient medicinal systems can be expected. Also physiologically, natural products with higher molecular weights and rotatable bonds with stereogenic centers are more readily absorbed compared to synthetic drugs (Newman *et al.*, 2003). Thus, bioactive compounds isolated from the active extracts of the plants used in traditional medicines can be used to develop novel drugs by way of semi-synthesizing analogs of lead compounds for higher activity and/or lower toxicity.

Human body is a complex network and the pathogenesis of any disease including diabetes mellitus involves multiple pathways. Modern medicines attempt to use a single compound to hit single target of a particular pathway for combating the related disease. This approach may be effective for an instantaneous relief but it may not prove to be a complete solution (Newman *et al.*, 2013). Further, a risk of unwanted side effects almost always is a part and parcel of modern medication. On the contrary, traditional medicines exert synergistic effects due to multi-constituents and multi-targets. Thus the traditional formulations can be used as readily available way for investigative new drugs (IND) for the development of potent efficacious medicaments.

1.2 Diabetes mellitus

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting

from defects in insulin secretion, insulin action, or both (WHO, 1999). The level of hyperglycaemia associated diabetes increases the risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to the related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. In 2000, an estimated 171 million people in the world had diabetes and this is projected to increase to 366 million by 2030 (WHO, 2006).

Diabetes mellitus is classified into insulin dependent diabetes mellitus (IDDM) or Type 1, and non-insulin dependent diabetes mellitus (NIDDM) or Type 2. Type 1 diabetes encompasses cases due to pancreatic islet beta-cell destruction and are prone to ketoacidosis. Type 1 includes those cases attributable to an autoimmune process, as well as those with beta-cell destruction and who are prone to ketoacidosis for which neither an aetiology nor a pathogenesis is known (idiopathic). Type 2 diabetes includes the common major form of diabetes which results from defect(s) in insulin secretion with a major contribution from insulin resistance (WHO, 1999).

Diagnosis of diabetes mellitus is based on measurement of glycated hemoglobin (HbA1C) level (6.1-7.0), fasting (7 mM or greater on two separate occasions) or random blood glucose level (11 mM or greater) if classic symptoms of diabetes such as polyuria, polydipsia, weight loss, blurred vision, fatigue are present, or two hour oral glucose tolerance testing (7.8-10.0mM) (Patel and Macerollo, 2010).

In conventional medical practice, the present therapies of diabetes mellitus are reported to have side effects. The glucose-lowering drugs include insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, metformin, thiazolidine-diones), α -

glucosidase inhibitors (miglitol, acarbose). The peptide analogs, such as exenatide, liraglutide and DPP-4 inhibitors, increase GLP-1 serum concentration and slow down the gastric emptying. Management of diabetes mellitus with insulin is associated with drawbacks such as insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment. In addition, insulin dependent diabetes mellitus is managed using drugs that control hyperglycemia such as amylin analogues. Sulphonylureas, an oral antidiabetic drug, act by closure of ATP dependent channel. Metformin, a biguanide oral antidiabetic acts by limiting intestinal glucose absorption. Besides the side effects associated with the use of insulin, the side effects of most oral glucose-lowering drugs may include severe hypoglycemia at high doses, lactic acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness and even death. Therefore, because of the side effects associated with the present antidiabetic drugs, there is need to develop effective, safe and cheap drugs for diabetes management. Such effective, safe and cheap drugs could be obtained by using medicinal plants which have been used by humans to prevent or cure diseases including diabetes since the dawn of civilization (Surendran *et al.*, 2011). These plant based herbal medicines are thought to be effective, safe and affordable to the common population in the underdeveloped and developing countries of the world.

1.3 Statement of the problem

The Mbeere community of Embu County and the Tigania community of Meru County, Kenya a developing country have used *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* to manage various diseases including diabetes mellitus since ancient times without any scientific evidence for efficacy, chemical composition and safety. The conventional injectable and oral blood glucose lowering drugs which are used throughout one's life are expensive, and have several side effects.

Therefore, because of the cost and side effects associated with the present antidiabetic drugs, there is need to develop effective, safe and cheap drugs for diabetes management for the resource poor communities of the developing countries including the Mbeere and Tigania communities of Kenya. Such effective, safe and cheap drugs could be obtained from medicinal plants.

1.4 Research questions

The research questions that guided this study were;

- i. Are the medicinal plants *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Lunea cornuta* antidiabetic?
- ii. Are the medicinal plants *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used in the management of diabetes safe?
- iii. What is the chemical composition of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta*?
- iv. What is the nutrient composition of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta*?

1.5 Research hypotheses

Four hypotheses concerning the bioprospecting of anti-diabetic compounds from selected medicinal plants used among Mbeere and Meru communities of Kenya were tested;

- i. *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used traditionally in management of diabetes mellitus have *in-vivo* hypoglycemic activity.

- ii. *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used traditionally in the management of diabetes mellitus are safe.
- iii. *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used traditionally in the management of diabetes mellitus have anti-diabetic compounds.
- iv. *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used traditionally in the management of diabetes mellitus in the Mbeere and Meru communities of Kenya are nutritive.

1.6 Objectives

1.6.1 General objective

The general objective of this study was to determine *in vivo* hypoglycemic activity and safety, chemical composition and the nutritive value of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used traditionally in the management of diabetes mellitus in Mbeere and Meru communities of Kenya.

1.6.2 Specific objectives

- i. To determine the *in-vivo* hypoglycemic activity of the ethylacetate and aqueous extracts of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta*.
- ii. To evaluate the safety of a high dose of the ethylacetate and aqueous extracts of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* using changes in body weight, hematological and biochemical parameters.

- iii. To determine the chemical composition of the ethylacetate extracts of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta*.
- iv. To determine the nutritive value of medicinal plants *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta*.

1.7 Justification

Diabetes mellitus has attained epidemic proportions worldwide with conventional drugs either being unaffordable, unavailable or having undesirable side effects. Herbal medicines are becoming an affordable, easily accessible by over 80% of people in developing nations, and are firmly embedded within wider belief systems of many people especially in developing nations, thus according them greater acceptance. This study will come up with invaluable information about *in vivo* hypoglycemic activity, safety, chemical and nutrient composition of *Pappea capensis*, *Senna spectabilis*, *Maytenus obscura*, *Ocimum americanum* and *Launaea cornuta* used among Mbeere and Meru communities in management of diabetes mellitus among others and help to validate their use. Based on the study findings, the plants can be used in development of various herbal products capable of managing diabetes mellitus effectively and safely.

CHAPTER TWO

LITERATURE REVIEW

2.1 Status of diabetes mellitus

In 2007, the global diabetes mellitus burden estimate was 246 million, and the International Diabetes Federation (IDF) estimates that this figure is likely to rise to 380 million by the year 2025 (WHO, 2009). The World Health Organization estimates that close to 347 million people are diabetic and this number is likely to rise to 438 million by the year 2030 (WHO, 2013), if proper control and management strategies are not put in place. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000, and is expected to rise to 4.4% in 2030 (WHO, 2002; Wild *et al.*, 2004). WHO (2002) estimated that globally 7.1 million deaths could be attributed to high blood pressure, 4.4 million to high cholesterol, and 2.6 million to excessive body weight.

In developing countries, those most frequently affected by type II diabetes are in the middle, productive years of their lives, aged between 35 and 64 years (WHO, 2002), and 55% deaths occur in women (WHO, 2009). The major factors identified for developing diabetes are inheritance (genetic predisposition) and environmental factors such as nutrition and chemical toxins (WHO, 1985).

Africa and Asia are identified as regions with greatest potential where diabetics could raise two to three folds above the present level (WHO, 2009). In sub-Saharan Africa, diabetes mellitus prevalence was estimated to be 0.01% in 2000 and was projected to rise to 0.12% by the year 2025. In Kenya, the prevalence of diabetes mellitus in 2010 was estimated to be 3.3% based on regional population, and is likely to be an underestimation because over 60% of the people diagnosed with diabetes mellitus usually present to the health care facility with seemingly unrelated complaints. Similarly, two thirds of Kenyans with

diabetes mellitus do not know they have the disease (Ministry of Public Health and Sanitation, 2010).

2.2 Pathogenesis of diabetes mellitus

The chronic hyperglycemic nature of diabetes is associated with prolonged damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (The Expert Committee on the Diagnosis and classification of Diabetes, 1997). Several pathophysiological processes are involved in the development of diabetes mellitus. These range from autoimmune destruction of the pancreatic β -cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficiency and insufficient action of insulin on target tissues leads to carbohydrates, fats and proteins metabolism abnormalities (The Expert Committee on the Diagnosis and classification of Diabetes, 1997).

The presenting symptoms of hyperglycemia include; polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision (Davis, 1996). Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia (The Expert Committee on the Diagnosis and classification of Diabetes, 1997). Acute life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome (Davis, 1996). Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, charcot joints and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction (The Expert Committee on the Diagnosis and classification of Diabetes, 1997).

Oxidative stress has been suggested as a contributory factor in the pathogenesis of diabetes mellitus (Riturparna and Neeraj, 2007). Diabetes increases the production of tissue damaging reactive oxygen species (ROS) by glucose autoxidation and / or non-enzymatic protein glycosylation (Brownlee, 1995). One of the major sites at which oxidative complications to diabetes take place is the vascular endothelium. Hyperglycemia has been found to increase production of ROS such as superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2) which reduce nitrogen oxide (NO) bioavailability in cultured endothelial cells (Riturparna and Neeraj, 2007), and in vascular tissue (Wild *et al.*, 2004). Endothelial dysfunction is a well-documented characteristic phenomenon in diabetes mellitus (Wild *et al.*, 2004), and is attributed to decreased vasorelaxant, and increased contractile responses to physiological, and pharmacological stimuli (Riturparna and Neeraj, 2007).

The number of people with diabetes mellitus is on constant rise globally due to: population growth, low birth weight, aging, urbanization, sedentary lifestyles with physical inactivity, over-processed diets, smoking, psychological stress and increase in prevalence of obesity (Wild *et al.*, 2004; Riturparna and Neeraj, 2007).

Diabetes mellitus (DM) is the most severe and challenging metabolic pandemic of the 21st century. This is because it affects essential biochemical activities in almost every cell in the body and increases the risk of cardiac and renal disorders. The worldwide survey reported that DM is affecting nearly 10% of the population (Siddharth, 2001). This pandemic is characterized by excessive sugar in the blood due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced to control blood glucose. This disorder affects carbohydrate, protein and fat metabolism (Davis and Granner, 1996), and chronic hyperglycemia causes glycation of body proteins that in turn leads to secondary complications that affects eyes, kidneys and nerves (Sharma, 1993).

2.3 Classification of diabetes mellitus

World Health Organization (WHO) classifies diabetes mellitus into insulin-dependent diabetes mellitus (IDDM) or type I, and non-insulin-dependent diabetes mellitus (NIDDM) or type II. Insulin-dependent diabetes mellitus, also referred to as juvenile onset diabetes, is usually first diagnosed in children, teenagers and young adults. In these patients the β -cells of the pancreas no longer make insulin because the body's immune system has destroyed them. Treatment for IDDM usually involves taking insulin shots or use of an insulin pump, wise food choices, regular exercise, controlling blood pressure and cholesterol (Greenspan and Gardner, 2001).

Type I diabetes mellitus, has multiple genetic predispositions and is related to environmental factors that are still poorly defined. It accounts for 5-10% globally of individuals with diabetes (The Expert Committee on the Diagnosis and classification of Diabetes, 1997). The rate of β -cell destruction is quite variable, being rapid mainly in infants and children and slow in adults (The Expert Committee on the Diagnosis and classification of Diabetes, 1997). Children and adolescents may present with keto-acidosis as the first manifestation of the disease, while others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or keto-acidosis in the presence of infection or other stress (Davis, 1996).

The other category is type II diabetes whose onset is usually after 40 years of age (Kaplan, 1989), and accounts for approximately 90-95% of the diabetes mellitus cases world-wide. It usually develops later in life though there is an increase in numbers of young patients. It is also called adults onset diabetes which affects individuals who have insulin resistance, and usually have relative insulin deficiency (Abel *et al.*, 2001). Insulin resistance is defined as an inadequate response to circulating insulin by insulin target tissues like

adipose, skeletal muscle and liver; and this usually precede the characteristic hyperglycemia in type II diabetes (Schenk *et al.*, 2008).

Most patients with type II diabetes are obese which causes some degree of insulin resistance (Abel *et al.*, 2001). This form of diabetes frequently goes undiagnosed for many years because hyperglycemia develops gradually and earlier stages are often not severe enough for the patients to notice any of the classic symptoms of diabetes (The Expert Committee on the Diagnosis and classification of Diabetes, 1997). Although patients with this form of diabetes may have insulin levels that appear normal or elevated, higher blood glucose levels would be expected to result in even higher insulin values had their β -cells function been normal (Abel *et al.*, 2001). Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. The risk of developing this form of diabetes increases with age, obesity and lack of physical activity.

Other forms of diabetes mellitus include; gestational diabetes and brittle diabetes (WHO, 1985). These forms are associated with monogenic defects in β -cell function inherited in an autosomal dominant pattern, and are frequently characterized by onset of hyperglycemia at an early age, generally before age of 22 years. They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action (WHO, 1985).

2.4 Major organs affected by diabetes mellitus

The physiologic effects of insulin in the body are far reaching. These also directly correlate to the effects seen in the body of either too much or too little insulin in circulation. The net effect of the hormone involves the storage, and utilization of carbohydrates, proteins and fats.

Most of the effects of insulin are seen in the adipose tissue, skeletal, cardiac and smooth muscle, liver and the pancreas. Adipose tissue is involved in the maintenance of normal glucose levels in the body. Its primary role is the storage of energy in form of triglycerides with glucose disposal being the primary role for the skeletal muscle (Huang and Czech, 2007). Adipose tissue has a number of glucose transporters such as GLUT 4, GLUT 8 and GLUT 12 that are responsible for shuttling glucose into the cells. GLUT 4 is the main hexose transporter and is highly expressed in the adipose tissue (Huang and Czech, 2007).

Visceral fat depots found in adipose tissue have very high lipolytic rates resulting in the release of large amounts of fatty acids into the system. Insulin normally suppresses the lipase and adipocytes triglyceride lipase enzyme that hydrolyses intracellular triglyceride but in the insulin resistant state, the activity of this enzyme is enhanced resulting in a free fatty acid flux (Duncan *et al.*, 2007). Adipose tissue releases large amounts of a protein known as tumour necrosis factor (TNF- α) that plays a major role in the repression of many genes in the body which are responsible for the uptake and storage of glucose as well as fatty acids. TNF- α also mediates the inflammatory process which is associated with obesity and type II diabetes (Ruan and Lodish, 2002).

Skeletal muscle stores glucose as glycogen which it oxidizes when needed to produce energy (Huang and Czech, 2007). It accounts for about 75% of the whole body insulin stimulated glucose uptake (Perriot *et al.*, 2001). About 500-600 g of glycogen is stored in the muscle tissue of a 70 kg man, but because of the lack of glucose-6-phosphatase in this tissue, it cannot be used as a source of blood glucose except by indirectly supplying the liver with lactate for conversion to glucose (Greenspan and Gardner, 2001).

Glucose is transported into the cells through a specialized transmembrane sugar transporter known as GLUT 4, which catalyses transport of glucose through the plasma membrane. This transporter works in tandem with others like GLUT 1, 5 and 12 to enhance glucose transport via facilitative diffusion. Insulin has many effects on the muscle, with the most important ones being the increased entry of glucose. When insulin binds to its receptors, tyrosine phosphorylation of protein substrates occurs and this activates the P13 kinase pathway.

Subsequent signaling pathways are activated with GLUT 4 eventually moving from its intracellular stores to the plasma membrane (Perriot *et al.*, 2001). This transporter has a unique characteristic such that its N and COOH terminals direct both endocytic and exocytic processes (Huang and Czech, 2007). Abel *et al.* (2001) demonstrated that sensitivity to insulin was markedly higher as was the response of uptake of glucose in transgenic mice which had higher expression levels of GLUT 4. In type II diabetic patients there is as much as a 90% reduction in levels of GLUT 4 that are responsive to insulin. Translocation of transporters and subsequent signaling pathways are interfered with. This is one way that results in the characteristic insulin resistant (IR) state found in type II diabetics. There is also an increased GLUT 4 expression in muscle in response to exercise (Huang and Czech, 2007).

The liver is the first major organ reached by insulin via the bloodstream. Insulin exerts effects on the liver by either promoting anabolism or inhibiting catabolism. The liver helps the body to maintain normal blood glucose concentrations in fasting and postprandial states. When insulin levels are low, then glycogenolysis and increased hepatic glucose production results (Lewis *et al.*, 2002).

The liver has a maximum storage capacity of 100-110 g of glycogen or approximately 440 kilo calories of energy. Insulin promotes glycogen synthesis and storage as well as inhibits breakdown of glycogen into glucose. These effects are mediated by changes in the activity of enzymes in the glycogen synthesis pathway. Insulin inhibits the expression of key gluconeogenic enzymes such as G-6-phosphatase leading to elevated levels of glucose production in the liver (Luca and Olefsky, 2007). Insulin increases both protein and triglyceride synthesis and very low density lipoproteins (VLDL) formation by the liver (Greenspan and Gardner, 2001). Individuals with type II diabetes have a higher incidence of liver function transferases (LFTs) abnormalities than individuals without diabetes. The most common abnormality is elevated alanine aminotransferase (ALT). Antidiabetic agents have generally been shown to decrease ALT levels as tighter blood glucose levels are achieved (Harris, 2005).

The human pancreas is made up of two types of tissues, namely exocrine and endocrine. The exocrine tissue (acini) secretes digestive enzymes that help to breakdown proteins, carbohydrates, fats and acids in the duodenum while the endocrine pancreas (islets of langerhans) has a hormonal function. It produces insulin, somatostatin, gastrin and glucagon. These hormones have important roles to play in maintaining glucose and salt homeostasis in the body (Harris, 2005).

2.5 Contributing factors for diabetes mellitus

The continued increase in prevalence of diabetes in the developing nations can be largely attributed to urbanization, westernization and economic development. The major contributing risk factors related to these are population ageing, obesity, sedentary lifestyles, over-processed diets, smoking, psychological stress and low birth weight (Riturparna and Neeraj, 2007). In obesity associated type II diabetes mellitus, there is an

increased accumulation of visceral fat which contains pro-inflammatory molecules such as α -tumour necrosis factor (TNF- α), which is involved in the regulation of insulin sensitivity in the body (Qatanani and Lazar, 2008). Other molecules such as adiponectin whose levels are low in obesity improves insulin sensitivity, reduce glucose output and fatty acid oxidation in the liver (Qatanani and Lazar, 2008).

Poor dietary choice is a major contributing factor to obesity and associated disorders like type II diabetes mellitus. Epidemiological evidence has demonstrated that saturated fatty acid intake is associated with increased risk of insulin resistance, diabetes and impaired glucose tolerance (Lichtenstein and Schwab, 2000). The inclusion of foods rich in *trans*-fatty acids and high ratios of saturated to unsaturated fats results in weight gain and predisposition to diabetes. Foods such as red meats, refined grains, sweets and high fat dairy products have been linked to risks of type II diabetes. In contrast, weight loss is characterized by reduction in fat cell mass especially visceral fat which contain inflammatory markers associated with insulin resistance and decreased insulin sensitivity. Reduced visceral fat due to weight loss is accompanied by decreased adipose TNF- α release resulting to improved insulin sensitivity (Mlinar *et al.*, 2006).

2.6 Diagnosis of diabetes mellitus

The accepted WHO criteria for diagnosis of diabetes mellitus are based on a venous plasma glucose concentration of > 11.1 mM, 2-hour after a 75 g glucose tolerance test (WHO, 2002). The criteria for abnormal glucose tolerance in pregnancy are those of Carpenter and Coustan (1982). Recommendations from the American Diabetes Association's Fourth International Conference on Gestational Diabetes Mellitus held in March, 1997 support the use of Carpenter and Coustan diagnostic criteria as well as the alternative use of a diagnostic 75-g, 2-hour oral glucose tolerance test (OGTT) (EC, 1997).

The new diagnostic criteria for diabetes mellitus have been greatly simplified. The OGTT which was previously recommended in 1979 by the National Diabetes Data Group (National Diabetes Data Group, 1979), has been replaced with the recommendation that diagnosis be based on two fasting plasma glucose levels of 2500 mg/L (13.8 mM) or higher (WHO, 2002; WHO, 2009).

Measurement of the fasting plasma glucose level is the preferred diagnostic test, but any combination of two abnormal test results can be used. Fasting plasma glucose is considered as the primary diagnostic test because it predicts adverse outcomes like retinopathy (WHO, 1985; Gutteridge, 1999).

2.7 Conventional management of diabetes mellitus

The mainstay of non-pharmacological treatment of diabetes is diet and physical activity (WHO, 2002). Other methods of treatment include; acupuncture, hydrotherapy, mineral supplementation and conventional drugs which include; exogenous insulin, oral hypoglycemic agents and transplantation (WHO, 2002).

Oral glucose lowering drugs belong to five classes of oral agents approved for the management of diabetes mellitus. Oral therapy is indicated in any patient in whom diet and exercise fail to achieve acceptable glycemic control. Although initial response may be good, oral hypoglycemic drugs may lose their effectiveness in a significant percentage of patients. The drug category includes; sulfonylurea, biguanide, α -glucosidase inhibitor, thiazolidinedione and meglitinide. These drugs have various side effects. For instance; sulfonylurea causes weight gain due to hyperinsulinemia, biguanide cause body weakness, fatigue, lactic acidosis and alpha glucosidase inhibitor may cause diarrhea while thiazolidinediones may increase LDL-cholesterol level (Pandey *et al.*, 2011). Insulin is

commonly included in an oral agent when glucose control is sub-optimal at maximal dose of oral medication. Weight gain and hypoglycemia are common side effects of insulin. Vigorous insulin treatment may also carry an increase in atherogenesis (Ribes *et al.*, 1986).

Oral glucose-lowering agent sulfonylurea, tolbutamide and glyburide act by enhancing insulin secretion from the pancreatic β cells (Kelly, 1995). These act on liver cells stimulating breakdown of glucose in glycolytic pathway and inhibiting glucose generation. Sulphonylureas acts by inhibiting K_{ATP} channels in plasma membranes of pancreatic β cells. The inhibition works to stimulate the secretion of insulin which is similar to that produced by glucose in the body but is of a distinct mechanism (Foye *et al.*, 1995). They may be used as first-line drugs in a case where oral hypoglycemic medication is required particularly in patients who cannot tolerate metformin. Newer drugs in this category such as glipizide and glimipramide appear to afford similar efficacy than older drugs such as gliclazide (Foye *et al.*, 1995).

All sulphonylureas drugs have a sulphonic acid-urea nucleus, and different chemical moieties are added at various positions on the nucleus to make different drugs. The action of the resultant drugs may have the desired effect, however, the potency and efficacy may differ significantly (Malender, 2004).

Sulphonylureas drugs are typically not indicated for type 1 diabetic patients since they require the functioning of the β cells to produce the desired effect on blood glucose. They have been found to be most effective in non-obese patients with mild maturity onset diabetes and whose high glucose levels have not responded appropriately to diet alterations (Greenspan and Gardner, 2001).

Biguanides such as metformin act by increasing glucose transport across cell membrane of the skeletal muscle. They act in the presence of endogenous insulin, and are effective only where there are residual functioning pancreatic islet cells (Zhou *et al.*, 2001). Metformin is widely used in treatment of patients with insulin resistance because it can be used safely as an adjunct to diet therapy in obese patients to control their high glucose levels especially those who are not responsive to other therapies. The exact mode of action of metformin is disputable. Zhou *et al.* (2001) indicated that it activates adenosine monophosphate protein kinase (AMPK) in liver cells leading to increased fatty acid oxidation and glucose uptake by cells. An overall reduction in lipogenesis and hepatic glucose production is normally observed. Metformin has antioxidant properties which are useful in its use in treatment of diabetes and cardiovascular disease. It has been demonstrated to inhibit xanthine oxidase and phosphodiesterase, advanced glycation end product formation and decreased production of tumour necrosis factor (Rahimi *et al.*, 2005).

The main problem with metformin is the risk of lactic acidosis which is particularly common in patients with renal insufficiency, cardiovascular disease, peripheral vascular disease, liver disease, pulmonary disease and in individuals over 65 years (Greenspan and Gardner, 2001). Weakness, fatigue, shortness of breath, nausea, dizziness and kidney toxicity are the side effects (Greenspan and Gardner, 2001).

Thiazolidinediones are known to act by increasing the sensitivity of peripheral tissues to insulin by affecting the expression of specific genes. They achieve this by binding and activating γ peroxisome proliferator-activated receptor (PPAR- γ), a nuclear receptor (Qatanani and Lazar, 2007). Some of the effects of this gene expression include the increase in the expression of the glucose transporters, decreased hepatic glucose output as

well as the increased differentiation of pre-adipocytes into adipocytes (Greenspan and Gardner, 2001). The high affinity of this drug to PPAR- γ is important in the management of insulin resistance since large adipocytes that differentiate from smaller ones produce TNF- α which increase insulin resistance. Thiazolidinediones therefore suppresses the expression of these adipokines involved in insulin resistance (Sharma and Staels, 2007).

Alpha-glucosidase acts by inhibiting alpha glucosidase enzyme in the brush border of the small intestine. This delays the absorption of glucose by decreasing the breakdown of complex carbohydrates by enteric digestive enzymes. Some of the most commonly used α -glucosidase inhibitors like acarbose have severe gastrointestinal side effects such as diarrhoea, flatulence and abdominal pains. This raises the need for other sources of these inhibitors that have fewer side effects (Greenspan and Gardner, 2001). The most obvious choice for these alternatives would be plants with ethnomedical uses in management of diabetes.

Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind especially in the line of medicine and pharmacology. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action in human body (Akinmoladun, 2007). The World Health Organization (WHO) has estimated that for some 3.4 billion people in the developing world, plants represent the primary source of medicines. This means that 88% of the world's population mainly relies on traditional herbal medicines for their primary health care (Farnsworth, 1985; Farnsworth, 1988). Between 1983 and 1994, 41% of all globally prescribed drugs were derived from natural products. The percentage of natural products-derived drugs was 40% in 2000 which has remained approximately constant at 26% after 2001 (Butler, 2004).

The phytomedicines have a long history and they are the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences of different cultures. Amerindians, black slaves from Africa and Caucasians whether explicable or not used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (Wheelwright, 1994). Most of the plant species in the world have not yet been subjected to phytochemical studies for possible biological active constituents. On the other hand, the majority of secondary metabolites that are identified in medicinal plants show a pleiotropic ability to interact with several targets (Wink, 2008).

Therefore, traditional medicine offers promising solutions to face the global increasing demands for new therapeutic agents. Insufficient data exist for most plants to guarantee their quality, efficacy and safety. Plants contain hundreds of constituents and some of them are very toxic such as the most cytotoxic anti-cancer plant-derived drugs, digitalis and the pyrrolizidine alkaloids (Farnsworth, 1988).

However, the adverse effects of phytotherapeutic agents are less frequent compared with synthetic drugs, but well-controlled clinical trials have now confirmed that such effects really exist (Brown, 1992). World Health Organization Alma-Ata Declaration in 1978 opened the door for a dialogue between traditional and modern health care on the understanding that unsafe practices should be eliminated and that only what is both safe and effective should be promoted. Safety should be the overriding criterion in the selection of phytomedicines. Screening, chemical analysis, clinical trials and regulatory measures should be undertaken in respect to phytomedicines.

2.8 Herbal management of diabetes mellitus

The systematic study of herbal medicines and the investigation of the biologically active principles of phytomedicines including their clinical applications, standardization, quality control, mode of action and potential drug interactions have emerged as one of the most exciting developments in modern therapeutics and medicine. Healthcare practitioners and medical scientists have come to accept that herbal medicines are different from the pharmacologically active molecules that they may contain (Rai and Carpinella, 2006).

Several comparative clinical studies have been published to show that herbal medicines could have full therapeutic equivalence with chemotherapeutic agents while retaining the simultaneous advantage of being devoid of serious adverse effects. Developments in molecular biology and information technology have enhanced the understanding of the mechanism of action of many herbal drugs and the associated phytomedicines which differ in many respects from that of synthetic drugs or single chemical entities (Rai and Carpinella, 2006). Herbal medicinal products are now generally available in both developed and developing countries.

Phytochemicals from roots of ginseng have been used for over 2000 years in Far East because of their health promoting effects in diabetic cases. The ginseng species most commonly used include *Panax ginseng* (Asian ginseng) and *Panax quinquefolius* (American ginseng) (Xie *et al.*, 2004). Their pharmacological activity is mostly attributed to ginsenosides, a family of steroids named steroidal saponins (Dey *et al.*, 2002). The most commonly reported side effects of ginseng are nervousness and excitation but these diminish with continued use or dosage reduction (Xie *et al.*, 2004).

The hypoglycaemic activity of some medicinal plants have been identified and experimentally demonstrated in *in-vivo* and *in-vitro* diabetic models and documented in several studies. These plants include; *Azadirachta indica* (Prabbakar *et al.*, 2013), *Cassia occidentalis* linn (Laxmi *et al.*, 2010), *Colocynthis citrullus* (Abdel-Hassan *et al.*, 2000), *Ocimum gratissimum*, *Momordica charantia* (Joseph and Jini, 2013) and *Zingiber officinale* (Asha *et al.*, 2011) among many others. The chemical composition and potency of herbal products depends on the plant extract derivative, the age of the plant part used season when harvested and the method of processing (Kunle *et al.*, 2012).

Spices commonly used as diet adjuncts that contribute to the taste and flavour of foods have been demonstrated to have hypoglycemic activity (John *et al.*, 2011). Among the spices, fenugreek seeds (*Trigonella foenumgraecum*), garlic (*Allium sativum*), onion (*Allium cepa*) and turmeric (*Curcuma longa*) have been experimentally documented to possess hypoglycemic potential (John *et al.*, 2011).

Herbal medicines are usually perceived by the public as being natural, safe and free from side effects and the rationale for their continued use has largely rested on long-term clinical experience (Wheelwright, 1994). Plants contain hundreds of constituents and some of them may have toxic side effects which make it necessary to carry out toxicity studies. The continued use of herbal medicines will necessitate a thorough scientific investigation, and should go a long way in validating their folkloric usage.

2.9 Essential anti-diabetic mineral elements from herbs

Medicinal plants are good and balanced sources of essential micronutrients minerals that are valuable in the management of diabetes mellitus. These micronutrients function as essential coenzymes and cofactors for metabolic reactions and thus help support basic

cellular reactions such as glycolysis, the citric acid cycle, lipid and amino acid metabolism required to maintain energy production and life (Shils, 1999).

Micronutrients have been investigated as potential preventive and treatment agents for both type I and type II diabetes and for common complications of diabetes (Mooradian, 1994). For instance, magnesium is a cofactor in the glucose-transporting mechanism of the cell membrane and various enzymes in carbohydrate oxidation, and is thought to play a role in the release of insulin. A deficiency of magnesium is significantly more common in type II diabetes than the general population (Mooradian, 1994). Magnesium deficiency has been associated with complications of diabetes particularly retinopathy. Studies have shown patients with most severe retinopathy also presents with the lowest magnesium levels (Pandey *et al.*, 2011). On the other hand, manganese is a cofactor of various enzymes critical in cellular biochemical reactions such as activation of manganese superoxide dismutase, an antioxidant enzyme involved in the protection of cell membranes and tissues from degeneration and disruption helping the body to catabolize carbohydrates, lipids, proteins and in energy production (George, 2004).

Zinc is involved in the regulation of insulin receptor-initiated signal transduction mechanisms and insulin receptor synthesis (Ezaki, 1989). Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells (Song, 1998). The abilities to synthesize and secrete insulin and use glucose are impaired in the zinc deficient state (Ezaki, 1989). Intestinal zinc absorption rates and plasma zinc levels are reduced in diabetic patients (Rosner, 1968).

The trace element trivalent chromium (Cr^{3+}) is required for the maintenance of normal glucose metabolism. Experimental chromium deficiency leads to impaired glucose

tolerance which improves upon the addition of chromium to the diet. Chromium plays an important role in glucose and lipid metabolism, and dietary deficiency can cause impaired glucose tolerance which is of great importance to diabetes. Oral supplementation with chromium corrects these problems in-patients with type II diabetes and in children with protein-energy malnutrition. Such supplements have no effect in people with normal chromium intakes.

Chromium is an essential micronutrient which functions as a cofactor in insulin-regulating activities. It facilitates insulin binding and subsequent uptake of glucose into the cell and therefore decreases fasting glucose levels, improves glucose tolerance and lowers insulin levels. It also lowers total cholesterol in normal, elderly and type II diabetic subjects (Mooradian, 1994; Baker, 1996). Without chromium, insulin's action is blocked and glucose levels are elevated (Mooradian, 1994). Chromium picolinate is a form of chromium that exhibits biological activity (Mertz, 1969).

Trivalent chromium has long been considered to be a safe nutritional supplement (Castro, 1998). Although the hexavalent form of chromium is a known human respiratory tract carcinogen when inhaled in high-exposure industrial settings, there is no evidence of any carcinogenic effects in humans from the trivalent form of chromium found in chromium supplements. A reasonable amount of supplemental chromium is 200 µg/day (Castro, 1998). The current adequate intake (AI) for chromium is 25 µg for women and 35 µg for men. No tolerable upper intake level (UL) which has been established. Previous recommendations placed a daily intake at ≤ 200 µg/day within a safe and adequate range (Anderson, 1998).

Another important mineral element is vanadium which is known to play critical role in regulation of intracellular signaling and as a cofactor of enzymes essential in energy metabolism. It reduces the rate of gluconeogenesis and increases glycogen deposition (Cohen, 1995). A reasonable amount of supplemental vanadium is 20 µg/day. Vanadyl sulfate at a dose of 100 mg/day is effective in improving insulin sensitivity (Cohen, 1995).

Molybdate is an effective anti-hyperglycemic agent in diabetics with severe insulin resistance. It is associated with substantial reduction of hyper-insulinaemia and an increase in pancreatic insulin stores. The glucose-lowering effect of molybdenum may be partly related to reduction of hepatic glucose production, and possibly to increased glucose usage (Reul, 1997).

Medicinal plants toxicities could be attributed to the high levels of mineral elements. For instance, trivalent chromium sources are not toxic. However, hexavalent chromium toxicity from industrial exposure through inhalation has been associated with increased incidence of lung cancer. In experimental animals, ingestion of chromate resulted in liver and kidney damage (Fishbein, 1988). Epigastric pain, diarrhea and vomiting have been observed from high zinc intake from food stored in galvanized containers. Supplements of as little as 25 mg of zinc have resulted in diminished absorption of copper, presumably because of competition (Kaplan, 1989). Lead toxicity produces neurological, gastrointestinal, renal, immunological, endocrinological and hematopoietic changes in humans (Kaplan, 1989). Supplementation of human volunteers with vanadyl compounds at oral doses of 50-125 mg/day caused cramps, loosened stools, green tongue in all patients, fatigue and lethargy in some individuals (Dimond, 1963).

2.10 Essential vitamins with hypoglycemic activity from herbs

The efficacy and safety of medicinal plants in the management of diabetes mellitus is further potentiated by presence of vitamins in appropriate amounts. These vitamins includes; α -tocopherol, retinol, ascorbic acids and carotenoids such as lycopene, β -carotene and β -cryptoxanthin.

2.10.1 Retinol

Retinol also termed as vitamin A refers to a sub-class of retinoic acids, long understood to help regulate immune functions and to reduce morbidity of infectious diseases (Bates, 1995). Vitamin A is required for normal functioning of the visual system, maintenance of cell function for growth, epithelial integrity, production of red blood cells, immunity, and reproduction (Bates, 1995). Different forms of vitamin A include β carotene, which is found in plants, and preformed vitamin A, which is found in animals. Vitamin A is an essential nutrient that cannot be synthesized so it must be obtained through diet (Bates, 1995).

Vitamin A deficiency increases vulnerability to a range of illnesses including diarrhoea, measles, and respiratory infections (Rice *et al.*, 2004). These are leading causes of mortality among children in low and middle income countries, where risk of infection and risk of mortality can be compounded by coexisting under-nutrition (Rice *et al.*, 2004). The bioavailability of provitamin A carotenoids in fruit and vegetables is lower than once believed (Tang, 2010), and it is difficult for children to fulfill their daily requirements through plant foods alone. Consequently, vitamin A deficiency is common among children whose families cannot afford eggs and dairy products (Bates, 1995). Preformed vitamin A (retinol, retinal, retinoic acid, and retinyl esters) is the most active in humans; it is usually used in supplements in the form of retinyl esters. High intake of synthetic vitamin A over

a prolonged period can lead to toxicity, but toxicity from food sources is rare. Periodic supplementation should not cause serious adverse effects (Bates, 1995).

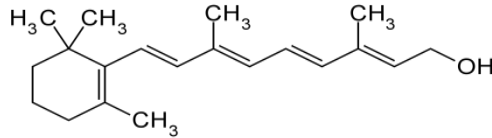


Figure 2.1: The chemical structure of vitamin A

2.10.2 Alpha-tocopherol

Alpha-tocopherol also called vitamin E is a collective name for a group of fat-soluble compounds with distinctive antioxidant activities that includes tocopherols and tocotrienols (Traber, 2006). It is found naturally in some foods, added to others, and available as a dietary supplement. Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma- and delta-tocopherol and alpha-, beta-, gamma- and delta-tocotrienol) that have varying levels of biological activity (Traber, 2006). The only form of tocopherol that is nutritionally important to humans is α -tocopherol (Health, 2011).

Serum concentrations of α -tocopherol depend on the liver, which takes up the nutrient after the various forms are absorbed from the small intestine. The liver preferentially re-secretes only α -tocopherol via the hepatic α -tocopherol transfer protein (Traber, 2006); the liver metabolizes and excretes the other vitamin E forms (Traber, 2007). As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of α -tocopherol and has been the subject of less research.

Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species (ROS) formed when fat undergoes oxidation. Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms, vitamin E might

help prevent or delay the chronic diseases associated with free radicals (Traber, 2006). In addition to its activities as an antioxidant, vitamin E is involved in cell signaling, regulation of gene expression and other metabolic processes (Traber, 2006). Usual dietary intakes are estimated at 7–11 mg/day. The recommended daily/dietary allowance (RDA) for alpha-tocopherol is 15 mg/day for people of 15 years of age and older. The UL for alpha-tocopherol is 1,000 mg/day from supplemental sources. Natural vitamin E (d-alpha tocopherol) has approximately twice the bioactivity of synthetic forms of the vitamin (dl-alpha tocopherol) (Sarubin, 2000).

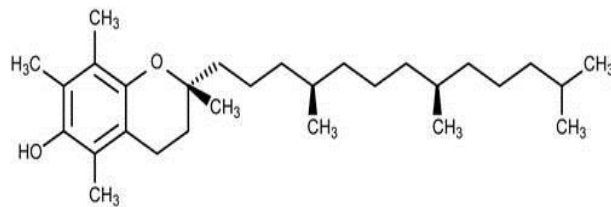


Figure 2.2: The chemical structure of vitamin E

2.10.3 Thiamine

Vitamin B1 is also termed as thiamine and has two primary functions; alpha-keto acid decarboxylation and transketolation. Decarboxylation reactions are an integral part of carbohydrate metabolism. Thiamine is involved in the alpha-keto acid decarboxylation of pyruvate, alpha-ketoglutarate and the branched-chain alpha-keto acids such as leucine, isoleucine and valine metabolites. Transketolation is involved in the pentose phosphate pathways (Fattal *et al.*, 2011).

Thiamine is converted to its active form, thiamine pyrophosphate. The thiamine-dependent enzymes are important for the biosynthesis of neurotransmitters and for the production of reducing substances used in oxidative stress defenses, as well as for the biosynthesis of pentoses used as nucleic acid precursors. Thiamine plays a central role in cerebral metabolism. Its deficiency results in dry and wet beriberi, a peripheral neuropathy, a

cardiomyopathy with edema and lactic acidosis and Wernicke–Korsakoff syndrome, whose manifestations consist of nystagmus, ophthalmoplegia and ataxia evolving into confusion, retrograde amnesia, cognitive impairment and confabulation (Fattal *et al.*, 2011).

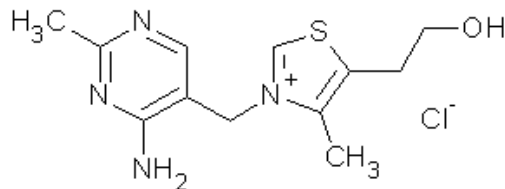


Figure 2 3: The chemical structure of vitamin B1

2.10.4 Nicotinic acid and Nicotinamide

Vitamin B3 occurs in two forms: nicotinic acid and nicotinamide. The active coenzyme forms, nicotinamide adenine dinucleotide (NAD) and NAD phosphate are essential for the function of hundreds of enzymes and normal carbohydrate, lipid and protein metabolism (Frank and Bantle, 1999). As a vitamin, the two compounds function similarly, but in pharmacological doses they have distinct effects (Frank and Bantle, 1999).

Nicotinic acid at a dose range of 1-3 g/day is an effective treatment for dyslipidemia, although its use in people with diabetes has been limited because of its negative effect on glycemic control (Sarubin, 2000). The daily requirements intakes (DRIs) for niacin are reported in niacin equivalents (NE) because niacin can be synthesized by the body from tryptophan. The recommended daily/dietary allowance (RDA) is 14 mg NE for women and 16 mg NE for men. The UL is 35 mg NE/day for adults (Sarubin, 2000).

Animal studies suggests that nicotinamide acts by protecting pancreatic β -cells from autoimmune destruction by maintaining intracellular NAD levels and inhibiting the

enzyme poly (ADP-ribose) polymerase (PARP), an enzyme involved in DNA repair. Nicotinamide also acts as a weak antioxidant of nitric oxide radicals (Kolb and Volker, 1999; Knip, 2000).

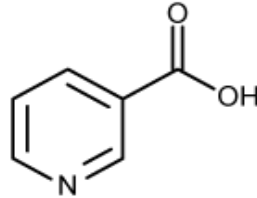


Figure 2.4: The chemical structure of vitamin B3

2.10.5 Ascorbic acid

Natural ascorbic acid is vital for the body performance (Gulfraz *et al.*, 2011). Vitamin C is an antioxidant which acts as an electron donor for 8 human enzymes; three of which participate in collagen hydroxylation and two in carnitine biosynthesis; of the three enzymes which participate in collagen hydroxylation, one is necessary for biosynthesis of the catecholamine and norepinephrine, the other is involved in amidation of peptide hormones, and the last one in tyrosine metabolism (Gulfraz *et al.*, 2011).

Vitamin C protects low-density lipoproteins *ex vivo* against oxidation and may function similarly in the blood. A common feature of vitamin C deficiency is anemia. The antioxidant property of vitamin C stabilizes folate in food and in plasma. Vitamin C promotes absorption of soluble non-haem iron by chelation or by maintaining the iron in the reduced (ferrous, Fe²⁺) form (Karau *et al.*, 2012). However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends on the amount of inhibitors, such as phytates and polyphenols, present in the meal (WHO, 2004).

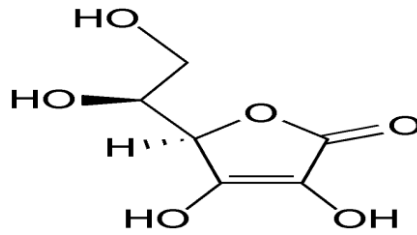


Figure 2 5: The chemical structure of vitamin C

2.11 Carotenoids from herbs

Among all the pigments present in living organisms, there is no doubt that the carotenoids are the most widely distributed in nature. They are found throughout the plant kingdom in both photosynthetic and non-photosynthetic tissues in bacteria, in fungi and in animals. The latter are unable to synthesize carotenoids and therefore they incorporate them from dietary plants (Goodwin, 1980).

Carotenoids are natural pigments synthesized by plants and microorganisms, but not by animals. These include; lycopenes which have two acyclic end groups and β -carotene which has two cyclohexene type end groups; oxygenated carotenoids also called xanthophylls. Examples of these compounds are; a) zeaxanthin and lutein (hydroxy), b) spirilloxanthin (methoxy), c) echinenone (oxo) and d) antheraxanthin (epoxy) (Goodwin, 1980).

Many epidemiologic studies have associated high carotenoid intake with a decrease in the incidence of chronic disease. However, their biological mechanism remains elusive. Multiple possibilities exist. Certain carotenoids i) can be converted to retinoids to have provitamin A activity, ii) can modulate the enzymatic activities of lipoxygenases such as proinflammatory and immunomodulatory molecules, iii) can have antioxidants properties which are well above what is seen with vitamin A, iv) can activate the expression of genes

which encode the message for production of a protein, connexin 43, which is an integral component of the gap junctions required for cell to cell communication. Such gene activation is not associated with antioxidant capacity and is independent of pro-vitamin A activity (Bendich, 1993).

Beta-carotene is the main compound with provitamin A activity (Olson, 1993). When incorporated into the diet, it is broken down into two molecules of retinol (vitamin A) by action of the enzyme β -carotene-15 15'-dioxygenase in the intestine. However, β -carotene is not the only carotenoid with provitamin A activity, and, any carotenoid with at least one unsubstituted β -ring can undergo similar cleavages and give rise to a vitamin A molecule. However, carotenoids such as α -carotene and β -cryptoxanthin can thus contribute substantially to the nutritional value of fruits and vegetables (Bendich and Olson, 1989).

Current attention is centered on the action of β -carotene as an antioxidant, as it may interfere with free radical oxidation such as the peroxidation of lipids, typical of many degenerative diseases. Although it has been clearly demonstrated that β -carotene has a significant antioxidant effect *in vitro*, there is still no real proof that it's *in vivo* function at the low concentrations in which it is found and under physiological conditions (Bendich, 1989).

There are 650 known naturally occurring carotenoids. Most of the carotenoids found naturally in fruits and vegetables present skeleton of 40 carbon atoms (C_{40}) and are biosynthesized from molecules of an intermediary C_{20} (geranylgeranyl diphosphate), giving rise to a phytoene as generic precursor of the whole wide range of carotenoids present in the plant kingdom. The phytoene molecule undergoes a series of successive desaturations (up to four), introducing new double bonds into the carbon chain, resulting

in spreading of the double bond conjugation and thus of the chromophore that is typical of these natural pigments and responsible for their chromatic properties (Britton, 1997).

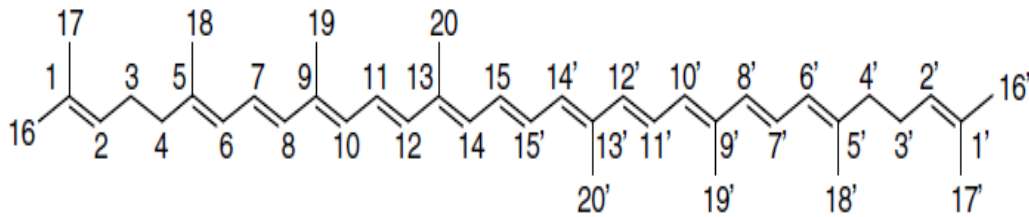


Figure 2.6: The chemical structure of lycopene

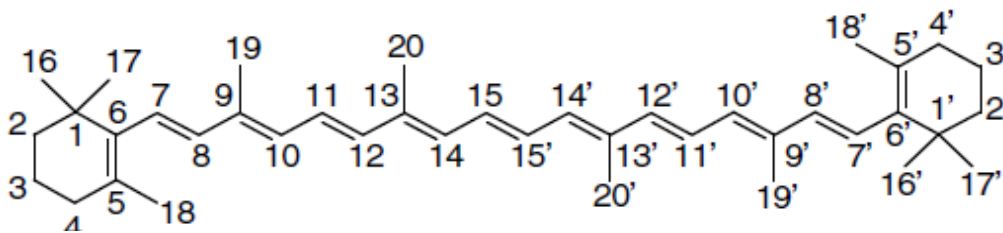


Figure 2.7: The chemical structure of β -carotene

Lycopene (ψ ψ -carotene), is an acyclic carotenoid containing 11 conjugated double bonds, is naturally present in *trans*-form in raw tomatoes and imparts red color to the tomatoes. It is one of the most abundant non-vitamin analogues present in human blood from food consumption. Among the common dietary carotenoids, lycopene has the highest single oxygen quenching capacity *in vitro* and its antioxidant properties are probably related to risk reduction of certain types of cancers. It has been found that after air-drying at 80°C, the number of hydroxyl phenol groups increases owing to the hydrolysis of flavonoid glycosides and / or the release of cell wall phenolics (Thadikamala, 2009). Processing promotes different side reactions that could affect the antioxidant activity of the plant products (Thadikamala, 2009).

2.12 Safety of herbal medicine

Systemic toxicity from the herbal extracts depends on the route, and site of exposure (Jothy *et al.*, 2011). Direct tissue damage is usually the result of cellular destruction and this may have a biochemical or immunological basis but many pathological lesions are of

unknown mechanism, particularly as regards the intermediate stages between the interaction of the toxin or its metabolite with cellular constituents, and the start of the final degenerative changes that lead to cell death (Jothy *et al.*, 2011).

Toxic effects may be detected by gross pathological examination in the post mortem or histopathological examination after toxicity studies have been carried out. Some may also be detected using clinico-chemical analysis of body fluids (Timbrell, 1996). Efficacy and safety of these medicinal plants have been studied by use of model animals such as BALB/c mice, albino mice, Winstar mice, rats and rabbits.

2.13 Alloxanized mice model for diabetes mellitus

The alloxan-induced diabetic male BALB/c mice represent one of the most useful tools available for evaluating the efficacy and safety of hypoglycemic medicinal plants (Szkudelski, 2001). Mice are widely used in scientific research due to their ease of handling as well as the degree of homology they share with human beings. Selective breeding has led to many strains and models of type I and II diabetes mellitus, obesity, and insulin resistance (Ghatta and Ramarao, 2004). New animal models continue to emerge with genetic manipulation, such as knock in, generalized knock out as well as tissue specific knockout mice (Rees and Alcolado, 2005). Mice models are invaluable tools for testing drug's efficacy, toxicity, dosage and routes of delivery (Ghatta and Ramarao, 2004).

The use of high fat diet fed mice model is ideal as a functional model for the evaluation of effects of excessive fat intake in humans, and it represents non-insulin dependent diabetes mellitus (NIDDM) model. The high fat diet rat fed model for insulin resistance has been extensively used in the elucidating mechanisms involved in the prevention and/or reversal

of insulin resistance (Ghatta and Ramarao, 2004). With high fat diet the energy intake is shifted from carbohydrate to fat (Ghatta and Ramarao, 2004).

The BALB/c mice belonged to albino stock acquired by H. Bagg in 1913, and therefore termed as Bagg albino or BALB. In 1923, they were inbred by MacDowell, Cold Spring Harbor, NY, USA and in 1936 at the F25 to Snell, who added the 'c' for the albino thus getting the name BALB/c (Ghatta and Ramarao, 2004).

2.14 Biochemical markers of medicinal plant extracts toxicity

Biochemical markers are mainly applied to recognize, characterize and monitor treatment-related responses following an exposure to xenobiotics. They play three main roles in toxicology, *viz a viz*; to confirm exposure to a deleterious agent, to provide a system for monitoring individual susceptibility to a toxicant, and to quantitatively assess deleterious effects of a toxicant to an organism or individual (Amacher, 2002). Because the liver is a general target for adverse effects of drugs and other chemicals, biomarkers of untoward hepatic response to xenobiotics are of particular interest to the pharmaceutical toxicologist (Abbott *et al.*, 1997).

Liver function markers refer to peripheral indicators of hepatic synthetic and secretory activities, enterohepatic function, or perturbations of the hepatic uptake and clearance of circulating biomolecules (Amacher, 2002). Liver injury biomarkers include various peripheral proteins released in response to a cellular damage or locally, proteins that are significantly altered within the liver. These include both circulating cytosolic, mitochondrial, or canalicular membrane markers, and the up-regulation or depletion of radical scavengers, modulators, and stabilizers of intracellular damage (Amacher, 2002).

Alanine aminotransferase (ALT), formerly serum glutamic pyruvic transaminase (SGPT) and aspartate aminotransferase (AST), formerly serum glutamic oxaloacetic transaminase (SGOT) are the most commonly used enzymes to assess hepatocellular damage (Hayes, 2008). ALT is the most frequently used hepatic serum biomarker in a variety of laboratory *in vivo* assays, although it is present in many tissues, it is concentrated in hepatocytes (Hayes, 2008). Significant elevations in ALT indicate release of ALT by hepatocytes. AST concentrations tend to mimic ALT activity with respect to liver damage, however AST is a less specific biomarker as high concentrations of the enzyme are found in a variety of other tissues (Hayes, 2008).

Blood urea nitrogen (BUN) and creatinine are nitrogenous end products of metabolism removed from the blood by the kidneys. BUN and creatinine together are the most commonly used clinical serum biomarkers of renal damage. Urea is derived primarily from dietary protein and protein turnover within the body and creatinine is the product of muscle creatine breakdown (Hayes, 2008). BUN concentrations vary greatly with individual protein intake, protein metabolism, state of hydration and renal urea excretion rates. Creatinine concentrations can vary with individual body muscle mass and measurement source (serum versus urine) and techniques. Elevations in BUN and creatinine can serve as clinical indicators of poor kidney function; however creatinine is more sensitive than BUN (Hayes, 2008).

2.14.1 Alkaline phosphatase (ALP)

Alkaline phosphatase is a ubiquitous enzyme in the body needed in small amounts to catalyze specific chemical reactions. When it is present in serum in large amounts, it may signify bone or liver disease or a tumor in these organs (Bigoniya *et al.*, 2009). In a

healthy liver, fluid containing ALP and other substances is continually drained away through the bile duct.

In a diseased liver, the bile duct is often blocked thus retaining fluid within the liver. ALP accumulates and eventually escapes into the blood stream. The cells lining the small bile ducts in the liver produce the ALP of the liver. Its origin differs from that of other diagnostic liver enzymes such as the aminotransferases (Bigoniya *et al.*, 2009). If the liver disease is primarily of an obstructive nature (Cholestatic), that is it involves the biliary drainage system, the ALP will be the first enzyme to be elevated. If on the other hand, the disease is primarily of the liver cells; the aminotransferases rise prominently.

2.14.2 Aspartate aminotransferase (AST)

This is a cytoplasmic and mitochondrial enzyme found in the liver, heart, skeletal muscles, kidney, pancreas, erythrocytes, lungs and brain tissue. When injury affects these tissues, the cells are destroyed and AST is released into the bloodstream. The amount of AST is directly related to the number of cells affected by the injury, but the level of elevation depends on the length of time that the blood is tested after the injury.

Serum AST levels become elevated eight hours after cell injury, peak at 24-36 hours, and returns to normal in three to seven days. If the cell injury is chronic (on-going), AST results remain elevated (Amacher, 2002). AST is a valuable aid in the diagnosis of liver disease. It is used in combination with other enzymes, for example, alanine aminotransferase (ALT); to monitor the cause of various liver disorders (Amacher, 2002).

2.14.3 Alanine aminotransferase (ALT)

This is a cytoplasmic enzyme found in high concentration in the liver and to a lesser extent in skeletal muscles, kidney and heart. A rise in plasma ALT activities is an indicator of damage to cytoplasmic membrane in the cell. Liver cells contain more AST than ALT and in most conditions damage to the cytoplasmic and mitochondria membranes lead to a relatively greater increase in plasma AST activity than that of ALT. However, ALT is confined to the cytoplasm and mitochondria and its high concentration is greater than that of AST (Zahra and Samaneh, 2012).

2.14.4 Creatinine kinase (CK)

Creatine kinase is the enzyme responsible for regeneration of ATP and in normal serum, at least 95% of the CK present is of skeletal muscle and is probably largely the result of leakage from skeletal muscle, particularly during physical activity (Kaplan, 1989). Because of this serum CK activity in healthy active persons shows asymmetrical distribution skewed towards higher values.

Age, sex and race affect CK activity in serum. It has been established that values are lower in women than in men and are lower in the morning than in the evening. Values tend to be lower in hospitalized patients, possibly because bed rest reduces the amount of enzyme released from muscle (Kaplan, 1989). The reference interval for total CK activity has been reported to be 130 -253 μ /L in humans (Kaplan, 1989). In view of the wide variation of the normal CK in animals, only high increases in its activity are significant clinically (Kaneko, 1989).

2.14.5 Blood urea nitrogen (BUN)

The major pathway of nitrogen excretion in humans is urea. Urea is synthesized in the liver from amino acids resulting from protein metabolism. The source of protein could be from diet or from tissues. Urea is released in the blood and cleared by the kidneys. The rate of urea production is accelerated by a high protein diet, by increased catabolism due to starvation, tissue damage or sepsis (Khajehdehi *et al.*, 1998). Blood urea nitrogen (BUN) is used as first line investigation of glomerular function. However, over 60% of glomeruli must be destroyed before blood urea nitrogen concentration significantly rises (McClellan *et al.*, 1997).

Low blood urea nitrogen is observed in a condition such as pregnancy (the most common cause in young women), over enthusiastic intravenous infusion, appropriate antidiuretic hormone (ADH) secretion. All these are caused by an increase in glomerular filtration rate (GFR). In children, it is due to decreased synthesis of proteins where amino acids are used for protein anabolism during growth, low protein intake, very severe liver disease and inborn errors of urea cycle (Joan *et al.*, 1988).

2.15 Plants under the study

2.15.1 *Pappea Capensis*

Jacket plum or wild plum, *Pappea capensis* L. tree belongs to the Litchi family Sapindaceae. The plant is called 'Muvaa' by people from Mbeere and 'Kivaa' by Akamba. The tree grows up to 3.9 m tall and can be deciduous or evergreen depending upon the prevailing environmental conditions (Mng'omba *et al.*, 2007). This plant is fairly adapted to a wide range of ecological areas and it is known to be drought-tolerant thus able to grow in marginal lands. The leaves are simple and oblong, hard-textured and wavy. New leaves

are an attractive pinky-bronze when they emerge in spring, and this contrasts well with the dark green of the old leaves (Mng'omba *et al.*, 2007). The photograph of this plant is shown in figure 2 8 a.

P. capensis is widespread in southern Africa from the Northern Cape through the drier Karoo, Eastern Cape, KwaZulu-Natal, to the Northern provinces, as well as Mozambique, Zimbabwe and northwards into eastern and southern tropical Africa (Mng'omba *et al.*, 2007; Fivaz and Robbertse, 1993). In Kenya, it is distributed in Lukenya hills, Ngong hills, northern Kapenguria and semi-arid regions of southern part of Embu County such as Siakago. It produces fleshy leaves which can be processed into vinegar, jelly and jam (Palmer and Pitman, 1972). Seeds contain 74% oil that is rich in edible, non-drying and fairly viscous oil constituents and is used for making soap and oiling guns and the plant is a good fodder for livestock. Among the Mbeere people, the boiled stem barks are used traditionally to treat whooping cough and the leaves are used in the management of diabetes mellitus.

2.15.2 *Maytenus obscura*

Maytenus obscura (A. Rich.) Cuf belongs to the genus *Maytenus* and family Celastraceae which is distributed worldwide, particularly in subtropical and tropical regions, Africa, China, Brazil, Paraguay, Uruguay and Argentina and in southern regions of Saudi Arabia (Alajmi and Perwez, 2014). The plant grows to 2.5-9 m tall and has with elliptic serrulate leaves. Flowers are greenish cream while fruits are reddish and ovoid shaped. The plant is found in riverine forest, drier forest margins and evergreen bushland. The local names are Muthunthi (Kamba), Muthuthi, Mutanda (Kikuyu) and Abukwa (Tugen). The genus *Maytenus* is rich in triterpenes, diterpenes, sesquiterpene alkaloids and spermidine

alkaloids (Gutierrez *et al.*, 2007). Other secondary metabolites isolate from different species of the genus *Maytenus*, including flavonoids and flavonoid glycosides, phenolic glucoside and agarofurans (Alajmi and Perwez, 2014).

Species belonging to this genus are extensively investigated for bioactive compounds as they are widely used in folk medicine such as antiseptic, antiasthmatic, fertility-regulating agents, antitumor and antiulcer (Alajmi and Perwez, 2014). More specifically, *M. heterophylla* and *M. senegalensis* are used to treat respiratory ailments and inflammation (da Silva *et al.*, 2011). The use of these plants as anti-inflammatory herbal drugs is also common in other African countries like Benin, Kenya, Zambia, Tanzania, Senegal and Zimbabwe. In Tropical and Southwestern Africa, *M. heterophylla* leaves are also employed in the treatment of dysmenorrhoea and *M. senegalensis* leaves are used to treat toothaches, in India (da Silva *et al.*, 2011). The photograph of *M. obscura* is shown in figure 2.8 c.

2.15.3 *Senna spectabilis* L

This rounded deciduous tree grows to 10 m and belongs to the family Caesalpiaceae; leaves are narrowly elliptic with softly hair below and are shed entirely, leaving the tree bare for months. Flowers are showy clusters of yellow. The tree is widely planted up to 2,000 m and very common around Nairobi, Kenya (Bum *et al.*, 2010). This is a quick-growing, flourishing even in poor or black-cotton soil, but not at its best if the site is too dry. It is spectacular in flower, deservedly popular and widely available in nurseries. The vernacular names are ‘Mukengeka’ or ‘Musingili’ (Kamba) and ‘Nyai-leka’ (Luo) (Bum *et al.*, 2010). This is shown in figure 2.8 e.

Senna spectabilis is found in tropical areas in Africa, Asia, Australia, Latino and South America. In Africa it is found in Angola, Burundi, Cameroon, Kenya, Nigeria, Tanzania, Togo, and South Africa. It is used in traditional medicine in Cameroon to treat constipation, insomnia, epilepsy, anxiety, among others (Bum *et al.*, 2010). The decoction is used in the treatment of epilepsy, malaria, dysentery and headaches. Some pharmacological studies showed that *S. spectabilis* was fully efficacious in reverting scopolamine-induced amnesia in mice (Viegas *et al.*, 2005). Among the Mbeere people of Embu County, the leaves and stem barks are used as an antidote to arrow poison, and decoctions from stems and leaves are used in the management of diabetes mellitus. Also, leaves decoctions mixed with soup are drunk to treat and manage internal injuries and tumours (Kokwaro, 2009).

2.15.4 *Launaea cornuta*

Launaea cornuta (Oliv. & Hiern) belongs to the family Asteraceae, and is widely distributed in almost all parts of Kenya, and farmers everywhere find it a troublesome weed. It is referred to as ‘Muthunka’ by Ameru and ‘Muthunga’ by people from Mbeere. It is an erect perennial with hollow stems up to 1.5 m high and creeping rhizomes. Leaves form a rosette at the base, alternate on the stem, sessile, up to 25 cm long by 3 cm wide, entire or with two to three pairs of lobes acute-pointed near the base. Inflorescence large, diffuse with numerous flower heads on peduncles about 2.5 cm long. This plant is shown in Figure 2.8 d.

Involucre up to 10 mm long by 4 mm across, glabrous or shortly pubescent, phyllaries in two to three rows, 2-4 mm long outside, up to 10 mm long inside. Florets 10-25, yellow up to 15 mm long, ligule often reddish outside. Seeds pale brown, elliptical, ribbed, 2-4 mm long with white pappus 5 mm long. *Launaea cornuta* is native in Africa (Cameroon,

Central African Republic, Ethiopia, Kenya, Malawi, Mozambique, Nigeria, Rwanda, Somalia, Sudan, Tanzania, Uganda, Zaire, Zambia, and Zimbabwe) (Jeffrey, 1966).

The boiled stem bark extracts are used to treat abdominal discomforts especially after birth, and to manage diabetes mellitus, among the Mbeere people of the Embu county.

2.15.5 *Ocimum americanum*

Ocimum americanum L. (syn. *O. canum* Sims) belongs to the family Lamiaceae is a widely distributed plant species in the tropics and subtropics of the Old and New World, but contrary to the name it is not native to America, but only to Africa and Asia (Paton *et al.*, 1999). It is referred to as Makuruthi by Ameru and Mutaa by Mbeere people. It is not often used as a culinary herb, unlike the related basil species *O. basilicum*, but more often as a medicinal plant. The essential oils found in this species have strong fungicidal activity against certain plant pathogens (Dubey, 1991).

In Africa, leaves of *O. americanum* have been used as an insecticide for the protection against postharvest insect damage especially that by bruchid beetles (Weaver *et al.*, 1991). The active ingredients are also likely to be associated with the essential oils. However, some medicinal properties may be associated with the external flavonoids, as some specimens produce very high levels of these compounds, especially nevadensin, which has antioxidant activity (Roberto *et al.*, 2003). The photograph of this plant is shown in figure 2.8 b.

Table 2.1: Medicinal plants, their local names, and their ethnomedicinal uses

S/No	Plant name (family)	Local names	Collection area and their ethnomedicinal use
1	<i>Maytenus obscura</i> (Celestraceae)	Muthunthi (Kamba)	Boiled stem extracts are used to treat abdominal discomforts especially after birth. The boiled extracts are used in the management of diabetes mellitus.
2	<i>Senna spectabilis</i> (Caesalpiniaceae)	Mukengeka (Kamba)	Leaves and stem bark are used as an antidote to arrow poison. Also the stems and leaves aqueous extracts are used in the management of diabetes mellitus.
3	<i>Launaea cornuta</i> (Asteraceae)	Muthunga (Embu) Muthunka (Meru)	The shrub is used as a vegetable and is claimed to manage diabetes among the natives.
4	<i>Pappea capensis</i> (Sapindaceae)	Muvaa or Kivaa in Mbeere and Kamba	The boiled plant extracts are traditionally used to treat whooping cough and manage diabetes mellitus.
5	<i>Ocimum americanum</i> (Lamiaceae)	Makuruthi in Meru Mutaa in Mbeere and Kamba	The plant decoction is used in management of diarrhoea, and diabetes mellitus.



(2.8 a). *Pappea capensis* (Jacket plum)



(2.8 b). *Ocimum americanum*



(2.8 c). *Maytenus obscura* (A Rich)



(2.8 d). *Launea cornuta*



(2.8 e). *Senna spectabilis*

Figures 2 8: **Photographs of the plants used in this study.**

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, and Testing Department, Kenya Bureau of Standards, Kenya from January 2011. Kenyatta University is 23 km from Nairobi off Thika Road. Kenya Bureau of Standards is 11 km off Mambasa Road along Popo Road.

3.2 Collection of plant materials

Stem barks and leaves of *Maytenus obscura*, *Senna spectabilis* and *Pappea capensis* were collected in February and March 2011 from Kambara Village, Kianjiru Location, Gachoka Division, Mbeere District, Embu County of Kenya. Leaves and stems of *Launea cornuta*, and *Ocimum americanum* were collected from Mbeu Village, Tigania, Meru County of Kenya.

These medicinal plants were collected from their geo-ecological habitats based on their folkloric use as was obtained from traditional medical practitioners from the areas. The plants were authenticated by a taxonomist at the Department of Plant and Microbial Sciences, Kenyatta University, Kenya and a voucher specimen deposited at the Kenyatta University Herbarium for future reference.

3.3 Processing of plant materials

The leaves and stems were harvested and the stem barks peeled off while still fresh, cut into small portions, and dried at room temperature under shade for one month. The dried plant materials were ground using an Electric mill (Christy and Norris Ltd, England). The powdered plant materials were kept at room temperature away from direct sunlight in closed, dry plastic bags.

One hundred grams of plant material was extracted in 1 liter of distilled water at 60°C in a metabolic shaker for 6 hours. After extraction, the extract was decanted into a clean dry conical flask and then filtered through folded cotton gauze into another clean dry conical flask. The filtrate was then freeze dried in 200 ml portions using a Modulyo Freeze Dryer (Edward England) for 48 hours. The freeze-dried material was then weighed and stored in an airtight container at -20°C until used for bioassay. Similarly, 100 g of plant material was extracted in 1 litre of ethyl acetate at room temperature in a metabolic shaker for six hours. The extracts were filtered with a Whatman Filter Paper No 540 and the filtrate concentrated in a Rotary Evaporator. The extracts were weighed and stored in airtight amber containers at 4°C ready for use.

3.4 Experimental animals

The study used male BALB/c mice, 3-5 weeks old that weighed 20-30 g with a mean weight of 25 g. The animals were allowed to acclimatize for a period of two weeks in the animal house at the Department of Biochemistry and Biotechnology, Kenyatta University prior to the study. The mice were housed in polypropylene cages, maintained under standard laboratory conditions of 12 hour light and dark normal photoperiodicity, at ambient temperature of $25 \pm 2^\circ\text{C}$ and 35-60% humidity. The animals were fed with standard mice pellets obtained from Unga Feeds Limited, Kenya, and water *ad libitum*.

The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

3.4.1 Identification of the optimum alloxan-monohydrate dose to induce diabetes

The identification of the intraperitoneal optimum dose of alloxan to induce diabetes was performed using a logarithmic scale (Thomson, 1985) that ranged from 50 to 480 mg/kg body weight with 6 dose levels at 50 (1.25 mg), 77.6 (1.94 mg), 120.4 (3.01 mg), 186.9 (4.67 mg), 290.0 (7.25 mg) and 480.0 (12 mg) mg/kg body weight, respectively. The doses were intraperitoneally administered in 0.1 ml physiological saline once for each level to five male BALB/c mice. The animals were monitored for changes in blood sugar within 24 to 48 hours. Any fatality, body weakness or abnormality observed at any dose level was recorded. After 48 hours, the diabetic animals were examined for suitability in the bioassays by measuring blood glucose levels after every 2 hours consistently for a period of 24 hours.

3.4.2 Induction of hyperglycemia

Hyperglycemia was induced experimentally by intraperitoneal administration of a single dose of 186.9 mg/kg body weight (4.6725 mg in 0.1 ml physiological saline) of a freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma (Steinheim, Switzerland) as determined in section 3.4.1. The optimal dose was obtained at the section 3.4.1. Forty-eight hours after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 2000 mg/L were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours (Szkudelski, 2001) but allowed free access to water until the end of this experiment.

3.4.3 Experimental design

The experimental animals average weight 25 g were randomly divided into six groups of five animals each. Group I consisted of normal mice orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic mice orally administered with 0.1 ml physiological saline; Group III consisted of alloxan induced diabetic mice orally administered with 0.075 mg of glibenclamide (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic mice orally administered with 1.25 mg extract (50 mg/kg body weight) in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice orally administered with 2.50 mg extract (100 mg/kg body weight) in 0.1 ml physiological saline; and Group VI consisted of alloxan induced diabetic rats either intraperitoneally or orally administered with 5 mg extract (200 mg/kg body weight) in 0.1 ml physiological saline.

3.4.4 Blood sampling and blood glucose determination

Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment and repeated after 1, 2, 3, 4, 12 and 24 hours. The blood glucose levels were determined with a glucose analyser model (Contour® TS, Bayer Pty, Ltd; Healthcare Division, Japan).

3.5 *In vivo* single dose toxicity test

The mice were randomly divided into nine different groups of five mice each. Group I consisted of untreated control mice orally administered daily for 28 days with 0.1 ml physiological saline. Group II, III, IV, V, VI, VII, VIII and IX consisted of normal control mice orally administered with aqueous and ethyl acetate extracts of the five plants at 25 mg (1 g/kg body weight) in 0.1 ml physiological saline daily for 28 days. During this period, the mice were allowed free access to mice pellet and water and observed for any

signs of general illness, change in behaviour and mortality. At the end of 28 days, the mice were sacrificed.

3.5.1 Determination of body weight

The body weight of each mouse was assessed after every seven days during the dosing period up to and including the 28th day and the day of sacrifice. On the day of sacrifice, all the animals were euthanized and blood samples taken by cardiac puncture of each sacrificed animal divided into two parts: one part for estimation of hematological parameters and the other part for estimation of biochemical parameters.

3.5.2 Determination of hematological and biochemical parameters

On the day of sacrifice, all the animals were euthanized and blood samples taken by cardiac puncture of each sacrificed animal divided into two parts. The blood sample collected in K3-EDTA tubes was used to estimate hematological parameters. Blood was examined using standard protocols (Jain, 1986; International Committee for Standardization, 1978). Red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) and platelets (PLT) were determined using the Coulter Counter System (Beckman Coulter®, ThermoFisher, UK).

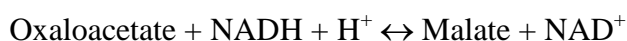
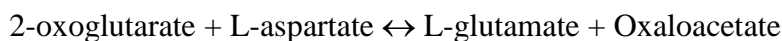
Differential white blood cell count for neutrophils (N), lymphocytes (LYM), and monocytes (M) were determined from stained blood films using a hemocytometer (Jain, 1986). Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphologies, respectively.

The blood collected in plastic test tubes was allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at -20°C until required for biochemical parameter analysis. The biochemical parameters determined on the sera specimen using the Olympus 640 Chemistry Auto Analyser were alanine aminotransferase (AST), aspartate aminotransferase (ALT), alkaline phosphatase (ALP), creatinine kinase (CK), and blood urea nitrogen (BUN).

3.5.2.1 Determination of the activity of aspartate aminotransferase (AST)

AST reagent was used to measure aspartate aminotransferase activity by an enzymatic kinetic UV rate method. In the reaction, aspartate aminotransferase catalysed the reversible transamination of L-aspartate and α -ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of reduced β -nicotinamide adenine dinucleotide (NAD). 10 μ l of the sample was reacted with 110 μ l of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of AST. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

The principal of the method is as follows:

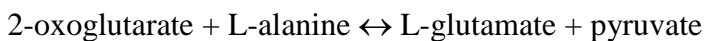


3.5.2.2 Determination of the activity of alanine aminotransferase (ALT)

The ALT reagent was used to measure alanine aminotransferase in the sample by an enzymatic kinetic UV rate method. In the assay reaction, the ALT catalyzed the reversible transamination of L-alanine and α -ketoglutarate to pyruvate and L-glutamine. The pyruvate then reduces to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β -Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β -Nicotinamide Adenine Dinucleotide (NAD).

Pyridoxal-5-phosphate was required in this reaction as a cofactor that was required for transaminase activity by binding to the enzyme using Schiff-base linkage. 10 μ l of the sample was reacted with 110 μ l of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of ALT. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

The principal of the reaction is as follows:

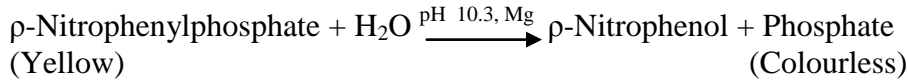


3.5.2.3 Determination of the activity of alkaline phosphatase (ALP)

Alkaline phosphatase reagent was used to measure alkaline phosphatase activity by a kinetic UV method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction alkaline phosphatase catalyzed the hydrolysis of the colorless organic phosphate ester substrate, *p*-nitrophenylphosphate to the yellow colored product, *p*-nitrophenol and phosphate. The reaction occurred at an alkaline pH of 10.3. 5 μ l of the sample was reacted with 250 μ l of the reagent. The change in absorbance was monitored at 410 nm and this

change was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37°C for three minutes.

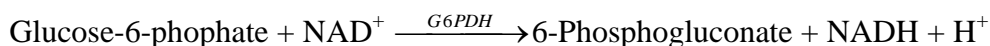
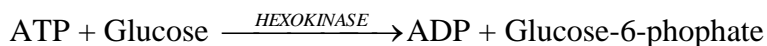
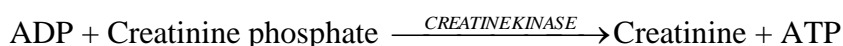
The principal of the reaction is as follows:



3.5.2.4 Determination of the activity of creatine kinase (CK)

Creatine kinase activity was determined using the method described by Oliver and Rosalki which is based on the primary reaction that is catalyzed by CK resulting in production of creatine and ATP. The ATP produced in the primary reaction is then used in a coupled enzymatic glucose assay using hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD). The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5 µL: 250 µL reagent). The machine calculated and expressed the activity in IU/L. The reaction took place at 37°C for three minutes. The production of NADPH in the indicator reaction is monitored at 340 nm and is related to CK activity within the patient specimen (Rosalki, 1967).

The principal of the method is as follows:



3.5.2.5 Determination of the blood levels of urea (BUN)

BUN was analyzed using the Glutamate Dehydrogenase (GLUDH) method. BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction, urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate

dehydrogenase (GLDH) catalysed the condensation of ammonia and α -ketoglutarate to glutamate with the concomitant oxidation of reduced β -nicotinamide adenine dinucleotide (NADH) to oxidized β -nicotinamide adenine dinucleotide (NAD). 3 μ l of sample was reacted with 300 μ l of reagent and the change in absorbance was monitored at 340 nm, due to NADH oxidation. This change was directly proportional to the concentration of BUN in the sample and was used to calculate and express concentration in mmol/L. The reaction took place at 37°C for one minute.

The principal of this method is as follows:



3.5.3 Quality control (QC)

Precinorm U (normal upper) and precipath U (pathological upper) for all the parameters from Roche Diagnostics were the quality control materials used during the study period. Before use, a QC bottle was carefully opened and exactly 3 ml distilled water pipetted carefully into the bottle, closed, and carefully dissolved by gentle swirling within 30 minutes. This was then aliquoted into six cryovials and stored at -20°C. Calibrator used the same types of tubes and racks as samples. A refrigerated rack position in the machine improved the stability of on-board controls. The system performed controls automatically according to the specifications in the test definition.

3.6 Determination of phytochemicals

3.6.1 Qualitative screening of phytochemicals

A phytochemical screening of phenolics, alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, cardiac glycosides, glycosides/reducing sugars, and phylobatannins present in plant extracts was performed using standard methods.

The phenolics were determined as follows: to 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicates phenolics (Houghton and Raman, 1998). For the alkaloids, to the 2 ml methanolic filtrate of the extract, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayers reagents/Wagner's reagent/Dragendorff reagent was added. Formation of Orange precipitate was an indication of the presence of alkaloids (Krishnaiah *et al.*, 2009).

To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of magnesium turnings was added. After 3 minutes magenta red or pink color indicated the presence of flavonoids (Jigna and Sumitra, 2007). Aqueous extract of 2 g powder of the plant material was made and subjected to frothing test. Frothing persistence indicated presence of saponins (Houghton and Raman, 1998), then the froth was mixed with few drops of olive oil and the formation of emulsion confirmed the presence of saponins (Houghton and Raman, 1998). To 2 ml of aqueous extract, 2 ml of 5% FeCl₃ was added. Formation of yellow brown precipitate indicated that tannins are present (Jigna and Sumitra, 2007).

The terpenoids were screened by adding to 2 ml of aqueous extract, 5 ml chloroform, 2 ml acetic anhydride and concentrated H₂SO₄ was added carefully to form layer. Reddish brown coloration of interface indicates terpenoids (Krishnaiah *et al.*, 2009). For steroids, 200 mg of each aqueous extract was boiled in 10 ml of chloroform and filtered. To 2 ml of

the filtrate, 2 ml of acetic anhydride and 2 drops of H_2SO_4 were added. Gradual appearance of blue-green ring indicated presence of steroids (Trease and Evans, 2002).

Cardiac glycosides were screened in the plant material as follows; to 2 ml alcoholic filtrate, 1 ml glacial acetic acid and 1-2 drops of FeCl_3 was added followed by 1 ml of concentrated H_2SO_4 . The appearance of brown ring at the interface indicated presence of cardiac glycosides and a violet ring may also appear below the brown ring (Krishnaiah *et al.*, 2009). For the presence of glycosides, to 5 ml of the Fehling's solution mixture, 2 ml of each plant extract was added and the solution was boiled in a water bath for five minutes. Appearance of brick-red precipitate was an indication of free reducing sugar/glycosides (Brain and Turner, 1975); for the presence of phylobatannins, 10 ml of aqueous extracts of each plant sample was boiled with 1% HCl acid in a test tube or conical flask. Deposition of a red precipitate indicated the presence of phylobatannins (Trease and Evans, 2002).

3.6.2 Quantitative determination of phytochemicals

The amounts of various phytochemicals screened were determined as follows; the total phenolic content was determined using Folin-Ciocalteu reagent and gallic acid as the standard according to the method by Rasineni *et al.* (2008). About 500 mg of milled plant material powder was weighed and homogenized in 10 ml of n-hexane. The homogenate was centrifuged at $10,000 \times g$ for 20 minutes and the supernatant was used in the determination of total phenols as follows. About 0.5 ml of Folin-Ciocalteu 2 N reagent was added to 2.5 ml of the supernatant and then 2 ml of 10% sodium carbonate in ethanol. The mixture was incubated for 5 minutes at 20°C and then the absorbance read in triplicates at wavelength of 650 nm.

The gallic acid standard was prepared by dissolving 100 mg of gallic acid (SD'S Lab-Chem Industry Bombay-India), in 100 ml of distilled water to make the standard stock. This was serially diluted into the working range of; 0.5, 1, 2, 4, 8 and 16 mg/100 ml. To each 2.5 ml of the serially diluted standard, 2 ml of the 10% w/v sodium carbonate solution and 0.5 ml of Folin-Ciocalteu 2 N were added and incubated for 5 minutes. The absorbance was read at 750 nm before 15 minutes. The mixture of distilled water, 10% sodium carbonate and Folin-Ciocalteu 2 N was used as the blank. The absorbances were read from UV-VIS spectrophotometer (UV-1700 Pharmaspec, UV-VIS Spectrophotomer, Shimadzu Japan). The total phenol content was expressed as mg/g dry weight gallic acid equivalent.

The flavonoids were determined by the method according to Kumaran and Karunakaran. (2007), with slight modifications. 5 ml of 2% aluminium trichloride in methanol with the same volume of aqueous plant extract. The absorbance was then read at 420 nm after one hour of incubation against a blank sample of 5 ml extract solution with 5 ml methanol without aluminium trichloride. The total flavonoid was determined using a standard curve of pyrocatechol ranging from 0.5 to 16 mg/100 ml, and the results expressed as mg/g pyrocatechol equivalent.

Alkaloid were determined according to the method by Edeoga *et al.* (2005) with slight modifications as follows: 2.5 g of the plant material powder was extracted using 100 ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed (Edeoga *et al.*, 2005).

Similarly, saponin was determined as per the method by Edeoga *et al.* (2005) with slight modifications: 10 g of sample was mixed with 100 ml of 20% aqueous ethanol. The mixture was kept for 4 hours on water bath shaker at 55°C. Filtrate was again extracted in same manner. The combined extract were concentrated to 40 ml over water bath at 90°C. Concentrate obtained was transferred into a separating funnel and 10 ml of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ether layer was discarded. The process was repeated and to the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10 ml 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Latter it was dried in oven to a constant weight (Edeoga *et al.*, 2005).

The tannins were determined as follows: 2 g of plant powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant was removed. Different aliquots were taken and final volume to 3 ml was adjusted by distilled water. The solution after vortexing were mixed with 1 ml of 0.016M $K_3Fe(CN)_6$, followed by 1 ml of 0.02 M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. The stabilizer was prepared in the ration of 3:1:1 of water, H_3PO_4 and 1% gum arabic, and then 5 ml was added to the mixture followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001 M gallic acid (Gurib-Fakim, 2006).

3.7 Determination of phytonutrients

3.7.1 Determination of vitamins A and E by HPLC method

Vitamin A and E are sensitive to UV-radiation and to oxidizing agents such as atmospheric oxygen. Therefore it is necessary to exclude UV-light and oxygen by nitrogen flushing when handling it.

Approximately 2 g of milled medicinal plant material was weighed in a round bottomed flask and dissolved in 50 ml of methanol. To the mixture, 0.25 g of ascorbic acid or butylated hydroxylated toluene (BHT) was added and 5 ml of 50% sodium hydroxide. The mixture was blanketed with nitrogen and saponified in a water bath at 55 to 60°C for 1 hour with intermittent shaking after every 20 minutes. During saponification process the flasks were protected from direct sunlight to prevent degradation of vitamins E and A. Then the flasks were cooled in a running stream of cold water. 50 ml of distilled water was added to the sample and then transferred into a separating funnel.

Retinol was extracted from the sample using 70 ml of n-hexane containing 30 µg/g BHT. For optimal extraction the separating funnel was gently shaken while avoiding pressure build up. The phases were allowed to separate and the aqueous phase drained to the round bottomed flask and the hexane layer into a conical flask covered with aluminium foil. The procedure was repeated two times with 50 ml of n-hexane containing 30 ppm of BHT. The extract was then evaporated in a rotary evaporator under reduced pressure and not more than 50°C. The remaining extract was reconstituted in 10 ml of methanol, filtered and injected into HPLC for the determination of alpha tocopherol and retinol.

The remaining extracts were reconstituted in 10 ml of methanol, filtered, and 10 µL injected into HPLC for the determination of α-tocopherol and retinol. 100 mg of α-

tocopherol standard (Fluka Biochemica, purity $\geq 97.0\%$ HPLC grade) was dissolved in 100 ml of absolute ethanol. The concentration was determined using UV-VIS spectrophotometer (UV-1700 Pharmaspec, UV-VIS Spectrophotometer Shimadzu, Japan) at 291 nm, the wavelength of maximum absorbance for α -tocopherol dissolved in absolute ethanol. The molar extinction coefficient of α -tocopherol in absolute ethanol is 75.6. The concentration of the stock standard solution was determined using the formula:

$$\text{Concentration of } \alpha\text{-tocopherol } (\mu\text{g/ml}) = \frac{\text{Mean Absorbance} \times 10^4}{75.8}$$

Three determinations of absorbance were made and the mean absorbance recorded. The concentration of the working standard solution for α -tocopherol was found to be 1.17 $\mu\text{g/ml}$.

For vitamin A, 100 mg of vitamin A-palmitate standard (Fluka, Lot 1319695 of 92% purity) was dissolved in 100 ml of absolute ethanol. Three determinations of absorbance were carried out, and the mean absorbance at a wavelength of 324.3 nm was recorded for determination of the actual concentration of the standard. The molar extinction coefficient of 1830 for vitamin A-palmitate in absolute ethanol was used and the working concentration determined using the following expression;

$$\text{Concentration of retinol } (\mu\text{g/ml}) = \frac{\text{Mean Absorbance} \times 10^4}{1830}$$

The concentration of the working stock solution for vitamin A was found to be 2.09 $\mu\text{g/ml}$. The HPLC system was set as follows: flow rate of 1.0 ml/minutes, column oven temperature 35°C, and injection volume of 10 μl and run time of 6.0 minutes. The UV-VIS detector wavelength of 325 nm was used. The standard working solution and the samples

were injected three times and the mean peak area was used in the determination of the concentrations of retinol from the medicinal plants.

HPLC mobile phase was prepared by mixing methanol and HPLC grade water in the ratio of 70:30. The mobile phase was sonicated and then filtered into a reservoir ready for use. Waters Spherisorb ODS-1 column of particle size 5 μm , 250 mm long and internal diameter 4.6 mm was used. The column was balanced with a mobile phase until a good baseline suitable for analysis was obtained. Concentration of α -tocopherol, and vitamin A in the medicinal plant materials were determined using the formula;

$$\text{Concentration (mg/100g)} = \frac{A_S \times \text{Cal}_T \times V_S \times V_{st} \times 100}{A_T \times M \times V_{is} \times 1000}$$

where: A_s is the peak area of the sample; Cal_T is the concentration of the standard solution in $\mu\text{g/ml}$; V_s is the total volume of sample test solution in ml; V_{st} is the injection volume of the standard solution in μl ; A_T is the peak area of the standard solution in $\mu\text{g/ml}$; M is the mass in grammes of the sample; V_{is} is the injection volume for the sample test solution in μl ; **1000** is the conversion factor from μg to mg; **100** is the factor for the calculation of the mass concentration per 100 g.

α -tocopherol was determined in triplicates by injecting 10 μl of the filtered sample and the standard. The HPLC system was set as follows: flow rate of 1.2 ml/minutes, column oven temperature 25°C, and injection volume of 10 μl and run time of 11.50 minutes. For the fluorescence detector, the excitation wavelength of 290 nm and emission wavelength of 330 nm were set. Between the standards and samples, a blank comprising of filtered mobile phase was injected to prevent carry over. The standard working solution and the samples were injected three times and the mean peak area was used in the determination of the concentrations of the α -tocopherol.

3.7.2 Determination of β -carotene, β -cryptoxanthin and lycopene

The saponified samples were analyzed for β -carotene with HPLC-UV method. Mobile phase was composed of methanol and acetonitrile in the ratio of 90:10 with 0.05% v/v of triethanolamine. The flow rate was set at 2.5 ml/min, column oven at 25°C and detector wavelength at 451 nm. 2 mg of β -carotene (Sigma, purity \geq 93%) standard was dissolved in 10 ml absolute ethanol and actual concentration determined spectrophotometrically. The wavelength of maximum absorbance, mean absorbance and the molar extinction coefficient of β -carotene in absolute ethanol were recorded and used in the determination of the actual concentration of the working standard. The molar extinction coefficient of β -carotene in absolute ethanol was found to be 2560.

The concentration of the working standard concentration was calculated using the following equation:

$$\text{The working concentration of } \beta\text{-carotene } (\mu\text{g/ml}) = \frac{\text{Mean Absorbance} \times 10^4}{2560}$$

The samples and the standards were analyzed in triplicates and the mean peak area, standard deviations and % coefficient of variation were determined.

1 mg of lycopene standard purchased from Sigma Aldrich, USA (L9879, > 90% purity, Lot No 040M5162V) was dissolved in 5 ml of n-hexane and vortexed until it dissolved completely. The solution was scanned in a UV-VIS spectrophotometer at a wavelength range of 450 to 510 nm to determine the wavelength of maximum absorbance. Then absorbance was determined in triplicates and the mean determined using molar extinction coefficient of lycopene in n-hexane as 3450. 1 ml of the standard stock was diluted 10 times to make a working solution. 1 mg of β -cryptoxanthin standard purchased from

Sigma Aldrich, USA (CAS472-70-8; C₄₀H₅₆O, > 98% purity) was dissolved in 5 mL of absolute ethanol (Merck Chemicals Ltd, South Africa), and vortexed until it dissolved completely. The solution was scanned in a UV-Vis spectrophotometer at a wavelength range of 430-490 nm to determine the wavelength of maximum absorbance. The absorbance at the wavelength of maximum was determined in triplicates, and the mean calculated for determination of the actual concentration of the standard using molar extinction coefficient of β -cryptoxanthin in absolute ethanol as 2356. 1 ml of the standard stock was diluted 10 times to make a working solution.

Mobile phase for HPLC was prepared by mixing methanol, acetonitrile, and tetrahydrofuran in the ratio of 70:25:5 (v/v). The mixture was sonicated to remove air bubbles. The extraction solution was prepared by mixing methanol, and tetrahydrofuran in 50:50 v/v, and this was also used as an experimental blank. The Waters Spherisorb (ODS-5 μ , Lot No 122, Part No 8364, length 250 mm x 4.6 mm, Serial No 05021098.1) HPLC column was conditioned at oven temperature of 25°C, flow rate of 1.0 mL/min, and wavelengths 451 nm (β -carotene), 471 nm (lycopene), and 452 nm (β -cryptoxanthin). The standards and the samples were analyzed in triplicates, and mean peak areas, standard deviation and % coefficient of variation recorded. Single point calibration was used in quantitation, and the amounts were reported in mg/100g of dry matter \pm standard deviation.

3.7.3 Determination of ascorbic acid

Ascorbic acid was determined in the extracts as total L+ and D+ ascorbic acids with HPLC-UV method in 2% metaphosphoric acid and ascorbic acid standard of 99.7% purity was used as the reference. Briefly, 1 g of the milled plant material was extracted in 10 ml of 2% metaphosphoric acid for 1 hour. The extraction was done in amber flasks covered

with aluminium foil and sonicated at room temperature. The extract was filtered with Whatman filter paper No 540 and further filtered with 0.54 µm membrane filter ready for injection into the HPLC. 50 mM of potassium dihydrogen phosphate was prepared in HPLC grade water and the pH adjusted to 2.4 with phosphoric acid. The mobile phase was filtered, and then sonicated to remove air bubbles.

The wavelength was set at 265 nm, flow rate at 2.0 ml/minute and oven temperature at 15°C. C₁₈ Phenomenex column 175 mm x 3.20 mm x 5 µm internal diameter was used. The baseline was attained by balancing the column with mobile phase, and extraction solution as a blank. Serial dilution of the ascorbic acid standard was prepared at a concentration range of 0.4 to 5.3 mg/100 g. The linearity of the curve, the limit of detection and quantitation were determined from the standards. Then 10 µl of the samples were injected into the HPLC system and peak areas recorded and used in determination of the amounts of ascorbic acid from the medicinal plants materials.

3.7.4 Determination of thiamine and niacin

Thiamine and niacin were determined by dissolving 1 g of the powdered plant material in 25 ml of extraction solution which comprised of 50 ml acetonitrile and 10 ml of glacial acetic acid, and topped up to 100 ml with HPLC grade water, while shaking on a water bath at 30°C for 40 minutes. The samples were cooled, filtered and the final volume adjusted to 50 ml with the extraction solution.

A standard for vitamin B1 was prepared by dissolving 0.1 g of thiamine hydrochloride (thiamine HCl, Lot No 36020, Serra Heidelberg, Germany) in 100 ml of HPLC grade water. The linear range for the working dilutions of the standard was 5-30 mg/kg. For

vitamin B3, 0.1 g of nicotinamide (Niacinamide, Lot 37F-0018, Sigma Aldrich, USA) was dissolved in 100 ml of HPLC water and working solutions ranged from 5 to 30 mg/kg.

The HPLC mobile phase comprised of 0.023 M H₃PO₄ prepared by dissolving 2.25 g of H₃PO₄ in 1 L of HPLC grade water, and pH adjusted to 3.54 with NaOH. The mixture was filtered and degassed ready for use. A Cronus HPLC column (Lot No CC011569, NF-00909 Lichrospher 100RP-18EC 5 μ \times 25 \times 0.46 mm) was used in an isocratic mobile phase system, flow rate set at 1.7 ml/min for vitamin B3 and 1.5 ml/min for vitamin B1 and the UV-Vis detector wavelength was 261 and 234 nm, for vitamin B3 and B1, respectively. The oven temperature was set at 30 and 25°C for vitamin B3 and B1, respectively. For each standard dilution level and medicinal plant extracts, 1 μ l was injected three times and peak areas recorded at the retention times for vitamins B1 and B3, respectively and these were used in determination of the amounts in medicinal plants using the equation of line from the serial dilution of the standards.

3.8 *In vitro* antioxidant activities of the plant extracts

3.8.1 Free radical scavenging activity by DPPH assay

The free radical scavenging activities of the medicinal plants was determined by 11-diphenyl-2-picrylhydrazyl (DPPH) method according to Brand-Williams *et al.* (1995). In this method, a stock solution was prepared by dissolving 2.4 mg of DPPH free radical in 100 ml of methanol. The solution was kept at 20°C until required. The working solution was prepared by diluting DPPH stock solution with methanol till the absorbance was noted to be 0.98 ± 0.02 at 517 nm. Then, 3 ml of the working solution was mixed with 100 μ l of a methanol extract of the medicinal plant (1 mg/ml). After incubating the mixture in the dark for 30 min, absorbance was measured at 517 nm.

The scavenging activity was calculated by using the formula:

$$\text{Percent scavenging activity} = \frac{(\text{Absorbance of the blank} - \text{Absorbance of the sample})}{\text{Absorbance of the blank}} \times 100\%$$

The blank contained all reagents except the medicinal plant extract. Ascorbic acid at a concentration of 1 mg/ml was used as reference.

3.8.2 Reducing power assay

The reducing power assay was carried out by the method of Oyaizu (1986), with some modifications. A plant extract or gallic acid solution (25-800 µg/ml; 2.5 ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 2.5 ml of trichloroacetic acid solution (100 mg/l) was added. The mixture was centrifuged at 650 rpm for 10 min, and 5 ml of the supernatant was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride solution. The absorbance was measured at 700 nm and recorded.

3.8.3 Total antioxidant activity by phosphomolybdate assay

To carry out phosphomolybdate assay, the procedure described by Umamaheswari and Chatterjee (2008) was used. The phosphomolybdate reagent was prepared by mixing equal volumes (100 ml) of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Test samples were prepared by dissolving 1 mg of medicinal plant methanolic extract in 1 ml of methanol. Then, 0.1 ml of the sample was dissolved in 1 ml of reagent solution in a test tube which was capped with silver foil and incubated in water bath at 95°C for 90 min. After cooling the sample to room temperature, the absorbance was observed at 765 nm against a blank. Ascorbic acid was used as a standard antioxidant with concentration ranging from 10 to 50 mg/l. The ascorbic acid absorbances were used in the

construction of the standard curve. The results were expressed as μg of ascorbic acid equivalent (AAE) per mg of the dried weight of the sample as determined from the equation of the standard calibration curve and the following expression was used to calculate the AAE:

$$\text{Ascorbic acid equivalent} = \frac{\text{Absorbance at } 765\text{nm}}{0.0034} \text{ } (\mu\text{g}/\text{mg of dried matter}).$$

3.9 Proximate composition

Proximate composition includes moisture, crude protein, ether extract for fat content, crude fiber, ash and nitrogen free extract (NFE). The dried medicinal plant materials were weighed into various proportions for proximate analysis.

3.9.1 Determination of the moisture content

Moisture was determined by oven drying method (AOAC, 2000). Medicinal plant powder were well-mixed and 2 g was accurately weighed in clean, dried crucible. The crucible was allowed in an oven at 100-105°C for 6-12 hour until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 minutes to cool. After cooling it was weighed again, the percent moisture content was calculated by the following formula:

$$\% \text{ moisture} = \left(1 - \frac{\text{Weight Dry Sample}}{\text{Weight Wet Sample}} \right) \times 100$$

3.9.2 Determination of ash content

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccator and then weight of empty crucible was noted (W1). One gram of each of the medicinal plant powder was taken in crucible (W2). This was then ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550°C for 2-4 hour. The appearances of gray

white ash indicate complete oxidation of all organic matter in the medicinal plant material. After ashing furnace was switched off. The crucible was cooled and weighed (W3). Percent ash was calculated by following formula:

$$\% \text{ Ash} = \frac{\text{Difference in weight of Ash}}{\text{Weight of the medicinal plant powder}} \times 100$$

Difference in weight of ash = W3 - W1

3.9.3 Determination of crude protein content

Protein in the sample was determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H₂SO₄) in the presence of digestion mixture. The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated. To 1.0 g of dried samples in digestion flask 15 ml of concentrated H₂SO₄ was added and 8 g of digestion mixture composed of potassium sulphate and copper sulphate in the ration of 8: 1

The flask was swirled in order to mix the contents thoroughly and then placed on heater to start digestion till the mixture became clear (blue green in color) for 2 hours. The digest was cooled and transferred to 100 ml volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus (Khalil and Manan, 1990). Briefly, 10 ml of digest was introduced in the distillation tube then 10 ml of 0.5 N NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH₃ produced was collected as NH₄OH in a conical flask containing 20 ml of 4% boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color appears due to NH₄OH.

The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink color. A blank was also run through all steps as above. Percent crude protein content of the sample was calculated by using the following formula:

$$\% \text{ Crude Protein} = 6.2 * \times \% N .$$

(Whereby * stands for the Correction factor).

$$\% N = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V}$$

Where: S = sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of the medicinal plant material after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

3.9.4 Determination of crude fat content

Dry extraction method for fat determination was applied. It consisted of extracting dry medicinal plant materials with some organic solvent, since all the fat materials like fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together therefore, the results are frequently referred to as crude fat. Fats were determined by intermittent Soxhlet extraction apparatus. Crude fat was determined by ether extract method using Soxhlet apparatus.

Approximately 1 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into the apparatus. Turned on water and heater to start extraction. After 4-6 siphoning ether was allowed to evaporate

and disconnect beaker before last siphoning. Transferred extract into clean glass dish with ether washing and evaporated ether on water bath. Then placed the dish in an oven at 105°C for 2 hrs and cooled it in a desiccator. The percent crude fat was determined by using the following formula:

$$\% \text{ Crude fat} = \frac{\text{Weight of ether extract}}{\text{Weight of medicinal plant material}} \times 100$$

3.9.5 Determination of crude fiber content

A moisture free and ether extracted sample of crude fiber made of cellulose was first digested with dilute H₂SO₄ and then with dilute KOH solution. The undigested residue collected after digestion was ignited and loss in weight after ignition was registered as crude fiber. The medicinal plant material was weighed (W₀ of 0.153 g) and transferred to porous crucible. The crucible was then placed into Dosi-fiber unit and the valve kept in “OFF” position. After that 150 ml of preheated H₂SO₄ solution was added and some drops of foam-suppresser to each column.

The cooling circuit was then opened and turned on the heating elements (power at 90%). When it started boiling, the power was reduced to 30% and left for 30 min. Valves were opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid from sample. The same procedure was used for alkali digestion by using KOH instead of H₂SO₄. Then the sample was dried in an oven at 150°C for 1 hour and then allowed to cool in a desiccator and weighed (W₁). The samples were kept in crucibles in muffle furnace at 55°C for 3-4 hours. The samples were cooled in a desiccator and weighed again (W₂). The percent crude fiber was calculated as follows:

$$\% \text{ Crude Fiber} = \frac{W_1 - W_2 \times 100}{W_0}$$

3.9.6 Determination of nitrogen free extract

Nitrogen free extract (NFE) was calculated by difference after analysis of all the other items in the proximate analysis. The following equation was applied:

$$\text{NFE} = (100 - \% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fiber} + \% \text{ ash}).$$

3.9.7 Determination of the energy content

The percent calories in the medicinal plants powders were calculated by multiplying the percentage of crude protein and carbohydrate with 4 and crude fat with 9 as Atwaters factors. The values were then converted to calories per 100 g of the sample.

3.10 Chemical compounds from the ethylacetate extracts of the medicinal plants

Chemical compounds in the organic plant extracts was performed by gas chromatograph interfaced with mass spectrometer (GC-MS) triple quad system (Agilent Technologies 7890A fitted with Agilent 7693 auto-sampler, and installed with Mass Hunter Workstation Software for qualitative analysis version B.05.00 build 5.0.519.0, service pack I © Agilent technologies 2011).

A capillary column DB-5 (30 m long × 0.25 mm ID and 0.25 μm composed of 100 % dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.9999% purity) was used as the carrier gas at the constant flow rate of 1 ml/min and an injection volume of 2 μl was employed (split ratio of 10:1); injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programed from 110°C (isothermal for 2 min), with an increase of 10 to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. The

chemical compounds present in the extracts were identified based on their mass spectra by deconvolution and matching with the compounds in the NIST 2011 database.

3.11 Elemental analyses by energy dispersive X-ray fluorescence spectroscopy

Energy dispersive X-ray fluorescence spectroscopy (EDXRF) system was used to determine the content of sodium, aluminium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, selenium and molybdenum in plant materials, and the amounts were expressed as $\mu\text{g/g}$. About 100 mg of homogenous sample was pressed into 1 mm thick and 10 mm diameter and placed onto the sample tray. Total reflection X-ray fluorescence system analysis consists of an x-ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd^{109} (half-life, $T_{1/2} = 453$ days and activity = 10 mCi) are incident on the sample that emits the characteristic X-rays.

These X-rays are detected by Si (Li) detector (EG & G Ortec, $30 \text{ mm}^2 \times 10 \text{ mm}$ sensitive volume, 25 μm Be window) with an energy resolution of 200 eV at 5.9 keV Mn K_{α} - line. The spectral data for analysis were collected using personal computer based Canberra S-100 multi-channel analyzer (MCA). The acquisition time applied in the TXRF measurement was 1000 seconds. For data analysis, the X-ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS, 1992) that is based on the fundamental parameters method (FPM). With this method, if the type and properties of all elements contained in a sample are known, then the intensity of each fluorescent X-ray is derived theoretically. By using this method, the composition of unknown sample is extrapolated by its fluorescence X-ray intensity of each element. The concentration values obtained were corrected by multiplying with the respective dilution factors. The final values were expressed as mg/kg dry matter.

3.12 Data management and statistical analysis

Data was entered in the Microsoft® Excel spread sheet, cleaned and then exported to Statistical Package for Social Sciences (SPSS Version 17.0) software for analysis. Results were expressed as Mean \pm Standard Deviation (SD) of the blood glucose levels per the number of mice used in every study point.

One-way ANOVA and post-ANOVA (Bonferroni-Holm) test was used to compare the means of untreated normal control mice with diabetic mice treated with physiological saline, diabetic mice treated with the conventional drug and diabetic mice treated with plants extracts at doses of 50, 100 and 200 mg/kg body weight. Student's t-test was used to compare the means of body weight and body weight changes, haematological and biochemical parameters of normal control mice administered with 0.1 ml physiological saline with those of normal mice administered with 1g/kg body weight plants extracts. $P < 0.05$ was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Yields of ethyl acetate and aqueous crude plants extracts

Pappea capensis aqueous stem bark extract yielded a brown paste at a concentration of 160 mg/g dry weight while the ethyl acetate stem bark extract of the same plant yielded a light red brown lyophilate at a concentration of 28.08 mg/g dry weight. The ethyl acetate leaf extracts of *P. capensis* yielded a 32.84 mg/g dry weight light green paste, while the aqueous leaf extracts of the same plant yielded 176.92 mg/g dry weight light green paste of concentration (Table 4.1).

The ethyl acetate stem bark extract of *Senna spectabilis* yielded a black paste of 315.6 mg/g dry weight while the aqueous stem bark extract of the same plant yielded a black paste of 146 mg/g dry weight. The ethyl acetate leaf extract of *S. spectabilis* yielded a black paste of 278 mg/g dry weight while the aqueous leaf extract of the same plant yielded a black paste of 186 mg/g dry weight.

For the *Ocimum americanum*, the ethyl acetate and aqueous extracts yielded a dark brown paste and a light brown paste, respectively, of concentrations, 215.7 and 168.0 mg/g dry weight, respectively. For the *Launea cornuta*, the ethyl acetate and aqueous extracts yielded a light green paste and a dark brown paste, respectively, of concentrations 183.12 and 50.0 mg/g dry weight, respectively. For the *Maytenus obscura*, the ethyl acetate and aqueous leaf extracts were dark green and dark brown, respectively, of concentrations 73.96 and 120.27 mg/g dry weight, respectively. For the *M. obscura*, the ethyl acetate and aqueous stem bark extracts were dark brown and light brown, respectively, at concentrations of 201 and 153.21 mg/g dry weight, respectively. The yields for all crude plants extracts are shown in table 4.1.

Table 4.1: Yield of crude plants extracts (mg/g dry weight)

Plant	Solvent	Leaves	Stem barks
<i>P. capensis</i>	Ethyl acetate	32.84	28.08
	Water	176.92	160
<i>S. spectabilis</i>	Ethyl acetate	278	315.6
	Water	186	146
<i>O. americanum</i>	Ethyl acetate	215.7	
	Water	168	
<i>M. obscura</i>	Ethyl acetate	73.96	153.21
	Water	120.27	201
<i>L. cornuta</i>	Ethyl acetate	183.12	
	Water	50.0	

4.2 Effects of oral administration of extracts on blood glucose levels

4.2.1 Optimum dose for induction of hypoglycemia in BALB/c mice

As shown in table 4.2, the optimum intraperitoneal dose of alloxan-monohydrate required to induce diabetes in male BALB/c mice is 186.9 mg/kg body weight after 48 hours. Doses below 186.9 mg/kg body weight had no effect on the blood glucose levels even after 48 hours; the blood glucose levels of the experimental BALB/c mice were similar to those of the control mice for all the tested doses. Intraperitoneal alloxan doses of 290.0 and 480 mg/kg body weight induced blood glucose levels to levels similar to those induced by the dose of 186.9 mg/kg body weight; however, one mouse died at the 480 mg/kg body weight dose on the second day post alloxan administration.

Table 4.2: Optimal intraperitoneal dose of alloxan-monohydrate required to induce hypoglycemia in male BALB/c mice

Dose and mice groups (in mg/kg body weight)	Body weight (g)	Blood glucose (mM) per day		
		0	1	2
Control	24.20±4.66	7.83±1.00	7.91±0.25	7.57±0.40
50.0	19.80±1.92	7.22±0.73	6.30±0.67	7.13±2.21
77.6	21.20±2.59	8.34±1.28	8.38±1.11	8.00±1.70
120.4	16.67±3.21	7.41±0.37	6.97±0.91	6.46±1.50
186.9	15.00±3.24	7.42±1.26	8.38±0.93	32.54±6.25 ^{Abc}
290.0	17.00±1.87	7.51±1.20	7.66±0.14	32.98±12.73 ^{Abc}
480.0	17.20±1.64	7.42±1.26	9.62±1.94	30.68±13.55 ^{Abc}

Results are expressed as Mean ± Standard Deviation (SD) for five animals per dose. Means followed by similar upper case letters and without letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

4.2.2 Effects of oral administration of extracts of *P. capensis*

Oral administration of ethyl acetate leaf extracts of *P. capensis* at 100 and 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the fourth hour through to the 8th hour in a dose independent manner. In the fourth hour, the ethyl acetate extracts at 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 86.5% and 68.3%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 72.4% within the same hour. By the eighth hour, the ethyl acetate extracts at 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 55.4% and 34.5%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 21.9% within the same hour (Table 4.3; Figure 4.1).

Oral administration of ethyl acetate stem bark extracts of *P. capensis* at 100 and 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the fourth hour through to the 8th hour in a dose independent manner. In the fourth hour, the ethyl acetate stem bark extracts at 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 43.5% and 35.4%, respectively, compared to glibenclamide

treated diabetic mice whose blood sugar levels was lowered to 22.3% within the same hour. By the eighth hour, the ethyl acetate extracts at 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 36.9% and 38.1%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 19.4% within the same hour. It was also found that the ethyl acetate leaves extracts demonstrated significantly high hypoglycemic activity compared to the ethyl acetate stem barks extracts of the *P. capensis* (Table 4.4; Figure 4.2).

Table 4.3: Hypoglycemic effects of oral administration of ethyl acetate leaf extracts of *P. capensis* in alloxan-induced diabetic male BALB/c mice

Mice Group	Treatment	Blood glucose levels (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.5±0.3	6.3±0.3	6.2±0.1	5.8±0.3	5.1±0.1 ^g
Diabetic control	Saline	24.8±3.1 ^A	26.6±3.2 ^A	27.0±2.6 ^A	27.7±2.8 ^A	29.3±2.6 ^C
Diabetic control	Glibenclamide (3 mg/kg body weight)	28.3±1.4 ^B	24.8±2.0 ^A	20.5±2.0 ^A	11.3±2.0 ^{cf}	6.2±0.8 ^{dgi}
	Extracts					
Diabetic treated	50 mg/kg body weight	28.0±1.9 ^B	26.6±1.6 ^A	25.7±1.3 ^A	24.3±1.3 ^A	22.0±1.3 ^B
	100 mg/kg body weight	32.7±0.4 ^B	31.0±0.6 ^A	28.3±0.9 ^{Ab}	24.7±1.4 ^{Acf}	18.1±0.7 ^{Bdgi}
	200 mg/kg body weight	28.4±1.4 ^B	22.7±2.3 ^A	19.4±1.9 ^{Ab}	15.0±2.0 ^c	9.8±0.6 ^{Adgi}

Results were expressed as Mean ± Standard Error of Mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^b $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour; ^c $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour; ^d $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at 8th hour; ^f $p \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 6th hour; ^g $p \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 8th hour; ⁱ $p \leq 0.05$ when blood glucose levels at the 4th hour is compared to blood glucose at the 8th hour; ^j $p \leq 0.05$ when blood glucose levels at the 6th hour is compared to blood glucose at the 8th hour by ANOVA and post ANOVA (Bonferroni-Holm) test.

Table 4.4: Hypoglycemic effects of oral administration of ethyl acetate stem bark extracts of *P. capensis* in alloxan-induced diabetic BALB/c mice

Mice Group	Treatment	Blood glucose levels (mM)				
		0 hr	2 hr	4 hr	6 hr	8 hr
Normal control	Saline	5.1±0.6	6.3±0.3	6.1±0.6	4.7±0.3	5.7±0.5
Diabetic control	Saline	16.5±0.3 ^A	21.2±1.9 ^B	23.8±1.7 ^{Abe}	19.3±2.5 ^A	30.2±1.1 ^{Adgi}
Diabetic control	Glibenclamide (3 mg/kg body weight)	20.6±2.9 ^A	15.3±2.4 ^B	4.6±0.7 ^{be}	4.5±0.4 ^{cf}	4.0±0.4 ^{dg}
	Extracts					
Diabetic treated	50 mg/kg body weight	17.4±0.5 ^A	14.5±1.7 ^B	14.5±4.9	11.7±4.4	13.9±4.9
	100 mg/kg body weight	16.8±0.5 ^A	7.6±0.2 ^{Aa}	7.3±0.5 ^b	6.5±0.5 ^c	6.2±0.3 ^{dg}
	200 mg/kg body weight	18.1±2.2 ^A	11.6±0.8 ^B	6.4±0.6 ^{be}	6.1±0.4 ^{cf}	6.9±0.3 ^{dg}

Results were expressed as Mean ± Standard Error of Mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by One-Way ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour; ^b $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour; ^c $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour; ^d $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 8th hour; ^e $p \leq 0.05$ when blood glucose levels at the 2nd hour is compared to blood glucose at the 4th hour; ^f $p \leq 0.05$ when blood glucose levels at the 2nd hour is compared to blood glucose at the 6th hour; ^g $p \leq 0.05$ when blood glucose levels at the 2nd hour is compared to blood glucose at the 8th hour; ⁱ $p \leq 0.05$ when blood glucose levels at the 4th hour is compared to blood glucose at the 8th hour by ANOVA and post ANOVA (Bonferroni-Holm) test.

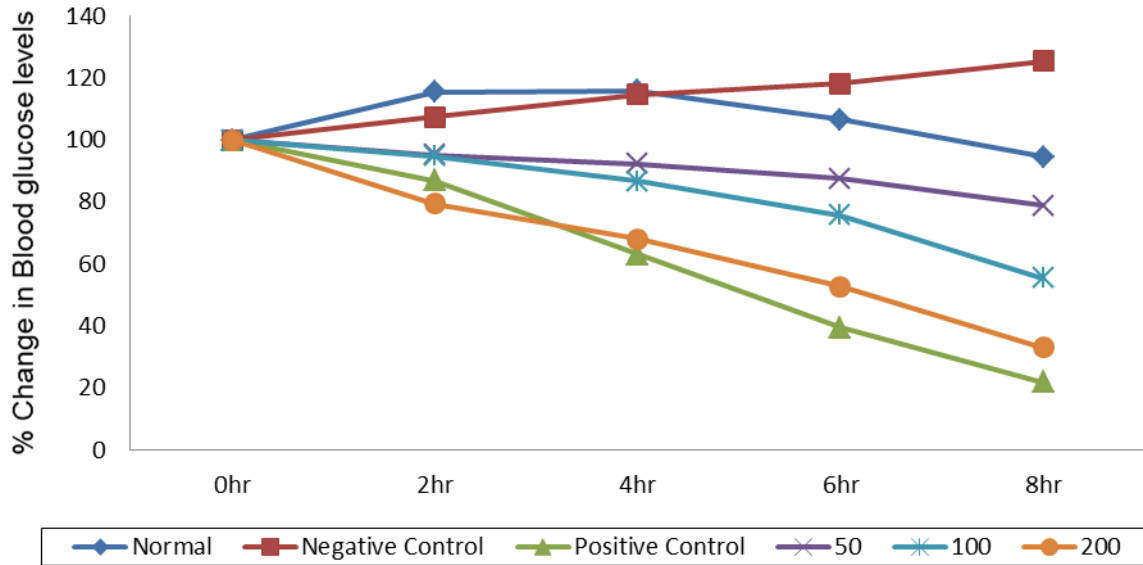


Figure 4.1: Mean percentage change in blood glucose levels after oral administration of ethyl acetate leaf extracts of *P. capensis* in alloxan-induced diabetic male BALB/c mice. Values are expressed as % means for five animals at each time point.

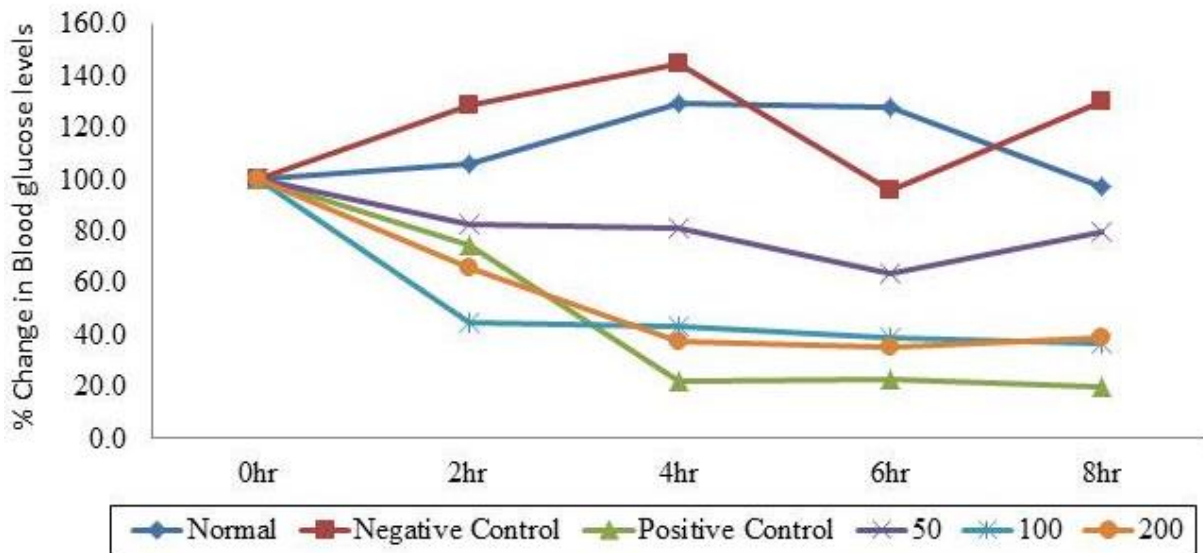


Figure 4.2: Mean percentage change in blood glucose levels after oral administration of ethyl acetate stem barks extracts of *P. capensis* to alloxan-induced diabetic male BALB/c mice. Values are expressed as % mean for five animals at each time point.

4.2.3 Effects of oral administration of extracts of *S. spectabilis*

Oral administration of ethyl acetate leaves extracts of *S. spectabilis* at 50 and 100 mg/kg body weight to mice significantly decreased the blood glucose levels from the sixth hour through to the 8th hour in a dose independent manner. In the sixth hour, the ethyl acetate leaves extracts at 50 and 100 mg/kg body weight lowered blood glucose levels in mice to 74.7 and 66.0%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 29.0% within the same hour.

By the eighth hour, the ethyl acetate leaves extracts at 50 and 100 mg/kg body weight lowered blood glucose levels in mice to 51.9 and 53.4%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 25.0% within the same hour (Table 4.5; Figure 4.3). However, the ethyl acetate leaves extracts at 200 mg/kg body weight in mice did not lower blood sugar levels to levels similar to those produced by the conventional drug, glibenclamide.

Oral administration of ethyl acetate stem bark extracts of *S. spectabilis* at 200 mg/kg body weight to mice decreased the blood glucose levels from the fourth hour through to the 8th hour in a dose independent manner. In the fourth hour, the ethyl acetate stem bark extracts at 200 mg/kg body weight lowered blood glucose levels in mice to 79.1% compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 40.2% within the same hour.

By the eighth hour, the ethyl acetate stem bark extracts at 200 mg/kg body weight lowered blood glucose levels in mice to 49.8% compared to glibenclamide treated diabetic mice whose

sugar levels was lowered to 22.5% within the same hour (Table 4.6; Figure 4.4). However, the ethyl acetate stem bark extracts at 200 mg/kg body weight in mice did not lower blood sugar levels to levels similar to those produced by the conventional drug, glibenclamide.

Table 4.5: Hypoglycemic effects of oral administration of ethyl acetate leaf extracts of *S. spectabilis* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mmol/L)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.7±0.1	5.6±0.3	5.8±0.1	5.7±0.5	5.8±0.5
Diabetic control	Saline	25.5±3.9 ^A	26.9±2.7 ^B	28.3±1.7 ^B	29.4±0.9 ^B	30.1±0.8 ^C
Diabetic	Glibenclamide (3 mg/kg body weight)	22.4±2.8 ^{Ac}	17.6±2.2 ^{Ab}	9.2±1.5 ^{Aa}	6.5±0.8	5.6±0.5
Diabetic	Extracts					
	50 mg/kg body weight	23.7±2.5 ^{Aa}	23.4±3.1 ^{Ba}	23.0±3.3 ^{Ba}	17.7±2.9 ^A	12.3±2.2 ^A
	100 mg/kg body weight	24.7±3.3 ^{Aa}	23.0±2.2 ^{Ba}	21.8±1.8 ^{Ba}	16.3±2.7 ^A	13.2±1.5 ^A
	200 mg/kg body weight	29.0±3.1 ^A	27.2±3.7 ^B	25.7±3.6 ^B	23.5±0.8 ^A	22.2±1.9 ^B

Results were expressed as Mean ± Standard Error of Mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour; ^b $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour.

Table 4.6: Hypoglycemic effects of oral administration of ethyl acetate stem bark extracts of *S. spectabilis* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.6±0.3	5.7±0.5	5.7±0.5	6.1±0.6	5.7±0.5
Diabetic control	Saline	24.4±2.3 ^A	26.3±1.4 ^B	27.8±1.4 ^B	29.3±0.7 ^{Aa}	30.0±0.3 ^{Ba}
Diabetic	Glibenclamide (3 mg/kg body weight)	24.9±1.8 ^{Ac}	19.0±2.6 ^{Ab}	10.0±0.8 ^{Aa}	5.8±0.5	5.6±0.5
Diabetic	Extracts					
	50 mg/kg body weight	24.6±2.5 ^A	26.5±1.5 ^B	26.7±0.7 ^B	25.6±1.3 ^A	24.5±0.9 ^A
	100 mg/kg body weight	26.2±1.8 ^A	30.1±2.0 ^B	29.6±0.7 ^B	25.4±2.6 ^A	22.7±2.9 ^A
	200 mg/kg body weight	27.3±2.1 ^A	26.3±1.4 ^B	21.6±0.5 ^B	14.1±0.9 ^A	13.6±1.1 ^A

Results were expressed as Mean ± Standard Error of Mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour; ^b $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour; ^c $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour.

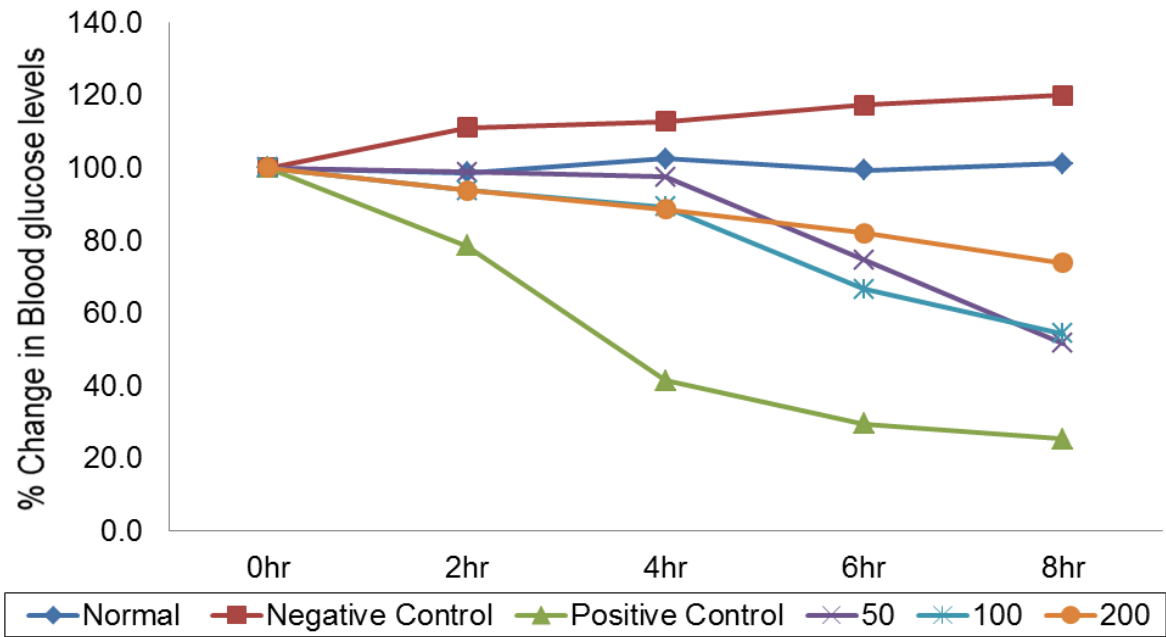


Figure 4.3: Hypoglycemic effects of ethyl acetate leaves extracts of *S. spectabilis* on alloxan-induced diabetic male BALB/c mice.

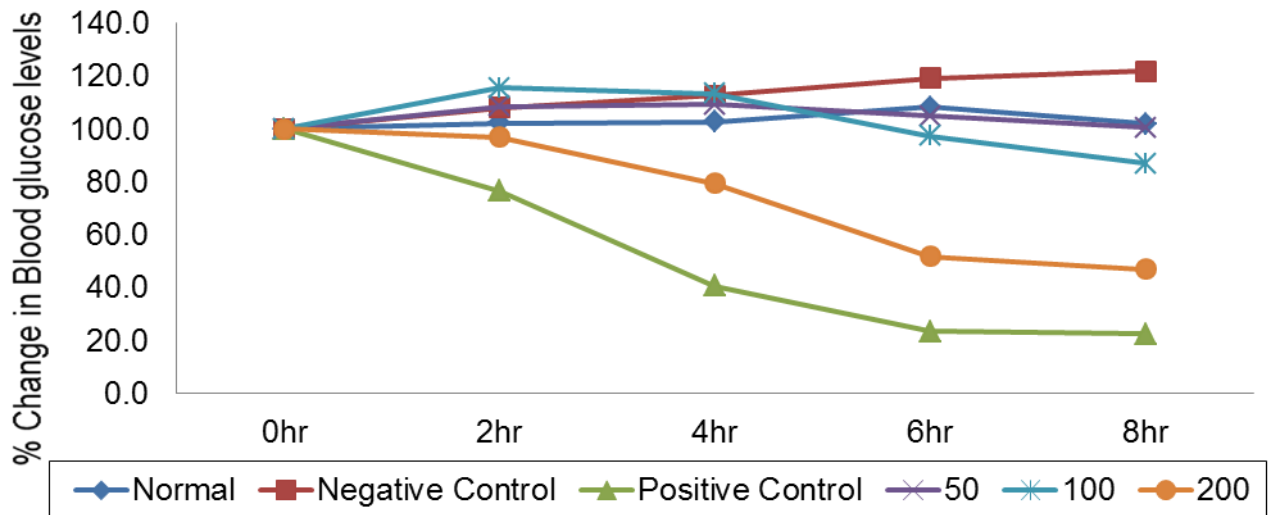


Figure 4.4: Hypoglycemic effects of ethyl acetate stem bark extracts of *S. spectabilis* on alloxan-induced diabetic male BALB/c mice.

Oral administration of aqueous stem bark extracts of *S. spectabilis* at 200 mg/kg body weight to mice decreased the blood glucose levels from the fourth hour through to the 8th hour in a dose independent manner, although the activity was not significant. However, oral administration of aqueous stem bark extracts of *S. spectabilis* at 100 mg/kg body weight to mice significantly decreased the blood glucose levels from the sixth hour through to the 8th hour in a dose independent manner. In the sixth hour, the aqueous stem bark extracts of *S. spectabilis* at 100 mg/kg body weight lowered blood glucose levels in mice to 59.0% compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 30.2% within the same hour.

By the eighth hour, the aqueous stem bark extracts of *S. spectabilis* at 100 mg/kg body weight lowered blood glucose levels in mice to 41.0% compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 26.5% within the same hour (Table 4.7; Figure 4.5). However, the aqueous stem bark extracts of *S. spectabilis* at 100 mg/kg body weight in mice lowered blood sugar levels to levels similar to those of mice treated with the conventional drug, glibenclamide and the normal control mice. In the eighth hour, oral administration of aqueous stem bark extracts of *S. spectabilis* at 50 mg/kg body weight in mice lowered blood sugar levels to levels similar to those of mice treated with the conventional drug, glibenclamide and the normal control mice.

Oral administration of aqueous leaves extracts of *S. spectabilis* at 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the sixth hour through to the 8th hour. In the sixth hour, the aqueous leaves extracts of *S. spectabilis* at 200 mg/kg body weight lowered blood glucose levels in mice to 53.9% compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 24.6% within the same hour. By the eighth

hour, the aqueous leaves extracts of *S. spectabilis* at 200 mg/kg body weight lowered blood glucose levels in mice to 37.8% compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 23.3% within the same hour (Table 4.8; Figure 4.6). However, in the eighth hour, the aqueous leaves extracts of *S. spectabilis* at 200 mg/kg body weight in mice lowered blood sugar levels to levels similar to those of mice treated with the conventional drug, glibenclamide and the normal control mice.

Table 4.7: Hypoglycemic effects of oral administration of aqueous stem bark extracts *S. spectabilis* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Mice Group	Treatment	Blood glucose levels (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.8±0.5	5.8±0.4	5.6±0.6	5.4±0.6	5.4±0.5
Diabetic control	Saline	24.9±2.5 ^A	28.2±2.6	26.4±1.8 ^B	25.1±3.8 ^B	25.8±2.6 ^A
Diabetic control	Glibenclamide (3 mg/kg body wt)	18.9±3.8 ^{Ab}	11.0±1.0 ^{Ab}	9.4±3.3 ^{Aa}	5.7±0.7	5.0±0.8
Diabetic treated	50 mg/kg body weight	17.1±1.6 ^A	15.6±2.0 ^A	10.9±1.5 ^A	7.6±0.9 ^A	8.0±1.3
	100 mg/kg body weight	15.6±1.1 ^{Aa}	15.7±2.7 ^{Aa}	11.9±2.6 ^{Aa}	9.2±1.1	6.4±1.1
	200 mg/kg body weight	17.6±2.2 ^A	16.8±1.3 ^A	9.7±0.8 ^A	7.3±1.6 ^A	7.4±1.7 ^A

Results were expressed as Mean ± Standard Error of Mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour; ^b $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour.

Table 4.8: Hypoglycemic effects of oral administration of aqueous leaf extracts of *S. spectabilis* at 50, 100, and 200 mg/kg body weight

Mice Group	Treatment	Blood glucose levels (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.0±0.5	5.4±0.4	5.4±0.3	5.3±0.2	5.6±0.4
Diabetic control	Saline	23.4±4.2 ^{Aa}	25.1±3.2 ^{Aa}	27.1±2.7 ^{Ca}	28.1±1.6 ^C	29.2±1.1 ^B
Diabetic control	Glibenclamide (3 mg/kg body weight)	23.2±4.8 ^A	17.6±5.3 ^A	10.0±1.1 ^A	5.7±0.6	5.4±0.5
Diabetic treated	50 mg/kg body weight	20.4±4.7 ^A	17.6±2.1 ^A	15.6±2.5 ^B	15.7±2.0 ^B	14.8±2.9 ^A
	100 mg/kg body weight	25.4±5.0 ^A	24.7±2.2 ^A	22.2±2.3 ^C	17.0±1.4 ^B	12.7±2.5 ^A
	200 mg/kg body weight	18.0±1.5 ^{Ab}	15.1±1.9 ^{Ab}	12.5±1.9 ^{Ab}	9.7±0.8 ^{Aa}	6.8±1.1

Results were expressed as Mean ± Standard Error of Mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour.

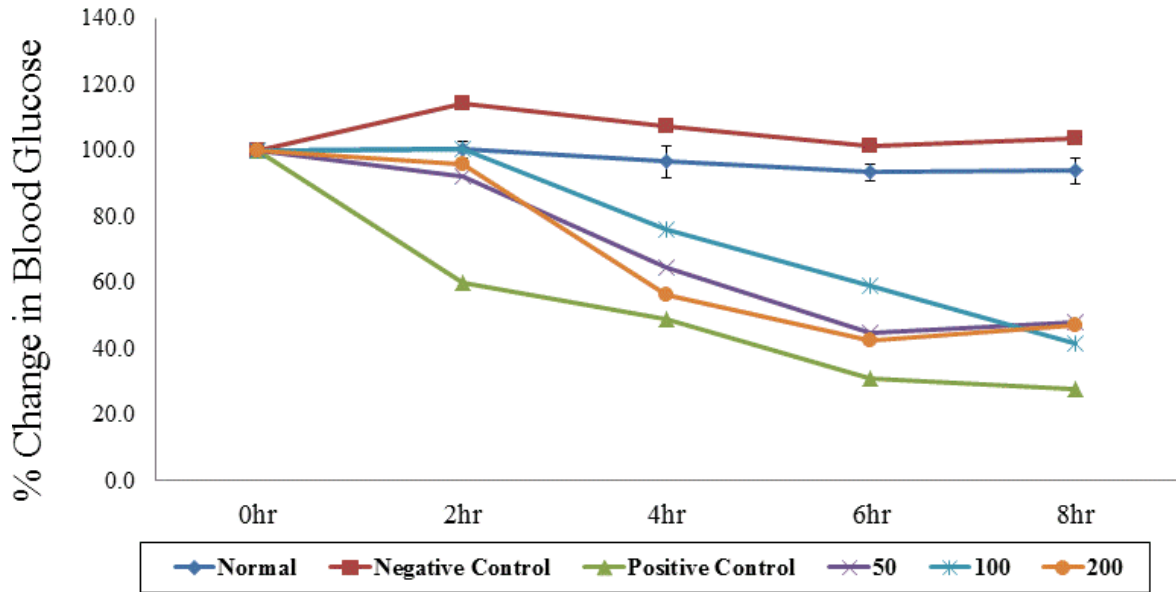


Figure 4.5: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous stem bark extracts of *S. spectabilis* at 50, 100 and 200 mg/kg body weight in alloxan induced diabetic male BALB/c mice.

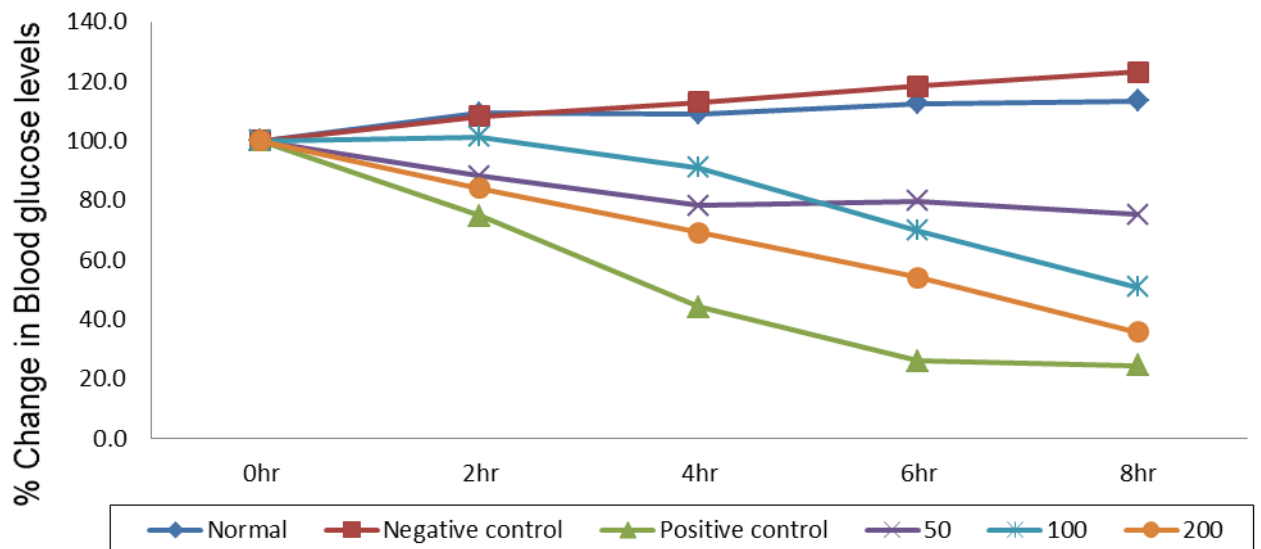


Figure 4.6: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous leaves extracts of *S. spectabilis* at 50, 100 and 200 mg/kg body weight in alloxan induced diabetic male BALB/c mice.

4.2.4 Effects of oral administration of extracts of *M. obscura*

Oral administration of ethyl acetate leaves extracts of *M. obscura* at 50 and 100 mg/kg body weight to mice significantly decreased the blood glucose levels from the second hour through to the 8th hour in a dose independent manner. In the second hour, the ethyl acetate leaves extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 58.8, 77.7 and 93.0%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 62.5% within the same hour.

In the fourth hour, the ethyl acetate leaves extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 27.6, 51.8 and 82.0%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 40.5% within the same hour. By the eighth hour, the ethyl acetate leaves extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 17.8, 29.9 and 54.5%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 22.4% within the same hour (Table 4.9; Figure 4.7).

Oral administration of ethyl acetate stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the second hour through to the 8th hour in a dose independent manner. In the second hour, the ethyl acetate stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 97.1, 80.5 and 72.6%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 64.9% within the same hour.

In the fourth hour, the ethyl acetate extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 79.4, 71.1 and 63.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 72.7% within the same hour. By the eighth hour, the ethyl acetate extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 50.7, 37.1 and 32.9%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 25.5% within the same hour (Table 4.10; Figure 4.8).

Table 4.9: Hypoglycemic effects of oral administration of ethyl acetate leaf extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.1±0.1	5.1±0.1	5.1±0.2	5.2±0.2	5.3±0.1
Diabetic control	Saline	24.0±0.8 ^A	25.0±0.8 ^B	27.7±0.9 ^{Ab}	28.7±0.9 ^{Ab}	29.9±0.9 ^{Bb}
Diabetic	Glibenclamide (3 mg/kg body weight)	23.2±0.7 ^A	14.5±0.9 ^{Aa}	9.4±0.6 ^{Bb}	5.8±0.6 ^c	5.2±0.1 ^{Ad}
Diabetic	Extracts					
	50 mg/kg body weight	17.4±1.0 ^B	10.0±0.7 ^{Ca}	4.8±0.5 ^b	3.3±1.0 ^{Bc}	3.1±0.3 ^C
	100 mg/kg body weight	19.7±0.8 ^C	15.3±0.8 ^{Aa}	10.2±0.3 ^{Cb}	6.7±0.7 ^B	5.9±0.6
	200 mg/kg body weight	24.4±3.7 ^C	22.7±0.8 ^A	20.0±0.9 ^C	15.3±0.5 ^B	13.3±0.7 ^A

Results were expressed as Mean ± Standard deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour. ^b $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour. ^c $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour and ^d $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 8th hour.

Table 4.10: Hypoglycemic effects of oral administration of ethyl acetate stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	4.8±0.5	5.2±0.2	5.2±0.2	5.5±0.4	5.4±0.3
Diabetic control	Saline	23.2±3.5 ^A	24.6±2.7 ^{Aa}	25.8±2.5 ^{Ab}	27.9±1.6 ^{Ac}	29.0±1.0 ^{Ad}
Diabetic	Glibenclamide (3 mg/kg body weight)	23.1±4.0 ^A	15.0±0.6 ^{Ba}	16.8±1.5 ^{Bbe}	7.5±0.9 ^{Bcfh}	5.9±0.6 ^{dgij}
Diabetic	Extracts					
	50 mg/kg body weight	20.9±1.8 ^A	20.3±0.7 ^{Ca}	16.6±1.2 ^{Cbe}	12.8±1.0 ^{Ccfh}	10.6±0.6 ^{Bdgij}
	100 mg/kg body weight	25.6±0.7 ^A	20.6±0.9 ^{Ca}	18.2±0.7 ^{DBe}	13.5±0.9 ^{Ccfh}	9.5±0.9 ^{Bdgij}
	200 mg/kg body weight	23.4±1.3 ^A	17.0±0.8 ^{Da}	14.9±0.8 ^{Cbe}	11.9±0.6 ^{Ccfh}	7.7±0.5 ^{Cdgij}

Results were expressed as Mean ± Standard deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour. ^b $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour. ^c $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour and ^d $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 8th hour. ^e $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 4th hour. ^f $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 6th hour. ^g $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 8th hour. ^h $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 6th hour, and ⁱ $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 8th hour ^j $P \leq 0.05$ when blood glucose levels at 6th hour is compared to blood glucose at the 8th hour.

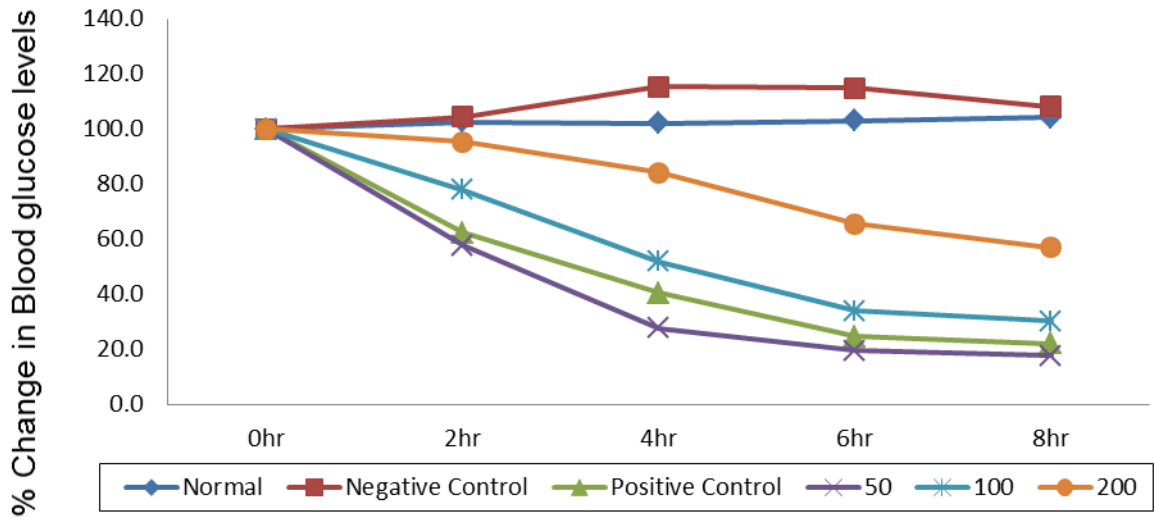


Figure 4.7: Percentage reduction in blood glucose levels at varying times after oral administration of ethyl acetate leaves extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

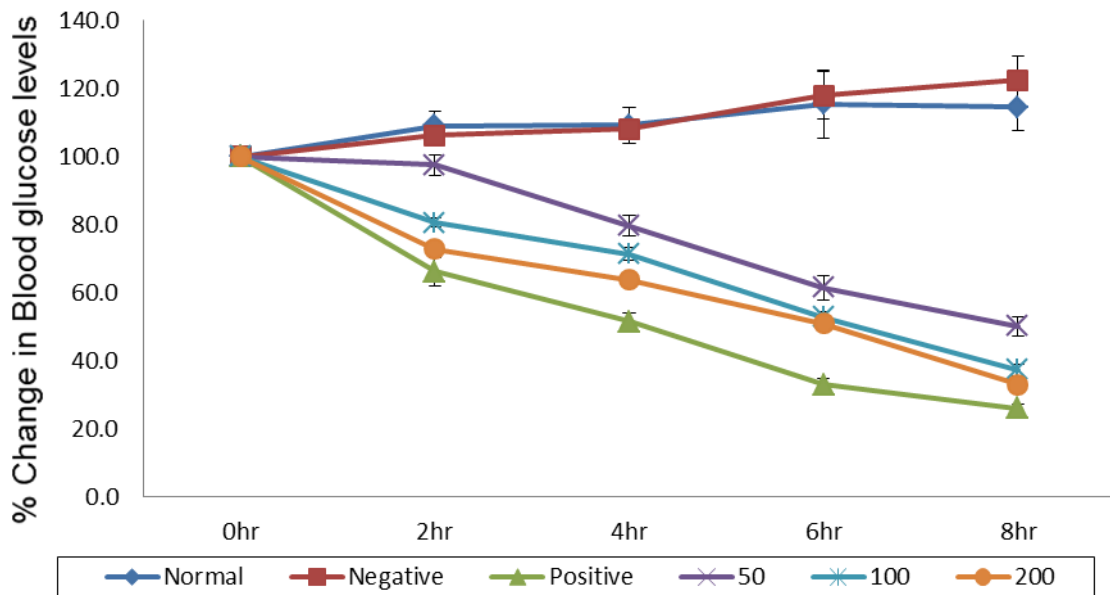


Figure 4.8: Percentage reduction in blood glucose levels at varying times after oral administration of ethyl acetate stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

Oral administration of aqueous stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the second hour through to the 8th hour in a dose independent manner. In the second hour, the ethyl acetate extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 75.8, 70.0 and 49.6%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 85.1% within the same hour.

In the fourth hour, the aqueous stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 70.0, 35.6 and 22.6%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 40.4% within the same hour. By the eighth hour, the aqueous stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 80.6, 37.2 and 19.8%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 18.7% within the same hour (Table 4.11 and Figure 4.9).

Oral administration of aqueous leaves extracts of *M. obscura* at 50 and 100 mg/kg body weight to mice significantly increased the blood glucose levels from the second hour through to the 8th hour compared to the zero hour in a dose dependent manner. In the second hour, the aqueous leaves extracts of *M. obscura* at 50 and 100 mg/kg body weight increased blood glucose levels in mice to 121.5 and 129.8%, respectively, compared to the zero hour. In the sixth hour, the aqueous leaves extracts of *M. obscura* at 50 and 100 mg/kg body weight increased blood glucose levels in mice to 114.5 and 131.5%, respectively, compared to the zero hour. By the eighth hour, the aqueous leaves extracts of *M. obscura* at 50 and 100 mg/kg body weight increased blood glucose levels in mice to 109.9 and 128.7%, respectively, compared to the zero hour (Table 4.12 and Figure 4.10).

Table 4.11: Hypoglycemic effects of oral administration of aqueous stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.2±0.1	5.2±0.1	5.2±0.1	5.2±0.3	5.1±0.2
Diabetic control	Saline	22.5±2.2 ^A	23.9±1.9 ^A	25.7±2.5 ^A	27.4±1.7 ^A	28.7±0.7 ^{Agh}
Diabetic	Glibenclamide (3 mg/kg body weight)	16.1±1.4 ^B	13.7±1.0 ^{Ba}	9.1±1.1 ^{be}	6.5±0.8 ^{cth}	4.7±0.5 ^{dgi}
Diabetic	Extracts					
	50 mg/kg body weight	22.7±1.1 ^A	17.2±0.8 ^{Ca}	16.4±1.7 ^{Bb}	15.9±1.0 ^{Bc}	18.3±1.8 ^{Bd}
	100 mg/kg body weight	25.3±3.2 ^A	17.7±1.5 ^{Ca}	10.8±0.8 ^{be}	9.0±0.8 ^{Ccth}	9.4±0.9 ^{Cdg}
	200 mg/kg body weight	25.2±0.8 ^A	12.5±0.6 ^{Ba}	8.1±0.7 ^{be}	5.7±0.6 ^{cth}	5.0±0.6 ^{dgi}

Results were expressed as Mean ± Standard Deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour. ^b $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour. ^c $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour and ^d $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 8th hour. ^e $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 4th hour. ^f $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 6th hour. ^g $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 8th hour. ^h $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 6th hour, and ⁱ $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 8th hour ^j $P \leq 0.05$ when blood glucose levels at 6th hour is compared to blood glucose at the 8th hour.

Table 4.12: Hypoglycemic effects of oral administration of aqueous leaves extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.2±0.1	5.2±0.4	5.1±0.1	5.2±0.1	5.1±0.2
Diabetic control	Saline	14.9±1.6 ^A	22.7±0.3 ^B	21.1±0.8 ^{Ba}	21.6±1.3 ^{Ba}	23.2±0.5 ^{Aa}
Diabetic	Glibenclamide (3 mg/kg body weight)	17.1±1.4 ^A	14.9±1.6 ^{Aa}	9.3±1.2 ^{Ab}	6.8±1.1 ^c	4.7±0.5 ^d
Diabetic	Extracts					
	50 mg/kg body weight	17.2±0.8 ^A	20.9±0.8 ^{Ba}	20.4±1.2 ^{Ba}	19.7±2.2 ^{Aa}	18.9±2.2 ^{Aa}
	100 mg/kg body weight	17.1±2.1 ^A	22.2±1.6 ^{Ba}	22.0±1.5 ^{Ba}	22.5±1.4 ^{Aa}	22.0±1.3 ^{Aa}
	200 mg/kg body weight	22.3±1.6 ^B	23.3±1.5 ^B	24.8±1.2 ^C	24.7±1.3 ^B	21.7±0.5 ^A

Results were expressed as Mean ± Standard Deviation (SD) of five mice per group. Means on the same column followed by the same upper case letters are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means on same row followed by the same lower case letters are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

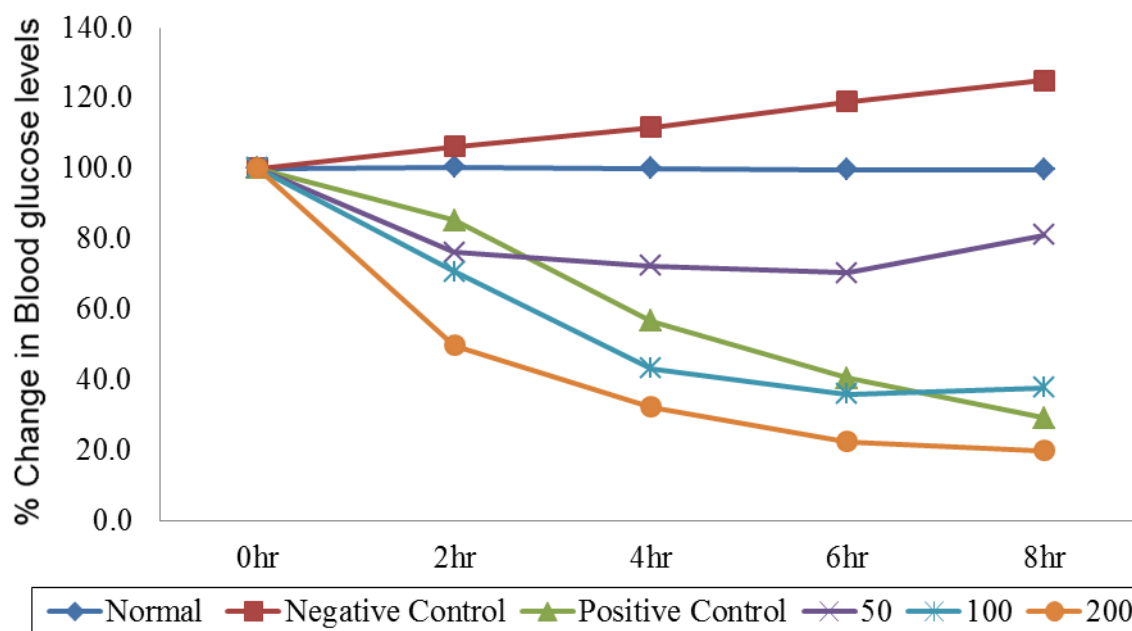


Figure 4.9: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous stem bark extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

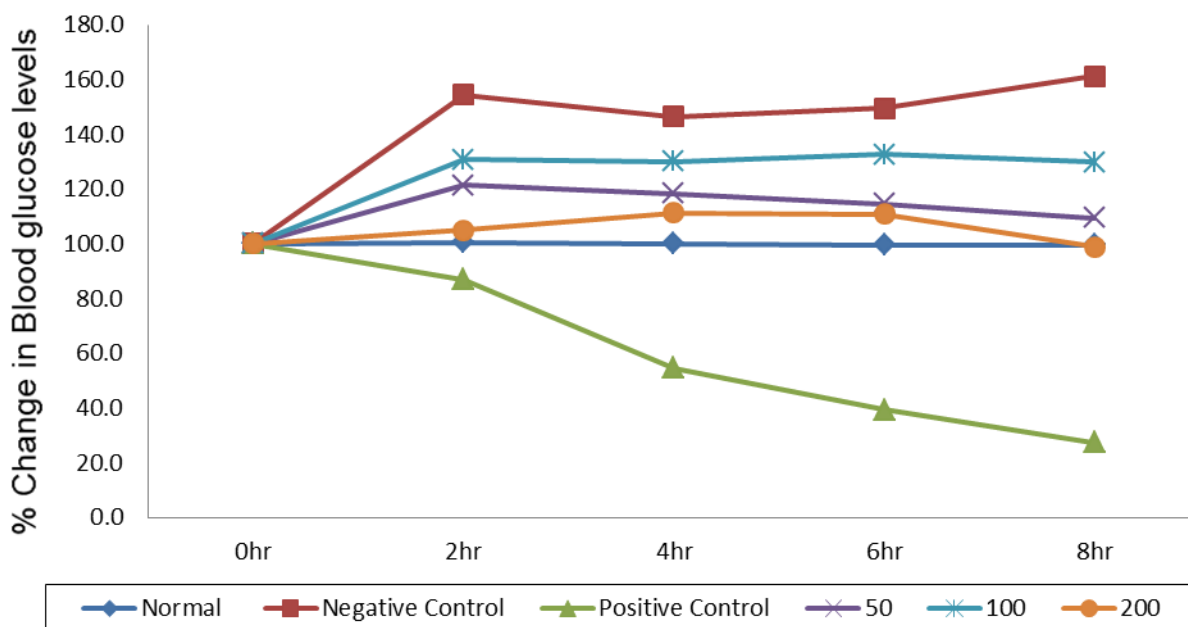


Figure 4.10: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous leaves extracts of *M. obscura* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

4.2.5 Effects of oral administration of *O. americanum*

Oral administration of ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the second hour through to the 8th hour in a dose independent manner. In the second hour, the ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 97.2, 79.9 and 74.4%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 73.6% within the same hour. In the fourth hour, the ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 88.3, 61.1 and 60.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 49.8% within the same hour.

By the eighth hour, the ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 48.4, 27.5 and 30.8%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 25.5% within the same hour (Table 4.13 and Figure 4.11). The glucose levels induced by the oral administration of ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight, respectively, were comparable to those induced by glibenclamide in the sixth and those induced by glibenclamide and in normal in the eighth hour, respectively.

Oral administration of aqueous extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight to mice decreased the blood glucose levels from the second hour through to the 8th hour compared to the zero hour in a dose dependent manner. In the sixth hour, the aqueous extracts of *O. americanum* at 100 and 200 mg/kg body weight significantly decreased blood glucose levels in mice to 50.2 and 50.9%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 37.6% within the same

hour. By the eighth hour, the aqueous extracts of *O. americanum* at 100 and 200 mg/kg body weight significantly decreased blood glucose levels in mice to 68.5 and 66.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 25.2% within the same hour (Table 4.14 and Figure 4.12).

Table 4.13: Hypoglycemic effects of oral administration of ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	4.8±0.5	5.3±0.3	5.2±0.2	5.5±0.2	5.5±0.3
Diabetic control	Saline	23.2±3.5 ^A	24.6±2.6 ^A	25.8±2.5 ^A	27.4±1.7 ^A	29.0±1.0 ^A
Diabetic	Glibenclamide (3 mg/kg body weight)	23.1±4.0 ^B	17.0±4.7 ^B	11.5±2.7 ^{Bb}	8.2±1.6 ^{Bcf}	5.9±0.6 ^{dgi}
Diabetic	Extracts					
	50 mg/kg body weight	21.3±3.0 ^B	20.7±3.0 ^B	18.8±2.8 ^C	13.9±4.8 ^B	10.3±3.1 ^{dgi}
	100 mg/kg body weight	22.9±4.4 ^B	18.3±4.1 ^B	14.0±4.4 ^C	10.0±2.3 ^{Bc}	6.3±2.0 ^{dg}
	200 mg/kg body weight	23.4±3.1 ^B	17.4±3.4 ^{Ba}	14.2±2.7 ^{Cc}	10.0±1.7 ^{Bcf}	7.2±1.1 ^{dgi}

Results were expressed as Mean ± Standard deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^a $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour. ^b $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour. ^c $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour and ^d $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 8th hour. ^f $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 6th hour. ^g $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 8th hour, and ⁱ $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 8th hour.

Table 4.14: Hypoglycemic effects of oral administration of aqueous extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.0±0.3	5.2±0.3	5.1±0.1	5.2±0.4	5.3±0.3
Diabetic control	Saline	22.6±3.6 ^A	24.0±3.3 ^A	25.2±2.7 ^A	28.8±1.4 ^A	30.0±0.9 ^{Bd}
Diabetic	Glibenclamide (3 mg/kg body weight)	21.0±2.8 ^A	19.3±3.8 ^A	14.2±3.2 ^{Ab}	7.9±1.4 ^{Bcjh}	5.3±0.6 ^{dgi}
Diabetic	Extracts					
	50 mg/kg body weight	26.1±2.3 ^A	25.0±2.9 ^A	22.3±2.1 ^A	23.7±2.7 ^C	21.9±1.7 ^A
	100 mg/kg body weight	27.3±2.1 ^A	23.6±1.9 ^A	20.0±1.2 ^{Ab}	13.7±2.0 ^{Cc}	18.7±0.8 ^A
	200 mg/kg body weight	21.6±2.4 ^A	21.1±2.7 ^A	17.5±4.6 ^A	11.0±2.3 ^{Cc}	14.4±1.4 ^A

Results were expressed as Mean ± Standard Deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same row are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. ^b $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour. ^c $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour and ^d $P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 8th hour. ^f $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 6th hour. ^g $P \leq 0.05$ when blood glucose levels at 2nd hour is compared to blood glucose at the 8th hour. ^h $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 6th hour, and ⁱ $P \leq 0.05$ when blood glucose levels at 4th hour is compared to blood glucose at the 8th hour. ^j $P \leq 0.05$ when blood glucose level at 6th hour is compared to blood glucose at the 8th hour.

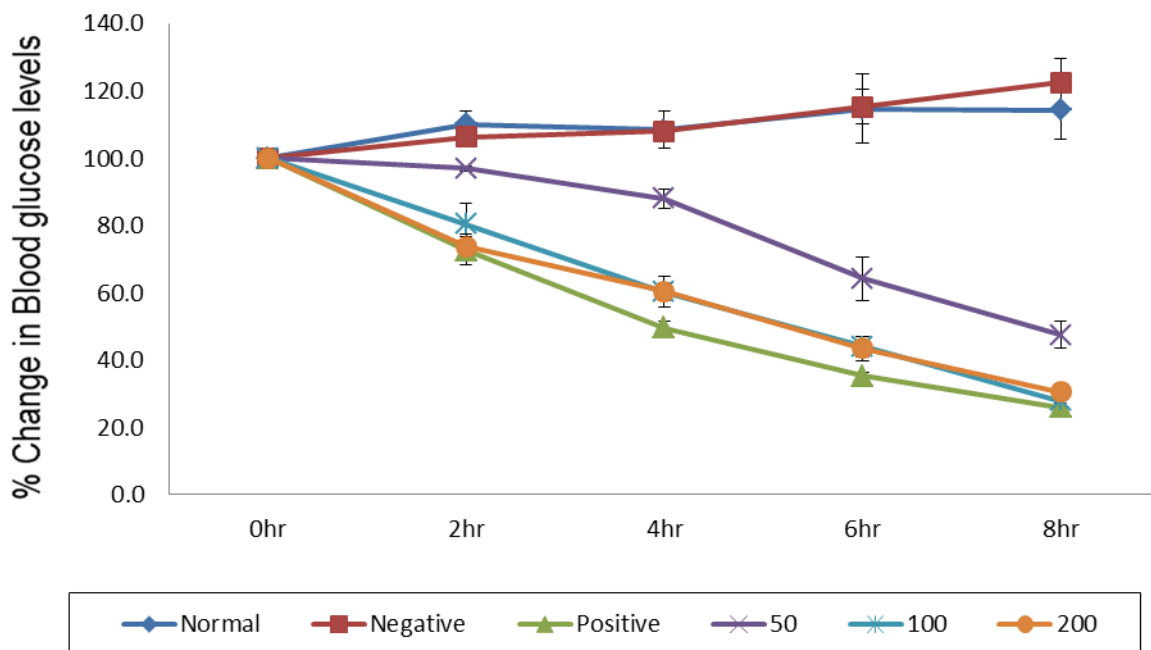


Figure 4.11: Percentage reduction in blood glucose levels at varying times after oral administration of ethyl acetate extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

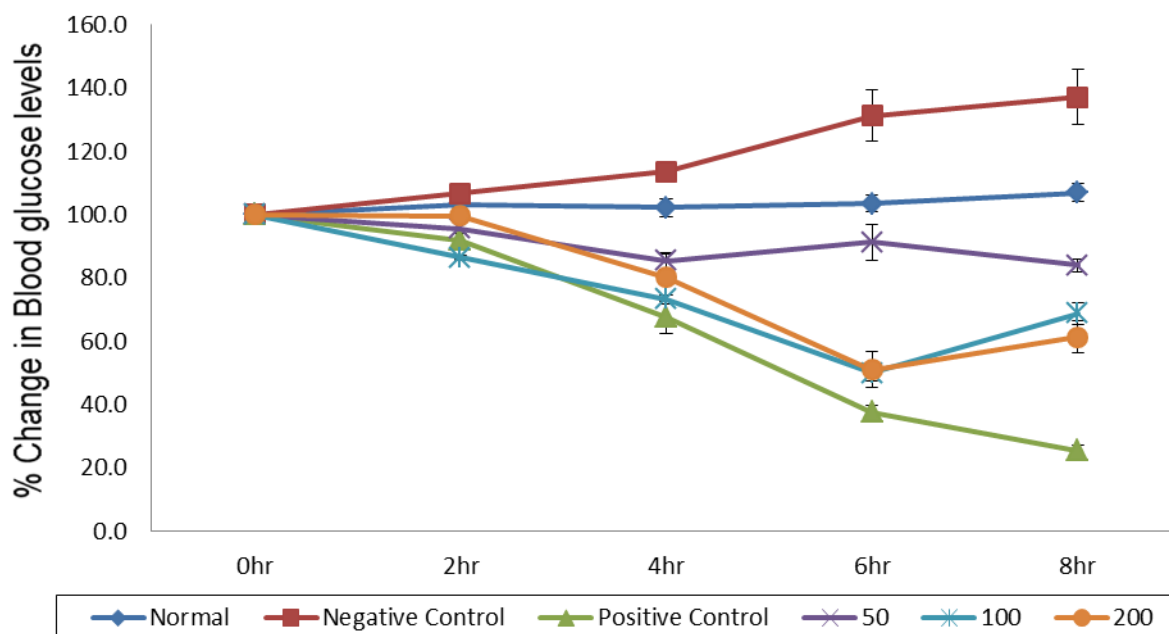


Figure 4.12: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous extracts of *O. americanum* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

4.2.6 Effects of oral administration of *L. cornuta*

Oral administration of ethyl acetate extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight to mice significantly decreased the blood glucose levels from the fourth hour through to the 8th hour in a dose independent manner. In the fourth hour, the ethyl acetate extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 43.6, 60.4 and 58.4%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 58.2% within the same hour. In the sixth hour, the ethyl acetate extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 24.8, 38.2 and 41.1%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 32.3% within the same hour.

By the eighth hour, the ethyl acetate extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight lowered blood glucose levels in mice to 22.4, 36.1 and 34.1%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 22.8% within the same hour (Table 4.15; Figure 4.13). The glucose levels induced by the oral administration of ethyl acetate extracts of *L. cornuta* at 50, and 100 mg/kg body weight, respectively, were comparable to those induced by glibenclamide and those in the normal animals in the sixth and eighth hour, respectively. In contrast, the glucose levels induced by the oral administration of ethyl acetate extracts of *L. cornuta* at 200 mg/kg body weight were higher than those induced by glibenclamide and the control animals in the sixth and eighth hour, respectively.

As depicted in table 4.16 and figure 4.14, oral administration of aqueous extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight to mice had no effect on the blood glucose levels from the second hour through to the 8th hour when compared to that in untreated diabetic mice. In the second hour, the aqueous extracts of *L. cornuta* at 50 and 200 mg/kg body weight increased

blood glucose levels in mice to 119.1 and 131.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 75.3% within the same hour. In the fourth hour, the aqueous extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight increased blood glucose levels in mice to 138.8, 113.1 and 166.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 49.5% within the same hour.

By the sixth hour, the aqueous extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight increased blood glucose levels in mice to 157.9, 138.1 and 206.3%, respectively, compared to glibenclamide treated diabetic mice whose sugar levels was lowered to 41.9% within the same hour. By the eighth hour, the aqueous extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight increased blood glucose levels in mice to 167.1, 146.9 and 202.4%, respectively, compared to glibenclamide treated diabetic mice whose glucose levels was lowered to 25.8% within the same hour.

Table 4.15: Hypoglycemic effects of oral administration of ethyl acetate extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.1 ± 0.1	5.2 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	5.4 ± 0.1
Diabetic control	Saline	23.3 ± 2.8 ^C	24.1 ± 2.7 ^D	25.8 ± 2.2 ^C	28.9 ± 1.1 ^B	30.1 ± 1.1 ^{Ca}
Diabetic	Glibenclamide (3 mg/kg body weight)	23.2 ± 3.7 ^C	20.6 ± 0.9 ^C	13.5 ± 1.2 ^B	7.5 ± 1.0 ^a	5.3 ± 0.5 ^b
Diabetic	Extracts					
	50 mg/kg body weight	16.5 ± 0.9 ^B	10.2 ± 1.6 ^A	7.2 ± 0.4 ^{Aa}	4.1 ± 0.8 ^a	3.7 ± 0.5 ^{Ab}
	100 mg/kg body weight	14.4 ± 0.6 ^A	11.9 ± 0.5 ^A	8.7 ± 0.9 ^{Aa}	5.5 ± 1.3 ^b	5.2 ± 0.8 ^c
	200 mg/kg body weight	21.4 ± 1.1 ^C	15.5 ± 0.7 ^B	12.5 ± 0.4 ^{Ba}	8.8 ± 1.2 ^{Ab}	7.3 ± 1.7 ^{Bc}

Results were expressed as Mean ± Standard Deviation (SD) of five mice per group. Means on the same column followed similar upper case letters are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means on the same row followed similar lower case letters are not significantly different at $P \leq 0.05$.

Table 4.16: Hypoglycemic effects of oral administration of aqueous extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice

Group	Treatment	Blood glucose levels at varying times (mM)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.2 ± 0.2	5.2 ± 0.2	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
Diabetic control	Saline	16.0 ± 1.9 ^A	17.7 ± 0.8 ^A	20.1 ± 1.4 ^{Aa}	22.6 ± 1.2 ^{Aa}	24.3 ± 1.1 ^{Aa}
Diabetic	Glibenclamide (3 mg/kg body weight)	19.8 ± 1.9 ^A	14.9 ± 0.9 ^{Ba}	9.8 ± 1.1 ^{Bb}	8.3 ± 1.3 ^{Bc}	5.1 ± 0.3 ^d
Diabetic	Extracts					
	50 mg/kg body weight	15.2 ± 1.6 ^A	18.1 ± 1.8 ^{Aa}	21.1 ± 1.7 ^{Aa}	24.0 ± 1.0 ^{Aa}	25.4 ± 1.0 ^{Aa}
	100 mg/kg body weight	16.0 ± 1.6 ^A	15.6 ± 2.3 ^{Aa}	18.1 ± 1.3 ^{Aa}	22.1 ± 2.1 ^{Aa}	23.5 ± 0.5 ^{Aa}
	200 mg/kg body weight	12.6 ± 1.4 ^A	16.6 ± 2.5 ^A	21.0 ± 1.6 ^A	26.0 ± 2.7 ^A	25.5 ± 1.1 ^A

Results were expressed as Mean ± Standard Deviation (SD) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means followed by similar lower case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test

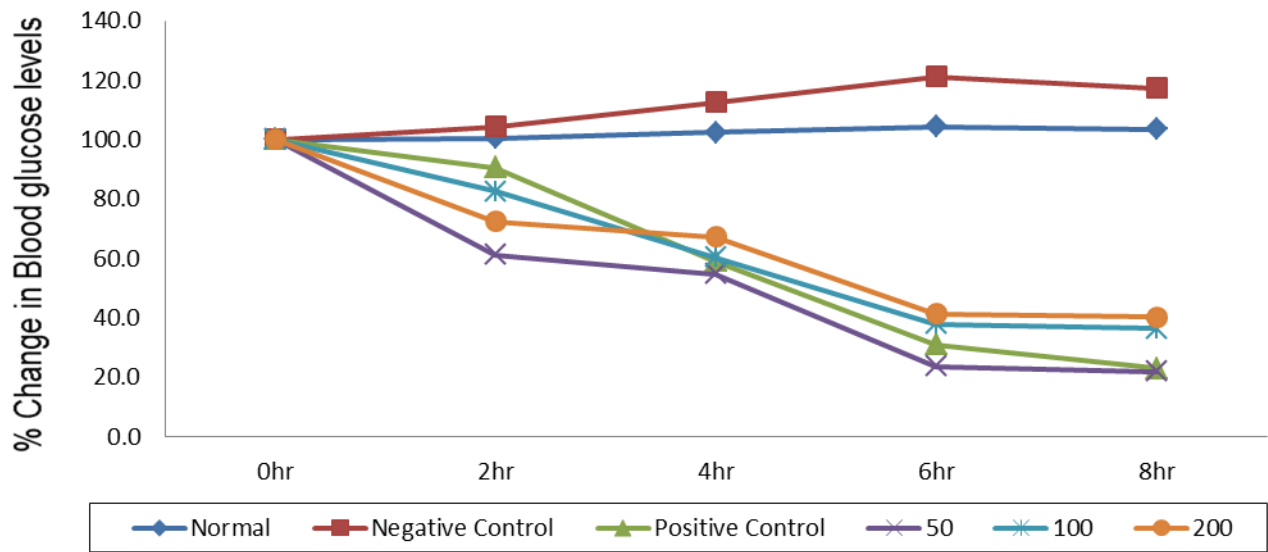


Figure 4.13: Percentage reduction in blood glucose levels at varying times after oral administration of ethyl acetate extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

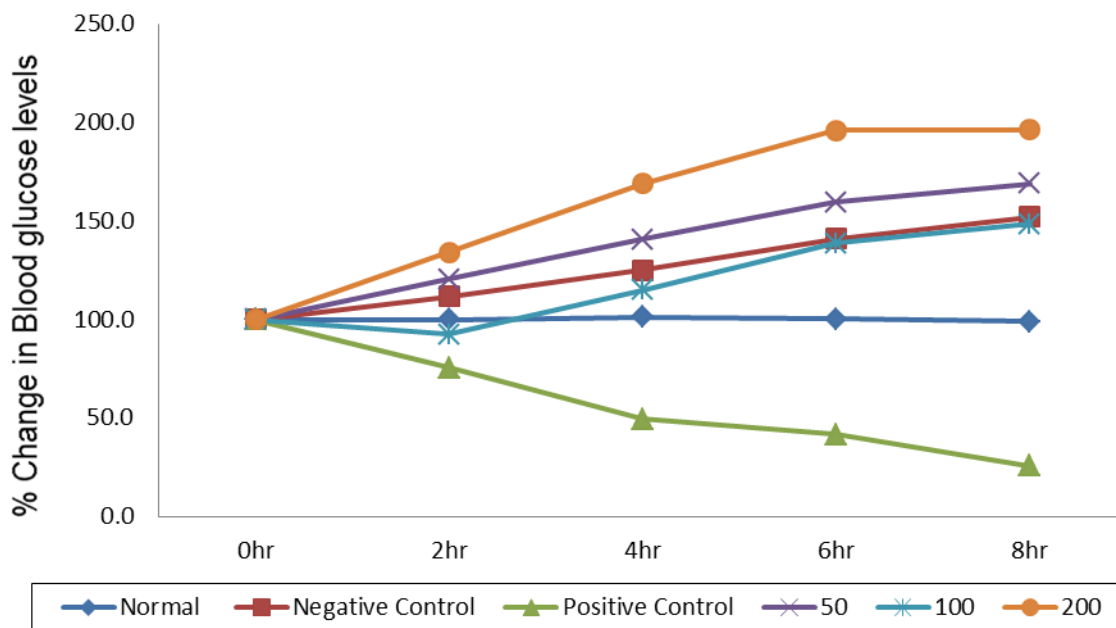


Figure 4.14: Percentage reduction in blood glucose levels at varying times after oral administration of aqueous extracts of *L. cornuta* at 50, 100 and 200 mg/kg body weight in alloxan-induced diabetic male BALB/c mice.

4.3 *In vivo* single dose toxicity of the ethyl acetate and aqueous extracts

4.3.1 Effect of oral administration of aqueous plants extracts on body weight and mean weekly body weight change

As depicted in table 4.17 and 4.18, oral administration of ethyl acetate and aqueous extracts of *P. capensis* leaves and *O. americanum* extracts at 1 g/kg body weight daily in mice for 28 days significantly reduced the body weight and mean weekly body weight gain compared to that of the normal control mice. In addition, the ethyl acetate extracts of *S. spectabilis* at the same dose significantly lowered the body weight and mean weekly body weight gain compared to that of the normal control mice. For mice treated with the ethyl acetate and aqueous extracts of *P. capensis* SB, *S. spectabilis* SB and L, *M. obscura* SB and L, and *L. cornuta*, their body weights were significantly lower than those of the control mice but they grew at the same rate as the control mice as measured by the mean weekly body weight gain.

Table 4.17: Effects of oral administration of ethyl acetate plants extracts at 1g/kg body weight daily in mice for 28 days on body weight and mean weekly body weight change

Treatment	Weekly Weight of Mice (g)					Δ Weight/Week
	0	1	2	3	4	
Control	23.06±1.82	23.31±1.80	23.90±1.44	24.82±0.88	26.96±1.62	0.98±0.34
<i>P. capensis</i> SB	20.56±0.93*	21.58±0.91*	22.34±1.05*	23.14±0.99*	23.85±0.89*	0.85±0.11
<i>P. capensis</i> L	18.42±4.05	18.64±4.07*	19.14±4.15	19.48±4.15*	18.48±1.56*	0.43±0.23*
<i>O. americanum</i>	21.86±4.44	22.16±4.43	22.42±4.52	22.94±4.43	23.18±4.72	0.23±0.22*
<i>S. spectabilis</i> SB	18.52±1.16*	19.54±1.43*	20.60±1.60*	21.58±1.67*	22.20±1.83*	1.01±0.25
<i>S. spectabilis</i> L	21.36±3.89	21.66±3.94	22.00±3.90	22.24±3.86	23.68±4.26*	0.56±0.08*
<i>L. cornuta</i>	23.14±2.87	23.90±2.35	24.50±2.43	24.88±2.43	26.00±2.28	0.78±0.26
<i>M. obscura</i> SB	19.42±1.25*	20.06±1.33*	20.76±1.44*	21.62±1.34*	22.56±1.12*	0.79±0.23
<i>M. obscura</i> L	19.98±3.22*	20.72±3.39*	21.34±3.53	21.78±3.56*	22.22±3.95*	0.56±0.36

Results are expressed as Mean \pm Standard Deviation (SD) for five animals in each treatment. * $P < 0.05$ is considered significant when the Mean of the control animals is significantly different from that of the extract treated animals by student T-Test.

Table 4.18: Effects of oral administration of aqueous plants extracts at 1g/kg body weight daily in mice for 28 days on body weight and mean weekly body weight change

Treatment	Weekly Weight of Mice (g)					Δ Weight/Week
	0	1	2	3	4	
Control	23.06±1.82	23.31±1.80	23.90±1.44	24.82±0.88	26.96±1.62	0.98±0.34
<i>P. capensis</i> SB	17.86±0.89*	18.44±0.91*	19.2±0.85*	19.70±0.91*	20.78±0.75*	0.66±0.04
<i>P. capensis</i> L	16.72±3.13*	17.08±3.17*	17.38±3.21*	17.74±3.23*	17.35±3.18*	0.35±0.05*
<i>O. americanum</i>	18.24±1.68*	18.54±1.70*	18.86±1.76*	19.36±1.70*	20.43±1.30*	0.39±0.08*
<i>S. spectabilis</i> SB	19.74±0.88*	20.14±1.00*	20.64±1.00*	21.50±0.96*	22.58±0.67*	0.67±0.07
<i>S. spectabilis</i> L	19.80±1.30*	20.26±1.19*	20.76±1.11*	21.74±1.01*	22.88±0.63*	0.67±0.12
<i>L. cornuta</i>	18.00±2.17*	18.58±2.15*	19.06±2.22*	19.86±2.42*	20.40±2.83*	0.64±0.12
<i>M. obscura</i> SB	18.60±0.64*	19.06±0.73*	19.82±0.77*	20.54±0.87*	21.80±0.57*	0.80±0.05
<i>M. obscura</i> L	18.82±0.98*	19.24±0.92*	19.78±0.76*	20.80±0.84*	21.70±1.24*	0.72±0.21

Results are expressed as Mean \pm Standard Deviation (SD) for five animals in each treatment. * $P < 0.05$ is considered significant when the Mean of the control animals is significantly different from that of the extract treated animals by student T-Test.

4.3.2 Effects of oral administration of the ethyl acetate and aqueous plants extracts on hematological parameters

Oral administration of the eight aqueous plants extracts at 1g/kg body weight daily to mice for 28 days significantly decreased levels of red blood cells (*P. capensis* SB and L, *S. spectabilis* L, *L. cornuta*), and increased the levels of red blood cells (*O. americanum*), haemoglobin decreased significantly (*P. capensis* SB and L, *S. spectabilis* L, *L. cornuta*, and *O. americanum*), and increased the levels of haemoglobin (*S. spectabilis* SB, *M. obscura* SB), packed cell volume (*P. capensis* SB and L, *S. spectabilis* L, *L. cornuta*, *M. obscura* SB, and *O. americanum*), mean cell haemoglobin (*P. capensis* L, *S. spectabilis* L, *M. obscura* SB, and *O. americanum*), mean cell haemoglobin concentration (*P. capensis* SB and L, *S. spectabilis* SB and L, *L. cornuta*, *M. obscura* SB and L, and *O. americanum*), mean cell volume (*P. capensis* SB and L, *S. spectabilis* SB and L, *L. cornuta*, *M. obscura* SB and L, and *O. americanum*), and platelets (*S. spectabilis* SB and L, and *L. cornuta*) relative to that of the control mice (Table 4.19).

In addition, oral administration of the eight aqueous plants extracts at 1 g/kg body weight daily to mice for 28 days significantly increased levels of the white blood cells (*P. capensis* SB and L, *S. spectabilis* L, *M. obscura* SB and L, *L. cornuta*, and *O. americanum*), neutrophils (*M. obscura* SB), and decreased the levels of monocytes (*S. spectabilis* L), lymphocytes (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* SB and L, *L. cornuta*, and *O. americanum*), and monocytes (*P. capensis* SB and L, *S. spectabilis* L, *M. obscura* L, *L. cornuta*, and *O. americanum*) and significantly decreased the levels of monocytes (*S. spectabilis* SB, and *M. obscura* SB) relative to that of the control mice (Table 4.20).

Oral administration of the eight ethyl acetate plants extracts at 1g/kg body weight daily to mice for 28 days significantly decreased levels of red blood cells (*P. capensis* SB and L, *S. spectabilis* SB, *M. obscura* SB and L, and *O. americanum*), haemoglobin (*P. capensis* L, *S. spectabilis* L, *M. obscura* L, and *O. americanum*) and increased levels of haemoglobin (*S. spectabilis* SB), packed cell volume (*P. capensis* SB and L, *S. spectabilis* SB and L, *L. cornuta*, *M. obscura* SB and L, and *O. americanum*), mean cell haemoglobin (*P. capensis* L, and *O. americanum*), mean cell haemoglobin concentration (*P. capensis* SB and L, *S. spectabilis* SB and L, *L. cornuta*, *M. obscura* SB and L, and *O. americanum*), decreased mean cell volume (*P. capensis* SB and L, *S. spectabilis* SB and L, *L. cornuta*, *M. obscura* SB and L, and *O. americanum*), and platelets (*P. capensis* SB and L, *S. spectabilis* SB and L, *L. cornuta* and *O. americanum*) relative to that of the control mice (Table 4.21).

In addition, oral administration of the eight ethyl acetate plants extracts at 1g/kg body weight daily to mice for 28 days significantly increased levels of the white blood cells (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* SB and L, *L. cornuta*, and *O. americanum*), neutrophils (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* SB and L, *L. cornuta* and *O. americanum*), lymphocytes (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* SB, *L. cornuta*, and *O. americanum*), and monocytes (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* L, *L. cornuta* and *O. americanum*) relative to that of the control mice (Table 4.22).

Table 4.19: Effects of oral administration of aqueous plants extracts at 1 g/kg body weight daily in mice for 28 days on some end point hematological parameters in mice

Treatment	RBC ($\times 10^6/\mu\text{L}$)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)	PLT ($\times 10^3/\mu\text{L}$)
Control	9.92 \pm 1.13	13.66 \pm 1.32	56.24 \pm 0.47	13.80 \pm 0.63	23.32 \pm 1.15	59.28 \pm 2.82	732.00 \pm 202.27
<i>P. capensis</i> L	6.65 \pm 1.10*	8.33 \pm 1.69*	27.05 \pm 4.76*	12.48 \pm 0.84*	30.68 \pm 0.86*	40.73 \pm 2.78*	594.25 \pm 103.74
<i>P. capensis</i> SB	4.84 \pm 1.16 *	7.80 \pm 2.67*	28.84 \pm 5.31*	12.98 \pm 0.62	30.86 \pm 3.68*	44.52 \pm 4.32*	499.60 \pm 168.17
<i>S. spectabilis</i> L	6.10 \pm 2.23*	8.05 \pm 2.84*	29.65 \pm 0.52*	13.28 \pm 0.68*	30.58 \pm 2.25*	43.58 \pm 4.46*	303.65 \pm 155.23*
<i>S. spectabilis</i> SB	4.93 \pm 0.30*	19.10 \pm 0.80*	58.20 \pm 1.50	13.35 \pm 0.79	25.50 \pm 0.90*	42.35 \pm 3.72*	497.75 \pm 11.62*
<i>M. obscura</i> L	5.96 \pm 1.55*	7.52 \pm 2.28	41.96 \pm 1.50*	12.54 \pm 1.38	29.80 \pm 2.42*	41.96 \pm 1.50*	490.40 \pm 107.70
<i>M. obscura</i> SB	5.20 \pm 0.70*	16.40 \pm 1.60*	49.20 \pm 1.70*	12.02 \pm 1.22*	30.10 \pm 1.30*	40.72 \pm 1.12*	566.00 \pm 53.02
<i>L. cornuta</i>	5.53 \pm 0.37*	7.30 \pm 1.07*	24.94 \pm 1.93*	13.16 \pm 1.25	29.16 \pm 2.42*	44.74 \pm 3.17*	318.06 \pm 135.47*
<i>O. americanum</i>	10.74 \pm 1.05*	7.52 \pm 1.41*	25.70 \pm 1.48*	12.82 \pm 1.43*	30.32 \pm 2.62*	42.78 \pm 1.39*	526.80 \pm 44.21

Results are expressed as Mean \pm Standard Deviation (SD). * $P < 0.05$ significantly different from control and values without asterisk mean they are comparable with the normal control group. The comparison was performed using T-test for two sample means.

Table 4.20: Effects of oral administration of aqueous plants extracts at 1 g/kg body weight daily in mice for 28 days on white blood cells and differential white blood cell count.

Treatment	WBC ($\times 10^9/L$)	DLC ($\times 10^9/L$)		
		Granulocytes	Lymphocytes	Monocytes
Control	3.70 \pm 0.44	0.95 \pm 0.12	2.21 \pm 0.24	0.54 \pm 0.12
<i>P. capensis</i> L	11.93 \pm 1.25*	1.34 \pm 0.46	8.77 \pm 1.08*	1.81 \pm 0.26*
<i>P. capensis</i> SB	14.44 \pm 3.55*	1.18 \pm 0.41	10.84 \pm 2.55*	2.42 \pm 0.85*
<i>S. spectabilis</i> L	12.66 \pm 0.40*	0.48 \pm 0.20*	10.50 \pm 0.28*	1.68 \pm 0.15*
<i>S. spectabilis</i> SB	3.79 \pm 0.51	0.82 \pm 0.11	2.65 \pm 0.37*	0.32 \pm 0.03*
<i>M. obscura</i> L	11.36 \pm 1.55*	1.22 \pm 0.39	8.72 \pm 0.88*	1.41 \pm 0.45*
<i>M. obscura</i> SB	5.35 \pm 0.77*	1.51 \pm 0.25*	3.55 \pm 0.50*	0.29 \pm 0.05*
<i>L. cornuta</i>	8.93 \pm 1.59*	1.12 \pm 0.27	6.52 \pm 1.21*	1.29 \pm 0.37*
<i>O. americanum</i>	10.78 \pm 1.00*	1.13 \pm 0.18	8.30 \pm 0.88*	1.35 \pm 0.28*

Results are expressed as Mean \pm standard deviation (SD). * $P < 0.05$ significantly different from control and values without asterisk mean they are comparable with the normal control group. The comparison was carried out by T-test for two sample means.

Table 4.21: Effects of oral administration of ethyl acetate plants extracts at 1 g/kg body weight daily in mice for 28 days on some end point hematological parameters

Treatment	RBC ($\times 10^6/\mu\text{L}$)	PCV (%)	Hb (g/dL)	MCHC (g/dL)	MCV (fL)	MCH (pg)	PLT ($\times 10^3/\mu\text{L}$)
Control	9.92 \pm 1.13	56.24 \pm 0.47	13.66 \pm 1.32	23.32 \pm 1.15	59.28 \pm 2.82	13.80 \pm 0.63	732.00 \pm 202.27
<i>P. capensis</i> L	5.90 \pm 1.94*	27.40 \pm 4.94*	7.16 \pm 2.02*	31.94 \pm 2.21*	44.52 \pm 4.32*	12.42 \pm 0.90*	445.54 \pm 66.96*
<i>P. capensis</i> SB	4.86 \pm 0.35 *	43.50 \pm 1.30*	14.40 \pm 0.93	32.40 \pm 1.00*	47.24 \pm 3.18*	13.38 \pm 1.15	497.60 \pm 65.68*
<i>S. spectabilis</i> L	7.80 \pm 1.27	33.52 \pm 5.25*	10.44 \pm 1.57	33.04 \pm 2.24*	43.04 \pm 1.86*	13.10 \pm 0.74	450.60 \pm 125.28*
<i>S. spectabilis</i> SB	5.50 \pm 0.60*	48.80 \pm 1.20*	16.7 \pm 1.10*	32.00 \pm 2.40*	43.68 \pm 1.54*	14.08 \pm 0.77	515.60 \pm 67.64*
<i>M. obscura</i> L	6.09 \pm 1.03*	27.96 \pm 2.05*	7.90 \pm 1.89*	32.58 \pm 1.84*	43.40 \pm 2.27*	12.98 \pm 1.65	535.00 \pm 144.01
<i>M. obscura</i> SB	4.70 \pm 0.87*	42.20 \pm 2.30*	14.2 \pm 0.96	31.50 \pm 1.10*	43.44 \pm 1.65*	13.43 \pm 1.16	528.20 \pm 106.92
<i>L. cornuta</i>	8.48 \pm 0.84	35.22 \pm 3.62*	11.66 \pm 1.77	34.58 \pm 1.73*	42.70 \pm 2.08*	13.06 \pm 0.65	482.60 \pm 109.21*
<i>O. americanum</i>	5.02 \pm 1.46*	22.80 \pm 2.16*	6.68 \pm 2.11*	34.76 \pm 1.20*	42.40 \pm 2.29*	12.78 \pm 0.91*	475.80 \pm 80.16*

Results are expressed as means \pm standard deviation (SD). * $P < 0.05$ significantly different from control and values without asterisk mean they are comparable with the normal control group. The comparison was carried out by T-test for two sample means.

Table 4.22: Effects of oral administration of eight ethyl acetate medicinal plants extracts at 1 g/kg body weight daily in mice for 28 days on white blood cells and differential white blood cell count.

Treatment	WBC ($\times 10^9/L$)	DLC ($\times 10^9/L$)		
		G	L	M
Control	3.70 \pm 0.56	0.83 \pm 0.25	2.35 \pm 0.06	0.52 \pm 0.27
<i>P. capensis</i> L	15.52 \pm 3.89*	2.88 \pm 0.56*	10.32 \pm 2.71*	2.32 \pm 0.88*
<i>P. capensis</i> SB	5.74 \pm 0.51*	2.35 \pm 0.20*	3.07 \pm 0.33*	0.32 \pm 0.03
<i>S. spectabilis</i> L	12.41 \pm 1.81*	2.55 \pm 0.48*	7.94 \pm 1.42*	1.92 \pm 0.29*
<i>S. spectabilis</i> SB	5.32 \pm 0.61*	2.03 \pm 0.26*	3.03 \pm 0.33*	0.26 \pm 0.05*
<i>M. obscura</i> L	12.98 \pm 1.29*	0.71 \pm 0.12*	10.20 \pm 0.89*	2.07 \pm 0.45*
<i>M. obscura</i> SB	7.09 \pm 0.71*	2.25 \pm 0.36*	4.24 \pm 0.53*	0.60 \pm 0.12
<i>L. cornuta</i>	16.27 \pm 1.53*	1.75 \pm 0.20*	12.11 \pm 1.38*	2.41 \pm 0.32*
<i>O. americanum</i>	11.37 \pm 0.51*	1.64 \pm 0.17*	8.17 \pm 0.32*	1.56 \pm 0.21*

Results are expressed as Mean \pm standard deviation (SD). * $P < 0.05$ significantly different from control and values without asterisk mean they are comparable with the normal control group. The comparison was carried out by T-test for two sample means.

4.3.3 Effect of oral administration of aqueous plants extracts on some end point biochemical parameters

As depicted in table 4.23, oral administration of the eight aqueous plants extracts at 1g/kg body weight daily in mice for 28 days significantly elevated the levels of BUN (*S. spectabilis* SB), AST (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* SB and L, *L. cornuta*), ALT (*S. spectabilis* SB, *M. obscura* SB and L), ALP (*M. obscura* SB), and CK (*P. capensis* SB and L, *S. spectabilis* SB and L, *M. obscura* L, *L. cornuta*) and lowered the levels of BUN (*M. obscura* L, *O. americanum*), AST (*O. americanum*), ALT (*P. capensis* SB and L, *S. spectabilis* L, *L. cornuta*, *O. americanum*), and ALP (*P. capensis* SB and L, *S. spectabilis* SB, *M. obscura* L, *L. cornuta*), relative to values in the control mice.

Table 4.23: Effects of oral administration of aqueous plants extracts at 1 g/kg body weight daily in mice for 28 days on some end point biochemical parameters

Treatment	BUN (mM)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	CK (IU/L)
Control	7.50±0.30	68.10±0.60	82.90±0.70	176.80±3.50	130.70±2.70
<i>P. capensis</i> stem barks	6.80±0.10	103.00±1.30*	74.20±1.70*	143.20±1.90*	149.40±0.90*
<i>P. capensis</i> leaves	7.38±0.44	95.30±0.80*	65.00±1.30*	162.90±3.07*	160.80±1.00*
<i>S. spectabilis</i> Stem barks	10.20±0.10*	109.80±0.90*	83.40±1.80*	156.20±2.50*	255.60±1.50*
<i>S. spectabilis</i> leaves	8.80±0.30	82.50±0.50*	37.60±0.65*	169.50±5.40	250.20±2.70*
<i>M. obscura</i> leaves	4.50±0.80*	120.80±5.40*	170.50±2.30*	70.20±6.40*	136.20±3.00
<i>M. obscura</i> stem barks	7.20±0.20	125.80±3.50*	91.40±3.00*	207.80±2.30*	340.20±1.90*
<i>L. cornuta</i>	8.56±0.13	80.00±1.30*	81.90±1.10*	81.64±3.70*	302.80±2.00*
<i>O. americanum</i>	5.20±0.43*	63.30±0.50*	33.80±1.20*	165.20±3.9	129.08±0.64

Results are expressed as Mean ± Standard Deviation (SD). * $P \leq 0.05$ significantly different from normal control mice by paired mean comparisons by two way student T-test.

4.3.4 Effects of oral administration of ethyl acetate stem bark and leaves extracts on some end point biochemical parameters

As depicted in table 4.24, oral administration of the eight ethyl acetate plants extracts at 1 g/kg body weight daily in mice for 28 days significantly elevated the levels of BUN (*P. capensis* SB and L, *S. spectabilis* SB, *M. obscura* L), AST (*P. capensis* SB and L, *S. spectabilis* SB and L), ALT (*S. spectabilis* L, *M. obscura* SB), ALP (*P. capensis* L, *S. spectabilis* SB, *O. americanum*), and CK (*S. spectabilis* SB and L, *M. obscura* SB and L) and lowered the levels of BUN (*S. spectabilis* L, *L. cornuta*), AST (*M. obscura* SB and L, *L. cornuta*, *O. americanum*), ALT (*S. spectabilis* SB, *M. obscura* L, *O. americanum*), and ALP (*S. spectabilis* L, *M. obscura* L, *L. cornuta*), and CK (*P. capensis* L, *L. cornuta*, *O. americanum*) relative to values in the control mice.

Table 4.24: Effects of oral administration of ethyl acetate plants extracts at 1 g/kg body weight daily in mice for 28 days on some end point biochemical parameters.

Treatment	BUN (mM)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	CK (IU/L)
Control	7.54±0.70	68.06±1.35	82.90±1.70	176.80±7.80	130.70±6.10
<i>P. capensis</i> SB	14.00±1.90*	75.40±3.70*	79.80±2.60	176.60±1.90	132.40±8.60
<i>P. capensis</i> L	12.20±9.80*	177.60±4.70*	75.30±3.50	313.40±3.60*	65.72±9.80*
<i>S. spectabilis</i> SB	9.40±0.20*	81.50±2.70*	69.20±1.90*	247.60±4.40*	242.20±5.70*
<i>S. spectabilis</i> L	5.10±0.30*	84.50±6.80*	87.20±6.60	76.10±4.50*	279.50±6.60*
<i>M. obscura</i> L	15.30±3.20*	27.90±2.70*	67.80±5.10*	68.20±6.30*	195.60±5.50*
<i>M. obscura</i> SB	7.90±1.40	60.80±3.30*	87.40±3.40*	175.00±5.20	141.40±4.70*
<i>L. cornuta</i>	4.40±1.90*	56.70±3.90*	36.30±3.20	81.90±6.60*	84.92±3.30*
<i>O. americanum</i>	6.70±1.00	37.10±1.70*	39.00±1.50*	239.00±5.30*	67.30±0.75*

Results are expressed as Mean ± Standard Deviation (SD). * $P \leq 0.05$ significantly different from normal control mice by paired mean comparisons by two way student T-test.

4.4 Proximate composition of the plants leaves and stem barks powders

As depicted in table 4.25, the leaf powder of *P. capensis* contained significantly a higher total ash and lipid content and a significantly lower total protein and nitrogen free extract compared to the stem bark powders. The dry matter, moisture content, crude fiber and gross energy content were comparable for both the leaf and stem bark powders of the plant and were higher for *P. capensis* compared to other studied medicinal plants. The dry matter for all the studied powders were comparable and ranged from 87.05 ± 0.15 to 94.62 ± 0.53 m/m. Similarly, the moisture content was comparable.

The total ash values varied from one medicinal plant to another with *O. americanum* having the highest total ash at 14.41 ± 0.38 % m/m followed by the stem barks of *M. obscura* at 8.60 ± 0.34 % m/m, while *S. spectabilis* stem barks had the lowest at 0.65 ± 0.03 % m/m. the protein composition was highest in the *O. americanum* followed by *S. spectabilis* leaves and then *L. cornuta*. However, the stem barks of *S. spectabilis* had the lowest amount of protein. The rest of the medicinal plants had significantly high amounts of protein above 8.0% m/m. *L. cornuta* contained significantly high amounts of lipids compared to all other medicinal plants and was followed by *S. spectabilis* stem barks. The rest had significantly lower amounts ($P < 0.5$) of lipids and the lowest was observed in in the stem barks of *P. capensis* (0.67 ± 0.06 % m/m).

For the crude fiber *S. spectabilis* stem bark had the highest amounts followed by the leaves of *M. obscura* and then *L. cornuta*. The lowest amount of crude fiber was from *O. americanum* at 16.90 ± 2.01 % m/m. *P. capensis* stem barks had the highest amount of nitrogen free extracts followed by leaves of *P. capensis* with 58.42 ± 1.08 and 56.27 ± 1.04 % m/m, respectively. *M. obscura* stem barks had amounts of NFE comparable to that of *P. capensis* leaves and *S. spectabilis* stem barks had the lowest. On the gross energy *P.*

capensis stem barks had the highest followed by *M. obscura* stem barks and then *P. capensis* leaves. The *S. spectabilis* stem bark extracts had the lowest amount of gross energy when compared to all the studied medicinal plants.

Table 4.25: Proximate composition of the dry medicinal plants leaves and stem barks powders

Medicinal plants		Proximate composition							
		Dry matter	Moisture	Total Ash	Protein	Lipid	Crude fiber	NFE	Gross energy
<i>P. capensis</i>	Leaves	91.54±0.24	8.46±0.42	5.33±0.15	8.66±0.53	1.13±0.12	19.11±0.89	56.27±1.04	288.77±5.13
	Stems	91.38±0.02	8.61±0.01	3.33±0.07	9.72±0.41	0.67±0.06	18.17±0.52	58.42±1.08	299.20±3.91
<i>S. spectabilis</i>	Leaves	91.44±0.46	8.56±0.80	6.60±0.06	11.57±0.10	2.60±0.15	30.99±0.89	39.68±1.14	248.55±3.16
	Stems	92.01±0.62	8.03±1.09	0.65±0.03	3.68±0.11	7.00±0.20	49.06±1.02	31.62±0.45	172.16±2.25
<i>M. obscura</i>	Leaves	94.62±0.53	7.09±2.27	7.23±0.02	9.85±0.05	3.09±0.04	30.12±1.08	47.99±1.12	276.69±1.60
	Stems	92.25±0.13	9.14±3.55	8.60±0.34	8.29±0.33	2.14±0.26	17.03±1.97	56.19±2.76	291.66±4.75
<i>O. americanum</i>		87.05±0.15	12.95±0.26	14.41±0.38	13.77±0.12	1.89±0.10	16.90±2.01	40.08±0.86	255.88±3.71
<i>L. cornuta</i>		90.38±0.11	9.62±0.20	3.45±1.01	10.28±0.14	16.68±1.53	28.90±1.12	49.49±0.98	250.91±21.75

Results are expressed as the Mean ± Standard Deviation (SD) of the % m/m of the proximate composition components and the energy is in mega Joule per 100 g of the dry medicinal plant powders.

4.5 Phytochemical present in the plants leaves and stem barks powders

The phytochemicals detected in the leaf and stem bark powders of *P. capensis* (L) were tannins, phenolics, saponins, phylobatannins, terpenoids, flavonoids, steroids, cardiac glycosides (in trace amounts) and alkaloids. However, reducing sugars were not detected in the stem barks or leaf powders of the *P. capensis* (Table 4.26). The reducing sugars were present in the stem barks of *S. spectabilis*, *M. obscura*, *L. cornuta* and *O. americanum*. In contrast, reducing sugars were not detected in the leaves of the *M. obscura* and *S. spectabilis*. Phylobatannins were not detected in the leaves of the *S. spectabilis* but they were present in trace amounts from the leaf powders of *M. obscura*. Tannins, phenols and saponins were present in substantial levels in the eight studied medicinal plants powders.

As depicted in table 4.27, *P. capensis* leaves powders contained the highest amounts of total phenols at 7.43 ± 0.85 mg gallic acid equivalent per g of dry matter followed by the stem barks with 5.81 ± 0.41 . The stem bark of *M. obscura* contained 4.71 ± 0.18 and this was followed by leaves of *S. spectabilis* with 4.01 ± 0.03 . *L. cornuta* and *O. americanum* had the lowest amounts of total phenols. For the alkaloids the stem barks of *P. capensis* had the highest amounts of 67.3 ± 7.0 mg/g of dry medicinal material followed by *L. cornuta* with 62.67 ± 3.06 and *M. obscura* leaves had the lowest amounts of alkaloids at 18 ± 0.32 mg/g dry matter. *M. obscura* leaves had the highest amount of saponins at 242.0 ± 3.0 mg/g, followed by the leaves of *S. spectabilis* at 214.0 ± 4.0 mg/g and the lowest was 108.0 ± 6.25 mg/g from *L. cornuta*.

The highest amount of flavonoids was 127.3 ± 15.3 and 106.7 ± 20.8 mg pyrocatechol equivalent per 100 g of medicinal plant material from *P. capensis* leaves and stem barks, respectively. These were followed by the leaves of *S. spectabilis* with 14.71 ± 0.03 and the

lowest amount was determined in both *O. americanum* and *L. cornuta* with both 9.26 ± 0.03 mg pyrocatechol equivalent per 100 g. the leaves of *M. obscura* contained the highest amount of tannins followed by *L. cornuta* with 11.87 ± 0.01 and 11.82 ± 0.04 mg/kg gallic acid equivalent and the lowest amount was recorded in *P. capensis* leaves. Therefore, the amounts of phytochemicals quantified varied from one medicinal plant to another and from one plant part studied to the other.

Table 4.26: Qualitative screening of the phytonutrients in the medicinal plants powders

Phytochemicals	Dry medicinal plants powders							
	<i>P. capensis</i>		<i>S. spectabilis</i>		<i>M. obscura</i>		<i>L. cornuta</i>	<i>O. americanum</i>
	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf	Composite	Composite
Tannins	++	++	++	++	++	++	++	++
Phenols	++	++	++	++	++	++	+	++
Saponins	++	++	++	++	++	++	+	++
Phylobatannins	++	++	++	-	++	Trace	+	++
Terpenoids	++	++	++	++	++	++	++	++
Flavonoids	++	++	Trace	++	+++	++	+++	+++
Steroids	++++	+++	++	+	+++	++++	++	++
Cardiac glycosides	++++	+ trace	+ trace	+++	+++	++++	+	+
Glycosides/ reducing sugar	-	-	++	-	++	-	+	+
Alkaloids	+	+	+	+	+	+	+	+

Legend:

The sign:

- Phytochemicals not detected

Trace: Phytochemicals detected in low amounts

+ Phytochemicals detected slightly high than trace

++ Phytochemicals detected in moderate levels

+++ or ++++ Phytochemicals detectable in high amount

Table 4.27: Quantitative analysis of phytochemicals in the medicinal plants leaves and stem barks powders

Plant	Plant part	Class of phytochemicals and amounts in medicinal plants				
		Total phenols	Alkaloids	Saponins	Flavonoids	Tannins
<i>P. capensis L.</i>	Leaf	7.43±0.85	18.0±4.0	201.0±8.5	127.3±15.3	2.85±0.02
	Stem bark	5.81±0.41	67.3±7.0	125.0±3.6	106.7±20.8	5.06±0.03
<i>S. spectabilis</i>	Leaf	4.01±0.03	62.0±4.0	214.0±4.0	14.71±0.03	3.72±0.03
	Stem bark	1.78±0.04	62.0±6.0	142.0±6.0	10.51±0.03	3.59±0.03
<i>O. americanum</i>	Composite	1.54±0.06	60.7±12.9	114.7±11.5	9.26±0.03	4.70±0.04
<i>L. cornuta</i>	Composite	1.54±0.04	62.7±3.1	108.0±6.3	9.26±0.03	11.82±0.04
<i>M. obscura</i>	Leaf	2.18±0.04	18.0±0.3	242.0±3.0	10.67±0.04	11.87±0.01
	Stem bark	4.71±0.18	26.0±4.0	108.0±4.0	11.41±0.05	2.10±0.03

Quantities are expressed as Mean ± Standard Deviation (SD) of three determinations for each powder. The total phenols were expressed as mg gallic acid equivalents per g dry powder, and tannins as mg/kg of gallic acid standard, while other measured phytochemicals were expressed as mg per g of dry powder. The flavonoids were expressed as mg pyrocatechol equivalent per 100g of dry plant powder.

4.6 Phytonutrient levels of the leaves and stem barks powders

As shown in table 4.28, various macronutrients were found in varying amounts in the five medicinal plants powders studied. The leaves powders of *M. obscura* contained the highest amount of ascorbic acid, followed by *S. spectabilis* and *P. capensis* had the lowest at $19310 \pm 70 \mu\text{g}/100 \text{ g}$ of the dry plant material. Generally, the five medicinal plants were found to be good source of ascorbic acid. For thiamine, the *P. capensis* stem bark powders were found to have the highest amounts followed by *L. cornuta* and the *O. americanum* and in *S. spectabilis* the amounts were not detectable. For niacin, the leaves powder of *P. capensis* had the highest amount followed by leaves powders of *S. spectabilis* and then leaves of *M. obscura*. However, the *M. obscura* stem barks, *S. spectabilis* stem barks and *O. americanum* did not contain detectable amounts of niacin.

The amounts of ascorbic acid varied with the medicinal plant and also with the part studied. The leaves of *M. obscura* had significantly high amount of ascorbic acid compared to other extracts. This was followed by the leaves of *S. spectabilis*, and the leaves of *P. capensis* had the lowest amounts. In general, the leaves of the medicinal plants were found to contain more ascorbic acid than the stem bark extracts. *O. americanum* shrub contained the highest amount of ascorbic acid than the *L. cornuta*. Alpha-tocopherol was the highest phytonutrient ($47540 \pm 40 \mu\text{g}/100 \text{ g}$) in the leaves powders of *P. capensis*, while the least ($6.0 \pm 0.1 \mu\text{g}/100 \text{ g}$) amount of lycopene was observed in the stem powders of *P. capensis*. *O. americanum* shrub had the highest amount of β -carotene followed by the leaves powders of *S. spectabilis* with 65520 ± 14 and $10580 \pm 5 \mu\text{g}/100 \text{ g}$, respectively. *L. cornuta* shrub powders followed by *O. americanum* shrub powders contained the highest amounts of vitamin B1; while *P. capensis* leaves powders contained the highest amounts of vitamin B3.

Table 4.28: Phytonutrients composition of the leaves and stem barks powders of the studied medicinal plants

Medicinal plant	Plant part	Classes of phytonutrients							
		Vit C	Vit B1	Vit B3	Retinol	Tocopherol	Lycopene	β -carotene	β -Cryptoxanthin
<i>P. capensis</i>	Leaf	19.31 \pm 0.70	43.8 \pm 0.6	30.4 \pm 0.6	30 \pm 10	47540 \pm 40	10.0 \pm 2.0	650 \pm 3	20 \pm 6
	Stem bark	40.30 \pm 0.50	138.4 \pm 11.0	7.3 \pm 0.4	5 \pm 10	400 \pm 10	6.0 \pm 0.1	67 \pm 1	ND
<i>S. spectabilis</i>	Leaf	235.54 \pm 0.40	29.2 \pm 0.7	9.8 \pm 1.2	44070 \pm 2	17950 \pm 10	7050 \pm 0.1	10580 \pm 5	4790 \pm 4
	Stem bark	80.61 \pm 0.40	ND	ND	11 \pm 0.2	870 \pm 10	8.0 \pm 0.2	95 \pm 8	13 \pm 0.1
<i>M. obscura</i>	Leaf	352.26 \pm 1.44	13.4 \pm 1.7	7.8 \pm 0.2	15760 \pm 2	28480 \pm 10	6030 \pm 0.2	1630 \pm 18	ND
	Stem bark	36.46 \pm 0.24	8.6 \pm 0.6	ND	10 \pm 5	550 \pm 20	10.0 \pm 0.1	110 \pm 9	ND
<i>L. cornuta</i>	Composite	65.25 \pm 0.42	74.5 \pm 1.3	9.1 \pm 0.4	700 \pm 5	3270 \pm 50	140.0 \pm 1.0	1140 \pm 94	590 \pm 0.5
<i>O. americanum</i>	Composite	42.89 \pm 0.30	69.5 \pm 7.1	ND	84090 \pm 1	560 \pm 30	360.0 \pm 0.2	65520 \pm 14	140 \pm 2

Results are expressed as Mean \pm Standard Deviation (SD) of three determinations for each extract. Each vitamin concentration was expressed as μ g/100g dry matter except for vitamin B1 and B3 and C expressed as mean mg/100 g \pm SD dry matter and ND stands for not detected.

4.6.1 Free radical scavenging activity by DPPH assay of the leaves and stem bark powders

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. As demonstrated in table 4.29, *M. obscura* stem barks powders had the highest radical scavenging activity of $109.27 \pm 0.24\%$ which was higher than the standard and all other medicinal plants extracts. This activity was followed by that of the ascorbic acid standard and then *S. spectabilis* stem bark at 97.76 ± 0.03 and $90.95 \pm 0.01\%$, respectively. The stem barks powders of *P. capensis* had higher free radical scavenging activity than the leaves at 91.01 ± 0.10 and $90.85 \pm 0.40\%$, respectively. *O. americanum* was found to possess the lowest antioxidant activity at $50.47 \pm 0.03\%$. The observed activities indicate that the plant powders have a strong proton-donating ability and could serve as free radical scavengers, acting perhaps as primary antioxidants.

Table 4.29: Percentage free radical scavenging activity by DPPH assay for the dry leaves and stem bark powders of the studied medicinal plants

Plants studied	Free radical scavenging activity by DPPH assay per extract expressed as Mean \pm SD % activity	
	Leaves	Stem barks
<i>P. capensis</i>	90.85 ± 0.40	91.01 ± 0.10
<i>S. spectabilis</i>	88.98 ± 0.001	90.95 ± 0.01
<i>M. obscura</i>	90.03 ± 0.03	109.27 ± 0.24
<i>O. americanum</i>	50.47 ± 0.03	
<i>L. cornuta</i>	86.02 ± 0.001	
Ascorbic acid	97.76 ± 0.03	

Results are expressed as Mean \pm Standard Deviation (SD) powders of the studied in the medicinal plants

4.6.2 Total antioxidant activity by phosphomolybdate assay of the leaves and stem bark powders

The phosphomolybdate assay is based on the reduction of Mo (VI) to Mo (V) by the plants extracts and the ascorbic acid fractions and subsequent formation of a green phosphate Mo (V) complex at the acid pH. As depicted in table 4.30, the leaves powders of *S. spectabilis*

were found to contain the highest amount of ascorbic acid equivalent (206.81 ± 0.02 $\mu\text{g}/\text{mg}$), followed by the leaves of *P. capensis* (115.38 ± 0.09 $\mu\text{g}/\text{mg}$), and the stem bark of *M. obscura* with 115.17 ± 0.02 $\mu\text{g}/\text{mg}$ of the dried plant powders. In contrast, the stem barks of *P. capensis* contained the lowest amount of ascorbic acid equivalent at 39.25 ± 0.09 $\mu\text{g}/\text{mg}$. For *S. spectabilis* leaves there was a significantly higher amount of total phenols and a correspondingly high amount of ascorbic acid equivalent when evaluated by the phosphomolybdate assay. Similarly, the *P. capensis* leaves contained high amount of total phenols (7.43 ± 0.85 $\mu\text{g}/\text{mg}$) compared to the stem barks (5.81 ± 0.41 $\mu\text{g}/\text{mg}$), and these values are in agreement with the total antioxidant activity demonstrated by phosphomolybdate assay as 115.38 ± 0.09 and 39.25 ± 0.09 $\mu\text{g}/\text{mg}$ dry plant powder, respectively.

Table 4.30: Total antioxidant activity by phosphomolybdate assay expressed as ascorbic acid equivalent in $\mu\text{g}/\text{mg}$ of dried plant powder

Plants studied	Total antioxidant activity expressed as ascorbic acid equivalent (AAE) in $\mu\text{g}/\text{mg}$ dried plant powder as Mean \pm SD	
	Leaves	Stem barks
<i>P. capensis</i>	115.38 ± 0.09	39.25 ± 0.09
<i>S. spectabilis</i>	206.81 ± 0.02	94.77 ± 0.03
<i>M. obscura</i>	49.25 ± 0.21	115.17 ± 0.02
<i>O. americanum</i>	60.08 ± 0.11	
<i>L. cornuta</i>	78.83 ± 0.02	

Results are expressed as Mean \pm Standard Deviation (SD)

4.6.3 Reducing power of the plants powders

As depicted in figure 4.15, a sequential increase in reducing power demonstrated by increase in absorbances with increase in the concentration of gallic acid and plants extracts was observed. This is as a result of continued formation of a blue Fe^{2+} complex. With exception of *S. spectabilis* leaf extracts, all other plants extracts attained maximum reducing power at a concentration of 800 $\mu\text{g}/\text{mL}$. *O. americanum*, *P. capensis* stem barks

and *M. obscura* leaves, demonstrated a significantly high reducing potential, which reached the maximum level at the concentration of 400 $\mu\text{g/mL}$. *L. cornuta* had the lowest reducing activity that gradually increased to attain the maximum level when the concentration was raised to 800 $\mu\text{g/mL}$. For the *S. spectabilis* leaves, the reducing power did not reach maximum level even when the concentration was at 800 $\mu\text{g/mL}$. In contrast, *O. americanum* had the highest reducing potential compared to the rest of the extracts, and its reducing power was slightly lower than that of the gallic acid standard.

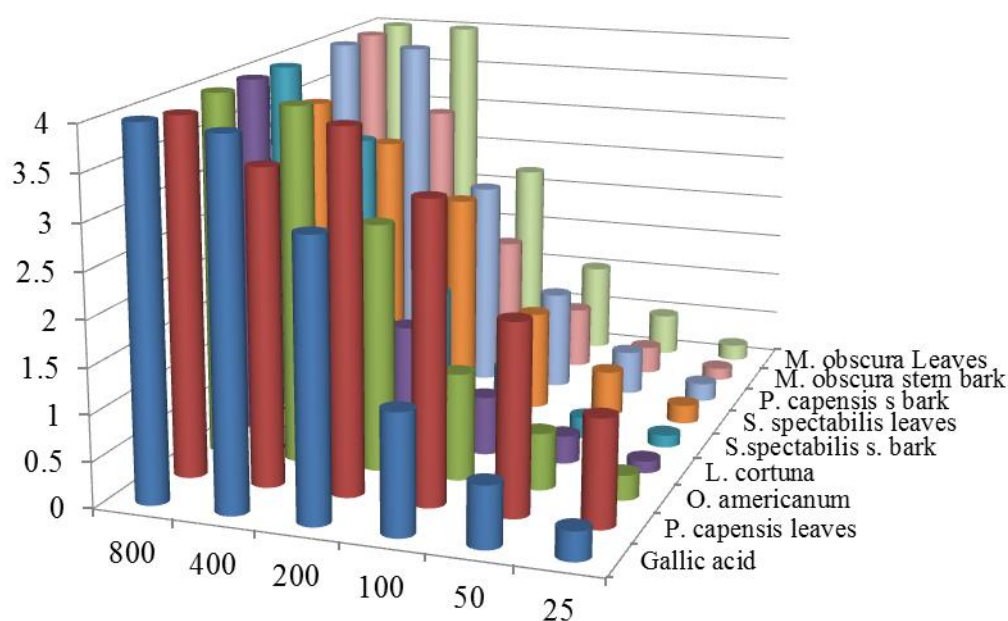


Figure 4.15: Plots of absorbance against concentration of the gallic acid and the eight medicinal plants powders ranging from 25-800 $\mu\text{g/mL}$

4.6.4 Compounds of ethyl acetate stem barks and leaves of *P. capensis*

As depicted in table 4.31, there were 29 chemical compounds detected from the ethyl acetate stem bark extracts of *P. capensis*. Of the 29 compounds identified, 12 were unknown indicating that they were not present in the NIST11 database and therefore their chemical names and structures remained unknown. The rest of the compounds were available in the database and their structures were known. Most of the compounds were terpenoids, phytosterols, fatty acids and phenylpropanoids.

Table 4.31: Compounds in the ethyl acetate stem bark extracts of *P. capensis*

S/N	RT (mins)	Compound name	Formula
1	4.0	Unknown	
2	4.1	Butane, 1,1'-[oxybis(2,1-ethanedioxy)]bis- (FAD)	C ₁₂ H ₂₆ O ₃
3	4.2	Sulfurous acid, 2- ethylhexyl hexyl ester (FAD)	C ₁₄ H ₃₀ O ₃ S
4	4.4	Unknown	
5	4.6	2-Propenoic acid, 6- methylheptyl ester- (FAD)	C ₁₁ H ₂₀ O ₂
6	5.1	Undecane, 6,6- dimethyl- (FAD)	C ₁₃ H ₂₈
7	5.5	Nonane, 3-methyl-5- propyl- (FAD/I)	C ₁₃ H ₂₈
8	5.6	Thymol (MT)	C ₁₀ H ₁₄ O
9	10.9	4-((1E)-3-Hydroxy-1- propenyl)-2-methoxyphenol (PP)	C ₁₀ H ₁₂ O ₃
10	13.2	n-Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
11	14.1	3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one (ST)	C ₁₅ H ₁₄ O
12	16.2	9-Octadecenoic acid, (E)- (FA)	C ₁₈ H ₃₄ O ₂
13-14	16.6-19.3	Unknown	
15	19.5	13-Docosenamide, (Z)- (FAD)	C ₂₂ H ₄₃ NO
16	26.3	2-Bromomethyl-1-isopropenyl-3-methyl-cyclopentane (MT)	C ₁₀ H ₁₇ Br
17	27.2	Tridecanol, 2-ethyl-2-methyl- (FAD)	C ₁₆ H ₃₄ O
18	28.2	Unknown	
19	28.5	Butanoic acid, 3-methyl-, 3,7- dimethyl-6-octenyl ester (MT/I)	C ₁₅ H ₂₈ O ₂
20	28.6	Unknown	
21	30.1	Dodecane, 6-cyclohexyl- (FAD)	C ₁₈ H ₃₆
22	32.4	Unknown	
23	33.1	Stigmasterol (S)	C ₂₉ H ₄₈ O
24-25	33.2-35.3	Unknown	
26	36.2	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1Hnaphthalen-2-one (ST)	C ₁₅ H ₂₂ O
27-29	36.3-36.8	Unknown	

Legend: FAD stands for fatty acid derivatives, FA/I for fatty acid / isoprene, MT for monoterpenoid, FA for fatty acid, PP for phenylprenoids, MT/I for monoterpenoid / isoprene, S for phytosterol and ST for sesquiterpenoid.

As shown in figure 4.16, the most prominent compounds were compounds numbers 11, 13, 20 and 28. Compound 13 with the highest peak is 3-phenylbicyclo (3.2.2) nona-3, 6-dien-2-one, and compound 17 is tridecanol, 2 ethyl-2-methyl-. Compound number 28 is unknown. The phytosterol stigmasterol is also detected in substantial amount as compound number 26.

As shown in table 4.32 and figure 4.17, 20 chemical compounds were detected from the ethyl acetate leaves extract of *P. capensis*. Out of these, only compound number 11 was of unknown nature since it could not be determined from the NIST database by chromatogram deconvolution. Other compounds names could be determined and they ranged from essential oils, esters, phytosterols, and hydrocarbonated compounds with various medicinal activities. As shown in figure 4.17, the highest peaks representing compounds with high composition in the extracts are; compounds 4, 6, 7, 8 and 21. Compound 20, had the highest amount and was found to be androstan-6-one, (5. alpha.)-.

Table 4.32: Compounds in ethyl acetate leaves extracts of *P. capensis*

S/N	RT (mins)	Compound name	Formula
1	4.2	Heptane, 4-ethyl-2,2,6,6-tetramethyl- (ST)	C ₁₃ H ₂₈
2	4.6	2-Propenoic acid, 6- methylheptyl ester (FAD)	C ₁₁ H ₂₀ O ₂
3	13.6	n-Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
4	15.8	Cyclohexanol, 5-methyl-2-(1- methylethyl)- (MT)	C ₁₀ H ₂₀ O
5	16.3	Oleic acid (FA)	C ₁₆ H ₃₄ O ₂
6	16.8	Hexadecanamide (FAD)	C ₁₆ H ₃₃ NO
7	19.6	9-Octadecenamide, (Z)- (FAD)	C ₁₈ H ₃₅ NO
8	26.3	Squalene (TT)	C ₃₀ H ₅₀
9	26.7	2-methyloctacosane (FA)	C ₂₉ H ₆₀
10	30.0	Pentadecane, 2,6,10,14- tetramethyl- (DTD)	C ₁₉ H ₄₀
11	32.4	Unknown	
12	33.1	Stigmasterol (S)	C ₂₉ H ₄₈ O
13	34.1	1R,4S,7S,11R-2,2,4,8- Tetramethyltricyclo[5.3.1.0(4, 11)] undec-8-ene (ST)	C ₁₅ H ₂₄
14	34.4	5.beta.-Pregnane-3.alpha.,20.alpha.-diol, bis(trifluoroacetate) (S)	C ₂₅ H ₃₄ F ₆ O ₄
15	34.5	2H-Cyclopropa [a] naphthalen-2-one (ST)	C ₁₅ H ₂₂ O
16	34.7	1,2,4,8-Tetramethylbicyclo[6.3.0]undeca-2,4-diene (ST)	C ₁₅ H ₂₄
17	35.1	beta-Amyrin (PCTT)	C ₃₀ H ₅₀ O
18	35.7	Lup-20 (29)-en-3-one (PCTT)	C ₃₀ H ₄₈ O
19	36.3	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl)- (TTD)	C ₃₃ H ₅₆
20	36.6	Androstan-6-one, (5. Alpha.)- (S)	C ₁₉ H ₃₀ O

Legend: FAD stands for fatty acid derivatives, MT for monoterpenoid, FA for fatty acid, S for phytosterol, ST for sesquiterpenoid, PCTT for pentacyclic triterpenoid, TTD for triterpenoid derivative and DTD for diterpenoid derivative.

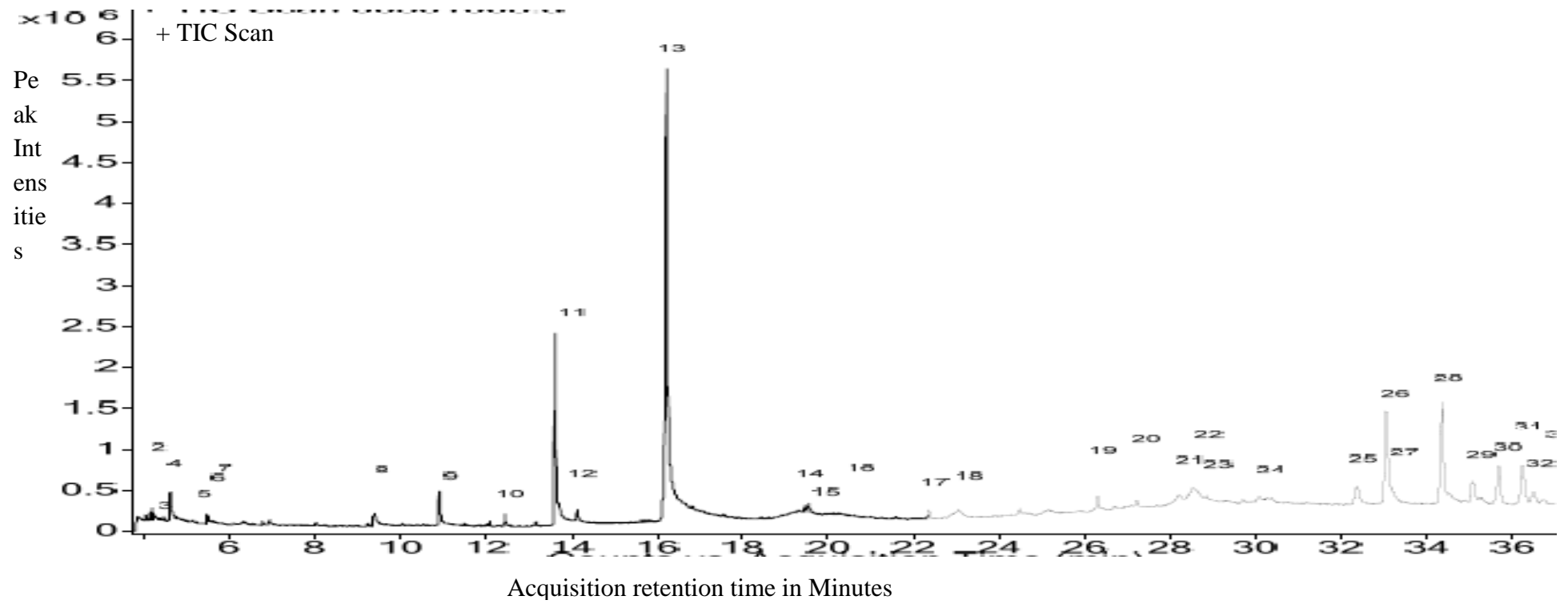


Figure 4.16: GC-MS chromatogram of *P. capensis* stem barks ethyl acetate extracts

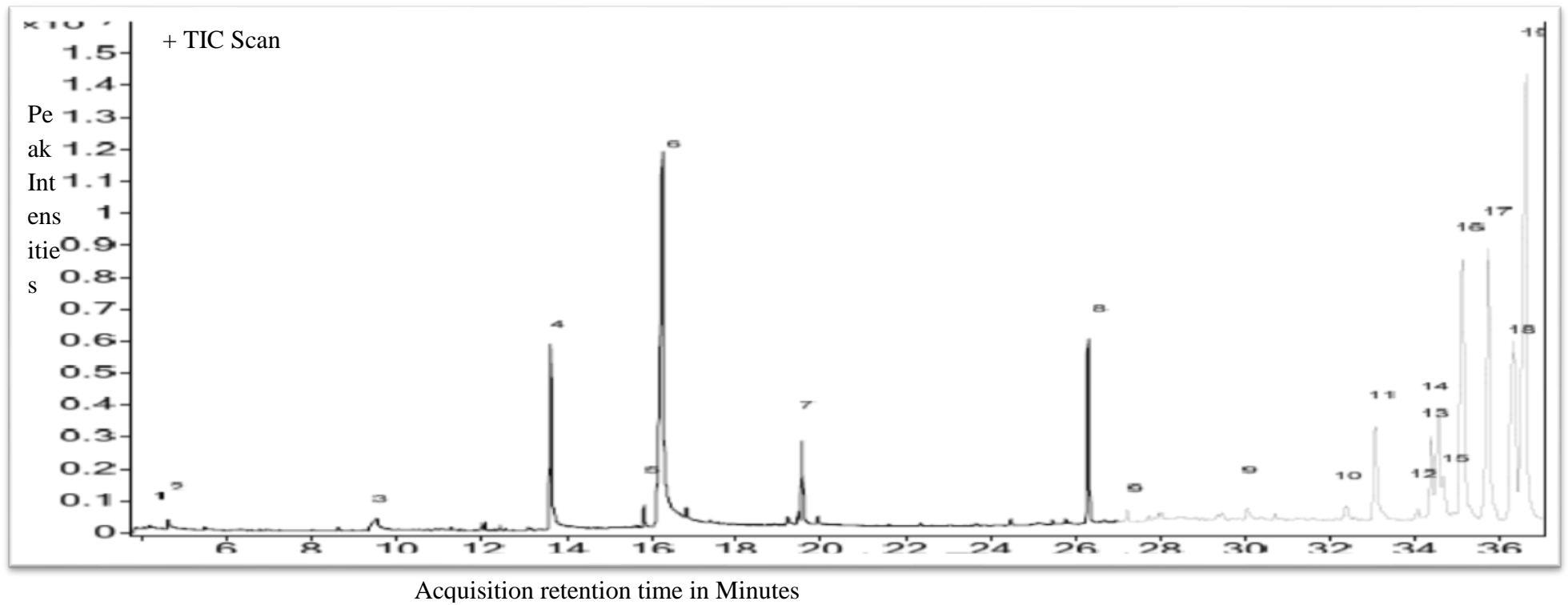


Figure 4.17: GC-MS chromatogram of the *P. capensis* leaves ethyl acetate extracts

4.6.5 Compounds from ethyl acetate stem bark extracts of *S. spectabilis*

As depicted in table 4.33 and figure 4.18, the ethyl acetate stem bark extracts of *S. spectabilis* contained 37 chemical compounds with 21 unidentified by the NIST2011 database. The results show that this plant part had very diverse chemical constituents which might greatly contribute to the observed activities in the bioassays. The compounds ranged from fatty acids derivatives, phenolics such as resorcinol and phytosterols.

Table 4.33: Compounds present in the ethyl acetate stem bark extracts of *S. spectabilis*

S/N	RT(min)	Compound name	Formula
1	4.12	(E)- 2-(2-Methoxyethoxy) ethyl 2-methylbut-2-enoate (FAD)	C ₁₀ H ₁₈ O ₄
2	4.23	A-amino, γ -butyrolactone (FAD)	C ₄ H ₇ NO ₂
3	4.40	Propanoic acid, propyl ester (FAD)	C ₆ H ₁₂ O ₂
4	4.48	n-propyl acetate (FAD)	C ₅ H ₁₀ O ₂
5	4.92	1,3-Dioxan-5-ol	C ₄ H ₈ O ₃
6	5.497	3-pentanone, 2-methyl- (MTD)	C ₆ H ₁₂ O
7	5.55	2-Heptanone, 3-methyl- (MTD)	C ₈ H ₁₆ O
8	5.75	<i>Sec</i> -butyl acetate (FAD)	C ₆ H ₁₂ O ₂
9	5.91	Toluene (AH)	C ₇ H ₈
10	7.34	Acetic acid, butyl ester (FAD)	C ₆ H ₁₂ O ₂
11	15.32	Benzofuran, 2,3-dihydro (SD)	C ₈ H ₈ O
12	16.19	Resorcinol (P)	C ₆ H ₆ O ₂
13	20.40	1,3-Benzenediol, 4-propyl (FAD)	C ₉ H ₁₂ O ₂
14	20.94	Methyl(methyl4-O-methyl-. alpha.-d-mannopyranoside) uronate (SD)	C ₉ H ₁₆ O ₇
15-21	32.50-34.38	Unknown	
22	34.90	Chondrillasterol (S)	C ₂₉ H ₄₈ O
23	35.13	Unknown	
24	35.46	.gamma.-Sitosterol (S)	C ₂₉ H ₅₀ O
25-37	36.99-42.24	Unknown	

Legend: FAD stands for fatty acid derivatives, S for phytosterol, SD for sugar derivative, P for phenolic and AH for Aromatic hydrocarbon.

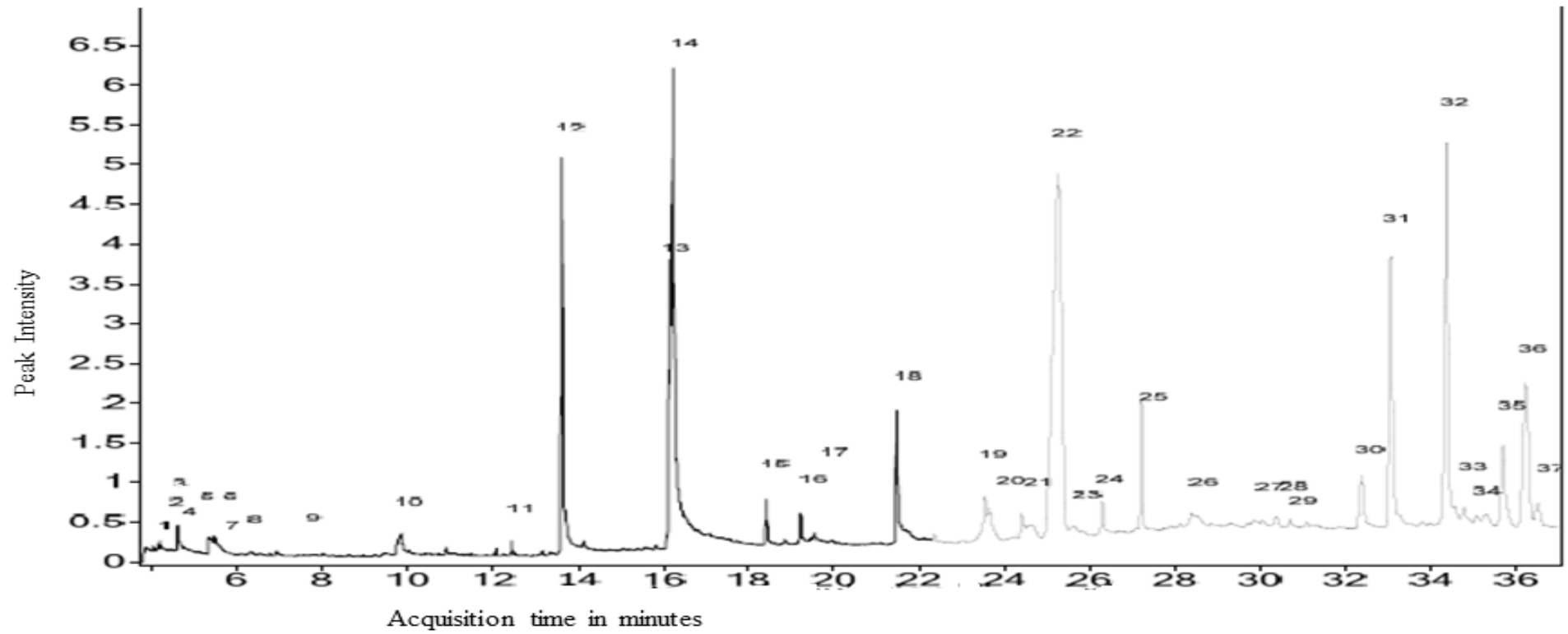


Figure 4.18: GC-MS Chromatogram of stem bark ethyl acetate extracts of *S. spectabilis*

As shown in table 4.34 and figure 4.19, the ethyl acetate leaves extracts of *S. spectabilis* were found to contain 47 chemical compounds ranging from 50 to 500 Da. Of these compounds, 17 were new compounds not present in the NIST 2011 chemical databases, while 30 were available in the database.

Table 4.34: Compounds from ethyl acetate leaves extracts of *S. spectabilis*

S/N	RT (mins)	Compound name	Formula
1	4.0	Unknown	
2	4.1	Propanoic acid, butyl ester (FA)	C ₇ H ₁₄ O ₂
3	4.2	Decane, 3,8-dimethyl- (TD)	C ₁₂ H ₂₆
4-5	4.4-5.1	Unknown	
6	5.3	Resorcinol (P)	C ₆ H ₆ O ₂
7	5.5	Decane, 3, 8-dimethyl (TD)	C ₁₂ H ₂₆
8	6.3	1,2-butanediol, 3, 3-dimethyl (ID)	C ₆ H ₁₄ O ₂
9-10	7.8-9.8	Unknown	
11	10.7	6-Tridecene, 7-methyl (FA)	C ₁₄ H ₂₈
12	11	Tetradecanoic acid (FA)	C ₁₄ H ₂₈ O ₂
13	11.3	Thiophene, 2-methyl-5-propyl- (FAD)	C ₈ H ₁₂ S
14	12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (DTD)	C ₂₀ H ₄₀ O
15	12.1	Methyl tetrahydroionol (STD)	C ₁₄ H ₂₈ O
16	12.6	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (DT)	C ₂₀ H ₄₀ O
17	13.1	Nerolidol 1 (ST)	C ₁₅ H ₂₆ O
18	13.6	n-Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
19	15.8	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.beta.,5.beta.)]- (MT)	C ₁₀ H ₂₀ O
20	16.1	9-Methylbicyclo[3.3.1]nonane (MT)	C ₁₀ H ₁₈
21	16.2	9-Octadecenoic acid, (E)- (FA)	C ₁₈ H ₃₄ O ₂
22	16.5	Unknown	
23	16.2	Bicyclo[4.3.0]nonane,2-methylene (MT)	C ₁₀ H ₁₆
24	19.5	Bis(2-ethylhexyl)hydrogen phosphate (FAD)	C ₁₆ H ₃₅ O ₄ P
25	19.9	Carbonic acid, methyl ester,[(E,E)-3,7,11-trimethyl-2,6,10 dodecatrien-1-yl] ester (STD)	C ₁₇ H ₂₈ O ₃
26	21.5	Fumaric acid, decyl pent-4-en-2-yl ester (FAD)	C ₁₉ H ₃₂ O ₄
27	21.6	Pentadecane, 2,6,10,14-tetramethyl- (DTD)	C ₁₉ H ₄₀
28	23	Hexadecane (FA)	C ₁₆ H ₃₄

S/N	RT (min)	Compound name	Formula
29	23.9	Unknown	
30	24.4	1-Cyclohexyl-1-(4-methylcyclohexyl)ethane (STD)	C ₁₅ H ₂₈
31	25.8	n-pentadecanol (FA)	C ₁₅ H ₃₂ O
32	25.9	Hexadecane, 2-methyl- (FAD)	C ₁₇ H ₃₆
33	26.3	Squalene (TT)	C ₃₀ H ₅₀
34	27.2	Heneicosane (FA)	C ₂₁ H ₄₄
35	27.5	Unknown	
36	27.7	Nerolidol 2 (ST)	C ₁₅ H ₂₆ O
37	28	2-Bromomethyl-1-isopropenyl-3-methyl-cyclopentane (MTD)	C ₁₀ H ₁₇ Br
38	28.4	Unknown	
39	29.4	.beta.-Tocopherol (TTD, Vit)	C ₂₈ H ₄₈ O ₂
40	29.7	Unknown	
41	30.1	Formic acid, dodecyl ester (FAD)	C ₁₃ H ₂₆ O ₂
42	32.4	Unknown	
43	34.1	1-Decanol, 2-hexyl- (FAD)	C ₁₆ H ₃₄ O
44	34.4	Ergost-22-en-3-ol, (3.alpha.,5.beta.,22E)- (S)	C ₂₈ H ₄₈ O
45	35.1	.beta.-Amyrin (PCTT)	C ₃₀ H ₅₀ O
46	36.1	Unknown	
47	36.5	Androstan-6-one, (5.alpha.)- (S)	C ₁₉ H ₃₀ O

Legend: FAD stands for fatty acid derivatives, MT for monoterpenoid, IS for isoprenoid derivative, FA for fatty acid, P for phenolics, ST for sesquiterpenoid, PCTT for pentacyclic triterpenoid, TTD, Vit for triterpenoid derivative vitamin, DTD for diterpenoid derivative, P for phenolic compound and STD for sesquiterpenoid derivative.

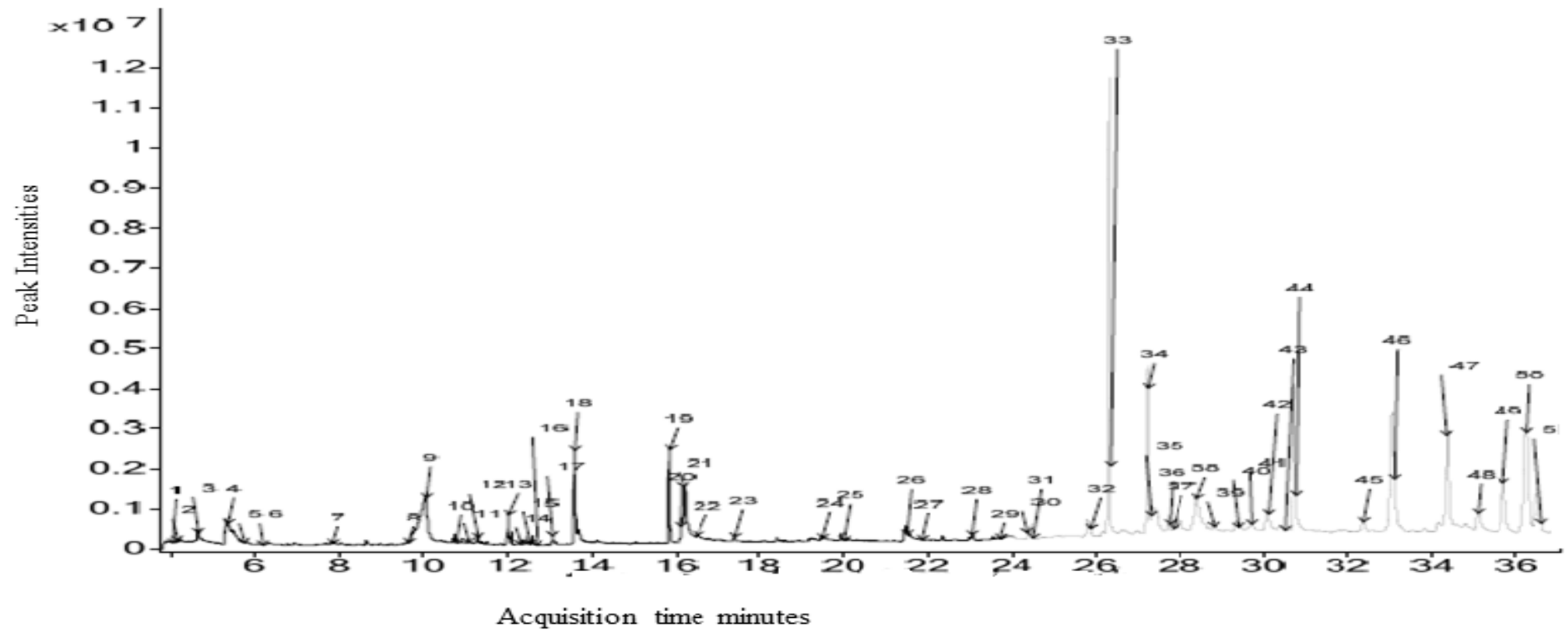


Figure 4.19: GC-MS Chromatogram of ethyl acetate leaves extracts of *S. spectabilis*

4.6.6 Compounds from ethyl acetate stem bark extracts of *M. obscura*

As shown in table 4.35 and figure 4.20, 32 compounds were detected in the ethyl acetate stem bark extracts of *M. obscura* which could be responsible for the observed efficacy and toxicity. Out of 32 compounds identified, 22 were available in the database and 10 were not present in the NIST 11 chemical database.

Table 4.35: Compounds in the ethyl acetate stem bark extracts of *M. obscura*

S/N	RT(mins)	Compound name	Formula
1	4.0	Unknown	
2	4.2	Heptane, 2,2,3,3,5,6,6- heptamethyl-	C ₁₄ H ₃₀
3	4.6	Unknown	
4	5.1	Nonane, 2,2,4,4,6,8,8- heptamethyl- (TTD)	C ₁₆ H ₃₄
5	5.5	Dodecane, 2, 6, 10-trimethyl- (TT)	C ₁₅ H ₃₂
6	9.9	Methyl(methyl 4- <i>O</i> -methyl-.alpha.-dmannopyranoside)uronate (SD)	C ₉ H ₁₆ O ₇
7	12	3,7,11,15-Tetramethyl-2- hexadecen-1-ol (DT)	C ₂₀ H ₄₀ O
8	13.6	n-Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
9	15.8	Cyclohexanol, 5-methyl-2-(1- methylethyl)-(MT)	C ₁₀ H ₂₀ O
10	16.2	9-Octadecenoic acid, (E)- (FA)	C ₁₈ H ₃₄ O ₂
11	16.5	Octadecanoic acid (FA)	C ₁₈ H ₃₆ O ₂
12	18.1	Unknown	
13	19.5	1,3,2-Oxazaborinane, 2-butyl-	C ₇ H ₁₆ BNO
14	19.7	Unknown	
15	20.0	3,5-Dimethoxy-7- methyl-naphthalen-2-ol (FAD)	C ₁₃ H ₁₄ O ₃
16-17	21.1-22.6	Unknown	
18	23.7	Benzimidazo[2,1- a]isoquinoline (IQA)	C ₁₅ H ₁₀ N ₂
19	24	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- (PCTT)	C ₃₁ H ₄₈ O ₃
20	24.5	Sulfurous acid, 2-ethylhexyl tridecyl ester (FAD)	C ₂₁ H ₄₄ O ₃ S
21	25.9	Hexadecane (FAD)	C ₁₆ H ₃₄
22	26.3	Squalene (TT)	C ₃₀ H ₅₀
23	27.2	Heneicosane (FAD)	C ₂₁ H ₄₄
24	27.7	<i>cis</i> -Thujopsene (ST)	C ₁₅ H ₂₄
25	28	2-Bromomethyl-1-isopropenyl-3-methyl-cyclopentane (MTD)	C ₁₀ H ₁₇ Br
26	28.5	Unknown	
27	30.1	1-Decanol, 2-hexyl (FAD)	C ₁₆ H ₃₄ O
28-30	30.7-34.7	Unknown	
31	35.1	.beta.-Amyrin (PCTT)	C ₃₀ H ₅₀ O
32	35.3	1,2,4,8-Tetramethylbicyclo[6.3.0]undeca-2,4-diene (ST)	C ₁₅ H ₂₄

Legend: FAD stands for fatty acid derivatives, MT for monoterpenoid, DT for diterpenoid, FA for fatty acid, ST for sesquiterpenoid, TT for triterpenoid, PCTT for pentacyclic triterpenoid, TTD for triterpenoid derivative, MTD for monoterpenoid derivative, IQA for isoquinoline alkaloid, and SD for sugar derivative.

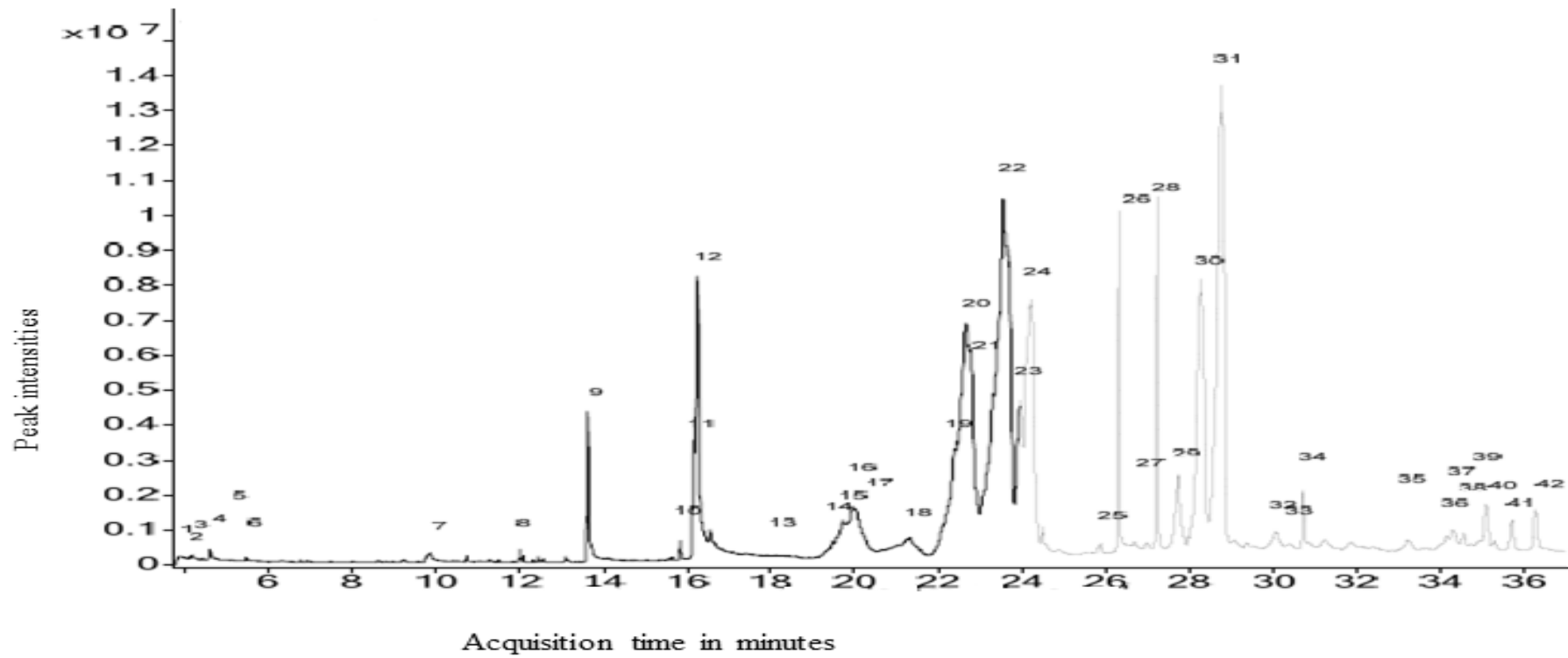


Figure 4.20: GC-MS Chromatogram of ethyl acetate stem bark extracts of *M. obscura*

As shown in table 4.36 and figure 4.21, 22 compounds were determined from the ethyl acetate leaves extracts of *M. obscura* with six (6) of unknown identity since they were not available in the NIST11 database. Squalene was found to be the most abundant compound and it eluted from the column at 26.3 minutes.

Table 4.36: Compounds from ethyl acetate leaves extracts of *M. obscura*

S/N	RT (mins)	Compound name	Formula
1-2	4.0-4.2	Unknown	
3	5.5	Nonane, 5-methyl-5-propyl (FAD)	C ₁₃ H ₂₈
4	6.5	Phenol, 2-propyl (PP)	C ₁₉ H ₁₂ O
5	12	3,7,11,15-Tetramethyl-2- hexadecen-1-ol (DT)	C ₂₀ H ₄₀ O
6	12.1	Methyl tetrahydroinol (STD)	C ₁₄ H ₂₈ O
7	13.6	Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
8	15.8	Cyclohexanol, 5-methyl-2-(1- methylethyl)-, [1S-(1.alpha.,2.beta.,5.beta.)]- (MT)	C ₁₀ H ₂₀ O
9	16.1	<i>cis</i> -bicyclo [4.2.0] octane (MTD)	C ₈ H ₁₄
10	16.2	Oleic acid (FA)	C ₁₈ H ₃₄ O ₂
11	19.5	2H-Pyran-2-one, tetrahydro-6- octyl- (FA)	C ₁₃ H ₂₄ O ₂
12	26.3	Squalene (TT)	C ₃₀ H ₅₀
13	27.2	Hexadecane (FA)	C ₁₆ H ₃₄
14	28	2-Methyl-3-(3-methyl-but-2- enyl)-2-(4-methyl-pent-3- enyl)-oxetane (ST)	C ₁₅ H ₂₆ O
15	30	Heinecosane (FA)	C ₂₁ H ₄₄
16	30.7	Vitamin E (TTD, Vit)	C ₂₉ H ₅₀ O ₂
17	34.6	Unknown	
18	35.1	.beta.-Amyrin (PCTT)	C ₃₀ H ₅₀ O
19- 21	35.3- 35.7	Unknown	
22	36.5	Androstan-6-one, (5.alpha.)- (S)	C ₁₉ H ₃₀ O

Legend: FAD stands for fatty acid derivatives, MT for monoterpene, DT for diterpene, FA for fatty acid, ST for sesquiterpene, MTD for monoterpene derivative, PCTT for pentacyclic triterpene, STD sesquiterpene derivative, TTD, Vit stands for triterpene derivative of the vitamin, PP for phenylpropanoid, TT for triterpene and S for phytosterol.

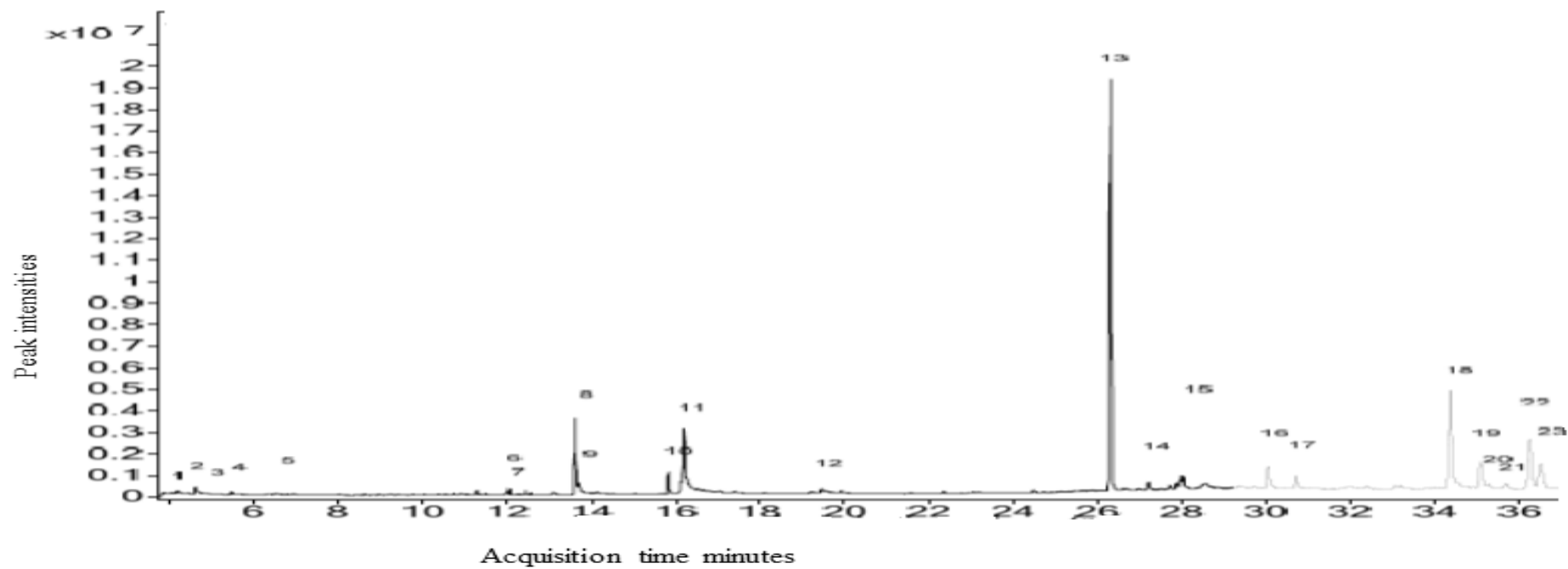


Figure 4.21: GC-MS Chromatogram of ethyl acetate leaves extracts of *M. obscura*

4.6.7 Compounds from ethyl acetate extracts of *O. americanum*

As depicted in table 4.37 and figure 4.22, 69 chemical compounds were detected from ethyl acetate of *O. americanum*. These compounds range from phytosterols, essential oils, antioxidants, vitamins and fatty acids among others. Nine (9) of the compounds were not available in the NIST11 database and therefore their chemical identity was unknown. Many compounds fragments were repeated and the entire scan revealed the largest number of compounds for new compounds compared to any other extract. This is the extract with the largest number of the chemical compounds which ranged from phytosterols, fatty acids and fatty acids derivatives, terpenoids, alkaloids and phenolics.

Table 4.37: Compounds from ethyl acetate extracts of *O. americanum*

S/N	RT (mins)	Compound name	Formula
1	3.8	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl- (MT)	C ₁₀ H ₁₆ O
2	4	.alpha.-Terpineol (MT)	C ₁₀ H ₁₈ O
3	4.3	Anethole (PP)	C ₁₀ H ₁₂ O
4	4.4	Furan, 2-ethyl-5-methyl- (FAD)	C ₇ H ₁₀ O
5	4.6	2-propenoic acid, 6-methylheptyl ester (FAD)	C ₁₁ H ₂₀ O ₂
6	5	Estragole (PP)	C ₁₀ H ₁₂ O
7		Benzaldehyde, 3-methoxy:- (P)	C ₈ H ₈ O ₂
8	6	5,6,7,8-Tetrahydroquinoxaline (A)	C ₈ H ₁₀ N ₂
9	6.3	Unknown	
10	6.7	2-propanone, 1-(4-methoxyphenol) (PP)	C ₁₀ H ₁₂ O ₂
11	6.9	2,3-methylenedioxyanisole (P)	C ₈ H ₈ O ₃
12	7.2	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- (ST)	C ₁₅ H ₂₄
13	7.4	Phenol, 2-(5-isoxazolyl)- (A)	C ₉ H ₇ NO ₂
14	7.5	Phenol, 2-methoxy-4-(1-propenyl)- (PP)	C ₁₀ H ₁₂ O ₂
15	7.7	1-Adamantyl bromomethyl ketone (STD)	C ₁₂ H ₁₇ BrO
16	8	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- (ST)	C ₁₅ H ₂₄
17	8.5	Copaene (ST)	C ₁₅ H ₂₄
18	8.6	Humulene (ST)	C ₁₅ H ₂₄
19	8.8	Benzene, 1,2,3-trimethoxy-5- (2-propenyl)- (PP)	C ₁₂ H ₁₆ O ₃
20	9.1	2-Hydroxy-2-(4-methoxyphenyl)- N-methyl-acetamide (A)	C ₁₀ H ₁₃ NO ₃
21	9.2	1,4-Methanobenzocyclodecene, 1,2,3,4,4a,5,8,9,12,12 adecahydro- (ST)	C ₁₅ H ₂₂
22	9.6	1-Methyl-6-(3-methylbuta-1, 3- dienyl)-7-oxabicyclo [4.1.0] heptane (STD)	C ₁₂ H ₁₈ O

S/N	RT (min)	Compound name	Formula
23	9.9	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- (PP)	C ₁₂ H ₁₆ O ₃
24	10.1	1,4-methano-1H-indene (ST)	C ₁₅ H ₂₄
25	10.5	dI-Isopulegol (MT)	C ₁₀ H ₁₈ O
26	11	Tetradecanoic acid (FA)	C ₁₄ H ₂₈ O ₂
27	11.3	Unknown	
28	11.7	5, 8-diethyl-6-dodecanol (FAD)	C ₁₆ H ₃₄ O
29	11.8	N-(1-Cyanoethyl)(7,7- dimethyl-2-oxobicyclo[2.2.1]hept-1-ylmethanesulfonamide (MTD)	C ₁₃ H ₂₀ N ₂ O ₃ S
30	12.1	Oxirane, decyl- (FAD)	C ₁₂ H ₂₄ O
31	12.7	1-Hydroxymethyladamantan-2-ol (MTD)	C ₁₁ H ₁₈ O ₂
32	13.1	<i>trans</i> -geranylgeraniol (DT)	C ₂₀ H ₃₄ O
33	13.4	Cyclohexanol,3,5-dimethyl (TTD)	C ₈ H ₁₆ O
34	13.7	n-Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
35	14.2	1-Dodecanol,3,7,11-trimethyl (ST)	C ₁₅ H ₃₂ O
36	15.8	Cyclohexanol, 5-methyl-2-(1-methylethyl)- (MT)	C ₁₀ H ₂₀ O
37	16.1	9, 12-Octadecadenoic acid, (Z, Z)- (FA)	C ₁₈ H ₃₂ O ₂
38	16.2	9-Octadecenoic acid, (E)- (FA)	C ₁₈ H ₃₄ O ₂
39	16.3	Spiro[2,5]heptane, 1,5-dimethyl-6-methylene (MT)	C ₁₀ H ₁₆
40	16.5	Octadecanoic acid (FA)	C ₁₈ H ₃₆ O ₂
41	17.5	3,7,11,15- tetramethyl-2-hexadecen-1-ol (DT)	C ₂₀ H ₄₀
42	17.8	Anethole (PP)	C ₁₀ H ₁₂ O
43	18.2	3,7-Dimethyl-2,3,3a,4,5,6- hexahydro-1-benzofuran (MT)	C ₁₀ H ₁₆ O
44	18.6	Octadecane, 1-bromo (FAD)	C ₁₈ H ₃₇ Br
45	19.3	Cyclooctane, tetradecyl (FAD)	C ₂₂ H ₄₄
46	19.9	Squalene (TT)	C ₃₀ H ₅₀
47-48	20.6-22.6	Unknown	
49	23.1	Benzamide, N-(3-nitrophenyl)-2-methoxy- (PA)	C ₁₄ H ₁₂ N ₂ O ₄
50	23.4	Ethanone, 2-hydroxy-1,2-bis(4-methoxyphenyl)-(P)	C ₁₆ H ₁₆ O ₄
51	24.5	Hexadecane (FA)	C ₁₆ H ₃₄
52	27.9	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20- pentamethyl-3,7,11,15,19- heneicosapentaenyl)-, (all-E)- (TT)	C ₃₀ H ₅₀ O
53	28	2,2-dimethyl-3-(3,7,16,20-tetramethylheneicosapentaenyl)-oxirane (TTD)	C ₂₉ H ₄₈ O
54	28.2	Dodecane, 1-iodo (FAD)	C ₁₂ H ₂₅ I
55	28.8	2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane (ST)	C ₁₅ H ₂₆ O
56	29.5	2-methylhexacosane (FAD)	C ₂₇ H ₅₆
57	30.7	Vitamin E (TTD, Vit)	C ₂₉ H ₅₀ O ₂
58	30.8	2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane (ST)	C ₁₅ H ₂₆ O
59	30.9	Unknown	
60	31.9	2-methyltacosane (FA)	C ₂₉ H ₆₀
61-63	32.5-33.4	Unknown	

S/N	RT (min)	Compound name	Formula
64	34.4	Ergost-25-ene-3,5,6-triol, (3.beta.,5.alpha.,6.beta.)- (S)	C ₂₈ H ₄₈ O ₃
65	34.8	Fucosterol (S)	C ₂₉ H ₄₈ O
66	35.1	.beta.-Amyrin (PCTT)	C ₃₀ H ₅₀ O
67	35.3	Lanosterol (S)	C ₃₀ H ₅₀ O
68	35.5	Unknown	
69	35.6	1,4-dimethyl-8- isopropylidenetricyclo[5.4.0.0(4,10)]decane (ST)	C ₁₅ H ₂₄

Legend: FAD stands for fatty acid derivatives, MT for monoterpenoid, FA for fatty acid, S for phytosterol and ST for sesquiterpenoid, PCTT for pentacyclic triterpenoid, TTD for triterpenoid derivative and DTD for diterpenoid derivative, A for alkaloids, PP for phenylpropanoids, PA for phenolic alkaloids, A for alkaloids, P for phenolics, TTD, Vit stands for triterpenoid derivative of the vitamin and TT for triterpenoid.

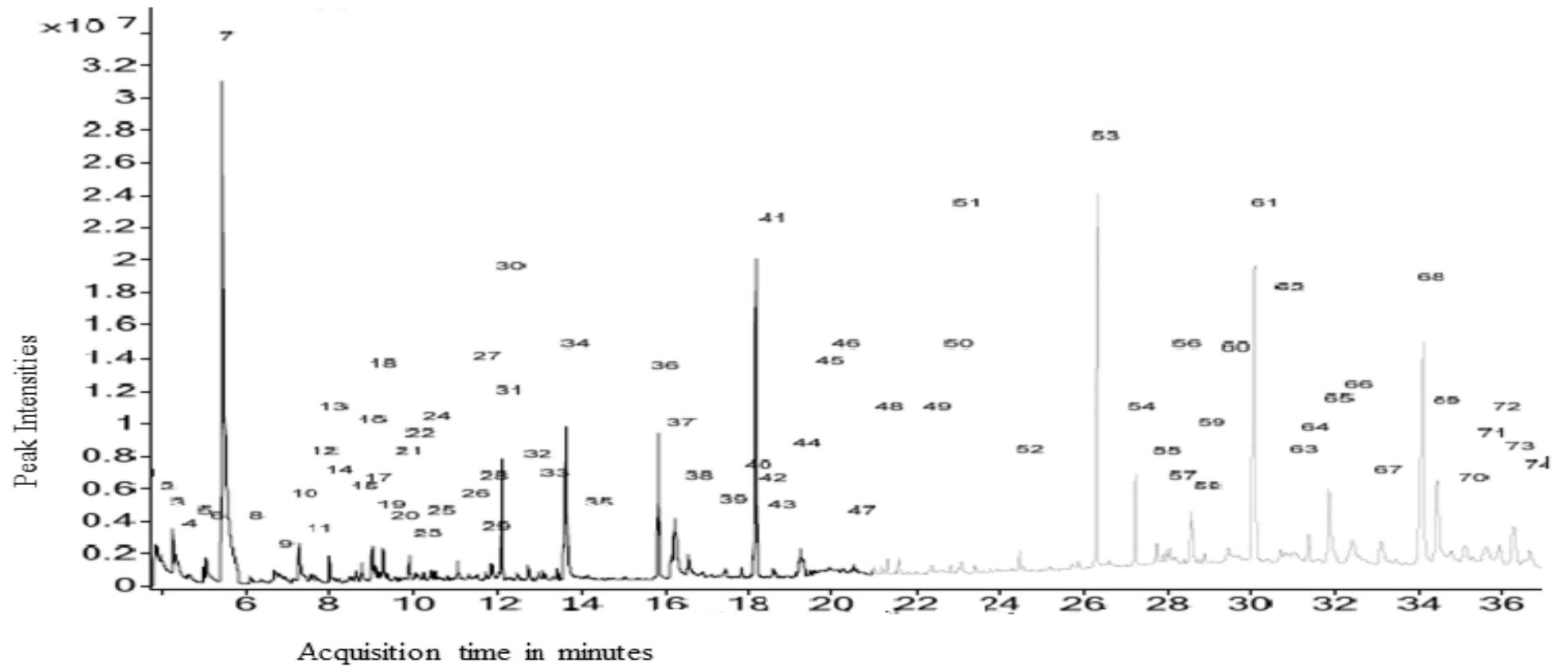


Figure 4.22: GC-MS Chromatogram of ethyl acetate extracts of *O. americanum*

4.6.8 Compounds from *L. cornuta*

As shown in table 4.38 and figure 4.23, 18 compounds were detected from the ethyl acetate extracts of *L. cornuta*. Only one compound which eluted at 34.9 minutes was not available in the NIST11 database. Seventeen (17) compounds were present in the database, and the most abundant compounds were 1-decanol, 2-hexyl- and n-Hexadecanoic acid as shown in the figure 4.23.

Table 4.38: Volatile compounds from ethyl acetate extracts of *L. cornuta*

S/N	RT (mins)	Compound name	Formula
1	4.6	2-Propenoic acid, 6-methylheptyl ester (FAD)	C ₁₁ H ₂₀ O ₂
2	12.1	Methyl tetrahydroionol (STD)	C ₁₄ H ₂₈ O
3	13.6	n-Hexadecanoic acid (FA)	C ₁₆ H ₃₂ O ₂
4	16.1	9, 12-Octadecadienoic acid (Z, Z)- (FA)	C ₁₈ H ₃₂ O ₂
5	16.2	9-Octadecenoic acid, (E)- (FA)	C ₁₈ H ₃₄ O ₂
6	16.5	Octadecanoic acid (FA)	C ₁₈ H ₃₆ O ₂
7	24.5	Heinecosane (FA)	C ₂₁ H ₄₄
8	27.3	1-Decanol, 2-hexyl- (FAD)	C ₁₆ H ₃₄ O
9	32.4	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate (S)	C ₂₈ H ₄₅ ClO ₂
10	33.1	Stigmasterol (S)	C ₂₉ H ₄₈ O
11	34.4	<i>trans</i> -Dehydroandrosterone, methyl ether (S)	C ₂₀ H ₃₀ O ₂
12	34.6	2H-1-Benzopyran-2-one, 6- acetyl-7-(acetyloxy)-4-methyl- (C)	C ₁₄ H ₁₂ O ₅
13	34.9	Unknown	
14	35.2	.beta.-Amyrin (PCTT)	C ₃₀ H ₅₀ O
15	35.3	Lanosterol (S)	C ₃₀ H ₅₀ O
16	35.7	Benzimidazo[2,1- a]isoquinoline (IQA)	C ₁₅ H ₁₀ N ₂
17	35.8	Lup-20(29)-en-3-one (PCTT)	C ₃₀ H ₄₈ O
18	36.7	Fern-7-en-3.beta.-ol (PCTT)	C ₃₀ H ₅₀ O

Legend: FAD stands for fatty acid derivatives, MT for monoterpenoid, FA for fatty acid, S for phytosterol and ST for sesquiterpenoid, PCTT for pentacyclic triterpenoid, TTD for triterpenoid derivative and DTD for diterpenoid derivative, IQA for isoquinoline alkaloids, TTD, Vit stands for triterpenoid derivative of the vitamin, C for coumarins and TT for triterpenoid.

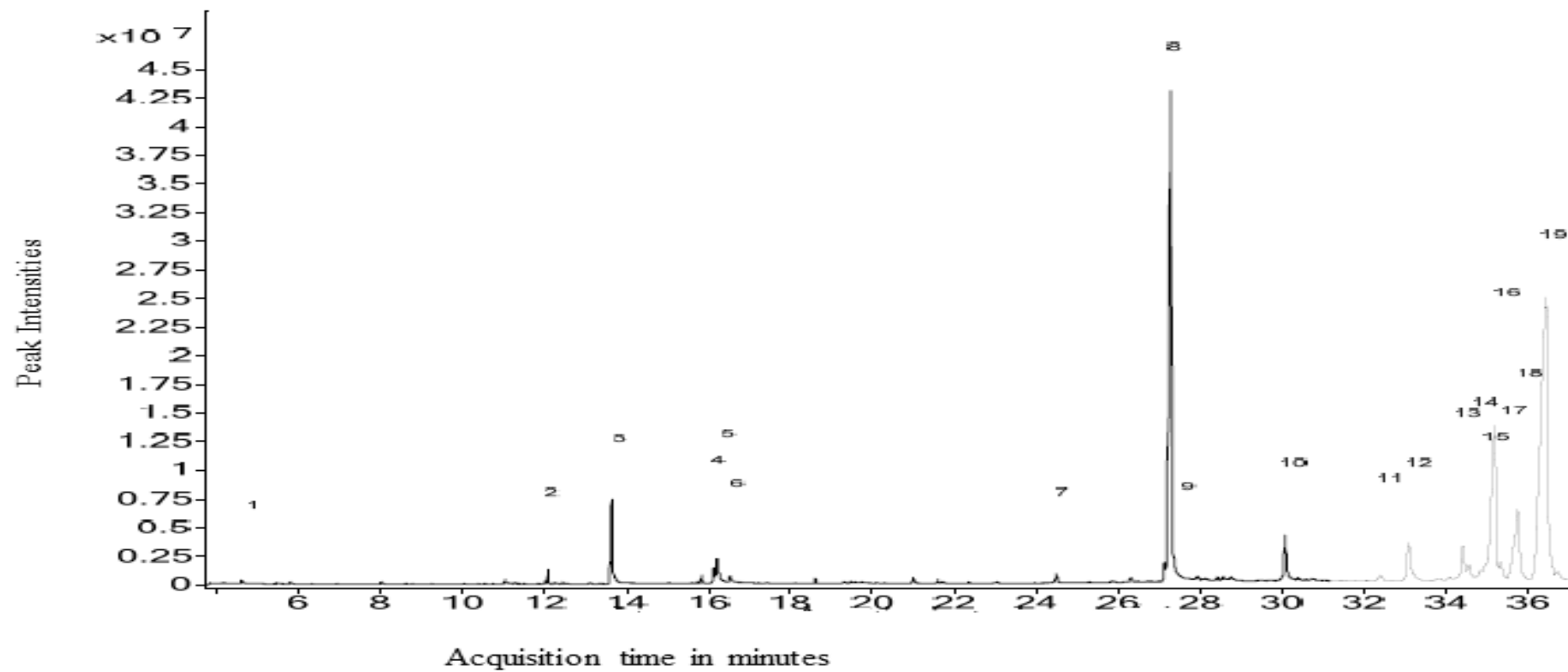


Figure 4.23: GC-MS Chromatogram of ethyl acetate extracts of *L. cornuta*

4.7 Mineral element composition of the plants powders

As depicted in table 4.39, among the mineral elements quantified Fe, Cr, Mg, Mn, and Ni were higher in the leaf than in the stem bark powders of *P. capensis* (L.); Se, Zn, Cu and Co were similar in both the leaf and stem bark powders of *P. capensis* (L.); while V and Mo were higher in the stem bark compared to the leaf powders of *P. capensis* (L.). The leaves and stem barks of *S. spectabilis* had highest amounts of Mo followed by Cr in the leaves and Co in the leaves and stem barks. The rest of the mineral elements in the leaves and stem barks of *S. spectabilis* were comparable in amounts.

The leaves and stem bark of *S. spectabilis* had the lowest amounts of Cu and V, respectively followed by selenium in the leaves and Cu in the stem bark. *M. obscura* had the highest amounts of Mo in the leaves and stem bark compared with other mineral elements, and this was followed by Cr in both leaves and stem barks. The lowest amount of mineral was Se detected in the leaves of *M. obscura*. In *L. cornuta*, the highest mineral element detected was Cr followed by Mo and then Ni, and the lowest amount was of Se and Zn. Similarly for *O. americanum*, the highest amount of mineral element was Cr followed by Mo, Co and then Ni and the lowest was Se and Zn. In general the mineral elements in the medicinal plants varied with the plant and the part studied and the amounts ranged from 0.11 to 55.67 mg/100 g of the medicinal plant material.

Table 4.39: Essential mineral element composition of the plants in mg/100 g dry matter

Element	Medicinal plants and the amounts of mineral elements in mg/100g of the plant material							
	<i>P. capensis</i>		<i>S. spectabilis</i>		<i>M. obscura</i>		<i>L. cornuta</i>	<i>O. americanum</i>
	Leaves	Stem	Leaves	Stem	Leaves	Stem		
Se	0.19±0.00	0.19±0.19	0.14±0.04	0.18±0.02	0.10±0.00	0.17±0.03	0.11±0.01	0.11±0.01
Fe	0.20±0.07	0.18±0.05	0.23±0.02	0.25±0.03	0.33±0.02	0.21±0.11	0.12±0.02	0.16±0.04
Zn	0.16±0.02	0.11±0.08	0.12±0.03	0.20±0.01	0.13±0.03	0.17±0.07	0.11±0.02	0.11±0.02
Cu	0.12±0.04	0.09±0.001	0.11±0.01	0.14±0.02	0.11±0.00	0.13±0.01	0.12±0.02	0.17±0.01
Cr	3.69±0.33	1.60±0.40	3.73±0.04	1.71±0.23	5.91±0.35	3.49±0.19	22.45±0.05	55.67±9.42
Ni	3.60±0.50	0.73±0.12	0.70±0.08	0.69±0.14	0.95±0.11	0.72±0.14	2.35±0.09	1.77±0.10
Mn	0.45±0.09	0.19±0.05	0.27±0.02	0.27±0.08	0.41±0.15	0.40±0.11	0.27±0.01	1.46±0.20
V	0.76±0.05	0.17±0.08	0.42±0.00	0.11±0.00	0.63±0.04	0.12±0.02	0.37±0.01	0.78±0.04
Co	1.75±0.16	1.85±0.13	1.77±0.06	1.90±0.11	1.83±0.14	2.04±0.05	1.96±0.06	2.21±0.10
Mo	9.16±0.05	8.68±0.19	8.89±0.09	8.79±0.14	9.22±0.10	8.80±0.06	7.68±0.08	10.09±0.25
Al	598.30±8.50	176.4±130.1	395.3±91.1	252.5±69.1	1138.9±15.9	33.20±17.9	117.0±0.14	1929.0±6.5
Pb	0.08±0.05	0.12±0.03	0.10±0.0002	0.10±0.004	0.05±0.07	0.11±0.01	ND	0.11±0.02
Hg	0.50±0.10	0.38±0.08	0.35±0.05	0.47±0.07	0.25±0.09	0.38±0.19	0.13±0.02	0.21±0.04

Results are expressed as Mean ± Standard Deviation (SD) for three determinations for each medicinal plant material using EDXRF. ND stands for not detected.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The optimal and safe intraperitoneal dose of alloxan-monohydrate of 186.9 mg/kg body weight that induced diabetes on 8-12 hours fasted mice in 48 hours post administration observed in this study, differs from the optimal and safe dose of 160 mg/kg body weight required to intraperitoneally induce diabetes in overnight fasted male albino rats in 96 to 120 hours as reported by Ashok *et al.* (2007). This could be explained by the fact that induction of diabetes depends on the animal species, route of administration and nutritional status. Animals kept on overnight fast are more susceptible to alloxan (Kastumata *et al.*, 1992), while glucose is known to protect the beta cells (Szkudelski, 2001). Alloxan destroys and reduces the β -cells via formation of reactive oxygen species like nitric oxide (Szkudelski, 2001). High glucose level prevents the superoxide derivative that causes cell damage (Martens *et al.*, 2005). However, the frequently used intravenous dose of alloxan to induce diabetes in rats is 65 mg/kg body weight (Boylan *et al.*, 1992). Additionally, when alloxan is given intraperitoneally or subcutaneously, its effective dose must be 2-3 times higher than the dose of 65 mg/kg body weight (Ashok *et al.*, 2007). This dose range for rats agrees with the optimal and safe alloxan dose of 186.9 mg/kg body weight observed in this study.

The death of one mouse (20%) observed on administration of alloxan at a dose of 480 mg/kg body weight could be associated with the fact that the range of the diabetogenic dose of alloxan is quite narrow and even light over-dosing may be generally toxic causing the loss of many animals (Szkudelski, 2001). This loss is suggested to be due to kidney tubular cell necrotic toxicity at high doses of alloxan (Lenzen *et al.*, 1996). Induction of diabetes using

alloxan-monohydrate to male BALB/c mice avails a functional and physiological model for the study of hypoglycemic agents. The alloxan-induced diabetic mice had a three to four fold increase in blood glucose (100 mg/dl to 200 mg/dl) relative to the normal control mice.

The observation that both the leaf and stem bark ethyl acetate and aqueous extracts of *P. capensis* (except the aqueous leaves and stem barks extracts), *S. spectabilis*, *M. obscura* (except the aqueous leaves extracts), *L. cornuta* (except the aqueous extracts) and *O. americanum* in various dosage modes lowered blood glucose indicates that these extracts have hypoglycemic constituents. These hypoglycemic constituents could be the polyphenols, alkaloids, flavonoids, saponins, tannins, and steroids present in these plants (Karau *et al.*, 2012b; Karau *et al.*, 2013a; Karau *et al.*, 2013b), among others. Phenols, flavonoids, alkaloids, tannins, terpenoids, phylobatannins, and sterols have been associated with hypoglycemic activity (Elliot *et al.*, 2000). As reported by Glauce *et al.* (2004), flavonoids like myricetin, a polyhydroxylated flavonol, has insulinomimetic properties and stimulates lipogenesis and glucose transport in the adipocytes, hence lowering blood sugar (Cao *et al.*, 1997; Elliot *et al.*, 2000). Similar studies on *Pterocarpus marsupium* found epicatechin and catechin flavonoids to have anti-diabetic properties (Subramanian, 1981).

The alkaloid 1-ephedrine promotes the regeneration of pancreatic islets following destruction of the beta cells, hence restoring the secretion of insulin and thus correcting hyperglycemia (Elliot *et al.*, 2000). The tannin epigallo-catechin-3-gallate exhibits anti-diabetic activity as demonstrated by Broadhurst *et al.* (2000). Terpenoids are used by patients with high blood pressure and diabetes because they help to reduce diastolic blood pressure and lower the sugar levels in blood (Hawkins and Ehrlich, 2006). The presence of terpenoids in the leaves and

seeds of *E. officinalis* makes them useful in the treatment of diabetes (Treadway, 1994; Chi-Mei and Jin-Yuarn, 2013).

The lowering of blood glucose levels by *P. capensis* in the same manner regardless of the dosage might suggest that the extracts may reflect uptake of the active constituents through saturable active transport; it may also reflect maximum hypoglycemic activity at the lowest dose used (50 mg/kg body weight). The observation that the three tested doses of aqueous leaf and stem bark extracts of *P. capensis* and aqueous leaves extracts of *M. obscura* could not significantly affect the blood glucose levels in alloxan induced diabetic male BALB/c mice indicates that these extracts lacked constituents with hypoglycemic activity or the active constituents might have been present together with antagonistic constituents. The observation that the aqueous extracts of *L. cornuta* at 50 and 200 mg/kg body weight dose raised the glucose levels to beyond that of the diabetic control mice suggests that these extract constituents may either have triggered gluconeogenesis together with glycogenolysis or could have had constituents which promoted further death of the pancreas.

The observed hypoglycemic activity could also be associated with minerals such as iron, chromium, manganese, vanadium, molybdenum and zinc which are present in these medicinal plants at various amounts (Karau *et al.*, 2012a). Iron influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver (Niederau *et al.*, 1984). Chromium functions as a cofactor in insulin-regulating activities. It facilitates insulin binding and subsequent uptake of glucose into the cell and therefore decreases fasting glucose levels, improves glucose tolerance, lowers insulin levels and decreases total cholesterol in type II diabetic subjects (Mooradian *et al.*, 1994;

Baker, 1996). Manganese is an activator and constituent of several enzymes like kinases and enzymes of oxidative phosphorylation (Friedman, 1987). Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells (Song *et al.*, 1998). Zinc also influences glyceraldehyde-3-phosphate dehydrogenase, the enzyme involved in glycolysis (Manuel *et al.*, 2002). Molybdate is an effective antihyperglycemic agent in diabetics with severe insulin resistance. It is associated with substantial reduction of hyperinsulinaemia and an increase in pancreatic insulin stores. The glucose-lowering effect of molybdenum may be partly related to attenuation of hepatic glucose production, and possibly also to increased glucose usage. Hence, molybdenum proves to be an effective blood glucose-lowering agent in severely diabetic patients (Reul *et al.*, 1997).

The antioxidants such as flavonoids, vitamin C, vitamin E, lycopene, β -carotene, and cryptoxanthin present in these plants may also be responsible for the glucose lowering activity; they do so by neutralizing the reactive oxygen species (hydroxy and superoxide radicals, hydrogen peroxide, lipid peroxides) generated during the induction of diabetes by alloxan by helping the pancreatic cells to regenerate and hence helping to maintain optimal cellular and systemic health and well-being of the mice (Battin and Brumaghim, 2009). These antioxidants interact and synergistically stabilize, or deactivate, free radicals before they attack cells. Vitamin C neutralizes hydroxyl and superoxide radicals, and hydrogen peroxide in the aqueous phase before lipid peroxidation is initiated (Battin and Brumaghim, 2009). Glutathione and vitamin C work interactively to quench free radicals and have a sparing effect on each other. Vitamin E neutralizes hydrogen peroxide, and lipid peroxides and is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane

fatty acids from lipid peroxidation. Vitamin C regenerates vitamin E. Beta carotene and other carotenoids also provide antioxidant protection to lipid-rich tissues by working synergistically with vitamin E. A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Flavonoids neutralize hydroxy and superoxide radicals, hydrogen peroxide and lipid peroxides thereby acting as protectors against a wide range of environmental stress and in humans and animals, they function as biological response modifiers. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages. Antioxidants are responsible for most of the biological activities of phytochemicals including antidiabetic, anti-inflammatory, anti-allergenic, antiviral, anti-aging and anti-carcinogenic activities (Percival, 1998).

Oral administration of aqueous and ethyl acetate extracts of *P. capensis* (leaves) and *O. americanum* and ethyl acetate leaves extracts of *S. spectabilis* at 1 g/kg body weight daily in mice for 28 days resulted in a significant reduction of weekly body weight gain when compared to that of the control mice indicating the presence of constituents in the extracts that retard growth. A loss of body weight of more than 10% such as that induced by *P. capensis* (L), *S. spectabilis* (L) and *O. americanum* is associated with protein-energy malnutrition which is associated with impaired physiological function such as impaired cell mediated and humoral immunity. Weight loss of more than 20% such as that induced by extracts of *P. capensis* (L), *S. spectabilis* (L) and *O. americanum* is associated with severe protein-energy malnutrition and is associated with pronounced organ dysfunction. Such constituents may include components of terpenoids, alkaloids, and flavonoids present in these extracts in addition to other undetermined constituents. Organ injury and damage are demonstrated by

altered levels of BUN (kidney), aspartate aminotransferase (liver, kidneys, heart and pancreas), alanine aminotransferase (liver), alkaline phosphatase (liver, kidney and spleen), and creatine kinase (heart and skeletal muscle) in the mice treated with *P. capensis* (L), *S. spectabilis* (L) and *O. americanum* at 1g/kg body weight daily for 28 days relative to those of the control mice. Protein-energy malnutrition causes structural and functional deterioration in several organs including the liver, kidney, lungs, heart, brain, spleen and eyes. Organ(s) injury and damage may partly be explained by leaky cell membranes which allow the movement of potassium and other intracellular ions into the extracellular space. The increased extracellular load in the interstitium causes water movement and edema in the affected organs. Injury to specific organs or tissues may partly account for increased levels of serum enzyme activities of mice treated with *P. capensis* (L), *S. spectabilis* (L) and *O. americanum* extracts at 1g/kg body weight daily to mice for 28 days.

Terpenoids such as forskolin, a diterpene reduces body weight by acting on adenylate cyclase that converts ATP to cyclic adenosine monophosphate (cAMP) (Chi-Mei and Jin-Yuarn, 2013). Cyclic adenosine monophosphate (cAMP) promotes lipolysis, increases the body's basal metabolic rate and increases use of body fat (Litosch *et al.*, 1982), and protein degradation and/or decreases protein synthesis. Alkaloids such as ρ -octopamine and synephrines may reduce body weight by exerting adrenergic agonist activity (Pellati *et al.*, 2002). Synephrines increase energy expenditure (EE) (resting energy expenditure [70%], thermic effect of feeding [10%], and energy expenditure of physical activity [20%]) and decrease food intake in addition to decreasing gastric motility (slows gastric emptying and intestinal transit) (Astrup, 2000; Tucci, 2010) and indirectly producing increased feeling of satiety and a decreased appetite. Nicotine an alkaloid induces weight loss by exerting its

effects through the central nervous system and metabolic actions by reducing appetite and altering feeding patterns. It increases metabolic rate and increases energy expenditure (EE) and hence decreases metabolic efficiency (Perkins *et al.*, 1991; Tucci, 2010). In the CNS, nicotine modulates the central nervous system pathways that regulate several aspects of food intake (Tucci, 2010). The flavonoid, chlorogenic acid reduces body weight by inducing reduction in body fat by reducing the absorption of glucose (energy source) leading to an increase in the consumption of fat reserves.

A major consequence of blocking digestion of carbohydrates in the proximal gut is colonic fermentation which leads to increased microbial production of gas in the bowel; gas production limits glucose utilization (Thom, 2007; Tucci, 2010). Catechins (flavonoids) such as epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin are associated with increase in sympathetic nervous system activity, thermogenesis and fat oxidation and hence accounting for the reduced body weight (Dulloo *et al.*, 1999; Nagao *et al.*, 2005). Catechins also induce reduction of body fat by inhibiting small intestine micelle formation and inhibiting α -glucosidase activity leading to decreased carbohydrate absorption (Muramatsu *et al.*, 1986; Tucci, 2010). Caffeine, an alkaloid, induces weight loss by decreasing energy intake (EI) (Jessen *et al.*, 2005) by acting through the central and peripheral nervous system mechanisms and by promoting thermogenesis and lipolysis (Acheson *et al.*, 1980; Tucci, 2010). The central nervous system effects of caffeine are due to its effects on the widely distributed adenosine α 1, α 2A, and α 2B receptors (Fredholm *et al.*, 1999; Quarta *et al.*, 2004).

Tannins which are present in these plants may reduce feed intake by decreasing palatability and by reducing feed digestion. Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Low palatability depresses feed intake. Digestibility reduction negatively influences intake because of the filling effect associated with undigested feedstuff. Tannins are divided into two: hydrolysable and condensed tannins. Hydrolysable tannins are converted by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid which are toxic. The major lesions associated with hydrolysable tannins poisoning are hemorrhagic gastroenteritis which decreases absorption of nutrients and necrosis of the liver and kidney.

Proanthocyanidins (PAs) (condensed tannins) retard growth by inhibiting feed intake and digestibility. Proanthocyanidins (PAs) which are not absorbed by the digestive tract, damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins, carbohydrates and essential amino acids such as methionine and lysine. They also increase excretion of proteins and essential amino acids and alter the excretion of certain cations (Clampitt and Hart, 1978).

A daily oral administration of ethyl acetate and aqueous extracts of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1g/kg body weight in mice for 28 days induced microcytic hyperchromic anemia. This anemic state may be caused by reduced production of red blood cells and white blood cells due to poisoning and suppression of bone marrow performance by constituents in these plants such as alkaloids, flavonoids, tannins and saponins which have previously been reported to reduce erythron

parameters (Barger, 2003). A daily oral administration of aqueous extracts of *S. spectabilis* (SB) at 1 g/kg body weight in mice for 28 days induced a reduction in red blood cells, mean cell volume and elevation of hemoglobin levels and mean cell hemoglobin concentration while a daily oral administration of aqueous extracts of *M. obscura* (SB) at 1 g/kg body weight in mice for 28 days induced a reduction in red blood cells, packed cell volume, mean cell volume and elevation of hemoglobin levels and mean cell hemoglobin concentration all of which are indicative of secondary polycythemia. Secondary polycythemia could be due to an abnormal increase in red cell production by the bone marrow (polycythemia vera) caused by some of the extract constituents. This leads to tissue hypoxia (Voet and Voet, 2004).

Tissue hypoxia causes most tissues to initially enlarge and as the swollen cells continue rupturing, the organ size reduces (organ atrophy) (Voet and Voet, 2004). During tissue hypoxia, cells which rely only on glycolysis for ATP production rapidly deplete the store of phosphocreatine (a source of rapid ATP production) and glycogen. As the rate of ATP production decreases below the level required by membrane ion pumps for the maintenance of proper intracellular ionic concentrations, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell. The overstretched membrane becomes permeable thereby leaking their enclosed contents. The decreased intracellular pH that accompanies anaerobic glycolysis because of lactic acid production permits the released lysosomal enzymes which are only active at acidic pH to degrade the cell contents. Among the degraded components are the initially elevated serum enzymes which are later reduced to values below the control values as the hypoxic state progresses. The reduced metabolic activity results in irreversible cell damage (Voet and Voet, 2004).

Injury of organs resulting from tissue hypoxia may also partly account for the altered serum levels of alkaline phosphatase (liver, kidney and spleen), alanine (liver) and aspartate aminotransferase (liver, kidneys, heart and pancreas), and creatine kinase (heart and skeletal muscle) in mice orally administered daily with extracts of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1 g/kg body weight daily for 28 days (Clampitt and Hart, 1978; Boyd, 1983; Kaneko, 1989).

The observed organ damage could also be accounted for by the toxicity of some of the phytochemicals and minerals present in these extracts. Saponins hemolyse red blood cells and cause cell death of many tissues. In the kidneys, saponins lead to haemorrhage in the glomeruli and focal destruction of the renal tubules (Clampitt and Hart, 1978). Toxic levels of saponins cause cardiac failure, acute hypoglycemia and hepatorenal damage leading to death. Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Clampitt and Hart, 1978).

The significant increase in white blood cells observed on oral administration of plant extracts of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1g/kg body weight daily in mice for 28 days indicates a more accelerated production of these cells and a boosted immunity to mice by these extracts (Kaushansky, 1995). This could be due to tissue damage caused by some constituents of the plants extracts.

The observed significant increase in percentage granulocytes (neutrophil, basophils, eosinophils) on oral administration of the plant extracts *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1 g/kg body weight

daily in mice for 28 days could partly explain the increased white blood cell count (Howard and Hamilton, 2002). Results showing a significant increase in percentage lymphocytes (main effectors cells of the immune system) on oral administration of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1 g/kg body weight daily in mice for 28 days indicate a possible stimulatory effect by these extracts on lymphocyte production. The observed significant increase in percentage monocytes on oral administration of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1g/kg body weight daily in mice for 28 days indicates a possible induction of an inflammatory condition by constituents of these plant extracts (Howard and Hamilton, 2002).

The presence of dry matter, crude fiber, moisture, total ash, total protein, lipids and soluble carbohydrate in *P. capensis* powder is an indication of the holistic nature of herbal medicine. The dry matter is an indication of total solids which include carbohydrates, fats, proteins, vitamins, minerals and antioxidants. The lipid content composed of triacylglycerols, phospholipids, cholesterol and cholesterol esters is hydrolyzed to fatty acids, lysophospholipids and 2-monoacylglycerols in the lumen of the intestine, absorbed and metabolized to yield energy. Cholesterol serves as a stabilizing component of cell membranes and as a precursor of bile salts and steroid hormones. The ash content is an indication of the mineral content; minerals are used by the human body for the proper composition of bone and blood and maintenance of normal cell function. They function along with vitamins as essential components in enzymes and coenzymes.

The proteins present in this plant powder are used to synthesize the numerous body proteins and their catabolism results in the supply of energy (Robinson, 1978). The crude fiber composed principally of indigestible cellulose, hemicellulose and lignins and pectins, gums and mucilages is nutritionally beneficial in the small intestines since it aids in the absorption of trace elements in the gut and reduces the absorption of cholesterol (Le Veille and Sanberlich, 1966), reduces the risk of coronary heart disease, obesity and diabetes mellitus (Monago and Uwakwe, 2009); digestion of soluble fiber, undigested starch and sugars by bacterial flora in the normal human gut yields short chain fatty acids which are absorbed by the colonic epithelial cells of the gut and some travel to the liver through the hepatic portal vein where they are metabolized to yield energy.

Carbohydrates composed principally of starch after digestion to monosaccharides are absorbed through the small intestines and catabolized to yield energy. Carbohydrates also provide the amino acids serine, glycine, alanine, glutamate, glutamine, aspartate and asparagine in addition to contributing to the sweetness, appearance and textural characteristics of many foods (Muhammad *et al.*, 2009). The low moisture content of *P. capensis* powder indicates the decreased perishability of this plant material since a high moisture content promotes susceptibility to microbial growth and enzyme activity which accelerates spoilage (Monago and Uwakwe, 2009). The caloric value (leaf powder, 28,877kJ/100 g; stem bark powder, 29, 920 kJ/100 g) of *P. capensis* shows that this plant could be a reliable source of energy and can provide a large portion of the daily requirement of 10,460 to 12,552kJ for adults if large quantities are consumed.

5.2 Conclusion

In conclusion, this study demonstrated that:

- i. Both the leaves and stem barks ethyl acetate and aqueous extracts of *P. capensis*, *S. spectabilis*, *M. obscura*, *L. cornuta* and *O. americanum* in doses of 50, 100 and 200mg/kg body weight possess *in vivo* antidiabetic activity.
- ii. The three tested doses of aqueous leaves and stem barks extract of *P. capensis* and aqueous leaves extracts of *M. obscura* did not possess antidiabetic activity.
- iii. The aqueous extracts of *L. cornuta* at 50 and 200mg/kg body weight dose raised blood glucose levels beyond that of the diabetic mice.
- iv. Oral administration of aqueous leaves extracts of *P. capensis*, and *O. americanum* and ethyl acetate leaves extracts of *S. spectabilis* at 1g/kg body weight daily in mice for 28 days significantly reduced the weekly body weight gain.
- v. Oral administration of aqueous leaves extracts of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* at 1g/kg body weight altered the red blood cell count, hemoglobin levels, packed cell volume, mean cell hemoglobin concentration, mean cell volume, platelets; white blood cell count and their differential counts; altered the levels of blood urea nitrogen and the activities of alanine and aspartate aminotransferases, alkaline phosphatase and creatine kinase.
- vi. The plants powders of *P. capensis* (L and SB), *S. spectabilis* (L and SB), *M. obscura* (L and SB), *L. cornuta* and *O. americanum* contained phytochemicals: phenols, tannins, saponins, flavonoids, alkaloids, terpenoids, phylobatannins, cardiac glycosides, sterols, fatty acids and reducing sugars; phytonutrients: vitamins C, E, B1, B3, retinol, β -carotene, lycopene and cryptoxanthin; minerals: selenium, iron, zinc,

copper, chromium, manganese, vanadium, molybdenum, cobalt and heavy metals nickel, aluminium, lead and mercury; can provide substantial metabolic energy from their carbohydrate, protein and fat content.

- vii. The observed hypoglycemic activity and / or slight toxicity in the bioactive plants extracts could be associated with the phytonutrients and minerals present in these plants.

5.3 Recommendations

- i. The mechanism behind hypoglycemic activity for these plant species other than antioxidant activity should be established.
- ii. Identification of phytochemical compound(s) directly associated with hypoglycemic activity of these plant species and assess whether the individual isolated compounds retain or lose their activity or are more active than their parent crude extracts.
- iii. Investigation of combination dosages of these plant extracts should be done to create a rationale for a multi herbal therapy in the management of diabetes mellitus.
- iv. After a thorough verification of bioactivity and safety, potentially easy-to-consume proprietary products fortified with bioactive extracts of target plant species should be developed for diabetic patients.
- v. Comprehensive toxicity (long term studies on reproductive, toxicokinetic profiling and carcinogenic tests) of the plant extract especially those that showed highest hypoglycemic activity should be done.
- vi. Investigation of the ratio of weights of various organs to the body weight should be done to determine which organs are affected by the plants extracts in safety studies.

- vii. Comprehensive screening and quantification of polar compounds should be carried out to determine the actual compounds responsible for observed hypoglycemic activity and toxicity. This could be done by LC-MS/MS. Determination of the chemical structures of unknown compounds by spectroscopic techniques should be carried out.

REFERENCES

- Abbott DB., Rosen MB. and Held G. (1997). Cellular, biochemical and molecular techniques in developmental toxicology; in Hood RD; developmental and reproductive toxicology: a practical approach. Taylor and Francis, New York: pp 599-619
- Abdel-Hassan I., Abdel-Barry J. and Tariq M. (2000). The hypoglycaemic and anti-hyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *Journal of Ethnopharmacology*, 71: 325-330.
- Abel E., Peroni O., Kim J., Kim Y., Boss O.H., Minnemann T., Shulman G.I. and Kahn B.B. (2001). Adipose selective targeting of the GLUT 4 gene impairs insulin action in muscle and liver. *Nature*, 409: 729-733.
- Acheson K.J., Zahorska-Markiewicz B., Pittet P., Anantharaman K. and Jequier E. (1980). Caffeine and coffee: Their influence on metabolic rate and substrate utilization in normal weight and obese individuals. *The American Journal of Clinical Nutrition*, 33, 989-997
- Akinmoladun A.C., Ibukun E.O., Afor E., Obuotor E.M. and Farombi E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Science Research Essay*, 2: 163-166.
- Alajmi F.M. and Perwez A. (2014). Anti-inflammatory activity and qualitative analysis of different extracts of *Maytenus obscura* (A. Rich) Cuf. By high performance thin layer chromatography method. *Asian Journal Pacific Journal of Tropical Biomedicine*, 4: 152-157.
- Amacher D. (2002). A toxicologist's guide to biomarkers of hepatic response. *Human and Experimental Toxicology*, 21: 253-262.
- American Diabetes Association. (2013). Standards of Medical Care in Diabetes-2013. *Diabetes Care*, 36: S1; 511-566
- Anderson A.R. (1998). Chromium, glucose intolerance and diabetes. *Journal of the American College of Nutrition*, 17: 548-555.
- Aragão G., Carneiro L., Junior A., Vieira L., Bandeira P., Lemos T. and desB Viana G.S. (2006). A possible mechanism for anxiolytic and antidepressant effects of alpha- and betaamyrin from *Protium heptaphyllum* (Aubl.) March. *Pharmacology Biochemistry and Behavior*, 85: 827-834.
- Asha B., Krishnamurthy K. and Siddappa D. (2011). Evaluation of anti hyperglycaemic activity of *Zingiber officinale* (Ginger) in Albino rats. *Journal of Chemical and Pharmaceutical Research*, 3: 452-456.

Ashok D., Shrimant N., Pradeep M. and Akalpita U. (2007). Optimization of alloxan dose is essential to induce stable diabetes for prolonged period. *Asian Journal of Biochemistry*, 2 (6): 402-408.

Association of Official Analytical Chemists (AOAC) (2000). *Official Methods of Analysis of AOAC International*, 17th edition; AOAC International: Gaithersburg, MD, USA.

Astrup A. (2000). Thermogenic drugs as a strategy for treatment of obesity. *Endocrine*, 13, 207-212.

Baker B. (1996). Chromium supplements tied to glucose control. *British Farmers Practice News*, 15: 5-7.

Balesubashini M.S., Rukkumani R., Viswanathan P. and Menon V.P. (2004). Ferrulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Research*, 18: 310-314.

Barger A.A. (2003). The complete blood cell count: a powerful diagnostic tool. *The Veterinary Clinic of Small Animal Practice*, 33: 1207-1222.

Bates C. J. (1995). Vitamin A. *Lancet*, 345: 31-35.

Battin E.E. and Brumaghim J.L. (2009). Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase and metal-binding antioxidant mechanisms. *Cell Biochemistry and Biophysics*, 55: 1-23.

Bendich A. and Olson J.A. (1989). Biological actions of carotenoids. *FASEB Journal*, 3: 1927-1932.

Bendich A. (1989). Carotenoids and the immune-response. *Journal of Nutrition*, 112-115.

Bendich A. (1993). Biological functions of dietary carotenoids. *Annals of New York Academy of Science*, 691: 61-67.

Bigoniya P., Singh C.S. and Shukla A. (2009). A comprehensive review of different liver toxicants used in experimental pharmacology. *International Journal of Pharmaceutical Sciences and Drug Research*, 1 (3): 124-135.

Boyd J. (1983). The mechanism relating to increase in plasma enzyme in diseases of animals. *Veterinary and Clinical Pathology*, 12: 9-24.

Boylan J., Brautigan D., Madden J., Raven T., Ellis L. and Gruppuso P. (1992). Differential regulation of multiple hepatic protein tyrosine phosphatases in alloxan diabetic rats. *Journal of Clinical Investigation*, 90: 174-179.

Brain K.R. and Turner T.D. (1975). Practical evaluation of phytopharmaceuticals. Wright-Scientific, Bristol. 1st Ed. 1975; pp. 144.

- Brand-Williams W., Cuvelier M.E. and Berset C. (1995). *Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss and Technology/Food Science and Technology*, 28: 25-30.
- Britton G. and Robins R.J. (1997). Carotenoids and colour in Fruit and vegetables. In: Tomas-Barberan F A, *Phytochemistry of Fruit and Vegetables* (pp 11-27). Oxford: Clarendon Press.
- Broadhurst C., Polansky M. and Anderson R. (2000). Insulin like biological activity of culinary and medicinal plant aqueous extracts *in vitro*. *Journal of Agricultural and Food Chemistry*, 48: 849-852.
- Brown R. (1992). Toxicity of Chinese herbal remedies. *Lancet*, 340: 673-674.
- Brownlee M. (1995). Advanced protein glycosylation in diabetes and aging. *Annual Review in Medicine*, 46: 223-234.
- Bum E.N., Nkantchoua G., Njikau N., Taiwe G., Ngoupaye G., Pelanken M., Nanga F.M., Maidawa F., Rakotonirina A. and Rakotonirina S.V. (2010). Anticonvulsant and sedative activity of leaves of *Senna spectabilis* in mice. *International Journal of Pharmacology*, 6 (2): 123-128.
- Butler M. (2004). The role of natural product chemistry in drug discovery. *Journal of Natural Products*, 67: 2141–2153.
- Cao G., Sofic E. and Prior R. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine*, 22: 749-760.
- Carpenter M.W. and Coustan D.R. (1982). Criteria for screening tests for gestational diabetes. *American Journal of Obstetrics and Gynecology*, 144: 768-773.
- Castro, R.V. (1998). Chromium in a series of Portuguese plants used in the herbal treatment of diabetes mellitus. *Biological Trace Element Review*, 62: 101-106.
- Chee T.Y. and Ridwans S. (1984). Fast growing species of trees suitable for urban roadside and shade planting. *The Malaysian Forester*, 47(4): 263-284.
- Chi-Mei K. and Jin-Yuarn L. (2013). Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. *Food Chemistry*, 144: 1104-1113.
- Clampitt R.B. and Hart R.J. (1978). The tissue activities of some diagnostic enzymes in ten mammalian species. *Journal of Comparative Pathology*, 88: 607-621.
- Cohen N., Dimondi E.G., Caravaca J., Benchimol A., Habberstam M., Shlimovich P., Chang C.J., Shamoon H. and Rossetti L. (1995). Oral Vanadyl sulfate improves hepatic and peripheral insulin sensitivity in patients with non-insulin dependent diabetes mellitus. *Journal of Clinical Investigations*, 95: 2501-2509.

da Silva G., Serrano R. and Silva O. (2011). *Maytenus heterophylla* and *Maytenus senegalensis*, two traditional herbal medicines. *Journal of Natural Science, Biology and Medicine*, 2: 59-65.

David H.V. and Israili Z. (1990). Alkaline Phosphatase and Gamma Glutamyltransferase. Walker H.K., Hall W.D. and Hurst J.W.I. *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd Ed. Butterworth Publishers, Boston. pp 28-35.

Davis S.N. and Granner D.K. (1996). *Insulin, oral hypoglycemic agents and the pharmacology of endocrine pancreas*. In: Goodman I S; Gilman AG, eds, *The Pharmacological Basis of Therapeutics*, 9th Ed. New York: Mc-Graw Hill; pp 1487-1517.

Dey L., Anoja S., Attele D. and Chun-Su Y. (2002). Alternative therapies for type II diabetics. *Alternative Medicine Review*, 7: 1-5.

Dimond E.G., Caravaca J. and Benchimol A. (1963). Vanadium: Excretion, toxicity, lipid effect in man. *American Journal of Clinical Nutrition*, 12: 49-53.

Dubey R.C. (1991). Fungicidal effect of essential oils of three higher plants on sclerotia of *Marcroponia phaseolina*. *Indian Phytopathology*, 44: 241-244.

Dulloo A.G., Duret C., Rohrer D., Girardier L., Mensi N., Fathi M., Chantre P., Vandermander J. (1999). Efficacy of a green tea extracts rich in catechin polyphenols and caffeine in increasing 24-hr energy expenditure and fat oxidation in humans. *American Journal of Clinical Nutrition*, 70: 1040-1045.

Duncan R., Ahmadian M., Jaworski L., Sarkadi-Nagy E. and Sul H. (2007). Regulation of lipolysis in adipocytes. *Annual Review in Nutrition*, 27: 79-101.

Edeoga H.O., Okwu E. and Mbaebie B.O. (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. *African Journal of Biotechnology*, 4: 685-688.

Elliot M., Chithan K. and Theoharis C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacological Reviews*, 52: 673-751.

Ezaki O. (1989). 11b group metal ions (Zn^{++} , Cd^{++} , Hg^{++}) stimulate glucose transporter activity by post-insulin receptor kinase mechanism in rat adipocyte. *Journal of Biological Chemistry*, 264: 16118-16122.

Farnsworth N.R., Akerele O., Bingel A.S., Soejarto D.D. and Guo Z. (1985). Medicinal plants in therapy. *Bulletin of the World Health Organization*, 63: 965-981.

Farnsworth N.R. (1988). *Screening plants for new medicines Chapter 9 in Biodiversity*, ed. E.O. Wilson. Washington, D.C: National Academy Press. pp. 61-73.

Fattal I., Friedmann N. and Fattal-Valenski A. (2011). The crucial role of thiamine in the development of syntax and lexical retrieval: a study of infantile thiamine deficiency. *Brain: A Journal of Neurology*, 134: 1720-739.

Fishbein L. (1988). Perspectives of analysis of carcinogenic and mutagenic metals in biological samples. *International Journal of Environmental Analysis*, 28: 21-69.

Fivaz J. and Robbertse P. (1993). *Pappea capensis*: monoecious, dioecious or androdioecious. *South African Journal of Botany*, 59: 342-434.

Foye W.O., Lemke T.L. and Williams D.A. (1995). *Principles of Medicinal Chemistry, 4th Edition*. New York: Wilkins and Wilkins.

Frank J.M. and Bantle J. (1999). Eds: *American Diabetes Association Guide to Medical Nutrition Therapy for Diabetes*. Alexandria Va: American Diabetes Association.

Frankel N.E., Kanner J., German B.J., Parks E. and Kinsella E.J. (1993). Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. *The Lancet*, 341: 454-457.

Fredholm B.B., Bättig K., Holmén J., Nehlig A., Zvartau E.E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews*, 51: 83-133.

Friedman B. (1987). Manganese balance and clinical observations in young men fed on a manganese-deficient diet. *Journal of Nutrition*, 117: 133-143.

Garfinkel D. and Garfinkel L. (1988). Magnesium and Regulation of Carbohydrate metabolism at Molecular Level. *Magnesium*, 7: 249-261.

George N., Konstantin M., Denko S. and Chaika P. (2004). Serum Manganese in children with type I diabetes mellitus. *Diabetologica Croatica*, 23: 33-42.

Ghatta S. and Ramarao P. (2004). Increased contractile responses to 5-Hydroxytryptamine and angiotensin II in high fat fed rat thoracic aorta. *Lipids in Health and Disease*, 3: 19.

Glauce S., Carolina C.M., Michelle R.L., Kalyne A.M., Tiago G.V. and Abreu F.M. (2004). Hypoglycemic and anti-lipidemic effects of the aqueous extract from *Cissus sicyoides*. *Biomedical Central Pharmacology*, 4: 9-12.

Goodwin T.W. (1980). *The Biochemistry of the Carotenoids*. Vol. 1 "Plants". New York: Chapman and Hall. pp. 167-200.

Greenspan F. and Gardner D. (2001). *Basic and clinical endocrinology*, 6th Ed. New York: McGraw-Hill. pp. 295-361.

Gulfranz M., Ahmad A., Asad M.J., Sadiq A., Afzal U., Imran M., Anwar P., Zeenat A., Sarfraz K.A., Maqsood S. and Qureshi R.U. (2011). Antidiabetic activities of leaves and root extracts of *Justicia adhatoda* Linn against alloxan induced diabetes in rats. *African Journal of Biotechnology*, 10: 6101-6106.

Gurib-Fakim A. (2006). Medicinal Plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27: 1-93.

Gutierrez F., Estévez-Braun A., Ravelo A.G., Astudillo L. and Zárate R. (2007). Terpenoids from the medicinal plant *Maytenus ilicifolia*. *Journal of Natural Products*, 70:1049–1052.

Harris E. (2005). Elevated liver function tests in type II diabetes. *Clinical Diabetes*, 23: 115-119.

Hawkins E. and Ehrlich S.D. (2006). *Gotu Kola*. Baltimore, USA: University of Maryland Medical Center.

Hayes A. (2008). *Principles and methods of toxicology*. New York: Informa Healthcare USA, Inc.

Health N.I. (2011). *Office of Dietary Supplements*. Retrieved April 28, 2011, from Strengthening Knowledge and Understanding of Dietary Supplements: <http://ods.od.nih.gov/factsheets/VITAMINE/>

Houghton P. and Raman A. (1998). *Laboratory handbook for the fractionation of natural extracts*. London: Chapman and Hall; 154-187.

Howard M.R. and Hamilton P.J. (2002). Haematology. Blood cells; 2nd Ed. Churchill Livingstone, Edinburgh. Harcourt Publishers Limited. pp 6-7.

Huang S. and Czech M. (2007). The GLUT 4 glucose transporter. *Cell Metabolism*, 5: 237-252.

International Committee for Standardization in Haematology (1978). Recommendations for reference method for haemoglobinometry in blood and specifications for international haemoglobincyanide reference preparation. *Journal of Clinical Pathology*, 31: 139-143.

Jain N. (1986). *Schalm's veterinary hematology* 4th Ed. Philadelphia: Lea and Febiger. pp 12-321.

Jeffrey C. (1966). Notes on Compositae I. The Cichorieae in East Tropical Africa. *Kew Bulletin*, 18: 427-486.

Jessen A., Buemann B., Toubro S., Skovgaard I.M. and Astrup A. (2005). The appetite-suppressant effect of nicotine is enhanced by caffeine. *Diabetes, Obesity and Metabolism*, 7: 327-333.

Jigna P. and Sumitra V. (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plant. *Turkey Journal of Biology*, 31: 53-58.

Joan F.Z., Peter R.P. and Phillip D.M. (1988). Liver disease and gall stones. In Arnold E. Edition. *Clinical chemistry in diagnosis and treatment*. 5th Ed. Singapore: P.G. Publishing Pte Limited, pp. 287-303.

John S., Chi-Tang H. and Fereidon S. (2011). *Nutraceutical Science and Technology: Functional foods of the East*. USA: CRC Press Taylor and Francis Group. pp. 87-123.

Joseph B. and Jini D. (2013). Antidiabetic effects of *Momordica charantia* (bitter lemon) and its medicinal potency. *Asia Pacific Journal of Tropical Disease*, 3: 93-102.

Jothy S.L., Zakara Z., Chen Y., Lau L.Y., Latha L.Y. and Sasidharan S. (2011). Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. *Molecules*, 16: 5268-5282.

Kaneko J.J. (1989). *Clinical Biochemistry of domestic animals*. San Diego: Academic Press, pp. 142-165.

Kaplan L.A. and Pesce A.J. (1989). Diabetes mellitus. *Clinical Chemistry: Theory, Analysis and Correlation*. St. Louis: Mosby. pp 613-641.

Karau G.M., Njagi E.N., Machocho A.K. and Wangai L.N. (2012a). Phytonutrients, mineral composition and *in vitro* antioxidant activity of leaf and stem bark powders of *Pappea capensis* (L). *Pakistan Journal of Nutrition*, 11 (2): 123-132.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N. and Kamau P.N. (2012b). Hypoglycemic activity of aqueous and ethyl acetate leaf and stem bark extracts of *Pappea capensis* in alloxan-induced diabetic BALB/c mice. *British Journal of Pharmacology and Toxicology*, 3 (5): 251-258.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N. and Kamau P.N. (2013a). Phytonutrients, minerals and *in vitro* antioxidant capacity of leaf and stem bark powders of *Senna spectabilis*. *Journal of Pharmacognosy and Phytochemistry*, 2 (2): 71-79.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N., Karau P.B., Kamau P.N. (2013b). Hypoglycemic effect of aqueous and ethyl acetate extracts of *Senna spectabilis* in alloxan induced diabetic male mice. *Journal of Pharmaceutical and Biomedical Sciences*, 31 (31): 1089-1095

Kastumata K., Kastumata K.J. and Kastumata Y. (1992). Protective effect of diltiazem hydrochloride on the occurrence of alloxan or streptozotocin-induced diabetes in rats. *Hormones and Metabolism Research*, 24: 508-510.

Kaushansky L. (1995). Thrombopoetin: the primary regulator of megakaryocyte and platelet production. *Thrombosis and Haemostasis*, 74: 521.

- Kawamura R. (1941). Intoxication by manganese in well water. *Kisasato Archives of Experimental Medicine*, 18: 145-169.
- Kelly G. (1999). Squalene and its potential clinical uses. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 4: 29-36.
- Khajehdehi P., Shariat A. and Nikseresht A. (1998). Acute renal failure due to severe Landry-Guillain-Barré syndrome. *Nephrology, Dialysis, Transplantation*, 13: 2388-2391.
- Khalil I.A. and Manan F. (1990). Text book of Chemistry I. Bioanalytical Chemistry 2nd Edition. Taj Kutab Khana Peshawar.
- Khan A., Bryden N.A., Polasky M.N. and Anderson R. A. (1990). Insulin-potentiating factor and chromium content of selected spices. *Biology of Trace Elements Research*, 24: 183-188.
- Knip M., Douek I.F., Moore W.P.T., Gillmor H.A., McLean A.E., Bingley P.J. and Gale E.A. (2000). For the ENDIT Group: Safety of high-dose nicotinamide: a review. *Diabetologia*, 43: 1337-1345.
- Kokwaro J. (2009). Diseases and the Plant Species used for Treatment. Medicinal Plants of East Africa, 3rd Ed. Nairobi: University of Nairobi Press. pp. 20-320.
- Kolb H. and Volker B. (1999). Nicotinamide in type 1 diabetes mechanism of action revisited. *Diabetes Care*, 22 (Suppl. 2): B16-B20.
- Krishnaiah D., Devi T., Bono A. and Sarbatly R. (2009). Studies on phytochemical constituents of six Malaysian. *Journal of Medicinal Plants Research*, 3(2): 67-72.
- Kunle O., Egharevba H. and Ahmadu P. (2012). Standardization of herbal medicines- A Review. *International Journal of Biodiversity and Conservation*, 4: 101-112.
- Laxmi V., Anirudh K., Basant K., Umesh K.P. and Rajesh S.P. (2010). Antidiabetic activity of *Cassia occidentalis* (Linn) in normal and alloxan-induced diabetic rats. *Indian Journal of Pharmacology*, 42: 224-228.
- Le veille G. and Sanberlich H. (1966). Mechanism of the cholesterol-dressing effect of pectin in the cholesterol fed rat. *Journal of Nutrition*, 3: 209-214.
- Lenzen S., Tiedge M., Jornas A. and Munday R. (1996). *Alloxan derivatives as a tool for the elucidation of the mechanism of the diabetogenic action of alloxan*. Birkhauser, Boston, In Lessons from Animal Diabetes. Shafrir, E. (Ed.). pp: 113-122
- Lewis G., Carpenier A., Khosvono A. and Giacca A. (2002). Disordered fat storage, and mobilization in the pathogenesis of insulin resistance, and type II diabetes. *Endocrinology Review*, 23: 201-229.

Lichtenstein A. and Schwab U. (2000). Relationship of dietary fat to glucose metabolism. *Atherosclerosis*, 150: 227-243.

Litosch I., Hudson T.H., Mills I., Li S.Y. and Fain J.N. (1982). Forskolin as an activator of cyclic AMP accumulation and lipolysis in rat adipocytes. *Molecular Pharmacology*, 22: 109-115.

Luca C. and Olefsky J. (2007). Inflammation, and insulin resistance. *Federation of European Biochemical Societies*, 582: 97-105.

Malender A. (2004). Kinetics-effect relations of insulin releasing drugs in patients with type II diabetes. *Diabetes*, 53: 151-55.

Manuel F.J, Abel L. and Wifredo R. (2002). Perspectives in diabetes; cross-talk between iron metabolism and diabetes. *Diabetes*, 51: 2348-2354.

Martens G., Cai Y., Hinke S., Stange G., Van de Castele M. and Pipeleers D. (2005). Glucose suppresses superoxide generation in metabolically responsive pancreatic beta cells. *The Journal of Biological Chemistry*, 280: 20389-20396.

McClellan W.M., Knight D.F., Karp H. and Brown W.W. (1997). Early detection and treatment of renal disease in hospitalized diabetic and hypertensive patients: important differences between practice and published guidelines. *American Journal of Kidney Diseases*, 29: 368-375.

Mertz B. (1969). Chromium occurrence and function in biological systems. *Physiological Review*, 49: 163-237.

Ministry of Public Health and Sanitation (2010). Kenya National Diabetes Strategy, 2010-2015, 1st Edition; WDF09-436, Retrieved from www.pubhealth.go.ke on 30th August, 2011.

Mlinar B., Marc J., Janez A. and Pfeifer M. (2006). Molecular mechanisms of insulin resistance and associated diseases. *Clinica Chimica Acta*, 375: 20-35.

Mng'omba S., Toit E.D., Akinnifesi F. and Venter H. (2007). Repeated exposure of jacket plum (*Pappea capensis*) micro-cuttings to indole-3-butyric acid (IBA) improved *in vitro* rooting capacity. *South African Journal of Botany*, 73: 230-235.

Monago C. and Uwakwe A. (2009). Proximate composition and *in vitro* anti-sickling property of Nigeria *Cyperus esculentus* (tiger nut sedge). *Trees for Life Journal*, 4 (2): 1-6.

Mooradian A., Faila M., Hoogwerf B., Maryniuk M. and Wylie-Rosett J. (1994). Selected vitamins and minerals in diabetes. *Diabetes Care*, 17: 464-479.

Muhammad S., Saghir A. and Nizamani S. (2009). Determination of nutrition values of Jamun fruit (*Eugenia jambolana*) products. *Pakistan Journal of Nutrition*, 8 (8): 1275-1280.

Muramatsu K., Fukuyo M. and Hara Y. (1986). Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *Journal of Nutritional Science and Vitaminology*, 32: 613-622.

Nagao T., Komine Y., Soga S., Meguro S., Hase T., Tanaka Y. and Tokimitsu I. (2005). Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *American Journal of Clinical Nutrition*, 81: 122-129.

National Diabetes Data Group. (1979). Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*, 28: 1039-57.

Niederau C., Berger M., Stremmel W., Starke A., Strohmeyer G. and Ebert R. (1984). Hyperinsulinemia in non-cirrhotic haemochromatosis; impaired hepatic insulin degradation. *Diabetologia*, 26: 441-444.

Noureddini H. and Byun J. (2010). Dilute-acid pretreatment of distillers' grains and corn fiber. *Bioresource Technology*, 101: 1060–1067.

Olson J.A. (1993). Vitamin A and carotenoids as antioxidants in a physiological context. *Journal of Nutrition Science and Vitaminology*, 39: 57-65.

Oyaizu M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition*, 44: 307-315.

Palmer E. and Pitman N. (1972). *Trees of Southern Africa covering all known indigenous species in the republic of South Africa, South-West Africa, Botswana, Lesotho and Swaziland*. Cape Town: AA. Ballkema 3v.

Pandey A., Tripathi P., Pandey R., Srivatava R. and Goswami S. (2011). Alternative therapies useful in the management of diabetes: A systematic review. *Journal of Pharmacy and Bioallied Sciences*, 3(4): 504-12.

Patel P. and Macerollo A. (2010). Diabetes Mellitus: Diagnosis and Screening. *American Family Physician*, 81: 863-870.

Paton A., Harley R.M. and Harley M.M. (1999). *Ocimum*-an overview of relationships and classification. In: Holm Y and Hiltunen R (eds), *Basil, the genus Ocimum. Medicinal and Aromatic Plants*. Harwood academic. Amsterdam, Netherland; pp. 1-38.

Pellati F., Benvenuti S., Melegari M. and Firenzuoli F. (2002). Determination of adrenergic agonists from extracts and herbal products of *Citrus aurantium* L. var. amara by LC. *Journal of Pharmaceutical and Biomedical Analysis*, 29: 1113-1119.

Percival M. (1998). Antioxidants. *Clinical Nutrition Insights*, 31: 1-4.

Perkins K.A., Epstein L.H., Stiller R.L., Fernstrom M.H., Sexton J.E., Jacob R.G. and Solberg R. (1991). Acute effects of nicotine on hunger and caloric intake in smokers and nonsmokers. *Psychopharmacology*, 103: 103-109.

Perriot M., Kono T., Whitesell R., Knobel S., Piston D., Grannen D.K., Powers A.C. and May J.M. (2001). Glucose uptake and metabolism by cultured human skeletal muscle cells. *American Journal of Physiology and Endocrinological Metabolism*, 281: 72-80.

Prabbakar P., Sudha P., Abhay M. and Sushilkumar V. (2013). Antidiabetic activity of alcoholic extract of neem (*Azadirachta indica*) root bark. *National Journal of Physiology, Pharmacy and Pharmacology*, 3: 142-146.

Qatanani M. and Lazar M. (2008). Mechanisms of obesity-associated insulin resistance: many choices on the menu. *Genes and Development*, 21: 1443-1455.

Qiu-xia M., Rebecca R.H. and Jane H.R. (2013). Ethnopharmacological and bioactivity guided investigation of five TCM anticancer herbs. *Journal of Ethnopharmacology*, 148: 229-238.

Quarta D., Borycz J., Solinas M., Patkar K., Hockemeyer J., Ciruela F., Lluís C., Franco R., Woods A.S., Goldberg S.R. and Ferré S. (2004). Adenosine receptor-mediated modulation of dopamine release in the nucleus accumbens depends on glutamate neurotransmission and N-methyl-D-aspartate receptor stimulation. *Journal of Neurochemistry*, 91: 873-880.

Rahimi R., Nikfan S., Larijani B. and Abdollahi M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine and Pharmacotherapy*, 59: 365-373.

Rai M. and Carpinella M.C. (2006). *Naturally Occurring Bioactive Compounds*. Elsevier Ltd. Maryland, USA: pp. 56-70.

Rasineni G.K., Siddavattan D. and Reddy A.R. (2008). Free radical quenching activity and polyphenols in three species of *Coelus*. *Journal of Medicinal Plants Research*, 2 (10): 285-291.

Rees D. and Alcolado J. (2005). Animal models of Diabetes mellitus. *Diabetes Metabolism*, 22: 359-370.

Reul B.A. (1997). Improvement of glucose homeostasis and hepatic insulin resistance in ob/ob mice given oral molybdate. *Journal of Endocrinology*, 155: 55-64.

Ribes G., Sauvaire Y., Da Costa C., Baccou J.C. and Loubatieres-Mariam M.M. (1986). Antidiabetic effects of subfractions from fenugreek seeds in diabetic dogs. *Proceedings of Society of Experimental Biology and Medicine*, 182: 159-66.

Rice A.L., West K.P. and Black R.E. (2004). Vitamin A deficiency. Global and regional burden of disease attributable to selected major risk factors. Volume 1. World Health Organization. Geneva, Netherlands.

Riturparna M. and Neeraj K.A. (2007). Artherosclerosis in DM: Role of inflammation. *Indian Journal of Medical Sciences*, 61: 292-306.

Roberto R.F., Renee J.G. and Paton J.A. (2003). Chemical profiling of *Ocimum americanum* using external flavonoids. *Phytochemistry*, 63 (5): 555-567.

Robinson H. (1978). *Fundamentals of Normal Nutrition* 3rd Ed. London: Macmillan Publishing Co., Inc ISBN 0-02-979590, pp 41-125, 272-284.

Rosalki S.B. (1967). An improved procedure for serum creatine phosphokinase determination. *Journal of Laboratory and Clinical Medicine*, 69: 696-705.

Rosner F. and Gorfien G. (1968). Erythrocyte and plasmic zinc and magnesium levels in health and disease. *Journal of Laboratory and Clinical Medicine*, 72: 213-219.

Ruan H. and Lodish H. (2002). Insulin resistance in adipose tissue direct, and indirect effects of tumour necrosis factor-alpha. *Cytokine Growth Factor Review*, 14: 2859-2865.

Ryter S.W., Kim H.P., Hoetzel A., Park J.W., Nakahira K. and Choi X.W. (2007). Mechanism of cell death in oxidative stress. *Antioxidant Redox Signaling*, 9: 49-89.

Sarubin A. (2000). *The Health Professional's Guide to Popular Dietary Supplements*. The American Diabetic Association. Chicago, USA.

Schenk S., Saberi M. and Olefsky J. (2008). Insulin sensitivity: modulation by nutrients and inflammation. *The Journal of Clinical Investigation*, 118 (9): 2992-3002.

Sen C.K., Khanna S. and Roy S. (2006). Tocotrienols: Vitamin E beyond tocopherols. *Life Science*, 78: 2088-2098.

Shahidi F. and Ho C. (2005). Phenolic compounds in foods and natural health products. In F. S. (Ed.), *ACS Symposium Series*. Washington, D C: American Chemical Society. pp. 1-8.

Sharma A.K. (1993). *Diabetes mellitus and its complications: An update*, 1st Ed. New Delhi: Macmillan India Ltd. pp. 92-205.

Sharma A.M. and Staels B. (2007). Review: Peroxisome proliferator activated receptor gamma and adipose tissue- understanding obesity related changes in regulation of lipid and glucose metabolism. *Journal of Clinical Endocrinology and Metabolism*, 92: 386-395.

Shils M.E., Olson J.A. and Shike M. (1999). *Modern Nutrition in Health and Disease*, 9th edition. Lea and Febiger, Philadelphia PA, USA.

Siddharth N.S. (2001). Containing the global epidemic of diabetes. *Journal of Diabetology*, 3: 11.

Song M., Rosenthal M., Naliboff B., Phanumas L. and Kang K. (1998). Effects of boirne prostate powder on zinc, glucose and insulin metabolism in old patients with non-insulin dependent diabetes mellitus. *Metabolism*, 47: 39-43.

Subramanian S. (1981). Epicatechin as an anti-diabetic drug. *Indian Drugs*, 18: 259-261.

Surendran S., Eswaran M.B., Vijayakumar M. and Rao C.V. (2011). *In vitro* and *in vivo* hepatoprotective activity of *Cissampelos pareira* against carbon tetrachloride induced hepatic damage. *Indian Journal of Experimental Biology*, 49: 939-945.

Szkudelski T. (2001). The Mechanism of alloxan and streptozotocin action in beta-cells of the rat pancreas. *Physiology Research*, 50: 536-546.

Tang H.F., Yang-Hua Y., Yao X.S., Xu Q.Z., Zhang S.Y. and Lin H.W. (2002). Bioactive steroids from the brown alga *Sargassum carpophyllum*. *Journal of Asian Natural Products Research*, 4: 95-101.

Tang G. (2010). Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *American Journal of Clinical Nutrition*, 91: 1468-1473.

Thadikamala S., Udayakiran D., Himabindu K., Sridevi P.L.D., Kezia D. and Bhojaraju P. (2009). HPLC method for the determination of lycopene in crude oleoresin extracts. *Asian Journal of Chemistry*, 21(1): 1-10.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997). Report. *Diabetes Care*, 20: 1183-1197.

Thom E. (2007). The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. *Journal of Internal Medicine and Research*, 35: 900-908.

Thomson E.B. (1985). *Drug Bioscreening: Fundamentals of drug evaluation techniques in pharmacology*. New York: Graceway Publishers Co. pp. 123-131.

Timbrell J.A. (1996). *Introduction to toxicology*. Toxicity testing and risk assessment. London: Taylor and Francis, pp. 55-149.

Traber M.G. (2007). Vitamin E regulatory mechanisms. *Annual Review in Nutrition*, 27: 347-362.

Traber M.G. (2006). Vitamin E. In: Shils M. E., Shike M., Ross A. C., Caballero B., Cousins R, *Modern Nutrition in Health and Disease*. 10th Ed Baltimore, MD: Lippincott Williams and Wilkins. pp. 396-411.

Treadway L. (1994). Amla: Traditional food and medicine. *HerbalGram: Journal of the American Botanical Council*, 31: 26.

Trease G.E. and Evan W.C. (2002). *Pharmacognosy*. 15th Edition. London Saunders Publishers; pp. 42-44, 221-229, 246-249, 391-393.

Tucci S.A. (2010). Phytochemicals in the control of human appetite and body weight. *Pharmaceuticals*, 3: 748-763.

Tulucku E., Yalcin H., Ozturk I. and Sagdic O. (2012). Changes in the fatty acid compositions and bioactivities of clary sage seeds depending on harvest year. *Industrial Crops and Products*, 39: 69-73.

Umamaheswari M. and Chatterjee T.K. (2008). *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *African Journal of Traditional, Complimentary and Alternative Medicines*, 5: 61-73.

Van Wyk B.E. and Gericke N. (2000). *People's Plants; A guide to useful plants of Southern Africa*. Briza Publications. Pretoria, South Africa.

Viegas J.C., Bolzani V.S., Primetel S.B., Castro N.G., Cabral R.F., Rodrigo S.C., Corinne F., Monica S.R., Maria C.M.Y., Eliezer J.B. and Carlos A.M.F. (2005). New selective acetylcholinesterase inhibitors designed from natural piperidine alkaloids. *Bioorganic and Medicinal Chemistry*, 13: 4184-4190.

Voet D. and Voet J.G. (2004). *Biochemistry, Biomolecules, mechanisms of enzyme action and metabolism. Electron transport and oxidative phosphorylation*; 3rd edition; John Wiley and Sons, Inc. New York, United States of America. pp. 742-797.

Weaver D.K., Dunkel F.V., Ntezurubanza L., Larry L.J. and Stock D.T. (1991). The efficiency of linalool, a major component of freshly-milled *Ocimum canum* (Lamiaceae), for protection against postharvest damage by certain stored products Coleoptera. *Journal of Stored Products Research*, 27: 213-220.

Wheelwright E. (1994). *Medicinal plants and their history*. Dover Publications, Inc. New York

WHO (1985). Diabetes mellitus report of a WHO study group. *WHO technical report series*, pp. 727.

WHO (1993). *Research guidelines for evaluating the safety and efficacy of herbal medicine*. World Health Organization, Geneva, Netherlands: pp. 1-86.

WHO (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus. WHO, Geneva.

WHO (2002). Laboratory Diagnosis and Monitoring of Diabetes Mellitus. World Health Organization Report. Geneva, Netherlands: pp. 5-7, 18-22.

WHO (2004). Vitamins and mineral requirements in human nutrition. A report of a joint FAO/WHO expert consultation, Bangkok, Thailand, 21-30 September, 1998. pp. 131.

WHO (2006). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. Report of WHO/IDF consultation. WHO, Geneva.

WHO (2009). Diabetes. World Health Organization Fact sheet Number 312. Geneva, Netherlands.

WHO (2013). Diabetes. World Health Organization Fact Sheet Number 312, Reviewed in October, 2013.

Wild S., Roglic G., Green A., Sicree R. and King H. (2004). Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27: 1047-1053.

Williams G. and Pickup J. (1991). New Drugs in the management of diabetes mellitus. In Pickup J.C. and Williams G. *Textbook of Diabetes* Oxford: II Blackwell. pp. 977-993.

Wink M. (2008). Evolutionary advantage and molecular modes of action of multi- component mixtures used in phytomedicine. *Current Drug Metabolism*, 9: 996-1009.

Xie J., Wu J., Mehendale S., Aung H. and Yuan C. (2004). Antihyperglycemic effect of the polysaccharides fraction from American ginseng berry extract from root in diabetic mice. *Phytomedicine*, 11: 182-187.

Zahra S. and Samaneh P.K. (2012). Toxicity of margarine on liver enzymes (Aspartate aminotransferase and Alanine aminotransferase) in rats. *Iran Journal of Toxicology*, 6 (17): 655-659.

Zhou G., Myers R., Li Y., Chen Y., Shen X., Fenyk-Melody J., Wu M., Ventre J., Doebber T., Fujii N., Musi N., Hirshman M.F., Goodyear L.J. and Moller D.E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *Journal of Clinical Investigation*, 108: 1167-1174.

APPENDIX I

1 External calibration curves and quality controls

1.1 Estimation of total phenolics calibration curve

The regression equation for quantitation of total phenols using gallic acid equivalent (GAE) is given as $Y = 12.12415x + 0.07706$.

Where; Y is the concentration of phenolics in mg/g, x is the absorbance, the slope is 12.12415, the y-intercept is 0.07706, and the line of best fit parameter ($R^2 = 0.9938$), the standard Error of the estimate = 0.01204

1.2 Preparation of the extracts for efficacy assay

50 mg/kg body weight for a BALB/c mice of average weight of 25 g, requires, 1.25 mg dissolved in 0.1% of physiological saline.

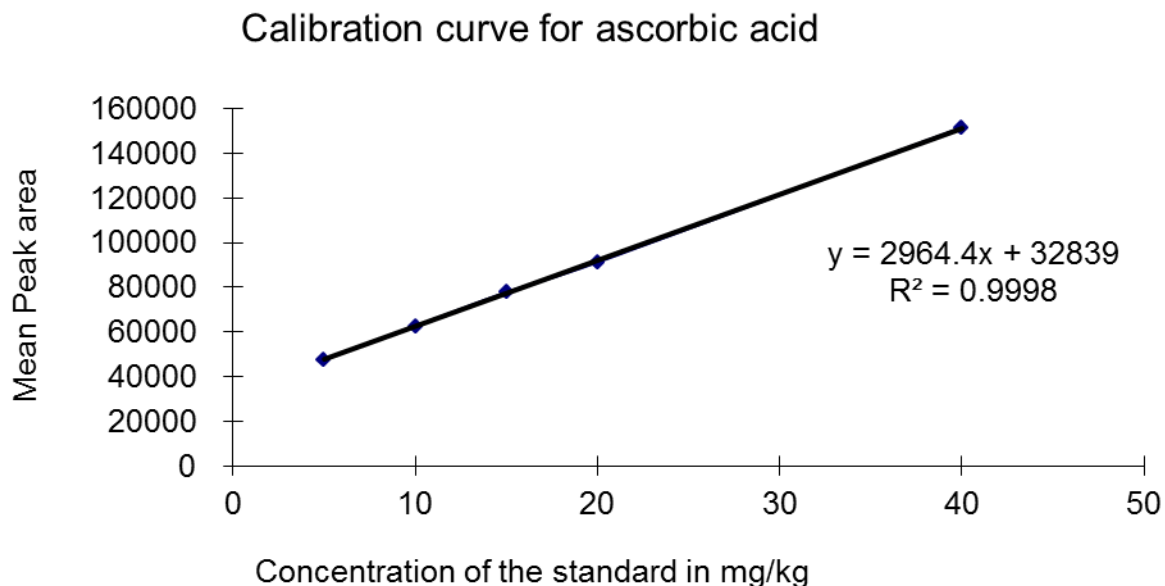
100mg/kg body weight will require, 2.5 mg dissolved in 0.1% of physiological saline

200mg/kg body weight required 5mg of extract dissolved in 0.1% physiological saline.

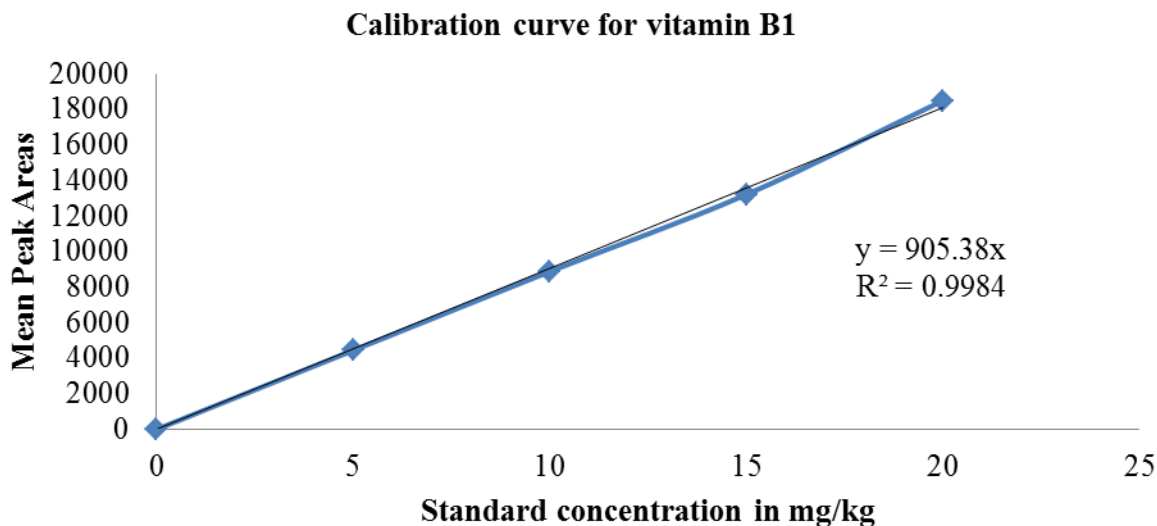
There are six groups of animals in the study as follows;

- 1) Normal controls to monitor the circadian rhythm (5 animals).
- 2) Diabetic control not treated with either oral or intravenous conventional drug or extracts (5 animals).
- 3) Diabetic group treated with either insulin or glibenclamide (5 animals).
- 4) Diabetic groups treated with:
 - i. 50mg/kg body weight (5 animals).
 - ii. 100mg/kg body weight (5 animals).
 - iii. 200mg/kg body weight (5 animals).

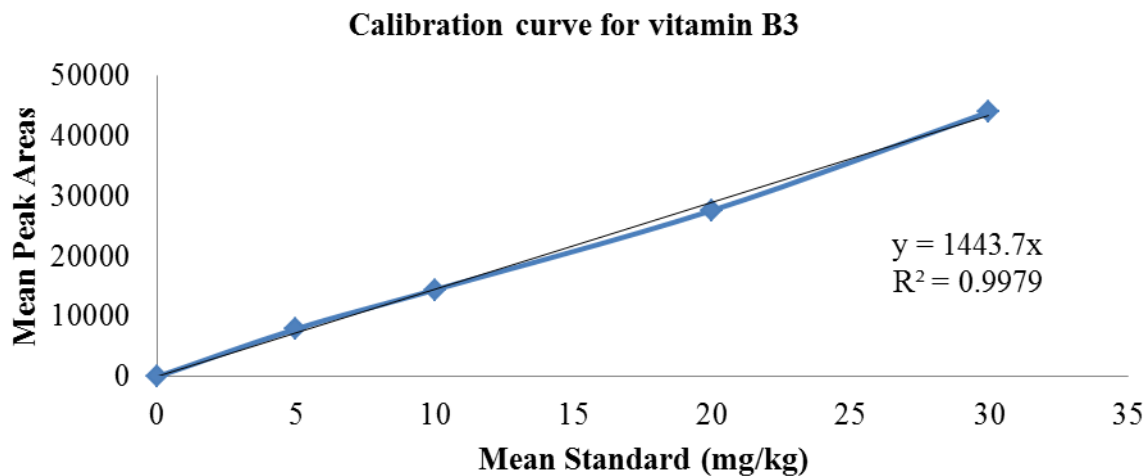
1.3 Calibration curve curves



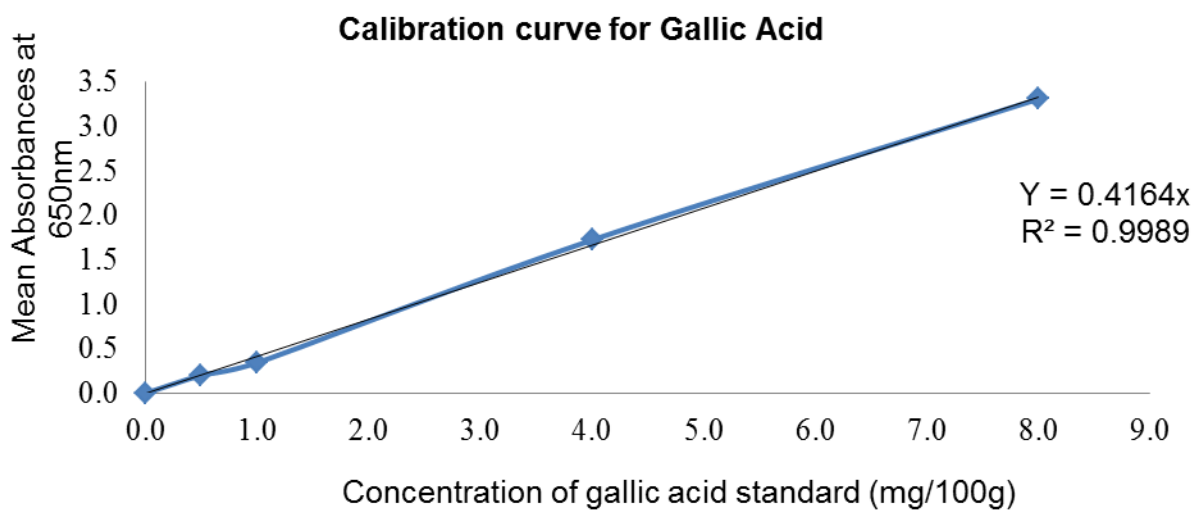
The linearity represented by R^2 was found to be 0.9998 and the injections were made in triplicates and were found to have percent coefficient of variation (% CV) of $\leq 2\%$ thus proving the method reliable for analysis. The retention time was 1.034 minutes. The slope is 2964.4, Y-intercept is 32839 and the equation used in determining the amount of ascorbic acid is Concentration of Ascorbic acid (mg/kg) = Mean peak areas/ 2964.4 – 32839.



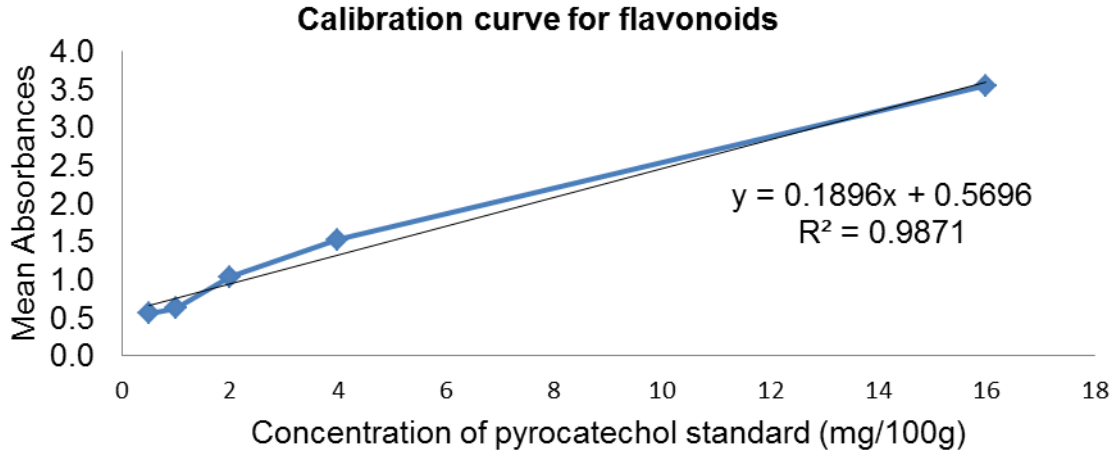
Linearity of the calibration curve was 0.9984, and every point was a mean of triplicates injection.



Linearity of the calibration curve was 0.9979, and every point was a mean of triplicates injection.

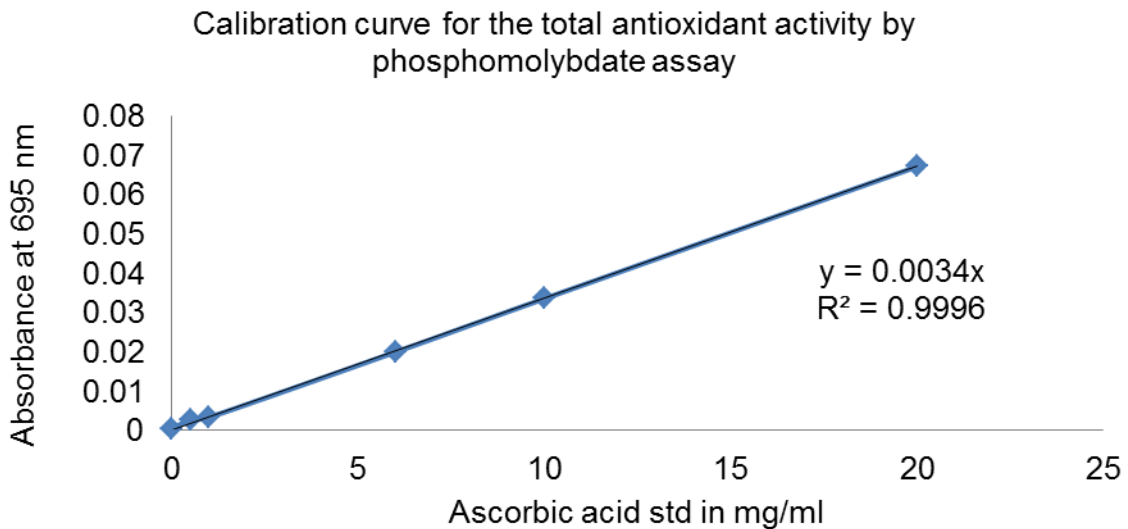


The total phenol was determined as gallic acid equivalent. A standard range of 0.5-8.0 mg/100g was used.



The flavonoids were determined as pyrocatechol equivalent, and the working range was 0.5-16 mg/100g.

The calibration curve for the determination of ascorbic acid equivalent (AAE) in phosphomolybdate assay



The expression for determining the ascorbic acid equivalent (AAE) is $Y = 0.0034X$ Where Y is the absorbance at 695 nm and X is the concentration of ascorbic acid in mg/ml. the R^2 is 0.9996.

APPENDIX II

Publications associated with the work

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N. (2014). Profiling of the chemical compounds in ethyl acetate extracts of *Launaea cornuta* Asteraceae by GC-MS. *Pharmacognosy Research*. **In press**.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N., Kamau P.N. (2014). Efficacy and Safety Assessment of *Launaea cornuta* Extracts in the Management of Diabetes Mellitus. *American Journal of Medicine and Medical Sciences*. 4(1): 1-7.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N. and Kamau P.N. (2013). Phytonutrients, minerals and *in vitro* antioxidant capacity of leaf and stem bark powders of *Senna spectabilis*. *Journal of Pharmacognosy and Phytochemistry* 2(2): 71-79.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N., Karau P.B., Kamau P.N. (2013). Hypoglycemic effect of aqueous and ethyl acetate extracts of *Senna spectabilis* in alloxan induced diabetic male mice. *Journal of Pharmaceutical and Biomedical Sciences* 31(31): 1089-1095

Karau G.M., Njagi E.N., Machocho A.K. and Wangai L.N. (2012). Phytonutrients, mineral composition and *in vitro* antioxidant activity of leaf and stem bark powders of *Pappea capensis* (L). *Pakistan Journal of Nutrition*, 11 (2): 123-132.

Karau G.M., Njagi E.N., Machocho A.K., Wangai L.N. and Kamau P.N. (2012). Hypoglycemic activity of aqueous and ethyl acetate leaf and stem bark extracts of *Pappea capensis* in alloxan-induced diabetic BALB/c mice. *British Journal of Pharmacology and Toxicology*, 3 (5): 251-258.