THE MAJOR ANTI-DIABETIC HERBAL MEDICATIONS USED IN BARINGO COUNTY KENYA, THEIR BIOCHEMICAL PROFILES, ACTIVITY AND SAFETY

BY

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DECLARATION

DECLARATION BY THE CANDIDATE;
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DEDICATION

This thesis is a special dedication to my family members; my wife Victoria, my daughters; Alexandria & Faith, my son; Victor, my sister; Jane and my late mother Rebecca Kobilo. Their immeasurable sacrifices, both socially and financially, contributed directly or indirectly to the success of this thesis.

ABSTRACT

The use of herbal medicine in the management of Diabetes Mellitus in the world today has been steadily increasing. In the African continent, approximately 80% of its inhabitants, use herbal products, whose' biochemical, efficacy, and safety are unclear. In the test for safety and efficacy for diabetic herbal medicine, rodents have traditionally been used, though recently, it has received criticisms from '*animal rights' advocates*, who see their use as unethical. Hence the need for an alternative protocol which is less ethically demanding, compared to the traditionally rat's protocol. This study therefore, identified the major anti-diabetic herbal medications used in Baringo County, Kenya, analyzed their biochemical profiles, and investigated two commonly used herbs for efficacy and safety, in the University of Eldoret (UOE) Biotechnology Centre, by firstly determining the safe dosages of the identified herbs using, Frog Embryo Teratogenic Assay-Xenopus 96HR protocol (FETAX), to guide the use of less numbers of rats, in succeeding experiments. A descriptive cross-sectional survey was adopted during the field work, whereby thirty-nine (39) diabetics, and twelve (12) herbalists, were recruited. A researcher-administered questionnaire was used to gather data from diabetics, while an interview guide was used to collect data from the herbalists. In UOE laboratories, the safety of the two most commonly used herbs, was firstly determined, using FETAX 96Hr protocol and using the LD_{50} obtained, and findings from previous studies, safe dosages was determined, and used in rats' protocol. Descriptive statistics, probit analysis, and regression analysis were used to analyze the frequency & use of major herbs, biochemical profiles, determination of LD_{50}'s, and efficacy, respectively. From the field studies, the commonly used antidiabetic herbs in Baringo county were *Urtica dioica* (UD) (75%) and *Carissa edulis* (CE) (58%), herbs. They were both endowed with the major known anti-diabetic phytochemicals (saponins +++, alkaloids ++, and flavonoids ++). On analysis of safety levels in vitro studies, CE extracts had and LD_{50} of, 4,321.67mg/l, and 14,706.3 mg/l and, UD extracts, and LD_{50} of 21,706mg/kg, and 3955mg/kg, for crude ethanolic and aqueous respectively, similar with dosages from previous studies. The identified dosages from in-vitro studies [FETAX], did not only manifest an antidiabetic activity of the herbs, but also portrayed some significant levels of safety, when extrapolated to in vivo studies. With reference to histological outcomes on the selected tissues of rats, no change was seen in those exposed to CE extracts, while a change was seen in those exposed to UD extracts- Kidney (*interstitial nephritis)'*, and liver (*inflammation*. In terms of antidiabetic activity -crude CE root extracts were much more efficacious compared to UD crude leave extracts [(CE p-value<0.001 (ethanolic) & p-value=0.011 (aqueous) and UD p-value <0.001 (ethanolic) & p-value =0.026 (aqueous)]. CE extracts purified forms showed a much more significant antidiabetic activity compared to its crude version, as opposed to UD extracts. In conclusion, CE extracts, are not only safe, but efficacious while UD extracts, though efficacious, they are not safe. The in-vitro (FETAX Assay) study used, helped in determination of the safe dosages used in, in-vivo(rats) studies, which significantly helped reduce the mortality of the animals [rats] used in in vivo studies, however, much more studies are needed with regards to UD extracts.

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ABBREVIATIONS ACRONYMS AND SYMBOLS

AIDS	Acquire Immune Deficiency Syndrome
ANOVA	Analysis of Variance
ALT	Alanine Transaminase
ASTs	Aspartate aminotransferase
AV	Average
AT	*Aloe tweedie*
BD	Twice a day
Ca	Calcium
cAMP	Cyclic Adenosine Monophosphate
CAP	Calpain like protease
CCT	Column Chromatography
CE	*Carissa edulis*
CMP	Compound
Ce6/CE6/PCE	Purified form of *Carissa edulis*
Cl^-	Chloride
CO_3^{2-}	Bicarbonate
UD	*Urtica dioica*
UD6/UD6/CUD6	Purified form of *Urtica dioica*
CBC	Complete Blood Cell Count
Conc.	Concentration.
DCT	Distal Convoluted Tubule
DCTV	Distal convoluted tubule vacuolization
df.	Degrees of Freedom
DM	Diabetes Mellitus
DMI	Diabetes Meditation Intuitive center
DMSO	Di-Methyl Sulphur Dioxide
DNA	Deoxyribonucleic Acid
EBH	Environmental Health and Biology
FETAX	Frog Embryo Teratogenesis Assay-Xenopus

Ft	Feet
EJ	*Eriobotrya japonica*
GAD	Glutamic Acid Decarboxylase
GDM	Gestational Diabetes Mellitus
GLUT	Glucose Transporters
GWAS	Genome-Wide Association Studies
H	Hydrogen
Ha	Alternative Hypothesis
HF	*Hypoestes forskaolii*
HAREC	Human and Animal Research and Ethics Committee
HCG	Human Chorionic Gonadotropin
HPLC	High-Performance Liquid Chromatography
HRIO	Health Records Information Office
HIV	Human Immunodeficiency Virus
Ho	Null Hypothesis
IAA	Insulin Autoantibody
IDF	International Diabetes Federation
IPBES	International Platform of Biodiversity Ecosystem Services
IU	International Units
IQR	Interquartile Range
Malf.	Malformation
MDS	Monogenic Diabetes Syndrome
MmHg	Millimeters of Mercury
Mg	Milligram
MF	*Mangifera foetida*
Mg/Kg	Milligrams per Kilogram
Ml /Mls	Milliliter/s
Mmol/l	Millimole per Litre
MODY	Maturity Onset Diabetes of the young
Na	Sodium
NACOSTI	National Commission for Science and Technology

OH	Hydroxide
PCT	Proximal Convoluted Tubule
PRN	When necessary
RBC	Red Blood Cells
RCT	Randomized Control Trial
SES	School of Environmental Studies
SO_4	Sulphate
SPSS	Statistical Package for Social Scientists
SH	Sulfurhydrl
SN	*Solanum nigrum*
Sq.Km.	Square Kilometers
Sq. mi	Square mile
STZ	Streptozotocin
TC	*Tinospora cordofolia*
TCF	Transcription Factor
TI	Teratogenic Index
TIn	*Tamarinda Indica*
TLC	Thin Layer Chromatography
SB	Sorghum Bicolor
SD	Standard Deviation
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for the Social Sciences
STATA	STAtistics and daTA
UOE	University of Eldoret
USA	United States of America
USD	United States Dollars
UV	Ultra violet
V	Version.
WHO	World Health Organization
ZC	*Zanthoxylum chalybeum*
ZO	*Zingiber officinale*

OPERATIONAL DEFINITION OF TERMS

Antidiabetic- ability to reduce high blood sugars.

Biodiversity- term given to the variety of life on Earth. The variety within and between all species of plants, animals, and micro-organisms and the ecosystems within which they live and interact (Swingland, 2001).

Biotechnology -the exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms for the production of antibiotics, hormones, etc.

Conservation-the careful utilization of a natural resources.

Diabetes Mellitus (DM) /Diabetes -A metabolic disorder of multiple causes manifested by chronic hyperglycaemia with disturbances of carbohydrates, fat, and protein breakdown resulting from defects in insulin secretion, insulin action or both (Chuhan et al., 2010; Ta, 2014).

Efficacy- The ability of an intervention or drug to produce the desired effect.
Embryotoxicity- adverse effects on the embryo (may be expressed as embryonic death).
Euglycemia/normal blood sugars – Sugar level between 3Mmol/l and 7Mmol/l (fasting blood sugars)](Dhatariya et al., 2012).

Herbal medicine- also may be referred to as Traditional herbal medicine or botanical medicine or phytomedicine. It is the use of plant's seeds, berries, roots, leaves, bark, or flowers for the management of diseases(Tulunay et al., 2015).

HI- Sugars higher than 33.3Mmol/l.
Histology- the microscopic study of selected animal tissues features - (brain, kidney, spleen, pancreas, liver and thyroid) with an aim of comparing and contrasting the normal with the abnormal structures in various disease states.

Hyperglycemia- a condition in which there is an excessive amount of blood sugars, circulating in the blood plasma. Generally a consistent two-hour postprandial random blood sugar level higher than 11.1 mmol/l (200 mg/dl or a consistent fasting blood sugars between ~5.6 and ~7 mmol/l (100–126 mg/dl) and above (Alberti et al., 2006).

Hypoglycemia - a condition in which there is low amount of blood sugars, circulating in the blood plasma (usually less than 3mmol/l).

Safety -the condition of being protected from or unlikely to cause danger, risk, or injury

Teratogenicity -congenital malformation/abnormalities.

Toxicity-The degree to which a substance (a toxin or poison) can harm humans or animals.

Postprandial- Blood glucose measurements taken two hours after eating a meal.

Pre-prandial- Blood glucose measurements taken two hours before a meal.

Xenopus - the African clawed frog, used in embryological research studies.

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CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

Diabetes Mellitus (DM) is a debilitating non-communicable disease that largely results from; either the production of insufficient quantity of insulin, unresponsiveness of body tissues to insulin, production of less potent insulin by the pancreas or inability of the body to mobilize sugar into the tissues, which leads to sustained hyperglycemia. In the absence of any interventions, hyperglycemia can result into cardiovascular damage, retinopathy, and nephropathy (Chuhan et al., 2010).

Currently, Diabetes is a major public health and economic problem and accounts for the largest proportion of non-communicable disease burden in the world. In the year 2019, an estimated 463 million (accounting for approx. 9.3% prevalence) people in the world, were diagnosed to have had Diabetes. (Saeedi et al., 2019) . In Africa, the accurate data of diabetic prevalence may not be available, but nevertheless in the year 2019, 19.4 million (approx. p of 3.9%) people, were estimated to have had diabetes (Saeedi et al., 2019), a figure that was estimated to increase to 47 million by the year 2045 (prevalence of 4.4%) (A-IDF 2019). Approximate number deaths recorded in the same year, (2019) were 366,200 and 79% of these deaths, occurred in ages 60 years and below—a proportion thought to be higher than in any other region in the world (A-IDF 2019). Kenya's prevalence of diabetes was approximately at 3.7 %, in the year 2018, a figure that was projected to 4.5% by the year 2025 (Mohamed et al., 2018)

The primary goal in the management of DM, is to regulate blood sugar levels within the acceptable limits, of 3 Mmol/l to7 Mmol/l (fasting) blood sugars, (Dhatariya et al., 2012) which will in turn help in relieving or prevent signs and symptoms, and associated health complications (Wiebe et al., 2016). This can be done through either; non-pharmacological (diet and physical exercises) or pharmacological approaches(conventional and non-conventional)(Dey et al., 2002).

Non-pharmacological approaches, denotes interventions that do not require the use of drugs in the management of Diabetes. One such approach is the nutritional intervention that involves the control of total calories and free carbohydrates. The major goal of this approach, is to moderately reduce the weight (≈7% of body weight). Low weight, can help improve blood glucose levels, an important component in the

prevention and treatment of diabetes (Chaudhury et al., 2017). Physical exercises can also control hyperglycemia, with or without a significant decrease in body weight. Such one physical exercise includes aerobics. Regular aerobics in patients diagnosed with Diabetes type II, increases skeletal muscle insulin sensitivity, hence sugar absorption by the tissues, thus promoting optimal glycemic control (Colberg et al., 2016).With reference to pharmacological approaches; there are two major ways, namely; conventional and non-conventional. Conventional way, is also referred to as western medicines or mainstream method, which refers to the formal way in which medical doctors and other healthcare professionals, treat signs and symptoms of diseases using drugs, radiation, or surgery. Pharmacologically (drugs), some of the antidiabetic conventional medical drugs include; insulin, sulphonylureus, thiazolidinedione's, biguanides, glinides and alpha-glucosidase inhibitors, insulin being the most commonly used drug (Alhadramy, 2016).

With regards to non-conventional drugs; these are the type of medications which are not formally prescribed by medical doctors or other healthcare professionals. More often than not, they are prescribed by herbalists and traditional medical healers. Ayurveda and herbal medications are some of the examples in this category. Ayurveda is a Hindu traditional medical system which is based on the concept of balance in bodily systems. It uses diet, herbal treatment, and yogic breathing exercises as the major form of treatment (Parasuraman et al., 2014).

Herbal medicines on the other hand refers, to the use of plant parts as medications which are believed to possess phytochemicals (polysaccharides, peptides, saponnins, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthones and flavonoids) - substances known to mediate healing processes (Septembre-Malaterre et al., 2018). In the management of diabetes for example, saponnins, have been found to activate adenosine monophosphate protein- kinase enzyme **(**AMPK), in calcium-independent channels, regulating gluconeogenesis and glucose uptake (El Barky et al., 2017) and flavonoids which are α-glucosidase inhibitors (Escandón-Rivera et al., 2012; Y. Q. Li et al., 2009), has also been found to prevent the digestion of carbohydrates and delay glucose absorption (Chen et al., 2015; Yoshida et al., 2008), thus lowering blood sugars. In general, as summarized by (Cefalu et al., 2011) , anti-diabetic herbal medications (phytochemicals) work by; either modulating adipocyte function and thus regulate endocrine secretions that play a role in enhancing the skeletal muscle insulin action, or regulate gluconeogenesis, or enhance the pancreatic β-cell function or

directly control the secretion of insulin in peripheral tissues e.g., in skeletal muscle and adipose tissue.

These phytochemicals are made available for consumption/use through a process known as extraction, -, the process of separating medicinally active plant portions from the inactive or inert components, using selective solvents. Extraction of herbal medication can be done using water (aqueous) or organic-based compounds such as methanol, ethanol, hexane etc. (Akter et al., 2010).

Most rural inhabitants in Kenyan communities, use water/aqueous solvents exclusively to extract herbal plant medication. This extraction, more often is not standardized, and seldomly do most herbalists and diabetics know what phytochemicals the substances they are prescribing, possess.

To establish herbal efficacy (anti-diabetic activity) and safety (toxicity), in controlling blood glucose, it is imperative to test them using laboratory animal models first before using them in human beings- a practice that has been a tradition in the development of pharmaceuticals. Such animals include; rats, mice and guinea pigs. Previously, such studies that used animals as study subjects for evaluating efficacy and safety of herbal medications, include but not limited to studies done in Canada ,USA and Africa on Fenugreek and American ginseng and Bidens pilosa (Neelakantan et al., 2014; Piero et al., 2012) respectively.

Nevertheless, the use of animals nowadays has received enough criticisms from 'animal rights' advocates, who see their use as unethical. If animals like rats have to be used (according to animal right advocates) , they must be used, on the framework of the three 'R's: -, which denotes; 'r'reducing the animal numbers to the least number as much as possible , 'r'efining experiments in such a way that ,at every step of the experiments, animal welfare is taken care of, as much as possible, and lastly 'r' eplacing the animals , if there is a suitable alternative (Schipani, 2015).

It is on this spirit, that, this study, used a less ethically demanding protocol, Frog Embryo Teratogenesis Assay (FETAX) to firstly estimate toxicity of the herbal extracts and secondly evaluate the safety of two commonly used herbs before extrapolating the findings to the rats-animal model (wistar male albino rats).

1.2 Statement of The Problem

There has been a great surge of public interest in the use of anti-diabetic plants (Smith-Hall et al., 2012). This use is based on the assumption that, the plants work and are safe. However, this may not be usually be the case.

Quite a number of Baringo County inhabitants, use herbs in management of their conditions, especially the chronic ones (like Diabetes, hypertension cancer etc.), though there is no verified scientific evidence on efficacy and safety of what they are using.

WHO (2011), recommended the evaluation of traditional plant treatments for Diabetes through a scientific validation exercises to prove their efficacy and safety (Wachtel-Galor & Benzie, 2011). A validation, which is often done using animals (mammals) like rats, guinea pigs etc. exclusively, often leading to unwarranted deaths of animals.

1.3 Justification

Because diabetes is a life-long disease and continuous medication is required, most people in rural areas in Africa lack resources or support systems to cover the high costs of drugs and therefore resort to looking for alternative cheaper ways to manage their ailments. One such way is the use of herbal medications, whose safety, and efficacy may not be known.

Our choice of study area was informed by a KDHS (2019) survey, that found out that the use of herbal medications in Baringo County is very common.

The use of rats and other rodents in toxicity and efficacy, which has been the tradition in a number of laboratories has attracted a fair share of condemnation from animal right advocates hence the need for a newer toxicity testing protocols that will hopefully complement the use of rats.

The findings of this study will not only help in generating scientific evidence on herbal medicines uses and safety, but also provide a better, faster and more ethically viable protocol of studying toxicity and safety of herbal medications.

1.4 Objectives

1.4.1 Broad Objective

To determine the major anti-diabetic herbs used in Baringo County Kenya, their biochemical profiles, and the anti-diabetic activities and safety of two commonly used herbs, using a less demanding protocol [FETAX 96hr] in relation to a more demanding protocol [Rats].

1.4.2 Specific Objectives

1). To investigate the major plants used in management of Diabetes in Baringo County, Kenya.

2). To characterize the biochemical properties of the most used anti-diabetic herbs in Baringo County, Kenya.

3). To establish anti-diabetogenic properties [efficacy] of both ethanolic and aqueous extracts of the two most commonly used antidiabetogenic herbs.

4). To determine the safety of the two most used anti-diabetic herbs by use of in vitro (FETAX studies) coupled with in vivo (rat-histology protocol) bioassays.

1.5 Study Research Questions

1) What are the major plants used in management of Diabetes Mellitus in Baringo County, Kenya?

2) What are the biochemical properties of the most applied anti-diabetic herbs used in Baringo County, Kenya?

3) What are the anti-diabetogenic properties/efficacy of both ethanolic and aqueous extracts of the two most commonly used anti-diabetic plants in Baringo County?

4) What could be the safety of two commonly used anti-diabetic herbal medications in, in vitro (Xenopus laevis) coupled with, in vivo (rat) [histology]) studies?

CHAPTER TWO
LITERATURE REVIEW

2.1 Diabetes Mellitus Introduction

Diabetes mellitus is a heterogeneous syndrome characterized by abnormalities in carbohydrate and fat metabolism caused by many factors which include genetic and environmental elements, that affect beta-cell function in the pancreas and insulin sensitivity in the muscles, liver and adipose tissues (Dunning, 2017; Kharroubi & Darwish, 2015).

2.1.1 Types of Diabetes Mellitus

There are three main types of Diabetes mellitus; Type I, Type II and Gestational Diabetes. Others are monogenic Diabetes syndromes, though not very common compared to the other 3 types aforementioned (Association, 2018).

2.1.2 Pathophysiology

2.1.2.1 Type I DM

This is an autoimmune disease in which the β-cells of the pancreas do not produce sufficient insulin as result of destruction; by combination of genetic (immune mediated) environmental or idiopathic factors. Genetic factors include antibodies ICA512/IA-2, insulin autoantibody (IAA) and glutamic acid decarboxylase (GAD). Environmental factors include enteroviruses, dietary factors and toxins.

2.1.2.2 Type II DM

Type II Diabetes mellitus occurs as a result of complex endocrine and metabolic dysfunctions which occurs due to the interaction between several genetic and environmental factors. The end result is a heterogeneous and progressive disorders with variable degrees of insulin resistance peripherally (liver and muscles) and pancreatic β-cell dysfunction. The environmental factors include, obesity, stress and toxicants (Skyler et al., 2017).

Genetically , over 500,000 single nucleotide polymorphisms (SNPs) is believed to be likely responsible for the occurrence of type II Diabetes; such nucleotides include calpain-like protease (CAPN10) and transcription factor 7-like 2 (TCF7L2)(Anderson, 2016). Type II Diabetes happens to be the major type of diabetes affecting more than 90% of diabetic populations.

2.1.2.3 Gestational Diabetes (GDM)

It occurs during pregnancy as a result of impaired glucose tolerance due to pancreatic β-cell dysfunction because of chronic insulin resistance. Some of the risk factors associated with GDM include obesity, advanced maternal age, a family history or any other type of diabetes. The major contributors to this type of diabetes, are the placental hormones namely, human placental lactogen, progesterone, cortisol, growth hormone and prolactin. These hormones cause decreased phosphorylation of insulin receptor substrate and thus causing the insulin resistance (Plows et al., 2018).

2.1.3 Signs and Symptoms of Diabetes Mellitus (DM)

The signs and symptoms of Diabetes mellitus can be classified, as either acute or chronic; acute symptoms occur mostly due to severe hyperglycemia. These symptoms include polyuria, polydipsia and polyphagia. Other signs, though not specific to this disease but under this subdivision include; weight loss, fatigue, itchy skin, slow healing of wounds and blurred vision.

Chronic signs and symptoms of Diabetes mellitus occur due to persistent high levels of sugar in the blood, thereby causing vascular damage, which in turn leads damage of body organs (Association, 2018).

2.2 Management of Diabetes Mellitus

2.2.1 Pharmacological

2.2.1.1 Conventional Drugs

This can also be referred to as western medicines or mainstream medications. Conventional medicines are the formal way in which medical doctors and other healthcare professionals, treat signs and symptoms diseases/health related states, using drugs, radiation, or surgery.

Pharmacologically , some of the antidiabetic conventional medical agents (drugs) include; insulin, Sulphonylureus, thiazolidinedione's, biguanides , glinides and alpha-glucosidase inhibitors. Insulin, being the most common drug compared to the rest aforementioned (Adunola et al., 2015; Alhadramy, 2016; DeFronzo et al., 2016; Jahangir et al., 2017).

Insulin is in a class of medications called hormones, that works just like the normal body insulin i.e. by helping move sugar from the blood into other body tissues and

stopping the liver from producing more sugar when sugar is excess in the body (American Society 2016.). Historically, insulin was generated exclusively from animal pancreases i.e. from pigs, cows and fish, but currently it is can also obtained through exploitation of biotechnology i.e. through the manipulation of E-coli bacteria in the environment (Quianzon & Cheikh, 2012). Side effects of insulin include hypoglycemia and weight gain.

Sulphonylureus – these are drugs that help one's body secrete more insulin. Some of the examples under this class' include glyburide (DiaBeta, Glynase), and glipizide. The possible side effects include low blood sugar and weight gain (Sola et al., 2015).

Thiazolidinediones - works by lowering body insulin resistance. It does this by reducing insulin levels acutely, and thus improve insulin sensitivity and/or reduced circulating fatty acids, which in the long run, arrest the decline in β- cell function- by protecting it from lipotoxicity. On their own, they tend to have a very slow onset of action. Their effects, begins to be seen in two weeks with the maximal benefit of treatment seen after three months of treatment (Brunmair et al., 2004; Kibiti & Afolayan, 2015)

Biguanides: The most common example is metformin. Works by decreasing intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. These drugs have been linked to such serious side effects like lactic acidosis. Other common side effects include diarrhea, cramps, nausea, vomiting, and increased flatulence (DeFronzo et al., 2016)

Glinides binds on sites of beta cells and stimulate them to release insulin. Its major side effects are weight gain and upper respiratory tract infections(Alhadramy, 2016).

Alpha-glucosidase inhibitors. As the name suggests, they work by inhibiting the alpha-glucosidases at the intestinal brush border and thus reducing carbohydrate absorption and postprandial blood glucose. Major side effects include but not limited to abdominal pain, bloating, flatulence, and diarrhea (Alhadramy, 2016).

2.2.1.2 Non-Conventional Drugs

2.2.1.2.1 Introduction

Non-conventional ways refer to the use of medical products and practices that are not part of standard care. They are the opposite of conventional medicine. One of the major ways in this category, is the use of unprocessed plant parts as medicines/ drugs. Commonly referred to as herbal medicines.

Herbal medicines refer to the use of plant parts as medications, commonly prescribed by traditional medicines and other non-conventional medical health care prescribers

2.2.1.2.2 Diabetic Herbal Medicines

From the ethno-botanical information, about 1200 hypoglycemic plants have been found so far, though less than a quarter of them have been fully studied to confirm their safety and hypoglycemic activities. Most of the studied plants have been reported in Asia , in countries like India and China, some parts of United States of America, United Kingdom and to a lesser extent in Africa (Mitra et al., 2016). Such plants include Ginseng species, Gymnema sylvestre, Trigonella foenum graecum, Ficus carica, Opuntia streptacantha, Momordica charantia, Salacia oblonga and Aloe vera.

American ginseng is an herb which its' roots are used to make medicine. This herb contains triterpenoid, saponnins and glycosides (ginsenosides or panaxosides) that affect insulin levels in the body and thus lower blood sugar. Its hypoglycemic effects have been shown in streptozotocin diabetic induced rat models (Yeh et al., 2003).

Gymnema sylvestre is a woody climber that grows in tropical forests of central and southern India. Preliminary human evidence suggests that Gymnema may be effective in the management of blood sugar levels in Type I and Type II Diabetes, as an adjunct to conventional drug therapy. It appears to lower serum glucose and Glycosylated Hemoglobin (HbA1c) levels following chronic use, but may not have significant acute effects (Ghorbani, 2013) .

Trigonella foenum graecum is a legume extensively cultivated in India, North Africa, and the Mediterranean. Its' seeds, which are rich in fiber, saponnins, and protein is believed to have hypoglycemic effects. It is claimed that it works by delaying gastric emptying, slowing carbohydrate absorption and inhibition of glucose transport from

the fiber content, as well as increased erythrocyte insulin receptors and modulation of peripheral glucose utilization (Neelakantan et al., 2014).

Ficus carica (fig leaf) is a popular plant used for patients with Diabetes in Spain and other areas in Southwestern Europe, though its active component remains largely unknown. Several studies in animal models with Diabetes have shown both short and long-term hypoglycemic effects, though human trials are lacking (Perez et al., 1996).

Opuntia streptacantha (nopal) which is also called the prickly pear cactus is found in arid regions throughout the western hemisphere, including the southwestern U.S. It is commonly used for glucose control by those of Mexican descendants. It has a high soluble fiber and pectin content, which may affect intestinal glucose uptake, partially accounting for its hypoglycemic actions (Shapiro & Gong, 2002).

Momordica charantia is a vegetable, indigenous to tropical areas, including India, Asia, South America, and Africa, also known as balsam pear, karela (karolla), or bitter melon. Reported preparations of the herb range from injectable extracts, to fruit juice to fried melon bits. Studies in alloxan-induced diabetic rabbits have suggested hypoglycemic effects (Patel et al., 2012; Yeh et al., 2003).

Aloe vera is the most well-known species of aloe, a desert plant resembling the cactus in the Liliaceae family. The dried sap and the gel are a traditional remedy for Diabetes in the Arabian Peninsula. A study done by Rajasekaran et al 2005, on streptozotocin diabetic induced rats, orally given Aloe vera gel extract at a concentration of 300 mg/kg showed a significant decrease on the levels of blood glucose. It is believed to work by preventing excessive formation of free radicals through various biochemical pathways (Rajasekaran et al., 2005).

Salacia oblonga is a strangling shrub native to India and Sri Lanka. Its aqueous and ethanolic extracts lowers acute glycemia in persons with Type II Diabetes after a high-carbohydrate meal. This plant appears to mimic the mechanisms of action of alpha-glucosidase inhibitors such as precose (acarbose) (Deepak et al., 2015; Jeykodi et al., 2016).

Some of the anti-diabetics found in Africa include but not limited to; Picralima nitida, Vernonia amygdalina, Hypoxis hemerocallidea Fisch (Hypoxidaceae), Catharanthus roseus, Bidens pilosa, Strychnos henningsii, Aspillia pluriseta, Catha edulis, and Erythrina abyssinica,

Picralima nitida (Apocynaceae family) - is an herbal medication found in most parts of South Africa. A study done by (Erharuyi et al., 2014; Teugwa et al., 2013), revealed that, this herb has an antihyperglycemic activities.

Vernonia amygdalina commonly known as bitter leaf, is a small tree growing up to 3 m high. It occurs wild in most countries of tropical Africa. Anecdotal reports and claims, support the antidiabetic activity of V. amygdalina (Owen et al., 2011).

With regards to Hypoxis hemerocallidea Fisch (Hypoxidaceae),very little scientific reports are available on the anecdotal evidence that claim that this herb is an anti-diabetic , though in a study done on evaluation of the antidiabetic activity of aqueous extracts of this plant on streptozotocin (STZ) induced diabetic rats (4), revealed a significant blood glucose concentration reduction (Erharuyi et al., 2014).

Catharanthus roseus is believed to have originated from Madagascar. Research findings have shown that while the aqueous leaf extract of the herb could lower blood glucose by about 20% in diabetic rats, dichloromethane and methanol extracts lowered blood glucose by 49%–58%. The hypoglycemic effects appeared to be the result of increased glucose utilization in the liver. No acute adverse effects were observed in the animals treated with the extract of this plant (Afolayan & Sunmonu, 2010).

Bidens pilosa, Strychnos henningsii, Aspillia pluriseta, Catha edulis, Erythrina abyssinica in a study done by (Piero et al., 2012) showed hypoglycemic activity in alloxan monohydrate diabetic induced rats. Mild signs of toxicity were noted in some of these plants/herbs.

In a study done by (Karau et al., 2012) on stem bark extracts of Pappea capensis, it was found that , this herb possess hypoglycemic activity, mimicking those of glibenclamide at 3 mg/kg body weight. No immediate harmful effects were seen in this study, though he did not rule out any in the long term.

2.2.1.2.4 Selected Previous Studies on Anti-Diabetic Plants/Herbs

Table 2.1, Below, describes how four randomly selected herbal studies from previous researches -, were analyzed, indicating some of the gaps (highlighted in yellow) missed during their analysis. Hence the need for more studies.

Table 2. 1; Selected diabetic herbal medicines; part extracted, animal used for test and solvent used for extraction.

Herb Scientific name	Part of the herb used	Animal used/ and chemical used for induction	Duration of test	Phytochemical in the plant	Efficacy	Safety	Extraction solvent used	Citation
1.Acacia arabica	bark	Rats' species not specified STZ	21 days Given once orally	Phenols, tannins Flavonoids	Sugars controlled	Not done	Chloroform	(Kooti et al., 2016)
2.Aegele mermelos	Leaves	Rats - species not specified STZ	35	Not indicated	Sugars Controlled	-	Aqueous	(Tiwari et al., 2018)
3.Gmelina arborea	Bark	Wistar albino rats Alloxan	Not indicated	Not indicated	Controlled the sugar	done	Aqueous	(Attanayake et al., 2013)
4.Languas galanga	Roots	Wistar albino rats Alloxan	Not indicated	Not indicated	Controlled the sugar	done	Aqueous	(Attanayake et al., 2013)

2.2.2 Non -Pharmacological Approaches

Non pharmacological approaches, means the use of no drugs in management of diabetes. One such approach, is through the consumption of healthy diet i.e., the control of total calories and free carbohydrates and consumption of complex dietary fiber and whole grains. The Major goal of this approach is to moderately reduce the weight (≈7% of body weight). Low weight, can help improve blood glucose levels, an important component in the prevention and treatment of diabetes (Chaudhury et al., 2017).

Physical exercises are also another non pharmacological way that can control hyperglycemia, with or without significant decrease in body weight. Such one physical exercise includes aerobics. Regular aerobics in patients diagnosed with diabetes type II, increases skeletal muscular insulin sensitivity, hence absorption and thus promoting glycemic control (Colberg et al., 2016).

2.3 Herbal Medicines Phytochemicals

Phytochemicals are bioactive substances found in plants. They play an important role in plant growth or defense against competitors and pathogens (Septembre-Malaterre *et al.*, 2018). These phytochemicals have been used in several parts of the world and especially by traditional/native medicine men in management of various ailments. Most of the current conventional medicines have their origin from phytochemicals.

Some of the phytochemicals found in plants, believed to be medicinal include polysaccharides, peptides, saponnins, alkaloids, glycopeptides, triterpenoids, amino acids, steroids, xanthones, flavonoids, lipids, phenolics, coumarins, iridoids, alkyl disulphides, inorganic ions and guanidine's. These compounds mediate their effects on the human body through processes identical to those already well-understood in conventional drugs (Tapsell *et al.*, 2006).

2.3.1 Some of the Phytochemicals that have Been Reported as Having Antidiabetic Activities

Table 2.2, below shows some of the phytochemicals and phytoconstituents of some plants, from previous studies.

Table 2. 2; Selected herbs/plants phytochemicals and its phytoconstituents of plants that have reported as antidiabetic propensities Adapted and modified from (B Gaikwad et al., 2014)

a. Alkaloids phytoconstituents		
Phytoconstituent	Herb /plant	Plant part
Berberine	Bereris spp. *Tinospora cordifolia*	Roots, stem-bark
Crytolepine	*Crytopelis sanguinolenta*	-
Jombosine	*Syzgium cumini*	Seeds, fruits, bark
b. Glycosides phytoconstituents		
Phytoconstituent	*Herb /plant*	*Plant part*
Kalopanax	*Kalopanax pictus*	Stem bark
Neomyrtillin	*Vaccinium myrillus leaves*	
Jamboline	*Syzgium cumini*	Seeds
c. Flavonoids phytoconstituents		
Phytoconstituent	*Herb /plant*	*Plant part*
Bengalenoside	*Ficus benghalensis*	Stem bark
Genistein	*Glycine spp.*	Soya beans
Prunin	*Amygdalus davidiana*	Stems
d. Terpenoids and steroids phytoconstituents		
Phytoconstituent	*Herb /plant*	*Plant part*
Basic acid	*Bumelia sartorium*	Leaves
Gymenic acid IV	*Gymnema sylvestre*	Leaves

These phytochemicals exert a wide range of antidiabetic activities through its different phytoconstituents. Mechanisms on how they work, varies from one phytoconstituent to another and also from one plant to another.

Mechanisms of some of the selected phytochemicals are described below:

Saponins are known to activate adenosine monophosphate protein- kinase enzyme **(**AMPK), in calcium-independent channels, regulating gluconeogenesis and glucose uptake. They also reduce blood sugars by increasing; insulin uptake by the body tissues, plasma insulin levels and induction of insulin release from the pancreas (El Barky *et al.*, 2017).

Flavonoids are α-glucosidase inhibitors (Escandón-Rivera *et al.*, 2012; Y. Q. Li *et al.*, 2009), that prevent the digestion of carbohydrates and delay glucose absorption (Chen *et al.*, 2015; Yoshida *et al.*, 2008). They are also involved in protecting normal cell structure and function by maintaining redox homeostasis, quenching free radicals that are believed to play a major role in the pathogenesis of diabetes mellitus (Al-Numair *et al.*, 2015; Lukačínová *et al.*, 2008).

Phenols and tannins, have been found to prevent oxidative damage of the cells and also control post-prandial hyperglycemia by inhibiting the digestive enzymes for example α-glucosidases (Ali Asgar, 2013; Goncalves & Romano, 2017).

Catechins are effective in controlling blood sugars by improving insulin sensitivity and reducing the risk factors for Type II Diabetes Mellitus, like oxidative stress, dyslipidemia, and obesity (Alipour *et al.*, 2018; Asbaghi *et al.*, 2019)

Coumarins phytochemicals protect pancreatic beta cells from damage, improve abnormal insulin signaling, reduce oxidative stress/inflammation, activate AMP-activated protein kinase (AMPK) and inhibit a-glucosidases hence diabetes management (H. Li *et al.*, 2017).

Plant sterols help in dietary management strategy for hypercholesterolemia in persons with type 2 diabetes(Lau *et al.*, 2005).

2.4 Extraction of Herbal Plant Materials

2.4.1 Introduction

Plant extraction is a process of partitioning therapeutically dynamic segments of plant utilizing suitably selective solvent. The solid object (the plant) is placed in contact with a (fluid) solvent which may be a liquid or a gas (water vapour or supercritical fluids). The plant components (dissolvable plant metabolites) of interest are then

solubilized and contained within the solvent. The solution (solvent mixture) thus obtained is the desired extract (Okoduwa *et al.*, 2016).

2.4.2 Principles Behind Herbal Plant Extraction

In thermodynamics, the solid object is believed to be homogeneous mixture in equilibrium. In absence of external disturbances, it will undergo no modification but when there is a disturbance, an exchange of thermic and mechanical energy with the surroundings (solvent) ensues, hence the mass transfer of solid contents to the liquid-a process usually occurring during extraction.

2.4.3 Common Methods for Herbal Plant Extraction

There are several methods of extraction which include;

2.4.3.1 Percolation- refers to the movement and filtering of fluids through porous materials.

2.4.3.2 Maceration- plant material is placed in ground form or powder in a container full of solvent and left to stand for two to three (72hrs) or more days. They can be placed in shaker to frequently shake until complete extraction of plant material. This is performed at room temperature and liquids that are most frequently used are water (aqueous) and alcohol- (Methanol, ethanol, chloroform, petroleum ether, hexane and ethyl acetate).

The choice of which alcoholic solvent to be used depends on polarity and which compound type you would want to extract. For instance, in the above given examples, in terms of decreasing polarity, methanol is more polar than ethanol then ethyl acetate then chloroform, then petroleum ether and last, hexane.

When water is used, they should not be put to stay for too long as this can present fungal contamination-unless extraction is done in an air tight container.

2.4.3.3 Digestion- is a form of maceration with slight warming during the extraction process, provided that the temperature does not alter the active ingredients of plant material and so there is greater efficiency in the use of menstruum (Azwanida, 2015).

2.4.3.4 Infusion- process of extracting chemical compounds or flavors from plant material in a solvent such as water, oil or alcohol, by allowing the material to remain suspended in the solvent over time (a process often called steeping). An infusion is also the name for the resultant liquid (Azwanida, 2015).

2.4.3.5 Decoction- method of extraction which involves boiling herbal or plant material to dissolve the chemicals in it (WHO, 2018).

2.5 Factors Influencing the Quality of Extract/Efficacy and Safety

1. Cultivation practices/geographical origin – This means where and what have been used to grow the plants. Certain herbal plants are known to absorb the chemical compounds of the soil they are growing in, thus affecting the quality or type of phytochemical inherent in it and hence the eventual efficacy and safety of the final extracted product (Andreas Tsipourlianos, 2018).

2. Preparation of the plant to be extracted; fresh or dry, whole herb/ part of the plant or ground, temperature used (Some plant constituents are heat labile and the plants containing them need to be dried at low temperatures.-, method of drying; Air-drying, microwave-drying, oven-drying and lyophilization of plants samples), storage (some active principles can be destroyed by enzymatic processes that continue for long periods of time after extract has been reconstituted) etc. (Azwanida, 2015).

3. Quality and type of the solvent used– the yield and quality, dependent on the nature of extracting solvent due to the presence of different antioxidant compounds of different chemical characteristics and polarities that may or may not be soluble in a particular solvent (Sukri, 2012). Also depending on the concentration of the solvent, and the quality (presence of contaminants), certain phytochemicals might or might not be extracted.

4. Choice of method of extraction- The content of some ingredients in herbs may increase, and others may decrease or even disappear depending on the choice of extraction method. This is because some solvents might slightly Change the chemical profile of some herbs depending on the amount of heat, storage and eventually influencing the efficacy of any particular herb.

2.6 Efficacy in Relation to Solvent Used to Extract an Herbal Product

A study done by (Adunola *et al.*, 2015) on the resultant efficacy of various extraction methods using different solvents showed that *C. lanatus* extracted from cold methanol had higher efficacy compared to hot chloroform and cold water extract. Cold chloroform extracts had no efficacy at all.

In another, study done on two medicinal plants (*Lawsonia inermis L*. and *Mimosa pudica L*) with regards to ethanol, petroleum ether, chloroform, methanol and aqueous extracts solvents on efficacy on antibacterial activity by (Akter *et al.*, 2010), ethanol solvent showed the highest antibacterial activity (efficacy) compared to the other solvents in the study. While in another study done with the same solvents (ethanol, petroleum ether, chloroform, and methanol), to determine the antitrypanosomally activity of *Artemisia macivarae linn*, no any statistical significance on the resultant efficacy of these solvents extracts was noted (Ene *et al.*, 2009).

2.7 Purification/Refinement of Herbal Extracts

Purification or refinement of an herbal compound is the act of concentrating the active compounds together. This can be achieved through various chromatographic techniques. Such techniques include; Thin layer chromatography (TLC), which is a quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture (Sasidharan *et al.*, 2011) and column chromatography (Ingle *et al.*, 2017).

2.8 Use of Rats for Laboratory Tests

The use of lab rats as animal model for human diseases studies, is highly recommended over many other animals' models. This is because the anatomy and physiology of rats resemble that of a human being. It is also because of its size, which allows for experimental manipulation e.g., serial blood draws and ease for surgical operations when need arises. If used as models for diabetes, they behave more or less like the diabetic human beings. Over a million publications in recent PubMed studies, have revealed that use of the rat has been done for over 100 years in various human disease with varied degree of success (Iannaccone & Jacob, 2009).

2.9 Drugs Used for Induction of Diabetes in Rats

Diabetogenic state in lab rats can be induced in many ways, but the most common method, is the use of chemicals; alloxan and streptozotocin (Radenković *et al.*, 2016).

2.9.1 Alloxan

It is one of the most commonly used drugs for induction of diabetogenic state in lab rats. Chemically alloxan is known as, alloxan (2, 4, 5, 6 -tetraoxypyrimidine; 2,4,5,6

pyrimidinetetrone). It is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution (Rohilla & Ali, 2012). It is a urea derivative which selectively necrotises the β- cells of pancreatic islets. It does this by oxidizing the essential sulfhydryl (-SH) groups, inhibiting glucokinase enzyme, generating free radicals and disturbing the intracellular calcium homeostasis. The severity of the diabetic state caused, vary depending on the dose of alloxan used and the animal model (Tripathi & Verma, 2014), but nevertheless the commonly used dosage for induction of diabetes in Wistar albino rats is usually between 140-150 mg/kg singly administered (Daradka *et al.*, 2014; Muralidharan, 2014) .

2.9.2 Streptozotocin

Streptozotocin is a chemical which is used to induce diabetes type1 in animal models and type II diabetes with multiple low doses. It's a monofunctional nitrosourea derivative, which works by preventing DNA (Deoxyribonucleic acid) synthesis in mammalian and bacterial cells. It reacts with cytosine groups, resulting in degeneration and destruction of DNA. It induces diabetogenic state by entering the pancreatic cell via a glucose transporter-GLUT2 (Glucose transporter 2), causing alkylation of DNA which induces activation of poly adenosine diphosphate ribosylation and nitric oxide release, resulting in pancreatic -cells destruction by necrosis (Tripathi & Verma, 2014).

NOTE: In the current study, alloxan monohydrate was used, because;

1. The diabetic effect that alloxan causes, usually lasts longer compared streptozotocin.
2. Alloxan is cheaper and easily available in the market, compared to streptozotocin.

2.10 Safety/Toxicity of Herbal Medicines

2.10.1 Introduction

An increase in the use of herbal medications in the world today has also resulted into an increasing concern about their safety/toxicity. These concerns may be either general or herb-specific, hence the need to study them. Test for toxicity, is very important for the development of new drugs and for the extension of the therapeutic potential of existing drugs. This toxicity of medicinal plants may be related to the mixtures of active compounds that they contain; their interactions with other herbs and drugs, contaminants, adulterants; or their inherent toxicity (Rodriguez-Fragoso *et al.*, 2008).

Toxicity which means the degree to which a substance (a toxin or poison) can harm humans or animals, can be classified into;

(a) Acute toxicity - harmful effects of a particular herb in an organism (given orally or Intradermal) through a specified single or multiple doses seen in a period not exceeding 14 days as described by (Bello *et al.*, 2016)

(b) Sub-acute/sub chronic toxicity - effects of a particular herb in an organism (given orally or intradermal) seen through a specified multiple or a single dose in a period not Exceeding 28 Days As Described By (Bello *Et Al.*, 2016)

(C) Chronic Toxicity - The Ability Of A Substance Or Mixture Of Substances To Cause Harmful Effects Over An Extended Period, Usually Upon Repeated Or Continuous exposure, sometimes lasting for the entire life of the exposed organism(Sathya *et al.*, 2012).

Worldwide, hepatotoxicity form of tissue toxicity, is the most reported type of toxicity in animals exposed to herbal medications. Other forms include kidney, brain, blood, heart dermatological effects and death (Saad *et al.*, 2006).

2.10.2 Tests of toxicity

Tests of toxicity can be determined, through animals -mammals (rats, guinea pigs, mice, rabbits, goats etc.-in vivo or through amphibians-xenopus laevis embryos -in vitro analysis, among many others.

2.10.2.1 Mammals

In mammals, you can check for the manifestations of toxicity through physical parameters (temperature, skin lesions, color, etc.), hematologic and biochemistry parameters, tissue changes, death etc.

(a) Hematology and biochemistry

Hematology refers to the study of blood and blood disorders, i.e., the number and morphology of the cellular elements; the red blood cells (erythrocytes), white blood cells (leucocytes) and the platelets (thrombocytes) while biochemistry is the study of protein and fat metabolism, carbohydrate metabolism, enzyme production, compound utilization etc. in order to assess the functioning of the major body organs such as the liver, kidneys and heart.

The aim of studying them, is to use the results in the diagnosis and monitoring of diseases which could be as a result of ingestion/inhaling/injection of toxic substances, (Jorum *et al.*, 2016).

Previous such studies on biochemistry and hematological toxicology, include a studies done by, (Davids *et al.*, 2016; Farsani *et al.*, 2016; Shyamal *et al.*, 2010; Sreshta & Babu, 2018) which used Wistar albino rats on examining blood and /or biochemistry parameters after orally/peritoneal injection of herbal products, for a period of between 14 to 90 days.

(b) Histology

Histology is the microscopic study of tissue features of animals or plants with an aim of comparing and contrasting the normal with the abnormal structures in various disease states. [But before the animals' tissues are examined, the animals must be killed in a humane way. One such procedure involves the use of volatile inhalational chemicals such as chloroform. This involves putting the rats into an appropriate receptacle with gauze or cotton wool soaked in the drug-chloroform for the rats to inhale. The animals usually dies within a period of between 10 to 15 minutes, after which, the desirable tissues are harvested (Close et al., 1997)-a procedure adopted in this study].

This is usually done by visualizing the tissue sample by either the use of light or electronic microscope after processing, embedding, sectioning, staining and mounting the specimen on a microscope slide (Anand & Pushpa, 2016; Kumar *et al.*, 2006).

1. Processing – It involves the immersion of the tissue/organ into a fixative solution with an aim of stopping the cells from breaking down and also prevent microorganism growth. Then later physically stabilizing by lyophilizing, use of microwave or by use of chemicals.

2. Embedding -This is firmly and deeply fixing the tissue in a surrounding mass. The tissue is placed in a mold and paraffin is poured over it and then solidified.

3. Sectioning- This refers to the cutting of the tissue into thin slices and mounting them in glass slides using a microtome (Usually the slices are cut into a thickness of

between 3-4µm). The thinly cut slices are then floated out into a water bath heated into about 10^0c melting point of paraffin. They are later placed in a 25x75x1mm glass microscope slide.

4. Staining – This is the use of dyes that binds and have affinity for certain components of the cell and extracellular. The major aim of doing this is to highlight structures in biological tissues for viewing, often with the aid of different microscopes (McCann *et al.*, 2014).

2.10.2.2. Frog Embryos

Frog Embryo Teratogenesis Assay- Xenopus (FETAX)

The frog embryo Teratogenesis assay Xenopus (FETAX) test is a developmental toxicity screening test. It is done using fertilized *Xenopus laevis* mid-blastula-stage eggs over the organogenesis period.

These eggs are usually opaque and can develop into transparent tadpoles within a couple of days, making it easy to study embryonic development. Also, since the xenopus embryos develop outside of the body, manipulation can be done easily, for example, in the treatment with proteins and chemicals that interfere with their development.

A Compound toxicity potential is determined after analysis of the mortality, growth and malformation observations on leaves after 96-hour period (Bantle *et al.*, 1989; Mouche *et al.*, 2017).

After a lot of validation, using known human teratogens, this test, has been found to predict potential mammalian teratogens by over 85%, making an important test in potential human toxicants (Bantle *et al.*, 1989; Mouche *et al.*, 2017) . Such validation studies, include studies done by (Fort, Stover, Farmer*, et al.*, 2000) on 'Assessing the predictive validity of frog embryo teratogenesis assay—*Xenopus* (FETAX) and that done by (Bantle *et al.*, 1990) on **'Evaluation of the Developmental Toxicity of Five Known Mammalian Teratogens and Non-Teratogens'**.

Though, this test has been there for quite some time, it has been seldom used in herbal medicine toxicity tests, which this study attempted to do.

2.11 Factors Influencing the Choices of Antidiabetic Herbal Medicines Use

WHO estimates that 80% of the world populations currently use herbal drugs for major health issues (Maiti *et al.*, 2017) , even in places where modern medication is accessible. This could be due to the fact that herbal products as perceived by the general population, as well tolerated , cheap, supplied in large quantities , effective and free of major adverse effects (Nasri, 2016). It could also be because of difficulties in accessing hospitals, inadequacy diabetic drugs in conventional government facilities and the easiness in obtaining these medicines (Rutebemberwa *et al.*, 2013).

In Kenya, and especially Baringo County, approximately over 80% of its residents use herbal medication in one way or another in management of their diseases, though the type, efficacy and the reasons of their use remains at large (KNBS, 2013).

2.12 Knowledge Gap Summary

Based on the above discussion, there is still no enough literature with regards to the types, safety and biochemical properties of most of the diabetic herbal medications used in the management of diabetes.

Secondly, the exclusive use of animal's models (rats) in efficacy and safety research studies, though highly recommended, requires a rigorous ethical approval and most times resulting in excess loss of animals used- [*commonly used being, mice and rats*], hence the need for a save, cheaper, quicker and less ethically demanding protocols.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

The field work study was done in Baringo County, and laboratory experiments were done in UOE, SES biotechnology laboratories center, and Moi University School of Medicine - Anatomy laboratories.

3.1.1 Description of the Study Site;

3.1.1.1 Location

Field Work

Field work activities were done in Baringo county, one of the 47 counties in Kenya. It covers an area of 11,075.3 km^2 and located at a latitude of 00°13" south and 1°40" north and longitudes of 35°6" and 36°30" east in mid-western Kenya. The administrative and economic headquarters of this County is Kabarnet town. The county has five constituencies, namely; Baringo Central, Baringo East, Baringo North, Eldama-Ravine, and Mogotio **(see Appendix iii).**

3.1.1.2 Socio Economic Status

The Baringo county census of 2019 showed an estimated population of about 666,763 people. Fifty-eight (58%) percent of this population lived below the poverty line. Small business enterprises and subsistence farming, including pastoralism and apiculture, were the most common forms of economic activities practiced in Baringo County (KNBS, 2019).

Its main Referral hospital, Kabarnet County Referral Hospital serves patients from lower category county health facilities like; Kabartonjo, Eldama-Ravine, Mogotio, and Marigat sub-district hospitals. It also offers curative, preventive, and specialized clinical services. One such specialized care service, is the management of diabetic patients.

Herbal remedies use is common in this county, with most herbalists operating from Kabarnet, Marigat, Barweza, Kabartonjo, and Eldama-Ravine towns. Usually, they do their business on either a Wednesday or a Saturday - during market days, though there are those who have clinics that operate throughout the week. Patients using herbs get their herbs from either their home surroundings or from designated herbalists.

It is in this phase where the herbalists and diabetic patients using antidiabetic herbs were interviewed. All the significant herbs were taxonomically identified at the department of Botany-UOE. Two commonly used herbs, were identified and further tested in the subsequent laboratory experiments – where their phytochemicals were identified and two commonly used herbs analyzed for efficacy and safety.

3.2 Study Design

In the field work, a cross-sectional descriptive survey was adopted. The study was further divided into two parts. Namely part 1 and 2

In Part 1; a Researcher administered questionnaire was administered on people with diabetes- using herbal anti-diabetic medications **(See questionnaire appendix 1 part I)** and in **Part 2;** a one-on-one interview were conducted on herbalists who have been prescribing anti-diabetic herbal medications to patients in Baringo County. **[See questionnaire appendix 1 part 2]**

Laboratory work; Was an Experimental study design. The primary objectives of the phase were;

(i) Herbal extraction and phytochemical qualitative screening.

(ii) To evaluate the toxicity of the two frequently used anti-diabetic herbs firstly using an in vitro (FETAX assay) study and secondly to determine the safe dosages to be used in, in vivo studies (rats).

To use the safe determined dosages in the evaluation of efficacy and toxicity of the two identified herbs in rats (in vivo) studies.

3.3 Study Populations

Field Work

3.3.1 Human Populations

Diabetic patients in Baringo County using antidiabetic herbal medicines.

(i) Herbalist prescribing anti-diabetic herbal medications in Baringo County.

Laboratory work

3.3.2 Animals

(i) *Xenopus laevis* fertilized mid-blastula-stage eggs

(ii) Rats; a total of 80 male Wistar albino rats

3.4 Inclusion and Exclusion Criteria

3.4.1 Subjects

Field work/survey: Human subjects

(i) Diabetics

Inclusion criteria

Diabetic patients

Diabetic patients who were 18 years and above, psychologically sound, using herbal antidiabetics and must have been, residents of Baringo County during the interview. Must also, have been seeking treatment in Baringo County for the previous six months before the day of commencing the study.

Exclusion criteria

Those unwilling to participate because of one reason or another.

(ii) Herbalists

Inclusion criteria

Herbalists managing diabetes and obtaining their herbs from Baringo County. They must have practiced as herbalists, in Baringo County for at least six months before the day of commencement of the study.

Exclusion criteria

Herbalists who were not able to communicate during the time of the interview and also those who were unwilling to divulge their knowledge of antidiabetic herbal medications, during the field work were not included.

3.4.2 Laboratory work: Animal Subjects

(a) Frogs

Inclusion criteria

Fertilized *Xenopus laevis* mid-blastula-stage eggs over the organogenesis period.

(b) Rats

Inclusion criteria

Health male Wistar albino rats of between 2-6months (8weeks-24weeks) months old.

3.5 Data Collection Instruments

Field work/survey: Interviewer administered questionnaire and one on one interviews (***see appendix I)***

Laboratory experiments; Tests and experiments.

3.6 Study Protocol, Materials and Methods

3.6.1 Sample Size Determination

A. Human Subjects

(A) Diabetic Patients

Fisher's formula was used

$$N=\frac{Z^2P\,(1-P)}{e}$$

Where N= Desired sample size.

Z^2 = is the abscissa of the normal curve that cuts off an area α at the tails (1.96).

P = is the expected proportion [(prevalence of diabetes in the country (Kenya) is estimated to be at 3.6%] (Mohamed *et al.*, 2018).

e = desired level of confidence, which was 95%

$$\text{Sample size was} = \frac{1.96^2 \text{x} 0.036 \text{x} 0.964}{0.05^2} = 53$$

Since the diabetic population is less than 10,000 according to (Baringo, 2015) a finite correction formula was used,

$$n = \frac{n_o}{1+\frac{(n_0 - 1)}{N}}$$

(Finite population correction factor adapted from (Kasiulevičius *et al.*, 2006)

Where; n-sample size

N-Population size

$$= \frac{53}{1+\frac{(53-1)}{600}}$$

$$= 48$$

A total of 48 diabetic patients were to be interviewed, but instead, 43 people with diabetes were interviewed, but because of inconsistencies, four patients were dropped, 39 remained which represented (39/48x100) 81.25% of the intended diabetics to be interviewed.

Sampling Technique

Diabetics: Snow ball sampling. The first patient was recruited at Kabarnet County Referral Hospital diabetic clinic. The same recruited patient directed me to the other patients. The later recruited patient, directed me further to another one. The same continued till a total of 43 diabetics number was reached.

(b) Herbalists

Sample size; A total of twelve (12) Herbalists dealing with diabetic herbal medications and willing to participate in the study were recruited.

Sampling technique – The identification of these twelve herbalists was done via Purposeful sampling technique.

B. ANIMAL SUBJECTS

(a) Amphibians-Frogs;

Three male and three female frogs of the species African clawed species-*Xenopus laevis* as described by (Dawson & Bantle, 1987) were collected from Lake Victoria; in Kisumu County, Kenya, and used to breed the eggs for FETAX tests.

(b) Mammals- Rats;

A total of 80 male Wistar albino rats were collected from the University of Nairobi department of biological sciences.

A total of sixty-seven (67) Wistar male albino rats were used. Thirty-five (35) rats were used in assessing the antidiabetic activity of the crude extracts of the commonly used herbs while eighteen (18) rats were used to test anti-diabetic activity of the purified version extracts of the two commonly used herbs. Other than the 35 rats that were exposed to crude extracts of commonly used herbs, (14) more rats were recruited for histopathological studies.

Five (5) rats were randomly caged in the 7 cages for crude ethanolic extracts and six (6) rats per cage for purified extracts. The numbers were determined based on the systematic published peered reviewed studies by (Ranasinghe *et al.*, 2012; Yeh *et al.*, 2003).

The protocol of handling the lab animals was based on the description by (Council, 2010).

3.6.2 Sourcing, Collection and identification of the herbs

All the herbs, that were described by the herbalists and diabetics were collected from the various places of their origin in Baringo county (*see appendix iii*). They were transported to UOE where they were taxonomically identified at the Department of Botany -University of Eldoret, Kenya, using their morphological characteristics (Agnew, 2013; Beentje *et al.*, 1994). About 2kg of each of the fresh plant materials were harvested, then dried in room temperature for a period of 72 hours and milled into a coarse powder using OHMS OCG-200 grinder.

3.6.3 Extraction

Introduction

Extraction of all the herbs was done using distilled water (aqueous) and ethanolic solution as the solvents because of their different levels of polarities. Ethanol is most commonly used solvent when it comes to laboratory studies and water is the commonly used solvent back in the community of origin.

Both the ethanolic and water solvents extraction were done concurrently.

Procedure

Five hundred grams (500g) of each of the herbs identified were separately soaked in 2 litres of ethanol (98 percent concentration analytical reagent) and distilled water, for a period of 72 hours. The resultant mixtures were then filtered using whatman filter paper (No.1) and the filtrate concentrated to approximately 40ml and 50ml for ethanolic extracted extracts and water extracts respectively, using vacuum-rotary evaporator machine R-3,000 at a temperature range of between 40^0C -50^0C for ethanol and 85^0C-90^0C for water.

3.6.4 Commonly used herbs

Based on the analysis of data obtained in phase 1, the two commonly used herbs were Carrissa *edulis* (CE) and Urtica *dioica* (UD). The two herbs were selected for further analysis, but firstly their extraction yield had to be determined.

3.6.5 Determination of extraction yield for the commonly used herbs (CE and UD).

The determination of the extraction yield, was done as described Qaid et-al 2020. But

in order do to that, the filtrates of CE and UD aqueous and ethanolic extracts ***(50 and 40 mls respectively), (see 3.6.3),*** had to be lyophilized first. Lyophilization was done with the help of '**Harvest Right freeze drier -USA'**. Lyophilization period was between 18 to 27 hours.

Formula:

Extraction yield= (Extracted dry quantity in mg / dry weight before extraction in mg x 100. (Qaid, 2020))

Table 3. 1 Amounts in mg and percentages extracted from the selected herbs

.

	CE		UD	
	Crude Ethanolic extract	**Crude Aqueous/water Extract**	**Crude Ethanolic extract**	**Crude Aqueous/water extract**
A. Dry quantity before extraction in mg of the herb (mg)	500,000	500,000	500,000	500,000
B. Extracted dry quantity after lyophilization in mg [nb, these were 50 mls and 40 mls for respectively for ethanolic and aqueous extracts] in mg	6,914.4	23,643	1,376	2,215.2
% yield extracted $=^{B}/_{A}$*100	1.38	4.73	0.28	0.44

The yielded powdered extracts of each herb, (CE and UD) were then dissolved in distilled water of equal amounts – 200 mls, to make the main crude concentrations -to be used in crude extracts tests phase.

Table 3. 2 Amounts of the herb in 200mls of distilled water used in the subsequent FETAX and Efficacy tests

	CE		UD	
	Crude Ethanolic extract	**Crude Aqueous/water Extract**	**Crude Ethanolic extract**	**Crude Aqueous/water extract**
Amount used to dilute	200mls	200mls	200mls	200mls
Concentration (mg/ml)	200mls =6914.4mg	200mls=23648mg	200mls=1376mg	200mls=2215.2
(What about 1ml)	1ml=34.572mg	1ml= 118.23mg	1ml= 6.88mg	11.08mg

Table 3.2 above formed the stock solution for used in crude extracts studies.

3.6.6 How the resultant crude extracted extracts of CE and UD were used

Table 3. 3 How the resultant crude extracts were used

	Herb extract	**Amounts in mls out of the 200mls**	**Test**
1	CE crude ethanolic extracts	50	96hr/FETAX
		60	21 days/Efficacy
		10	Phytochemical testing
		50	Column chromatography-purification
		Total=170	
2	CE crude Aqueous extracts	50	96hr/FETAX
		60	21 days/Efficacy
		10	Phytochemical testing
		Total=120	

3	UD crude ethanolic extracts	50	96hr/FETAX
		60	21 days/Efficacy
		10	Phytochemical testing
		50	Column chromatography-purification
		Total=170	
4	UD crude Aqueous extracts	50	96hr/FETAX
		60	21 days/Efficacy
		10	Phytochemical testing
		Total=120	
5	CE+UD (1:1) crude ethanolic mixtures	50	96hr/FETAX
		60	21 days/Efficacy
		Total=110	
6	CE+UD (1:1) crude Aqueous mixtures	50	96hr/FETAX
		60	21 days/Efficacy
		Total=110	

Table 3.3 above shows how the various extracts of crude CE and UD extracts.

3.6.7 Purification of CE and UD herbs crude ethanolic extracts.

Fifty milliliters [(50mls) (see table 3.3 above)] of ethanolic extracts of each of the selected herbs (CE and UD) were used in purification.

Purification technique - Column Chromatography (CCT).

Column Chromatography **(**CCT) has two major parts that assist in purification; the mobile phase and the stationary phase.

The mobile phase is usually a liquid/chemical-media with different polarities that aids in concentrating a compound to be separated as they move along the column. In the case of the current study, the mobile phase chemicals utilized were; 95% pure hexane, 99.9% pure ethyl acetate and 98% ethanol and 95% pure Methanol

The Stationary phase refers to the media in which the purification process takes place. It is usually made of different materials, with different meshes.

NOTE: For the current study, silica gel of 60-120 Mesh was used.

Silica gel (Stationary phase) and 98% ethanolic pure analytic reagent (solvent-mobile phase) was made into a slurry mixture and poured bit by bit into the column until it was ½ way full (column dimensions was 30cm long by 3cm wide), (the mixture was ensured that it was uniformly distributed).

NOTE: The stationary phase (silica gel) was ensured wet and uniform and, the upper-level uniform.

Then then compound to be separated [the 10mls of each compound mixed with silica gel (ethanolic extracts of CE or UD)] introduced to the column. They were added from the top of the column in such a way that the top level of stationary was not to be disturbed. See the figure 3.1 below.

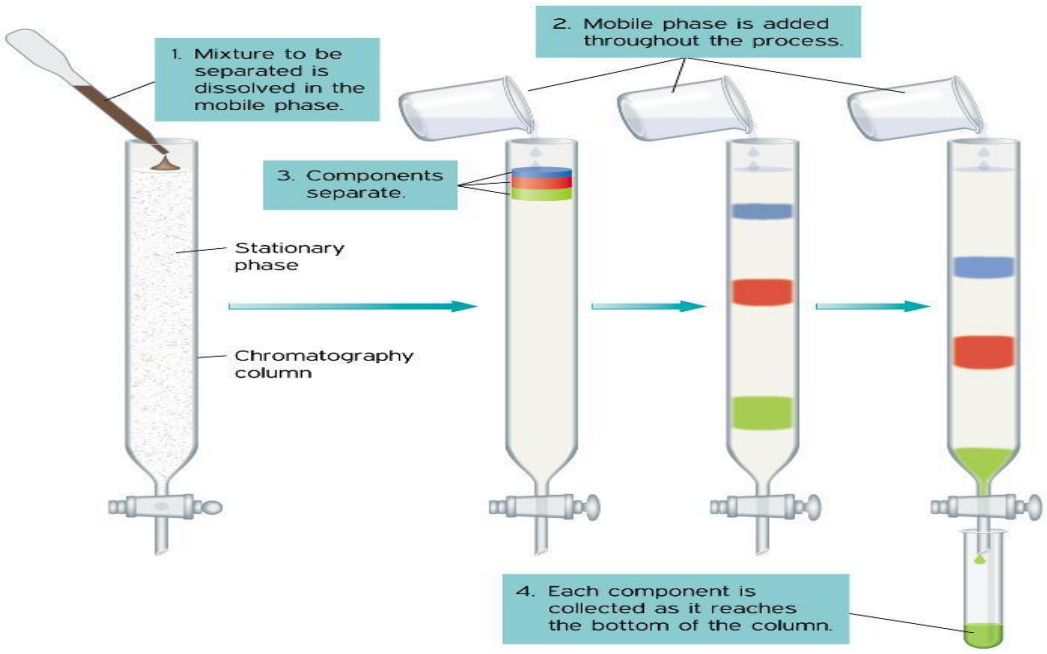

Fig 3. 1; Introduction of compounds in column chromatography;

Adapted from: https/Kromatografia.png

After the introduction, of the compound to be separated to the column, the different concentrations of the solvent (hexane and ethyl acetate) were then repeatedly added on top.

Technique of adding the mobile phases:

While introducing to the column 100 mls hexane and systematically lowering down by 5mls until it reached 0mls, the concentration of ethyl acetate was systematically increased by 5mls until 100mls. After which 300mls of 95% methanol-AR, was used to clear the column.

As this addition was going on, the compound mixture, was believed to be moving (separating) along with the eluent at different times depending on the polarity of the sample. The tap or clip on the lower end of the column was opened for the eluent to trickle into some several 20mls test tubes.

The different separated compounds collected in the 20mls tubes were identified via thin layer chromatography (TLC) plate technique.

Like the column chromatography, TLC plate has,

a) **A stationary phase** (a solid, or a liquid supported on a solid) and
b) A **mobile phase** (a liquid or a gas).

The mobile phase flows through the stationary phase and carries the components of the mixture along with it and as it does this, separation occurs in respect to their different polarities. A thin line was drawn using a pencil, one centimeter near the edge of the bottom end and another one centimeter near the top edge (upper side) of the TLC plate. A small drop [(spot) (from the different 20mls test tubes)] of the elutes is placed one centimeter apart on the bottom line of the TLC plate in such a way that one TLC plate, took 4 drops spots of 4 test tubes [the 20ml test tube].

Reading the TLC plate

A solution is prepared to aid in the reading

Reading solution -Preparation 1

Ten milliliters (10 mls) preparation of a solution containing; a ratio 3:7 of hexane to acetyl acetate is made (just to covered the bottom of the beaker) and covered using a piece of glass lined with a paper containing the mixture of the solvents (hexane and acetyl acetate) in it.

Reading solution -Preparation 2

Another 10mls of methanol to acetyl-acetate in a ratio 2:8 is prepared to be used in spreading the mixtures of either CE or UD, eluted with methanol. It is also covered using a piece of glass lined with a paper containing the mixture of the solvents in it. See figure 3.2 below.

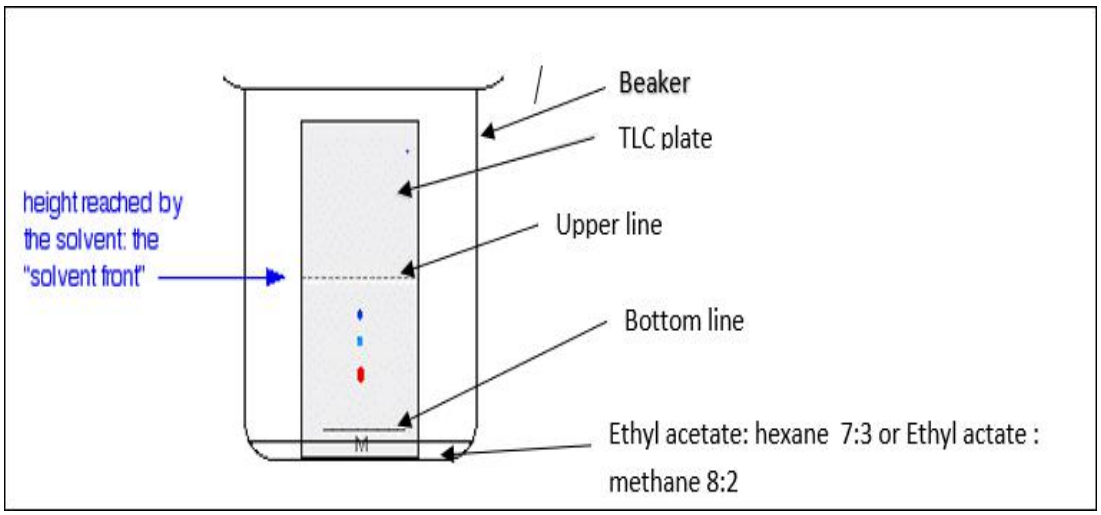

Fig 3. 2; Reading the TLC plate

Step 1 After the spotting, the plate is dipped carefully in the beaker containing the hexane and acetyl acetate in a way that the bottom line goes first

Same is done with the compounds eluted with methanol, but instead of using acetyl-acetate and hexane, acetyl acetate and methanol was used.

Step 2 The TLC plate is removed and dried carefully so that the contents movement was seen. A UV viewer can also be used, if colorless compounds are suspected.

The compound with the highest number of phytochemicals among the purified/separated compounds was selected, to be used in the safety and efficacy tests (in vivo) but, first -FETAX assay tests was done to predict the dosages.

3.6.8 Determination of Extraction Yield After Purification

After purification of the ethanolic crude extracts, they were also lyophilized and % yield extracted determined **(See table 3.4 below)**

Table 3. 4; Extracted Yield Amounts After Ethanolic Extracts Purification

	Extracts in mg	
	CE	UD
A. Ethanolic extracts subjected to purification= mg/50mls. (Where 1ml=34.572mls, as shown in *see Table 3.2*	A total 50mls = 1,728.6	A total 50mls =344
B. Amount in 50mls after purification [the lyophilized substance in mg]	627.85mg	134.4mg
% yield $=^{B}/_{A}*100$	36.32%	39.07%
mg/ml	12.5557mg	2.688mg

The yielded powder extracts of CE and UD purified, were dissolved 120mls of distilled water each and used in the purified extracts phase of the tests.

Table 3. 5 How the resultant purified extracts were used

	HERB EXTRACT	Amounts in mls out of the 200mls	Test
1	CE purified ethanolic extracts	50	96hr/FETAX
		55	21 days/Efficacy
		5	Phytochemical testing
		Total=110	
2	UD purified ethanolic extracts	10	96hr/FETAX
		55	21 days/Efficacy
		5	Phytochemical testing
		Total=70	
3	CE+UD (1:1) purified ethanolic mixtures	10	96hr/FETAX
		55	21 days/Efficacy
		Total=55	

Table 3.5 above shows how purified extracted extracts of CE and UD, were used in the subsequent tests.

3.6.9. Phytochemical screening of all the herbs protocol (qualitative analysis)

All the herbs identified herbs in the field were subjected phytochemical screening analysis.

Two (2) mls of each of the ethanol and aqueous extracts of all the herb extracts were further diluted into five (5) mls using distilled water and phytochemicals composition of each herb, determined as described below;

(a) **Test for Alkaloids:** Three (3) to five (5) drops of each ethanol or aqueous extracts was treated with 1% of Hcl mixture warmed and then added 2mls of *Wagner reagent* [a reagent composed of 1.27g of iodine and 2g of potassium iodide in 100mls of water] separately. The resultant mixture was stirred vigorously. A formation of reddish brown precipitate or coloration in each extract indicated the presence of alkaloids (Banu & Cathrine, 2015; Parekh *et al.*, 2006) .

(b) **Test for Flavonoids (alkaline reagent test):** Two mls of the extract's ethanol or aqueous extracts in a 10mls test tube were each treated with 3 to 5 drops of sodium hydroxide solution. A formation of an intense yellow color which becomes colorless on addition of dilute hydrochloric acid denoted the presence of flavonoids (Yadav & Agarwala, 2011).

(c) **Test for Phenols (ferric chloride test):** A ml [1ml] of ethanol or aqueous extracts were each treated with 2-3 drops of ferric chloride solution. Bluish intense coloration or black was an indication of phenols (Ghalem & Ali, 2017; Parekh *et al.*, 2006).

(d) **Test for Saponnins**: A separate ml [1ml] of each ethanolic or aqueous extracts were dissolved 3 mls of distilled water in a test tube that was covered well on top. Each test tube contents were then shaken vigorously for about 30 seconds after which the test tubes were allowed to stand in a vertical position and observed over a period 30 minutes. A persistent honey comb like froth formation above the surface of the liquids that lasts for about 30 minutes, indicated the presence of saponnins, (Banu & Cathrine, 2015).

(e) **Test for tannins (Braymer's test)** –was done based on the description by Ghalem et-al (Ghalem & Ali, 2017) . Two (2mls) of each ethanol or aqueous extracts were treated with 5% alcoholic ferric chloride solution separately. A generous bluish or greenish colour solution in each extract indicated the presence of tannins.

(f) **Test for Quinones**: 2-3 drops of concentrated sulphuric acid or aqueous sodium hydroxide solution were added in the test tube containing, aqueous extracts of each solution. A yellow precipitate or coloration formation denoted the presence of quinoid compounds (Krvavych *et al.*, 2014).

(g) **Test for oxalates**- 3 drops of each extract were added 2-3 drops of ethanolic acid glacial and observed for an appearance of appearance of greenish coloration (Ugochukwu *et al.*, 2013).

(h) **Test for terpenoids** 2mls of each ethanolic or aqueous extracts were dissolved in 1ml of chloroform, after which 2-3 drops of concentrated sulphuric acid was added. If a reddish brown precipitate was produced immediately, then the presence of terpenoids was indicated (Iqbal *et al.*, 2015).

(i) **Test for Glycosides**: A ml [1ml] of each ethanolic or aqueous extracts were dissolved in 1 ml of aqueous sodium hydroxide solution separately. Formation of yellow colour indicated the presence of glycosides.

(j) **Test for Steroids (Liebermann- Burchard Test)**: Two Mls. of ethanolic or aqueous extracts in a 10mls test tube container were each treated with 3-4 drops of acetic anhydride and a drop of concentrated sulphuric acid. Positive result or the presence of steroids was denoted by the appearance of purple colour, which changed to blue or green colour after 3-5 minutes (Iqbal *et al.*, 2015)

(k) **Test for Coumarins**: Three (3) drops of Ethanolic and aqueous extracts of each solution was added 2-3 drops of alcoholic sodium hydroxide and stirred. If a yellow colour appeared, it indicated the presence of coumarins (Savithramma *et al.*, 2011).

(l) **Test for Sterols and Triterpenoids**: Five Millilitres of aqueous and ethanolic extracts of were each placed in a small beaker and evaporated to dryness. The resultant residues of each extract were then dissolved in 0.5 ml of acetic anhydride and chloroform. The solutions were then later transferred into a dry test tube and 2 to 4 drops of concentrated sulphuric acid was added to each extract. A Brownish red or violet rings at the zone of the contact with the supernatant and green or

violet coloration denoted the presence of sterols and triterpenes respectively (Bhandary *et al.*, 2012; Kachkoul *et al.*, 2018).

(m) **Test for Xanthones**: Extract's solution was each added, 2-3 drops of concentrated nitric acid and then 2-3 drops of ammonia. If a red precipitate appeared, it indicated the presence of xanthones (Abate & Mengistu, 2018).

(n) **Test for catechins**; Two drops of ethanolic or aqueous extracts of the selected plant extracts were mixed with 2 drops of Ehrlich reagent and then one to two drops of concentrated hydrochloric acid added in each mixture. If a pink or purplish color appeared, an indication of the presence of catechins was noted (Iqbal et al., 2015).

1. Most of the above protocols was also described by (Muralidharan, L. 2015).

2. Crude extracts phytochemicals (ethanolic and aqueous extracted extracts) of all the herbs were done first (including the two commonly used), then later the purified extracted extracts.

3.6.10 Assessment of CE and UD acute toxicity using Frog embryo teratogenic assay-xenopus [(FETAX protocol (in vitro studies)]

a. Determination of the best (optimal) concentrations for FETAX studies

After determination of the actual amounts to be used in the subsequent type of studies; {see table 3.3 and table 3.5. i.e., crude and purified extracts respectively}, crude ethanolic extracts of CE and UD, were then subjected to six (6) random preliminary trials -prior to the actual FETAX tests with an aim of determining the best optimal FETAX concentration to be used in the study.

The eventual, optimal concentrations in mg/ml arrived after the 6 trials is has as shown in table 3.6 below.

The concentrations in table 3.6 below was obtained by firstly considering, what the totals in milligrams, the concentrations used in FETAX studies (tables 3.3 for crude and table 3.5 for purified), would have, then extrapolating the findings to what 400mls for CE, and 750mls of UD would contain.

Table 3. 6 ;Final concentration used in the FETAX studies

	CE extracts			***UD extracts***		
	Crude Ethanolic	***purified Ethanolic***	***Crude Aqueous /water***	***Crude Ethanolic***	***Purified Ethanolic***	***Crude Aqueous /water***
	A	B	C	D	E	F
Concentration(mg/ml) (amount obtained after extraction)	34.572	12.5557	118.215	6.88	2.688	11.076
Stock solution (ml) [Amounts of distilled water used for dilution]	400	400	400	750	750	750
Concentration in mg/ml	4.3215	1.56946	14.7769	0.4587	0.1792	0.7384

Table 3.6 above shows the concentrations of the various CE and UD in mg/ml used in the Fetax studies- prediction of CE and UD toxicity in rats.

But, before the study is done, a liquid media known as FETAX solution, had to be made. FETAX solution is known, to be the best optimal environment for *Xenopus laevis* embryos to thrive in (Dawson & Bantle, 1987)

b. FETAX solution preparation

FETAX solution was prepared using; 625mg of Nacl, 96mg of $NaHCO_3$, 30mg of Kcl, 15mg of $Cacl_2$, 60mg of $CaSo_4$ and 75mg of $MgSo_4$/ Litre. PH was regulated using 1N NaOH to between PH 7.8 and 8.1 as described by (Dawson & Bantle, 1987). Then the concentrations prepared as shown in table 3.7 below.

c. Concentrations for FETAX tests

Using the concentrations in table 3.6; Nine (9) different concentrations were made for each extract (3-CE extracts [ethanolic, aqueous and purified], 3-UD extracts [ethanolic, aqueous and purified] and 3-CE+UD extracts mixtures [ethanolic, aqueous and purified]). See the table 3.7, 3.8 and 3.9 below

CE extracts concentrations preparations

Table 3. 7: CE extracts

CONC /NO	% CONC	Distilled water in mls	CE extracts			FETAX solution(ml)	Total concentration subjected to frog embryos in mls
			Crude ethanolic extracted in mg	Purified ethanolic extracted in mg	Aqueous crude extracted in mg		
	A	B	C			D	B+D
1	75	7.5	32.411	11.771	110.827	2.5	10
2	50	5	21.608	7.847	73.884	5	10
3	25	2.5	10.804	3.924	36.942	7.5	10
4	12	1.2	5.186	1.883	17.732	8.8	10
5	6	0.6	2.593	0.942	8.866	9.4	10
6	3	0.3	1.296	0.471	4.433	9.7	10
7	1.5	0.15	0.648	0.235	2.217	9.85	10
8	0	0	0	0	0	10	10

UD extracts concentrations preparations

Table 3. 8: UD extracts

CONC /NO	% CONC	Distilled water in mls	UD extracts			FETAX solution(ml)	Total concentration subjected to frog embryos in mls
			Crude Ethanolic extracted In mg	Purified ethanolic extracted in mg	Aqueous crude extracted in mg		
	A	B	C			D	B+D
1	75	0.15	3.44	1.344	5.538	9.85	10
2	50	0.1	2.293	0.896	3.692	9.9	10
3	25	0.05	1.147	0.448	1.846	9.95	10
4	12	0.024	0.55	0.215	0.886	9.976	10
5	6	0.012	0.275	0.108	0.443	9.988	10
6	3	0.006	0.138	0.054	0.222	9.994	10
7	1.5	0.003	0.069	0.027	0.111	9.997	10
8	0	0	0	0	0	10	10

Mixtures UD+CE extracts concentrations preparations

Table 3. 9: Mixtures extracts

CONC/NO	% CONC	Distilled water in mls	Mixtures of CE and UD			FETAX solution(ml)	Total concentration subjected to frog embryos in mls
			CE+UD crude ethanolic extracted	CE+UD Purified ethanolic extracted in mg	CE+UD Aqueous crude extracted in mg		
	A	B	C			D	B+D
1	75	3.825	17.9255	6.5575	58.1825	6.175	10
2	50	2.55	11.9505	4.3715	38.788	7.45	10
3	25	1.275	5.9755	2.186	19.394	8.725	10
4	12	0.612	2.868	1.048	9.309	9.388	10
5	6	0.306	1.434	0.525	0.651	9.694	10
6	3	0.153	0.717	0.2625	2.3275	9.847	10
7	1.5	0.0765	0.393	0.131	1.164	9.9235	10
8	0	0	0	0	0	10	10

Tables 3.7, 3. 8 and 3.9 above shows the different concentrations of CE, UD and their mixtures extracts (ethanolic extracted, water extracted and purified ethanolic) used in the determination of various concentrations used in FETAX studies.

Column A represents, the percentage concentration, column B represents distilled water in milliliters used in the preparation and column D denotes the amount of FETAX solution used.

Column C represents the actual amount of the herb in milligrams used in the different concentrations (from 75% represented by 7.5mls to 0% represented by 0mls)

d. Obtaining the eggs embryos for FETAX tests

Three pairs (male and female) of adult frogs were injected with HCG (Human chorionic gonadotropin) hormone. The males were injected 600 I.U and female 1000 I.U as described by(Bantle *et al.*, 1989). Each pair was put in a separate aquarium as shown in the *figure 3.3*, below. Breeding tank holding water set at 30^0 c for 8 hours. Pair A, pair B and pair C.

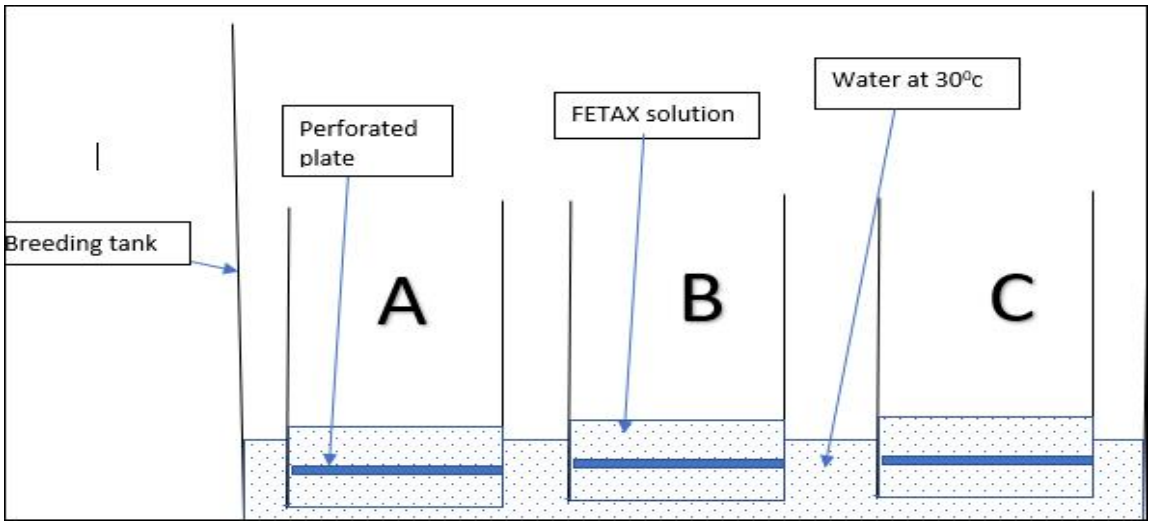

Fig 3. 3; Frog embryos breeding tank illustration

(The eggs they had laid were harvested after 8 hrs.).

Injection was done in the late evening (8pm) - laid eggs collected early in the morning (after approximately 8 hours)

The harvested fertilized eggs according to (Fort, Stover, Bantle, et al., 2000) had to have been normally pigmented on the top surface, The pigment must have been even in coloration and not mottled, They must have been not laid in strings, Less than 30% of the eggs should have not exhibited abnormal pigmentation when first laid, Greater than 70% should have rotated in such a way that the animal (dark) pole is facing up in

the dish and that Fertilization and normal cleavage rates must have been in excess of 70%.

De-jelling of the eggs

The eggs were then dejjeled using 2% cysteine solution.

e. Setting the FETAX tests

Twenty-five (25) fertilized eggs were then put in a petri dish with the various concentrations shown in table 3.7, 3.8 and 3.9 above of either CE ethanol, CE aqueous, UD aqueous, UD ethanol, Mixtures of CE and UD; both Ethanolic and aqueous, purified CE Ethanolic and purified UD Ethanolic. A triplicate of 25 eggs was made for each concentration and labelled 'A', 'B' and 'C'. They were then put in an incubator at temperature between 22^0c and 26 ^{0}c. The solution was changed after every 24hrs.

f. Staging of the xenopus laevis

Staging was done in every 24hrs in reference to normal table of xenopus laevis by (Nieuwkoop & Faber, 1994) as described in the figure below.

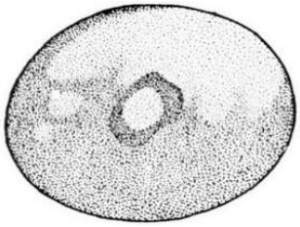

Fig 3. 4; One cell stage (1.4-1.5mm) - day 0, 0hr

One cell stage, shortly after fertilization. Pigmentation darker ventrally than dorsally (length 1.4-1.5mm).0hr (egg at the start of the FETAX test) of development animal view

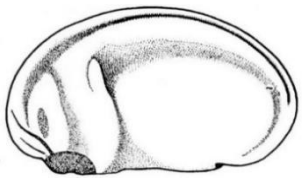

Fig 3. 5 ; Tail bud-discernible (Length 2.5-1.5mm). At the end 24hrs of development, lateral view (1 day)

Eyes protruding less far laterally than gill area. Gill area more prominent than jaw area. Tail bud-discernible (Length 2.5-1.5mm)._At the end 24hrs of development, lateral view (1 day)

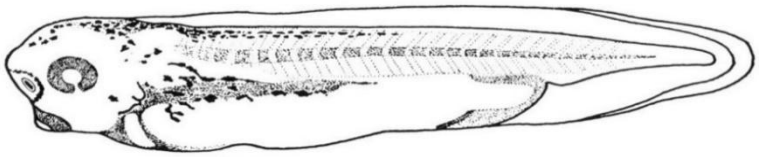

Fig 3. 6 ; Two gill rudiments (length 5.3-6.0mm). At the end 48hrs of development lateral (2day)

Stomodal invagination roundish. Eyes entirely black. Formation of two gill rudiments (length 5.3-6.0mm). At the end 48hrs of development lateral (2day)

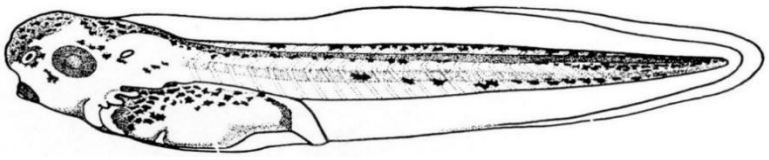

Fig 3. 7; Gills broader and flatter (Length is 6.7- 7.5mm). At the end of 72hrs of development (3days)

Gills broader and flatter, more laterally developed. (Length is 6.7- 7.5mm). At the end of 72hrs of development (3days)

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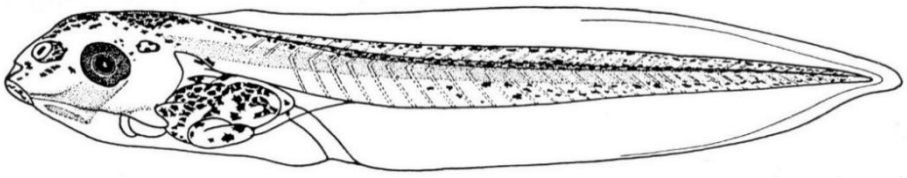


Fig 3. 8 *;* Operculum partly covers the gills (Length 8-10mm). At the end of 96 hours of development

Operculum partly covers the gills. Edge still straight. Intestine spiralized in ventral aspects. Beginning of feeding. (Length 8-10mm). At the end of 96 hours of development.

g. Parameters collected and analyzed in the FETAX [96hr] developmental tests

(a) **Mortalities/embryotoxicity** -Number mortalities at 24, 48, 72 and 96 hrs. counted.

(b) **Abnormalities/malformations/teratogenicity** -Abnormalities after 96 hours in reference to atlas of abnormalities(Bantle, Dumont*, et al.*, 1991). The pictures were taken using a dissecting microscope mounted with MOTIC digital camera.

(c) **Embryonic Development**-Embryonic development after 96 hrs. The length was measured using MOTIC software.

3.6.11 Assessment of the two selected herbs antidiabetic activity (Efficacy test protocol using the Wistar male albino rats)

3.6.11.1 Determination of the dosage used in efficacy studies

Safe dosages used in the in vivo studies were determined based on the outcome of the in vitro test (FETAX assay) and the already available literature.

Lethal Dose 50% as per FETAX assay (current study)

Table 3. 10 ; LD_{50} based on FETAX studies of the various extracts extracted using the different solvents exposed to the frog embryos/laevis as described in 3.6.10 c above [n/b LC_{50} obtained after FETAX studies was converted to LD_{50}. see *Table 4.13-chapter 4]*

	HERB	ETHANOLIC EXTRACTS	WATER/AQEOUS EXTRACTS	
UNPURIFIED (CRUDE)				
	LD_{50} in mg/kg		LD_{50} in mg/kg	
1	CE	4,322.7 mg/kg	4	14,433.3 mg/kg
2	UD	21,706.3 mg/kg	5	3,955 mg/kg
3	CE +UD	4,686.2 mg/kg	6	2,127 mg/kg
PURIFIED ETHANOLIC EXTRACTS				
	LD50 in mg/kg			
7	CE	1570 mg/kg		
8	UD	21 mg/kg		
9	CE+UD	171.56 mg/kg		

The table above shows the lethal doses of the various CE and UD crude ethanoic, purified, and aqueous extracts that killed 50% of the four-day tadpoles. According to Organization for Economic Cooperation and Development (OECD), guideline 423, on toxicity of herbal substances, a substance with an LD_{50} value from 300 to 2000 mg/kg, is considered dangerous, and any substance with an LD_{50} value higher than 2000 mg/kg is deemed to be safe (Alonso-Castro et al., 2017).

Previous studies

Previous studies, on LD50s , obtained on orally administered CE and UD ethanolic crude extracts, given to rats and mice , were 3,807.9 mg/kg (Ngulde et al., 2013) and 17,213 mg/kg (Fatima *et al.*, 2018)respectively. These findings are more or less similar to the findings of this current study- at crude ethanolic values of CE and UD, at 4,322.67mg/kg and 21,706.3 mg/kg, respectively. N/B that both values were above the 2000mg/kg threshold values stated by OECD.

Based on these findings a safe dosage was therefore, determined and used in the in-vivo tests (rat studies) as illustrated in **Table 3.11**

Determinations of the dosages, based on table 3.2, 3.4 and 3.10 above

Table 3. 11;Amounts in mg/kg predicted dosages exposed to the rats for efficacy safety experiments-in vivo studies

	Extracted extracts	Dosage that would kill 50% of a 200 mg weighted rat (using the LD 50 from FETAX studies) in mg	Dosage in mg given to the rats (a rat is approx. 200mg) 0.5mls/100mg/rat	Factor (times less)
Crude/unpurified extracts		*A*	*B*	*C*
1	CE ethanolic extracts	864.5	34.57	25
2	CE aqueous extracts	2,886.7	118.22	25
3	UD ethanolic extracts	4,341.3	6.88	630
4	UD crude aqueous extracts	791	11.08	71
5	CE+UD (1:1) ethanolic mixtures	4,686.3	20.73	22.1
6	CE+UD (1:1) aqueous mixtures	425.4	64.65	6.58
Purified extracts				
7	CE	314	12.56	25
8	UD	21	2.69	7.8
9	CE+UD (1:1)	34.3	7.62	4.5

Assumptions; Average weight of a rat = 200mg.

The dosages in table 3.11 above shows the determined dosages that were exposed to the rats -in vivo studies.

3.6.11.2 Efficacy studies (antidiabetic activity) exposure protocol

The protocol of exposures was divided into two; exposure of crude/unpurified extracts to the rats and exposure of purified extracts to the rats

(a) crude /Unpurified herbs

The first 35 rats out of the 80 of rats obtained from university of Nairobi, were randomly caged in seven cages of 5 rats each.

All the eighty rats put in an enclosed environment (open on top) and the seven cages put at determined positions in the enclosure, food and water put inside the cages and the doors left open.

The first five rats to enter each of the seven cages were recruited.

With regards to the herbal extracts to be given to the rats in the seven cages, a simple randomized technique was utilized.

The concentrations/dosages, given to the rats are as described in **Table 3.11** with exception of the last cage (cage seven , who were given metformin at 100mg/kg, as described by (Cheng *et al.*, 2006).

All the rats were diabetically induced with alloxan monohydrate 140mg/kg single dose. See the efficacy protocol of this exercise in **appendix IV-D;**

(b). Purified herbs protocol test of efficacy

As noted earlier, the ethanolic extracted extracts were the only ones which was purified. Controls for this part was same as that of the protocol in appendix IV-D. Eighteen (18) rats were utilized in this phase. The selection of the rats and what type of medications was also done randomly as described in 'a' above. **See appendix IV-E.** for the protocol and for dosages, **see also Table 3.11**

3.6.11.3 Baseline laboratory Information of the rats

Complete blood cell count, biochemistry, Liver function tests and Alanine Aminotransferase, were done both before and after the experiment-aim to determine their health status of the rats.

3.6.11.4 Induction of diabetic state in the rats

The diabetic state in the experimental rats (2-6 months old which were approximately 8weeks to 24weeks) weighing between 60grams to 214.9 grams were induced using a

single dose alloxan monohydrate 140mg/kg administered intraperitonially as described previously by (Daradka *et al.*, 2014; Muralidharan, 2014). This was after acclimatizing the rats in the lab environment for a period of 14 days (make them adapt to the laboratory environment). Upon induction, they were let to rest for 48 hours. During this period the animals were fed with glucose pellets from Unga Limited Kenya at a dosage of approximately between 5 to 6g/100mg/day of a rat (Klurfeld *et al.*, 2021) and provided with water *ad- libitum*. In addition, their physiologic state was monitored (that was the body temperature, appetite, weakness and any other signs of discomfort). After the 48 hours the rats were tested to confirm the development of diabetes mellitus using **Accu-check^{R} active glucometer from Roche industries** an approved glucose monitoring equipment. It was the same instrument that was used to measure the subsequent, rats blood sugars. Blood was taken by milking the rat's tail.

The first (1st) capillary blood sample for the testing of the sugars was taken 3 days before the experiment, the second (2nd) just before the subjects (albino wistar rats) are inducted with alloxan monohydrate, the (3rd) third test was taken after 48 hours of induction, (confirm the diabetic state).

3.6.11.5 Testing of efficacy procedure

This was done 3 times during the entire period of experiment i.e. On the fourth day of every week, from the time after the diabetic state was confirmed. This protocol was done as per description of (Piero *et al.*, 2011) on his study on '**Hypoglycemic Activity of Some Kenyan Plants Traditionally used to Manage Diabetes Mellitus in Eastern Province***'*. Twelve (12) hours before the actual experiment, the rats were given food and water ad-libitum. **See table 3.12**

Table 3. 12; Efficacy testing protocol

CASES 48 -dosages table 3.11			CONTROLS 5rats		
EXTRACTS - 1. Crude CE ethanolic, **2** Crude CE aqueous. **3**. Crude UD ethanolic, **4**. Crude UD aqueous, **5** Aqueous crude mixtures CE+UD. **6** Ethanolic crude mixtures CE+UD. **7** Purified CE .**8** Purified UD. **9** Purified mixtures CE+UD6			(Diabetic induced) 1. Controls given metformin 5		
Preliminaries					
Time line	TEST done	Tests T	Time line	TEST done	Test
3 days before the actual experiment	Baseline	Sugars,	3 days before the actual experiment	Baseline	Sugars
Just before induction		Sugars	Just before induction		Sugars
48 hrs. after induction (cases treated)	Sugars confirm diabetic state		48 hrs. after induction (controls treated)	Sugars- confirm diabetic state	
12 hrs. after the confirmation	Sugars		12hrs after the confirmation	Sugars	
Herbal treatments Protocol					
After the 12 hours of confirmation, till the 21 day-once Daily	-0.5mls/100g/ rat of the herb given daily. -Sugars done after every fourth days and efficacy		After 12 hours after confirmation till the 21 day-once Daily	-100mg/kg of metformin given daily Sugars done after every fourth days and efficacy	

On the day of experiment, sugars levels of the rats were taken and immediately fed on the herbal medication (0.5mls/100mg) orally of the selected herb, all the food in the cages withdrawn. Sugars of the rats were then taken at an interval of one hour, 3 times. The findings were then be recorded and analyzed. **See a sample of test results in appendix IV-B**

Cases were treated orally with doses of the identified herbs [(herb Carissa *edulis-CE* and Urtica *dioica-UD*) extracts (the doses are as shown in *Table 3.11*) 0.5mls/100mg - body weight once a day)] and their mixtures (Carissa *edulis-CE* & Urtica *dioica-UD*).

3.6.12 Histological tests

For histological tests, 2 rats randomly selected from the first 6 cases (cases) and 3 from last cage – cage 7, *[(controls) (n/b all the rats were diabetically induced) that were initially used in the test for crude extracts efficacy protocol*], and another additional 14 rats that were not induced but given; Carissa *edulis-CE* ethanolic *(2)*, Carissa *edulis-CE* aqueous *(2)*, Urtica *dioica-UD* ethanolic *(2)*, Urtica *dioica-UD* aqueous *(2)*, aqueous mixtures of Carissa *edulis-CE* + herb Urtica *dioica-UD (2)*, ethanolic mixtures of Carissa *edulis-CE* + herb Urtica *dioica-UD (2)* and metformin *(2)*, were recruited.

They were humanely curled as described by (Council, 2010 (*check 2.10b*) and their *pancreases, livers, kidney, thyroid glands, spleens and brains* harvested for histopathological studies. Collection, preparation and examination of the tissues and eventual examination was done in accordance to the description by (McCann *et al.*, 2014).

3.7 Ethical Considerations

Human participation was voluntary, they were at liberty to withdraw from the study at any time of their choice with no consequences bestowed upon them whatsoever.

Confidentiality of the participants information was ensured.
Guidelines of handling lab animals as described by (Care *et al.*, 1985; Council, 2010) was also observed to the later.

Permission to collect data in Baringo county was sought from the Baringo County administrators and the medical superintendent of Baringo County Hospital during the period of the field study. An informed consent was also signed by both the diabetic patients and herbalists identified and willing to participate in the study.

Scientific integrity was duly observed during the entire period of study i.e., in the field and also during the lab work.

Approval of the research was done by Human and Animal Research and Ethics Committee HAREC of University of Eastern Africa Baraton (UEAB) and NACOSTI (see appendix 2).

3.8 Data Analysis

Completeness of entry on forms, dual entry, cleaning and coding was conducted using Excel office 19. Data entry done in SPSS version 21 and analysis done in STATA TMP Software**.**

All the phytochemicals were tested and presented nominally in a table form, based on the color visualized, and presented into 4 different categorizes as either absent, low, moderate, or high as signified by -, +, ++, and +++ signs respectively.

Comparing of the differences between proportions and the significance of means was done using ANOVA

Linear regression graphs were used to assist in the interpretation and determination of efficacy of the selected herbs.

In determination of developmental toxicity, Abbot correction formula was used to adjust for abnormalities and deaths that might have occurred due to environment issues. Probit analysis method, was used to determine LC_{50} and EC_{50}, used to calculate the TI, with regards to FETAX tests. LC_{50}, later converted to LD_{50}.

The summarized data was presented using tables of frequencies, box plots, bar charts, pie charts and line graphs where applicable.

All tests were 2-tailed, where applicable and was considered significant at a p=0.05.

CHAPTER FOUR
RESULTS

4.1 Introduction

The results are divided into two (2) phases, namely; field work and laboratory work. Field work Results, are based on the survey done in Baringo County and laboratory work**,** are based on the findings of the laboratory experiments done in; University of Eldoret, Biotechnology Laboratory and Moi University, School of Medicine, anatomy laboratory.

4.2 Field Work/Survey-Identification of the Herbs

Phase one results were further divided into three (3) parts. Part one report is based on the researcher administered questionnaire- administered to the diabetics using herbal medications, part 2, is based on the one-on-one interviews with herbalists prescribing anti-diabetic herbal medications, and lastly part 3 is based on the description of the identified herbs.

4.2.1 Patients Using Anti-Diabetic Herbal Medications

The findings were based on 39 individuals using antidiabetic herbal medications. This response, were equivalent to (39/48x100) 81.25% of the anticipated population. Nine cases were discarded due to inconsistencies.

4.2.1.1 Demographic characteristics of diabetic patients

(i) Age, sex education, marital status and occupation

The age of the respondents ranged from 27 to 70 years old, with a mean of 46.8(SD 11.2) years. Fifty percent (50%) of the participants were below 47 years (IQR 37, 56). Males were slightly more 21(53.8%) than females. Majority 24(61.5%) were married. Those who had completed secondary school education were 12(30.8%), and tertiary level education level were 15 (38.5%). The age distributions were similar across all the sexes (p-value =0.936). Male had a mean of ($\bar{X} = 46.9; SD\ 9.5$) and females($\bar{X} = 46.6; SD\ 13.2$) See the table below

Table 4. 1; Age, sex, marital status, and education level

Variable			
Age		Mean -yrs.	Range- yrs.
		46.8(11.2)	27 – 70
	Category	$^{x}/_{39}$ (Frequency)	Percentage $^{x}/_{39} * 100$
Sex	Male	21	53.85%
	Female	18	46.15%
Marital status	Married	24	61.54%
	Single	5	12.82%
	Separated/widowed/divorced	10	25.64%
Education level	No formal education	2	5.13%
	Primary	10	25.64%
	Secondary	12	30.77%
	Tertiary	15	38.46%

(ii) Occupation

Most (11/39 =28%) of the respondents were businessmen and women followed by Farmers (9/39=23%) and teachers (6/39=15%). The least were prison wardens, policemen, drivers, and clinical officers', each at 2.5%, as shown in *figure 4.1* below.

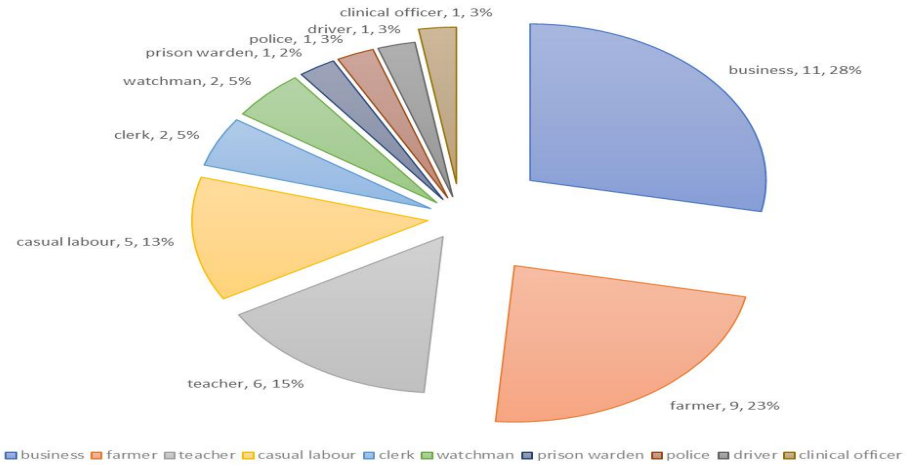
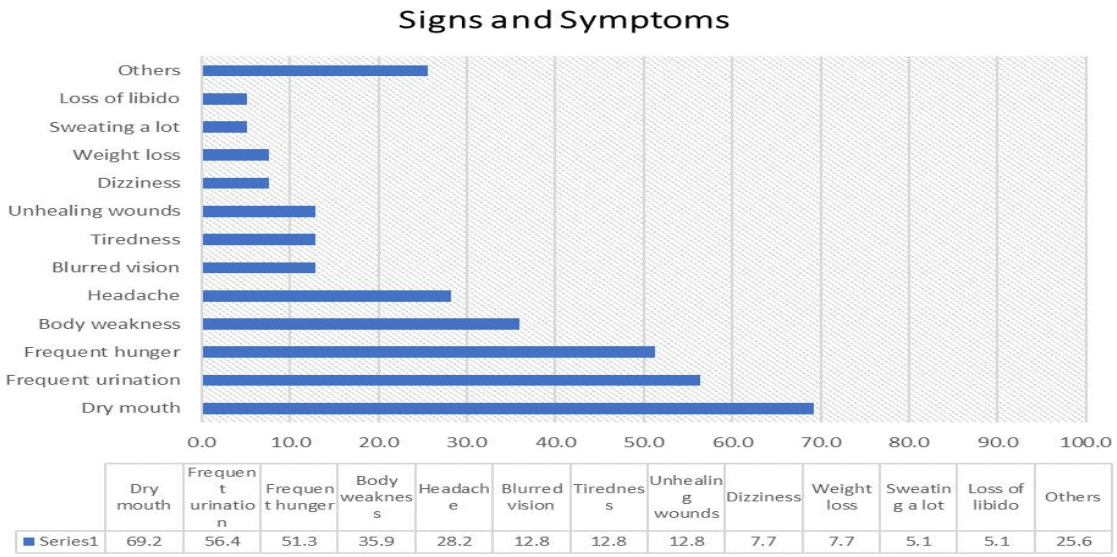


Fig 4. 1; Occupation of diabetics

4.2.1.2 Diabetic Knowledge

(i) Sign and symptoms

The top three reported signs and symptoms were dry mouth (69.2%), frequent urination (56.4%), and hunger at 51.3%. The others (10/39=25.6%) were; palpitation, itchy eyes, body numbness, stomach ache, poor eyesight, shivering, difficulty in breathing, confusion, high blood pressure, and fainting episodes. The least reported was the loss of libido at 5.1%. See the figure (4.2) below



	Dry mouth	Frequent urination	Frequent hunger	Body weakness	Headache	Blurred vision	Tiredness	Unhealing wounds	Dizziness	Weight loss	Sweating a lot	Loss of libido	Others
Series1	69.2	56.4	51.3	35.9	28.2	12.8	12.8	12.8	7.7	7.7	5.1	5.1	25.6

Fig 4. 2; Signs and symptoms as described by diabetics

ii) General Knowledge of diabetics on diabetes mellitus (DM) [what they know related to diabetes]

About 77% (30/39) reported that diabetes, as having no cure, management is psychologically disturbing and tedious. More than half [54% (21/39)] of the respondents reported that conventional management of diabetes is expensive. Forty-nine percent (49% [19/30]) reported diet control as a way of managing diabetes. Forty-six percent (46% [18/39]) emphasized on issue of drug compliance in the management of diabetes.

4.2.1.3 Medicinal remedies used by the interviewed diabetics in managing their diabetic status other than herbal medications

Other than herbal medications, most of the respondents were on oral hypoglycemic drugs (62%) and diet control (77%) (See Table 4.2 below).

Table 4. 2; Medical remedies used by the interviewed diabetics other than herbs

Variable	Category	$^{x}/_{39}$ (Frequency)	Percentage $^{x}/_{39} *100$
Pharmacologic conventional	Insulin	16	41%
	Oral hypoglycemic	24	62%
Non-pharmacologic	Exercise	23	59%
	Diet control	30	77%

4.2.1.4 Use of herbs vs other medications

(i) Singly or combination

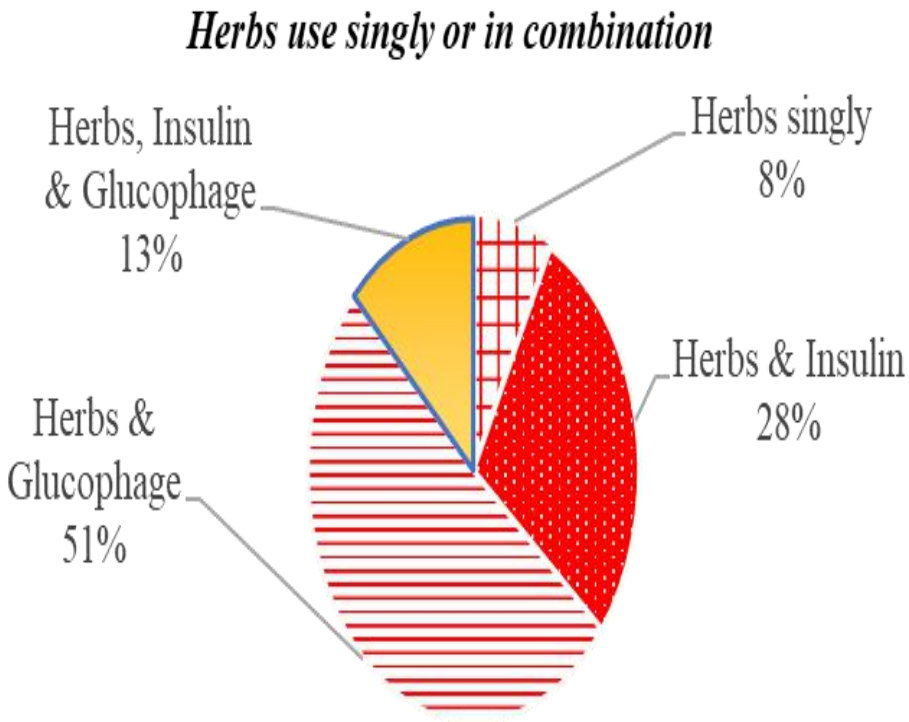


Fig 4. 3; Use of herbs singly or in combination with other medications

As depicted by the pie chart above (fig 4.3), the majority of the anti-diabetics combined their herbal medications with Glucophage (oral antidiabetic) (20/39, 51.28%)

followed by those who use insulin (11/39, 28.21%). Only eight percent (3/39, 7.69%) reported using herbal medications singly.

(ii) Reported side effects of pharmacologic conventional antidiabetics

Most (18/39=46%) of the respondents mentioned itchiness, followed by nausea (9/39=23%), and vomiting (6/39=15%). Diarrhea and stomach upset were the least reported (1/39= 2%). Refer to fig 4.4 below.

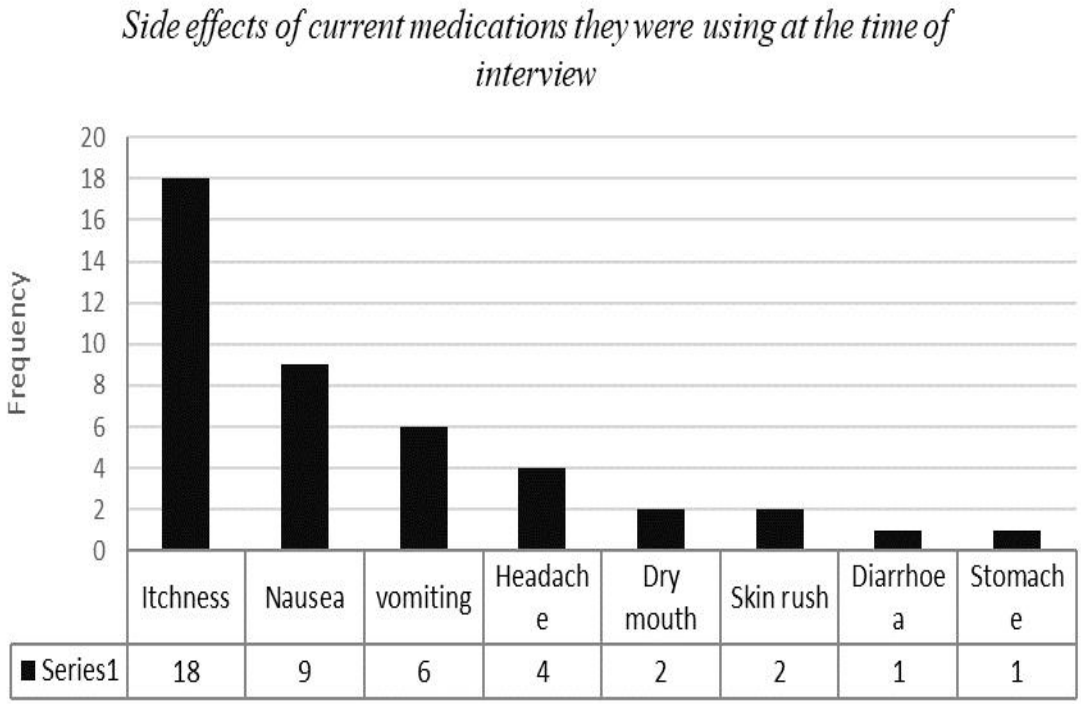


Fig 4. 4; Reported side effects of the antidiabetic medicines at the time of the interview

Sources of herbal medication information they were using at the time of interview

From the above table, it's clear that most of the respondents first got information on diabetic herbal medicine from the relatives, a friend, workmates 33/39=85%. Very few got from internet, radios and herbalists (3%). *See Table 4.3*

Table 4. 3; Source of information about herbal drugs

Information source	**Percentage $^{x}/_{39 * 100}$ Response**
1. Friend/neighbor/relative/workmate	85%
2. Posters	23%
3. Mobile phones	21%
4. Barazas-chiefs etc.	10%
5. Newspaper/written periodicals	5%
6. Internet	3%
7. Radio	3%
8. Herbalists	3%

Common herbal medications used by the patients

Table 4. 4; Herbs used by respondents

	Herb	**Part used**	**% ($^{x}/_{39 * 100}$) of patients using them**
1	Siwot (Urtica *dioica*)	Leaves and stems	67
2	Legetetwet *(*Carissa *edulis*)	Roots	64
3	Sirar *(*Hypoestes *forskaolii)*	Roots	59
4	Tengeretwet *(*Aloe *tweedie)*	Leaves	26
5	Ginger *(*Zingiber *officinale)*	Roots	15
6	Kipnyalil bei *(*Tinospora *cordofolia)*	Leaves and stems	15
7	Mosong -Sorghum *(*Sorghum *bicolor)*	Seeds	10
8	Cinnamon *(*Cinnamomum *verum)*	Bark	13
9	Arwo-Tamarinda *indica*	Seeds and pulp	56
10	Kokian (Zanthoxylum *chalybeum*)	seeds	56
11	Likwas- Loquat- *(*Eriobotrya *japonica)*	leaves	26
12	Maebyot- Mango (Mangifera	leaves	28

	foetida)		

The commonly used herbal medications in the management of Diabetes in Baringo by diabetics were *Urtica dioica* (UD) 67% (26/39) locally referred as Siwot, followed by *Carissa edulis* (Legetetwet) (CE) 64% (25/39) and Sirar (*Hypoestes forskaolii [HF]) 59% (23/39)*. Least reported herbal medication was Mosong -*Sorghum bicolor (Asbaghi et al.)* seeds at 10%. **Refer to table 4.4 above.**

Preparation of reported herbal medications.

Table 4. 5; How the herbs are prepared

Methods of preparation	***Percentage*** $^{x}/_{39 * 100}$
1. Crushed then dried and resultant powder put in boiling water (*Carissa edulis* roots and bark, *Urtica dioica* leaves, loquat leaves, *Hypoestes forskaolii* mango leaves, *Zanthoxylum chalybeum* seeds, and *Tamarinda indica* seeds).	84.61%
2. Taken raw/chewed (sorghum seeds, leaves of *Aloe tweedi*e, *Tamarinda indica pulp*).	25.64%
3. Sap squeezed into a cup (*aloe tweedie* leaves)	5.13%
4. Dried seeds- taken the way they are (sorghum seeds,)	2.56%
5. Chopped and boiled in water (*Hypoestes forskaolii)*	2.56%
6.Soaked in cold water for 3 days (*Tinospora cordofolia*, *Tamarinda indica*)	15%
7. Crushed raw into small pieces and mixed with beverages (ginger and cinnamon)	12.82%
8.Cooked as food (*Sorghum Bicolor*)	5.12%

The majority (33/39=85%) of the herbal medications were crushed, dried and later boiled before being taken. (10/39=26%) Others were taken (chewed) raw. About 2.56% were chopped and boiled (2.56%).

The patients view on efficacy on the herb's they were using

Majority 30(76.9%) of the diabetics believed that, the taking of the herb had enhanced their treatment, and 34(87.2%) would recommend herbs to somebody else.

Source of the herbs

Table 4. 6 ; Source herbs

Source of herbs	$^{X}/_{39}$ (Frequency)	Percentage $^{x}/_{39} * 100$
1. Forest	30	76.92%
2. Herbalist clinic	27	69.23%
3. Their own farm	9	23.08%
4. Own Home compound	3	7.69%
5. Market	3	7.69%

The sources of the herbs depended on which herb the respondent was describing. For example, those who mentioned sorghum and Ginger sourced them from either the market or their farms. It also depended on how many/much the herbs the patient was using.

As indicated on table 4.6 above, most (30/39=77%) of the respondents obtained their medications directly from the forest, followed by diabetic herbal clinics (27/39=69%). The least of the diabetics got their herbs from the marketplaces (3/39=8%).

Knowledge on diabetic medicinal plants conservation

With regards to their understanding of conservation of diabetic medicinal plants, (12/39), 30.7% of the respondents described conservation as '**using of medicinal plants wisely or efficiently and harvesting them only, if needed'**. (17/39) 43.59% understood conservation as planting more medicinal herbal trees/shrubs upon collecting them and teaching the future generation on the importance of medicinal plants so that they also could plant more. (2/39) 5.12% understood conservation as protecting medicinal plants from predators.

Other reasons for using herbs

Other than the belief that diabetic herbal medications help in reducing blood sugars (17/39, 43.59%), others believed that it enhances the work of other conventional drugs that they are using. Others believed that they were cheap (25/39, 64.10%), readily available, accessible culturally and socially (11/39, 28.20%) acceptable compared to conventional drugs, (31/39, 79.48%).

They also (6/39, 15.38%) believed that, the herbal medications they were using have very minimal side effects compared to conventional medicine.

Some (7/39, 17.94%) cited that they use the herbs as a desperate measure, because having used conventional medicine for quite some time, they were yet to receive healing. Hence, they use it, with a hope that it would bring a positive long-lasting change in their diabetic situation one day.

4.2.2 Major Identified Plants Used as Prescribed by Herbalists

4.2.2.1 Introduction

Twelve (12) purposefully identified herbalists were interviewed with regards to their experience in the management of diabetes mellitus.

4.2.2.2 Demographics

The age of the respondents ranged from 43 to 69 years old with an average of 53.8 (SD 8.0) years. Majority 8(66.7%) were women. About 58% (7/12) of the respondents had either no formal education 25% (3) or had attended adult education 33.3 % (4/12).

4.2.2.3 Knowledge about definition of diabetes

Twenty-five percent of the herbalist, 25% (3/12) defined diabetes as **'instability of sugars in the body**. About thirty-three 33.3% *(*4/12) defined diabetes as '**high and low blood sugar in blood'** while 16.7 % (2/12) defined diabetes as **'instability of blood sugar in the blood'**. Eight percent *8.3%* (1/12*)* defined diabetes as **'complications of sugars in human body, disease caused by unregulated sugar in the body' and 'when the sugar in once body is imbalance'**

4.2.2.4 Experience of the herbalists in managing diabetes

The number of years the herbalist had practiced and treated clients with diabetes ranged from 9 to 30 years with an average of 17.4(SD 7.1) years.

4.2.2.5 Knowledge on diabetes manifestation

Their knowledge on signs and symptoms ranged between 'body weakness (8/12, 66.67%), frequent thirsty (4/12, 33.33%), wounds that do not heal as faster as expected (3/12, 25%), frequent thirsty (3/12, 25%) to frequent urination (2/12 16.67%)

4.2.2.6 Herbal medication used in the management of diabetes.

Siwot (*Urtica dioica*) was the most frequently (9/12, 75%) prescribed herb followed by Legetetwet (*Carissa edulis* 7/12, 58.30%) and then sirar *(Hypoestes forskaolii)* and sorghum *(sorghum bicolor)* each at (6/12, 50.0%). Suchon (*Solanum nigrum*) was the least prescribed at (1/12, 08.33%) as shown in Table 4.7 below

Table 4. 7; Commonly prescribed herbal antidiabetic herbs by Baringo County herbalist

Antidiabetic herbs as prescribed by the herbalists/used by diabetics		
Herb/plant	Part used	% of the herbalist prescribing
1. Siwot *(Urtica dioica UD)*	Leaves and Stems	75
2. Legetetwet *(Carissa edulis CE)*	Roots	58
3. Sirar (*Hypoestes forskaolii- HF)*	Roots	50
4. Mosong -Sorghum *(Sorghum bicolor SB)*	Seeds	50
5. Cinnamon *(Cinnamomum verum CV)*	Bark	42
6. Arwo-Tamarind *(Tamarinda indica TI)*	Seeds and Pulp	42
7. Ginger (*Zingiber officinale ZO)*	Roots	33
8. Kokian tree *(Zanthoxylum chalybeum ZC)*	Seeds	33
9. Likwas (*Eriobotrya japonica EJ)*	Leaves	25
10 Maebyot *(Mangifera foetida MF)*	Leaves	25
11. Kipnyalil bei *(Tinospora cordofolia TC)*	Leaves and Stems	17
12.Tengeretwet *(Aloe tweedie AT)*	Leaves	17
13. Suchon *(Solanum nigrum SN)*	Leaves	08

4.2.2.7 Prescription of the herbs

(i) Singly or in combination

Most (8/12, 66.7%) of the herbalist administer their medications singly. Twenty-five percent (3/12, 25%) of the herbalist prescribed them in combination. Those combining different medication did so, because they believed that, by combining the different herbs, sugar reduction process was enhanced.

(ii) Dosage administration

The dosage of administration of the herbs varied from one herbalist to another. It also varied from one herb to another. But, nevertheless, most (7/12, 58.30%) of the herbalist, give their clients ground crushed powder of the selected herb.

Most preferred advising their patients to take two teaspoonfuls soaked in 250mls hot, then cooled water twice a day (i.e., morning and evening) while (5/12, 41.67%) others preferred the same two teaspoonfuls soaked in 500mls of in hot, then cooled water twice a day *(see table 4.8 below).*

(ii) Description of how the herbs were dosaged

Table 4. 8; Dosage of the herbs as described by the herbalists

Serial number	***Herb***	**Herbalist Dosages**	**The equivalent amount of herb dry weight consumed in grams**
1	*Carissa edulis roots and bark*	2 teaspoonsful in 500 mls twice a day	Approx. 16.5g of the herb B. D
		2 teaspoonsful in 250 mls twice a day	Approx. 16.5g of the herb B. D
2	*Sorghum bicolor seeds*	By chewing a handful of sorghum when the client has fatigue and general malaise (PRN)	Approx. 100g of the herb PRN
		Sorghum flour prepared as Ugali and taken once daily (OD)	Approx. 500g of the herb /day/OD
3	*Urtica dioica leaves*	Used as vegetables 3 times in a week	Approx. 152g of the herb /per serving x3= 456g/day
		As vegetables once daily.	Approx. 152g of the herb per serving x1= 152g/day/OD
		Once a day as vegetable/OD	152g of the herb /day/OD
		Boiling approx. 1kg the dried herb in 2litres of water till boiling point and taking 50mls of the resultant product	Approximately 33.3g/50mls twice a day/BD

		as herbal tea twice daily/BD	
4	*Hypoestes forskaolii stems & roots*	Taken once daily as herbal tea in a 250ml s cup- which as approximately 2-3 teaspoonful per cup/BD	5.8921g per teaspoonful 2 teaspoonfuls=11.78g (BD) 3 teaspoonful=17.67g (Junlapeeya et al.)
5	*Aloe tweedie leaves*	Around 50mls, sap squeezed into a cup and taken once daily	Approx. 40g/day/OD
6	*Zingiber officinale roots*	½ A teaspoonful mixed with 250mls of tea and taken once daily in the morning	Which equivalent to 3.625 grams of ZO per day
7	*Tinospora cordofolia stems*	About 500g of the herb soaked in 1000 mls of cold clean water for 48hrs, after which approximately 50mls taken twice daily	50mls is Approx. 10g of the herb /day
8	*Solanum nigrum*	As vegetables once daily.	Approx. 152g per serving x1= 152g/day
9	*Cinnamomum verum*	2 teaspoonsful mixed with a 250 mls cup of tea/coffee/milo etc. and taken once daily	6.5197 g per teaspoonful which approximately 13g/day of cinnamon
10a	*Tamarinda indica Pulp*	Approximately 500g of Tamarinda Indica sap (i.e., after removal of the seeds) was soaked in 1.5l of cold water	100mls was approximately = 33.3g

		for 48 hours. The resultant mixture was taken at a dose of 100mls three times a day	
10b	*Tamarinda indica Seeds*	The seeds crushed into coarse powder and two teaspoonsful added in 250 mls of hot water and taken twice a day	2 teaspoonful's is approximately=12.68g/day
11	*Zanthoxylum chalybeum seeds*	One teaspoonful of dried fruits crushed into coarse powder and added into 500l mls boiling water and taken twice a day	One teaspoonful is equivalent to 5.43g Therefore 2 teaspoonsful =10.86g/day
12	*Mangifera foetida Leaves*	Approximately 500g of Leaves boiled in 1.5 litres of water until boiling point and 50mls taken twice per day	50mls is approximately equivalent to 10g of the dried herb/day
13	*Eriobotrya japonica leaves*	Approximately 500g of Leaves boiled in 1.5 litres of water until boiling point taken once a day	50mls is approximately equivalent to 10g of the dried herb/day

(iii) Part of the herb used

Roots happens to be the most (75%) frequently used part (*Legetetwet and sirar*), followed closely by the leaves and stem (67%) (Siwot). The least used part are the seeds (08%).

4.2.2.8 Preparation of the herb

As mentioned above, the commonly used part of the herb is the root and the leaves. The method of preparation varied from one part of the herb to another and also from one herb to another. However, most (over 75%) of them were prepared by soaking in hot water for 3-5 minutes after which they were sieved and left to cool, before being taken. Some (58.30%) boil the herbs, -. i.e., the fresh leaves or the roots as soon as they get them, then they use/take the herb, until the original taste cannot be perceived. Others take them fresh from the farm e.g., Tengeretwet/ *Aloe tweedie*.

4.2.2.9 Efficacy on the herbal medication as reported by the herbalists

Around sixty seven percent *(8/12, 66.67%)* of the interviewed herbalist believed that the medications they are administering, stabilize the sugars. Twenty five percent (3/12, 25%) reported that, the purpose of their herbs was not only to stabilize or reduce the sugars, but to cure diabetes in the long run. The least- (08%, 1/12) reported that the herbs they were prescribing, alleviates pain as well as lowering the blood sugars.

4.2.2.10 Clients response on the efficacy of the prescribed herbs

About twenty-five percent (3/12, 25 %,) of the herbalist reported that their clients voiced complete healing of their diabetic condition, while seventy-five percent (9/12, 75%) reported sugar stabilization after taking their herbs.

4.2.2.11 Reported side effects/complaints with regards to the antidiabetic herbal medication.

Only two (16.7%) herbalists reported to have heard side effects/complaints from their clients in regards to their herbs. Such reported side effects included nausea, lack of appetite and stomach upset.

4.1.2.12 Source of antidiabetic herbal medications to the herbalists

All the herbalist obtained their herbal medicines from either their farms (3/12) 25% or a forest 75% (9/12). None of them reported buying medicines from a market or any other place.

4.2.2.13 Frequency of harvesting the medications

Nearly all (11/12, 92%) the herbalists reported harvesting their herbs twice in a month (every two weeks) or when the need arises.

4.2.2.14 Conservation

Only 2 (16.7%) of the respondents said they have never heard about the term 'conservation'. Those (10/12, 83.3%) who have heard about conservation gave different opinions on what they know about conservation. Such opinions include; 'to ensure that they have constant supply', 'those herbs which can be dried and stored are harvested in plenty enough during the rainy season to last till the next season 9/12 (75%)'. Some also believed that by keeping the knowledge to themselves is a way of conservation 2/12 (16.7%). Thirty-three percent 4/12 (33.3%) described that by doing responsible harvesting e.g., 'if one is harvesting roots in an herb, one has to harvest some and leave some to ensure that the herb does not die' denotes conservation.

4.2.3 Description of the Identified Herbs

All the identified herbs (taken by diabetics and prescribed by herbalists) were transported from the field (place of origin) to University of Eldoret, Botany department to be identified. The description of the identified herbs is as shown below;

a). Carissa edulis Vahl (CE)

Carissa Edulis Vahl., comes from the order, *Gentianales*, tribe *Carissa*, family *Apocynacea*e. It is a spiny shrub that rises to about 5 m high and found in dry deciduous forest, throughout the drier parts of Baringo County. The plant is characterized by multiple branching and the presence of stiff spines which are about 5 cm long.

Fig 4. 5; Carissa edulis vahl tree (Source : Author, 2019)

It has flowers which are white to purple. Its roots emit a strong smell of methyl salicylate when crushed. It is the latter part of this herb that is used often as medicine in Baringo County (Burkill, 1985.; Venter, 2007).

Fig 4. 6; Chopped pieces of CE roots (Part used as Medicine in Baringo County). (Source : Author, 2019)

Other than diabetes, CE is also used for abdominal related ailments.

b). Urtica dioica (UD)

Urtica dioica (stinging nettle) comes from the *Urticaceae* family. It is an erect, herbaceous perennial herb that has an extensive underground network of rhizomes that can spread up to an area close to 5 feet or more in a season.

Fig 4. 7; Stem and leaves of Urtica dioica (Part that is used as medicine) (Source : Author, 2019)

Its stems are usually unbranched, and grow between 3 to 6 ½ feet tall, covered with bristly stinging hairs. Its' flowers are tiny, greenish to white and are arranged in clusters. It also has branched spiked formations in its' leaves (Jakubczyk *et al.*, 2015). Other than diabetes, this herb is also used by hypertensive patients.

c). Tinospora cordofolia (TC)

Tinospora cordofolia is a climbing shrub which belongs to the family *Menispermaceae*. It is normally found in warm climatic regions of Baringo County, often near riparian regions. It is a large deciduous shrub with several coiling branches.

Fig 4. 8; Tinospora cordofolia chopped stems (Source : Author, 2019)

Its' stems are succulent often with long filiform. Aerial roots can arise from its' branches. It has also a creamy white to gray and deeply left spiraling bark. Leaves of this plant are simple, alternate, ex-stipulate, long-petioled approximately 15 cm. They are also round, pulvinate, heart-shaped, twisted partially and halfway round. It is the stems that are harvested for medicinal purposes in Baringo County, not only for diabetes but also for diarrheal and other abdominal related ailments.

d). Aloe tweedie (AT)

Aloe Tweedie is a short-stemmed shrub, an evergreen perennial succulent plant species of the genus *Aloe*, with a broad, glossy green, mottled leaves which are well-branched.

Fig 4. 9; Aloe Tweedie (Source : Author, 2019)

In Baringo County, *Aloe Tweedie* occurs naturally in many of the arid and semi-arid areas. It is the leaves that are harvested for medicinal purposes, for conditions such as diabetes and hypertension.

e). Solanum nigrum (SN)-African nightshade

African nightshade *(Solanum nigrum)* is an annual or occasionally perennial plant in the *Solanaceae* family. It grows between 15–60 cm tall and it usually has many branches. Its leaves are triangular to elliptic in shape and stems are circular and sometimes slightly hairy.

Fig 4. 10; African nightshade (Source : Author, 2019)

Its flowers are small, star-shaped and white. It is the stems and leaves that are harvested for food by many Baringo citizens and also for medicinal purposes.

f). Sorghum bicolor

Sorghum bicolor is an annual grass-like plant from the family *Gramineae*, subfamily *Panicoideae*. It grows in drought-like, warm humid conditions of Baringo County. The seeds are the ones which are harvested for food and its medicinal purposes.

Fig 4. 11; Sorghum seeds (Source : Author, 2019)

g). Hypoestes forskaolii

Hypoestes forskaolii is classified under the family, *Acanthacea*e. It is a shrub-like a plant which grows to a maximum height of 1-1.5 meters

Fig 4. 12; Leaves, flower, and stem of Hypoestes forskaolii shrub
(Source: Author, 2019)

It has pale pink or white flowers. The lower stems proximal to the ground and the roots are the ones that are harvested for medicinal purposes.

h). Tamarinda indica (TIn)

Tamarinda indica is a leguminous tree that belongs to the family of *Fabaceae*. It produces pod-like fruit that contains a brown, edible pulp. It is a long-lived, medium-growth tree, which attains a maximum crown height of 12 to 18 metres (39 to 59 ft.).

Fig 4. 13; *Tamarinda indica* tree(Source : Author, 2019)

The fruit is an indehiscent legume, which is aproximately12 to 15 cm (4.7 to 5.9 in) in length, with a hard, brown shell. This fruit has a fleshy, juicy, acidic pulp and black to reddish-brown seeds. It is the pulp (used as food also) and the seeds that are used medicinal purposes.

***Fig 4. 14; Tamarinda indica* fruit(Source : Author, 2019)**

i). Zanthoxylum chalybeum (ZC)

Zanthoxylum chalybeum is a deciduous shrub or tree from the family *Rutaceae*. It can grow up to 12 metres high. It grows in medium to low altitudes of dry woodland or grassland, which is found mostly in the lowlands of Baringo along Kerio Valley escarpment.

***Fig 4. 15; Zanthoxylum chalybeum* tree(Source : Author, 2019)**

Fig 4. 16; *Zanthoxylum chalybeum* dried seeds(Source : Author, 2019)

Its fruits are spherical, about 5 mm in diameter, reddish-brown, splitting to allow the shiny black seeds to partly protrude. It is these fruits that are harvested and used as an antidiabetic herbal medicine.

j). Eriobotrya japonica (EJ)

Loquat is botanically referred to as *Eriobotrya japonica*. It is a species of flowering plant in the family *Rosaceae*. The tree can grow to 5–10 metres (16–33 ft) tall, but is often smaller, about 3–4 metres (10–13 ft). The leaves are alternate, simple, 10–25 centimeters (4–10 in) long, dark green, tough and leathery in texture, with a serrated margin, and densely velvety-hairy below with thick yellow-brown pubescence. It is grown in most parts of Baringo climatic regions. The fruits become yellow and succulent when ripe. It is the young leaves which are harvested and used as an antidiabetic.

***Fig 4. 17; Eriobotrya japonica*(Source : Author, 2019)**

k). Mangifera foetida

Mango tree botanically referred to as *Mangifera foetida* belongs to the family *Anacardiaceae*. It is also grown as fruit in most of Baringo County. It is well distributed worldwide and it is one of the most widely cultivated fruits in the tropics. It grows to 35–40 m (115–131 ft) tall, with a crown radius of 10 m (33 ft). The leaves are evergreen, alternate, simple, 15–35 cm (5.9–13.8 in) long, and 6–16 cm (2.4–6.3 in) broad; when the leaves are young they are orange-pink, rapidly changing to a dark, glossy red, then dark green as they mature-it is, these young leaves that are harvested for medicinal use. *Mangifera foetida* grows in almost all climatic regions of Baringo County.

***Fig 4. 18; Mangifera foetida* (Source : Author, 2019)**

Herbs grown in Baringo County, but not natives of Baringo County

l.) *Zingiber officinale* –ZO (Ginger) (Source: Author, 2019)

Ginger plant is a grass-like plant that is classified in the family of *Zingiberaceae*. Botanically it is referred to as *Zingiber officinale*. It is an herbaceous perennial herb with pseudo stems. It is, the roots that are harvested as ginger root or ginger and used as medicine.

Fig 4. 19 ; Ginger root (Source : Author, 2019)

m.) *Cinnamomum verum-CV (Cinnamon)*

Cinnamon is a spice obtained from the inner bark of the tree genus *Cinnamomum,* which is an evergreen and aromatic plant belonging to the family of *Lauraceae.*

Fig 4. 20: Cinnamomum tree barks (Source : Author, 2019)

4.3 Laboratory Work-Biochemical Properties, Testing for Safety and Efficacy (Antidiabetic Activity) of the Two Most Used Antidiabetics

After taxonomical identification, description and extraction of all, the identified diabetic herbs of Baringo county, the two commonly used herbs (CE and UD), were further purified using column chromatography technique.

4.3.1 Process of Purification Of CEAnd UD Ethanolic Extracts

Column Chromatography

The figure below depicts how purification was done

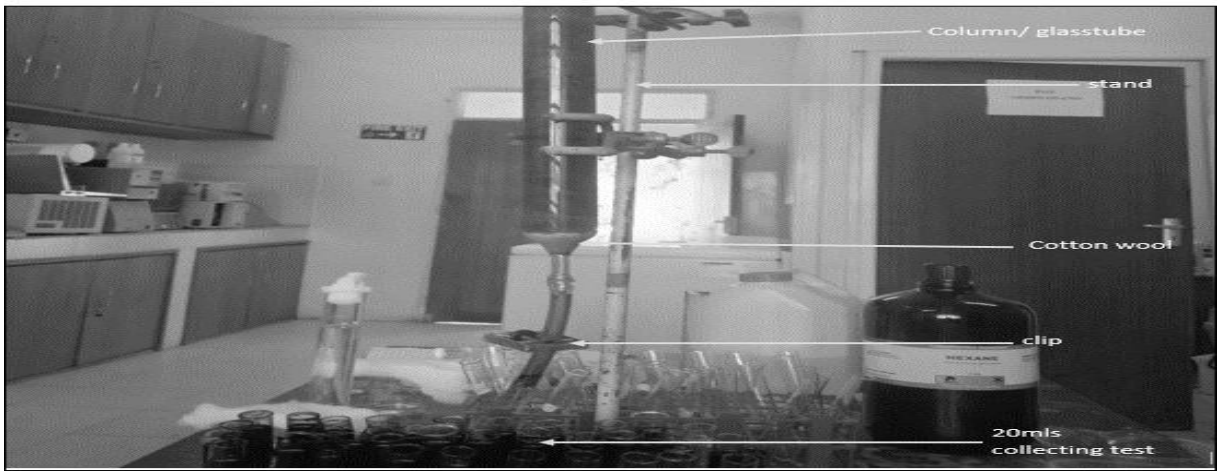


Fig 4. 21; An extract undergoing column chromatography

Figures 4.22 and 4.23, shows the final purified extracts of CE and UD

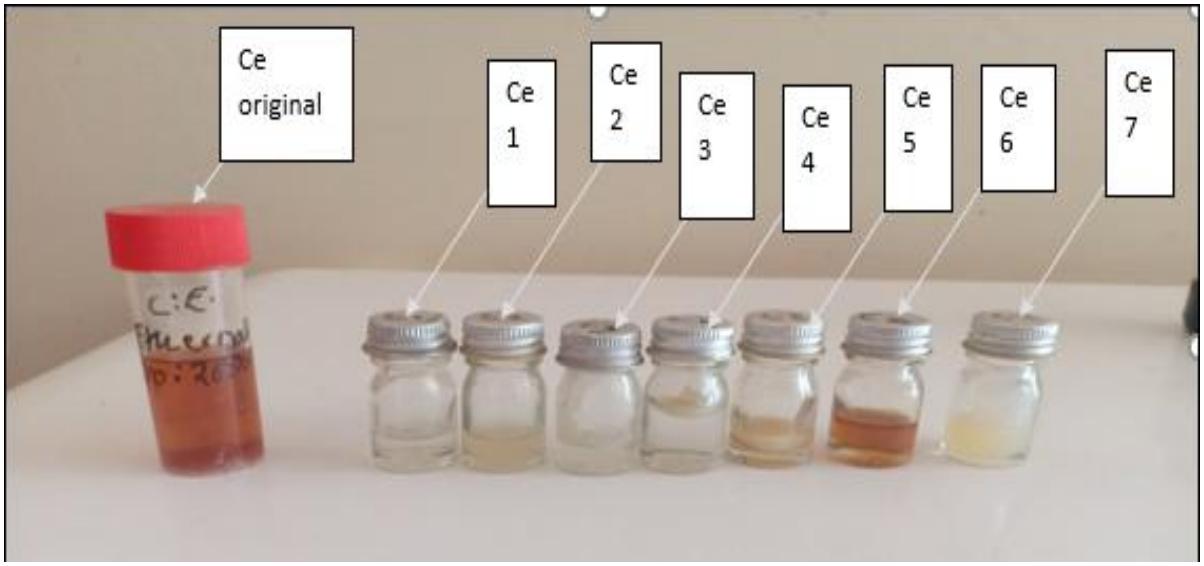


Fig 4. 22; Resultant purified ethanolic extracts CE

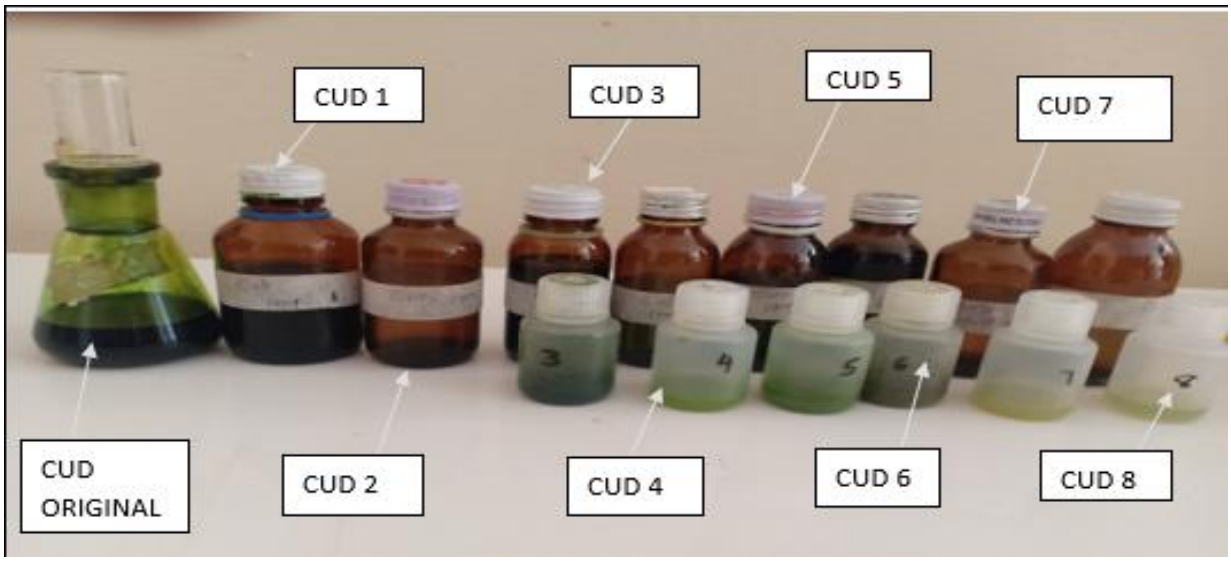


Fig 4. 23; Resultant ethanolic purified compounds of UD

Attached below is a sample of a TLC plate used to differentiate the various compounds of CE and UD.

Sample TLC used to differentiate the different purified compounds of CE and UD

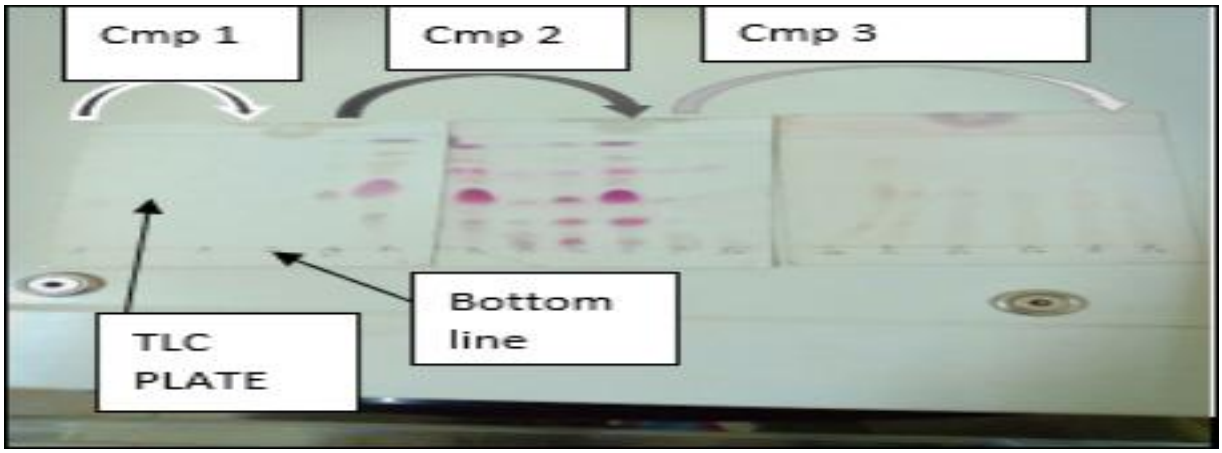


Fig 4. 24; One sample of thin layer Chromatography plate (TLC), used to distinguish the different compounds of CE and UD after column chromatography

Outcome of column chromatography purification

For ethanolic compound mixture of CE, seven (7) compounds were separated, as shown in the *figure 4.22* above and for UD ethanolic compounds, eight (8) different compounds were separated, as shown in the *figure 4.23* above.

The crude extracts of all the 13 herbs and the purified ethanolic versions of CE and UD, were then subjected to qualitative phytochemical analysis

4.3.2 Phytochemical Analysis Results

A. Crude Extracts of All the 13 Herbs

Results of the phytochemicals presented here, were based on the 13 herbs identified in Baringo County, each extracted using ethanolic and aqueous solvents (*procedure is described in 3.6.8).*

Table 4. 9; Phytochemicals results found in the diabetic herbs of Baringo county

		HERB																										
		WATER (AQEOUS) extracts														ETHANOL extracts												
		1	2	3	4	5	6	7	8	9	10	11	12	13a	13 b	1	2	3	4	5	6	7	8	9	10	11	12	13 a	13b
Phytochemicals		CE	U D	T C	A T	S N	Z O	C V	S B	H F	Z C	MF	EJ	TIn (s)	T1 n(p)	C E	U D	T C	AT	S N	ZO	CV	S B	H F	Z C	M F	EJ	TI (s)	T1(p)
1	Alkaloids	++	++	++	_	_	_	_	_	_	_	++	++	+	++	++	++	++	+	_	+	+++	_	_	+	+	+	+	++
2	Flavonoids	+++	+	++	++	_	+	+	_	_	+	++	+	_	+	++	++	++	++	_	+	+	_	_	_	++	_	_	+
3	Phenols	+++	_	_	+	++	_	_	_	+	_	++	_	+	_	++	_	_	++	_	_	++	++	_	_	_	_	+	_
4	Saponnins	+ +	+++	+++	+++	+++	++	++	+	+++	+++	+++	+++	+++	_	+++	+++	++	++	_	++	_	_	_	_	_	_	++	++
5	Tannins	+ +	_	_	_	++	_	_	+	++	+	_	_	+	_	_	_	_	+	_	_	++	+++	_	_	_	+	+	_
6	Quinones	_	_	++	+++	_	+	+	_	_	_	+	_	_	+	_	+	++	++	_	+	+	++	_	_	_	_	_	+
7	Oxalates	_	_	_	_	_	_	_	_	_	_	_	_	+	_	_	_	++	+	+	+	_	_	+	+	_	+	_	+
8	Terpenoids	+ +	_	_	_	+	_	+++	+	_	++	+	++	+	_	_	_	+	_	_	+++	++	++	+	+	_	_	++	+++
9	Glycosides	+++	+++	++	+++	_	+	+	_	_	+	+	+	_	+	+++	+	++	++	_	+	+	_	_	_	++	_	_	+
10	Steroids	+	+++	_	_	_	_	+	+++	_	_	_	_	+	_	+	_	+	_	_	+	+	++	_	+	+	+	+	_
11	Coumarins	+ +	+	++	+++	_	+	+	_	_	+	+	+	+	_	+	+	++	++	_	+	+	_	_	_	++	_	_	+
12	Sterols and Triterpenes	+	+	_	+	_	+	++	+	_	_	+	+	+	+	++	++	_	+	_	_	+++	++ +	_	++	+	+	+	_

13	Xanthones	++	_	+	_	+	_	++	_	++	+++	+++	+++	+++	++	_	_	++	++	_	++	++	_	++	_	_	++	++	+++
14	Catechins	++	_	_	_	_	++	_	_	_	_	++	_	+++	+	_	_	_	_	_	+++	+	+	_	_	_	+	++	+++
% presence of phytochemicals in reference to the total number of phytochemicals tested		12/14	7/14	7/14	6/14	5/14	7/14	9/14	5/14	4/14	7/14	11/14	8/14	11/14	7/14	8/14	7/14	9/14	11/14	1/14	11/14	12/14	7/14	3/14	5/14	6/14	7/14	9/14	10/14
		86	**50**	**50**	**43**	**36**	**50**	**64**	**36**	**29**	**50**	**79**	**57**	**79**	**50**	**57**	**50**	**64**	**79**	**7**	**79**	**86**	**50**	**21**	**36**	**43**	**50**	**64**	**67**

Key: CE –Carissa edulis, UD-Urtica dioica, AT-Aloe tweedie, ZO-Zingiber officinale, SB-Sorghum bicolor, CV-Cinnamomum verum, HF-Hypoestes forskaolii, SN- Solanum nigrum, TC- Tinospora cordofolia, MF-Mangifera foetida, ZC- Zanthoxylum chalybeum, EJ- Eriobotrya japonica, TIn (p) Tamarinda indica pulp and TIn (s) Tamarinda indica seed.

- A negative result, (denotes the absence of the phytochemical)

+ Positive low result

++ Moderate levels of the phytochemical

+++ High level of the phytochemical

4.3.2.1Analysis of the phytochemical screening results

Aqueous extracts, in general, extracted a greater number of phytochemicals compared to ethanolic extracts in the herbs tested.

Certain phytochemicals were better extracted in either water or ethanol. For example, alkaloids are better extracted in ethanol than water, while the opposite is true with saponnins.

In general, the most readily available phytochemical in aqueous extracted herbs was saponins. Saponins were detected in all the thirteen aqueous extracted herbs with either moderate (++) or high (+++) colour intensities. The least detected phytochemical in aqueous extracted extracts was oxalates. With regards to aqueous extracts, CE, MF and TI(s*)* recorded the highest number of phytochemicals tested while HF recording the least. Flavonoids, saponnins, phenols, and glycosides were most abundant in CE, while in MF and TI(s), saponnins and xanthones were the most abundant. In the ethanolic extracts, CV had the highest number of phytochemicals tested followed closely by ZO and AT. The least was HF. Terpenoids and catechins were rich in ZO while alkaloids and sterols were rich in CV. *Aloe tweedie* recorded moderate strength of flavonoids, phenols, saponnins, quinones, glycosides, coumarins and xanthones phytochemicals.

b. Purified compounds of CE and UD phytochemicals

(i) CE

Table 4. 10; CE purified extracts phytochemicals

	CE							
	Selected phytochemicals	**mobile phase -Hexane and Ethyl acetate**						**mobile phase - Methanol**
		CE original	**CE1**	**CE2**	**CE3**	**CE4**	**CE5**	**PCE**	**CE7**
1	Alkaloids	+ ++	+ +	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++
2	Flavonoids	+ ++	+ +	+ +	-	+	+ ++	+ ++	+ ++
3	Phenols	+ ++	-	-	-	-	-	+ ++	-
4	Saponnins	+ +	-	-	-	-	+ ++	+ ++	-
5	Tannins	+ +	+	+	-	-	+	+ ++	-
6	Quinones	-	-	-	-	-	-	-	-
7	Oxalates	-	-	-	-	-	-	-	-
8	Terpenoids	+ +	-	-	-	-	+ ++	+ ++	+ +
9	Glycosides	+ ++	+ +	+ ++	-	+	+ ++	+ ++	+ ++
10	Steroids	+	-	-	-	-	-	+	-
11	Coumarins	+ +	+ +	+ ++	-	+	+ ++	+ ++	+ ++
12	Sterols and Triterpenes	+	-	-	-	-	-	+	-
13	Xanthoproteins	+ +	+ +	+ +	+	-	+ +	+ ++	-
14	Catechins	+ +	-	-	-	+	+	+	-

Based on the above table compound CE6/[PCE], compared to other compounds had more phytochemicals. The most prevalent phytochemical in all the seven compounds was alkaloids, followed by flavonoids, glycosides and coumarins. Other compounds like sterols, catechins and steroids were present though in traces. No quinones and oxalates was found in this compound.

(i) UD

Table 4. 11; UD purified extracts phytochemicals results

Based on the above results compound UD 6 [PUD] had more phytochemicals properties compared to the rest of the compounds in group. These compounds include alkaloids which were the most prevalent followed by flavonoids, steroids and coumarins. Saponnins, oxalates, sterols and glycosides were also present though in traces.

	UD								
	Selected phytochemicals	**mobile phase Hexane and Ethyl acetate**						**Methanol as the mobile phase**	
		UD original	**UD 1**	**UD 2**	**UD 3**	**UD 4**	**UD 5**	**PUD**	**UD 7**	**UD 8**
1	Alkaloids	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++	+ ++
2	Flavonoids	+ +	-	-	-	-	+	++	-	-
3	Phenols	-	-	-	-	-	-	-	-	-
4	Saponnins	+	-	-	-	-	-	+	-	-
5	Tannins	-	-	-	-	-	-	-	-	-
6	Quinones	-	-	-	-	-	-	-	-	-
7	Oxalates	+	-	-	-	-	-	+	-	-
8	Terpenoids	-	-	-	-	-	-	-	-	-
9	Glycosides	+	-	-	-	-	-	+	-	-
10	Steroids	+ +	-	-	-	-	+	++	-	-
11	Coumarins	+ +	-	-	-	-	+	++	-	-
12	Sterols and Triterpenes	+	-	-	-	-	-	+	-	-
13	Xanthoproteins	-	-	-	-	-	-	-	-	-
14	Catechins	-	-	-	-	-	+	+	-	-

After determination of phytochemicals, both CE and UD ethanolic purified extracted extracts were selected, lyophilized, percentage yield determined and used in the subsequent studies

4.3.3 Toxicity Studies of CE and UD -FETAX Studies (In Vitro Studies)- Determining the Safe Dosages to be Used in Rats Protocol

4.3.3.1 Developmental Toxicity Results

The results were divided into THREE main groups namely;

1. CE extracts (Aqueous, ethanolic and purified ethanolic)
2. UD extracts (Aqueous, ethanolic and purified ethanolic)
3. Mixtures of CE+UD (Aqueous, ethanolic and purified ethanolic)

4.3.3.2 CE extracts

Parameters studied were;

a. Embryotoxicity/mortality
b. Teratogenicity and
c. Growth and development

Embryotoxicity/mortality

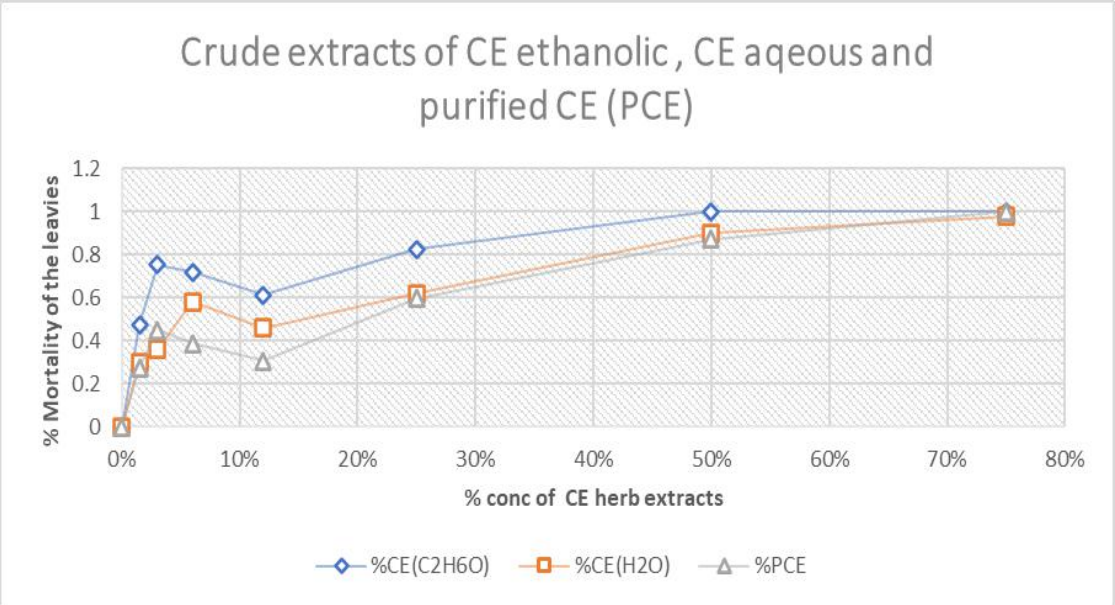


Fig 4. 25; Comparison of deaths from crude extracts of CE ethanolic, CE aqueous and purified CE (PCE)

Zero percent (0%) Concentration was the controls.

The figure 4.25 above, shows the results of mortality of frog embryos from; *Carissa edulis* extracted with ethanol, water/aqueous and the purified version of Carissa *edulis* extracts. Comparing the three solvents- water, ethanolic and purified ethanolic extracts; purified ethanolic extracts had the lowest mortality numbers across the board, followed by water extracted extracts. The highest mortalities were recorded in crude ethanolic extracts. LC_{50} calculated and converted to LD_{50}.

LD_{50} of CE crude ethanolic, Purified CE, and aqueous CE were 4,322.67mg/kg, 1,570mg/kg and 14,433.3mg/kg respectively.

Teratogenicity

Figure 4.26 below, depicts the abnormalities of the live embryos noted after the 96hr period, exposed to extracts of CE and UD

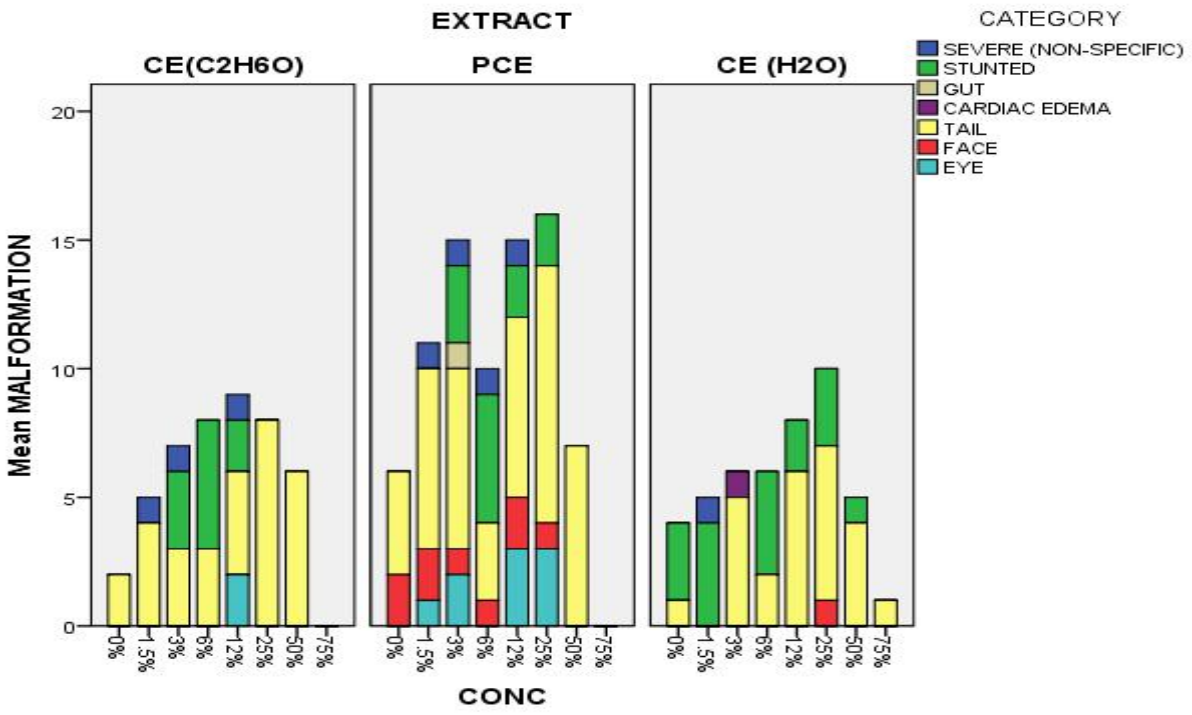


Fig 4. 26; Comparison of malformations of crude extracts of CE ethanolic, CE aqueous and purified CE (PCE)

The graphs above show the type of abnormalities observed after 96hrs of ethanolic aqueous and purified version of CE exposure. Note that abbot correction formula was used to adjust the abnormalities -to take care of other environmental factors that might have caused the deaths of the laevis.

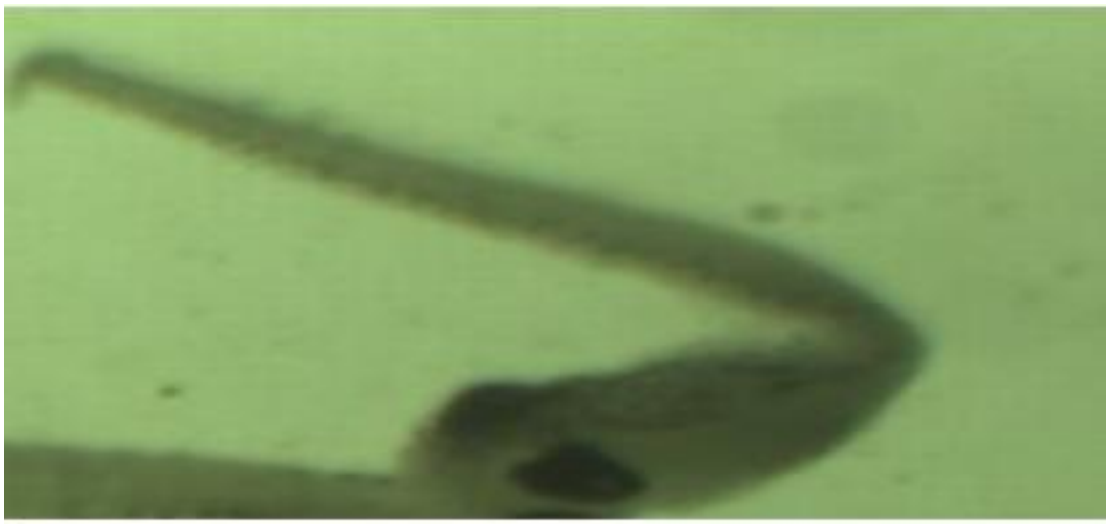
With regards to the types of malformations, tail malformations accounted for most of the abnormalities, followed closely with stunted growth and the least were recorded in the gut.

Purified extracts recorded the highest number of abnormalities, followed by CE water. EC_{50} concentration was recorded at 16.42%, 34.38% and 13.77% for CE ethanolic, CE aqueous and purified version of CE ethanolic respectively.

Some of the Malformations /abnormalities, noted from crude ethanolic and water concentrations of CE

a. <u>Ethanolic malformations</u>

(i)



Severe lateral flexure of the tail seen at 1.5% concentration

(ii)

(iii)

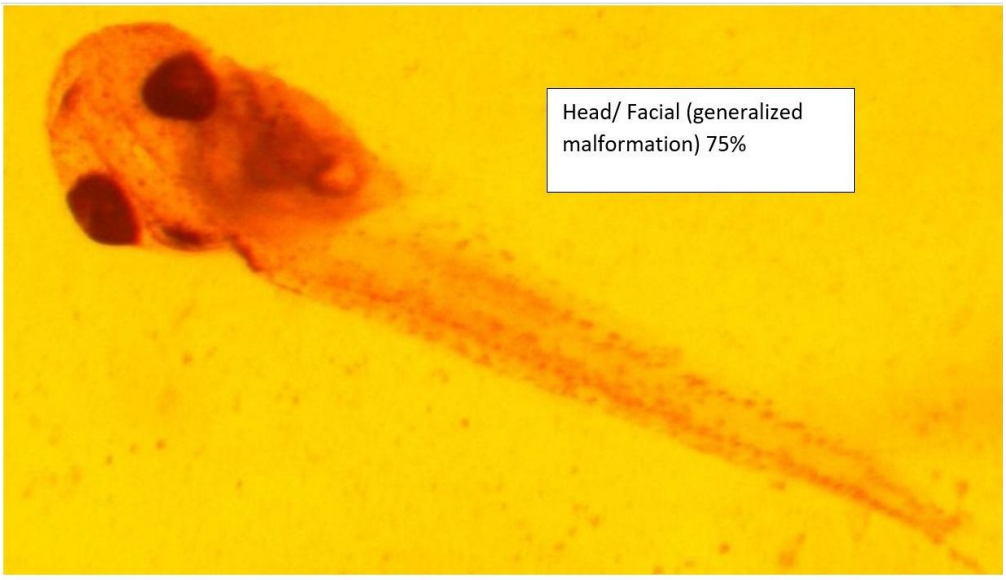


Fig 4. 27; CE ethanolic malformations

b. Aqueous CE malformations

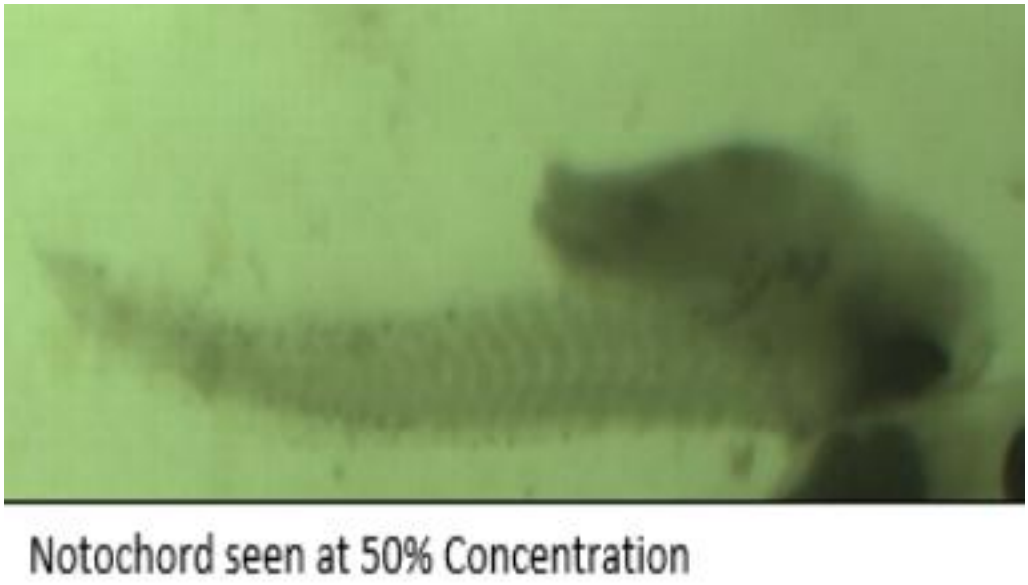


Fig 4. 28; Aqueous CE malformations.

c. Purified ethanolic extracts sampled malformations

Severely edematous face abnormality.50%

Fig 4. 29; PCE Edematous face seen 50%

Growth /development-length in mm

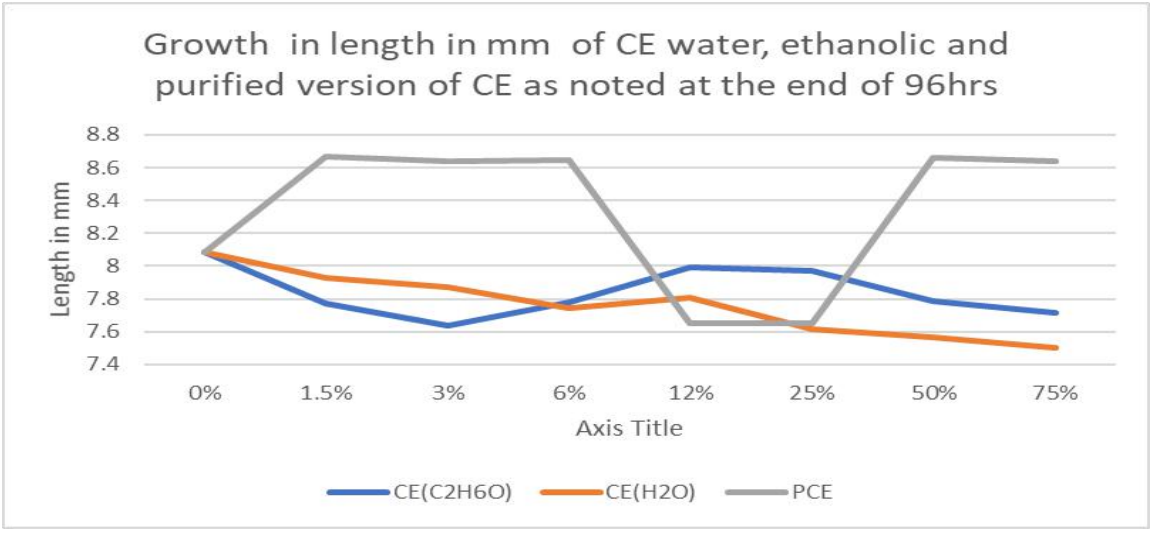


Fig 4. 30; Growth in length in mm of CE water, ethanolic and purified version of CE as noted at the end of 96hrs

The average length in the controls was recorded at 8.08mm and therefore used as base in reference to the concentration. Also in this experiment, 0% concentration was the controls.

In crude aqueous extracts of CE, steady decline was seen as you move from 0% concentration to 75% while in crude ethanolic extracts, the length decreased from 0%

conc to 3%, then a slight increase to conc 12%, and then a steady slight decrease to conc 75%.

Regarding the purified version of CE (PCE), other than 12% and 25%, concentration, the other concentration recorded lengths higher than controls at 8.08mm (the controls).

Teratogenicity index (TI)

Teratogenicity index (TI) with regards to crude ethanolic extracts was $=LC_{50}/EC_{50}$ 5.59/16.42= 0.34, crude water extracts was = LC_{50}/EC_{50} 5.79/34.58=0.167 and purified extracts at 0.66.

4.3.3.3 *Urtica* dioica (UD extracts

Three parameters were studied;

a. Embryotoxicity/mortality
b. Teratogenicity and
c. Growth and development

Embryotoxicity/mortality

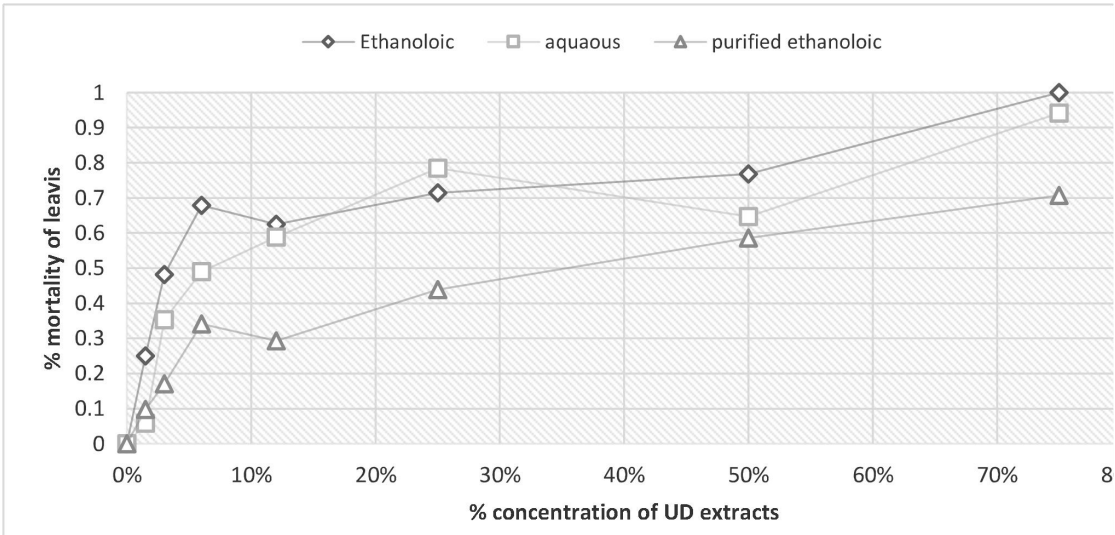


Fig 4. 31. Abbott adjusted proportionate mortality of embryos exposed to different concentrations of crude ethanolic &water and purified ethanolic extracts of UD

Zero percent (0%) was the controls

From the results there is a general steady increase of mortality between 0% and up to around 6%.

Highest mortalities were recorded in crude ethanolic, followed by aqueous and last the purified version of ethanolic extracts. As the concentration increases in all the extracts, the number of mortalities increases. Purified version of ethanolic extracts recorded the lowest number of mortalities while the crude version recorded the highest. LD_{50} of crude ethanolic extracts was at 21,706.3 mg/kg, water/aqueous extracts were at 3,955/kg and the purified ethanolic extract was at 21mg/kg.

Teratogenicity

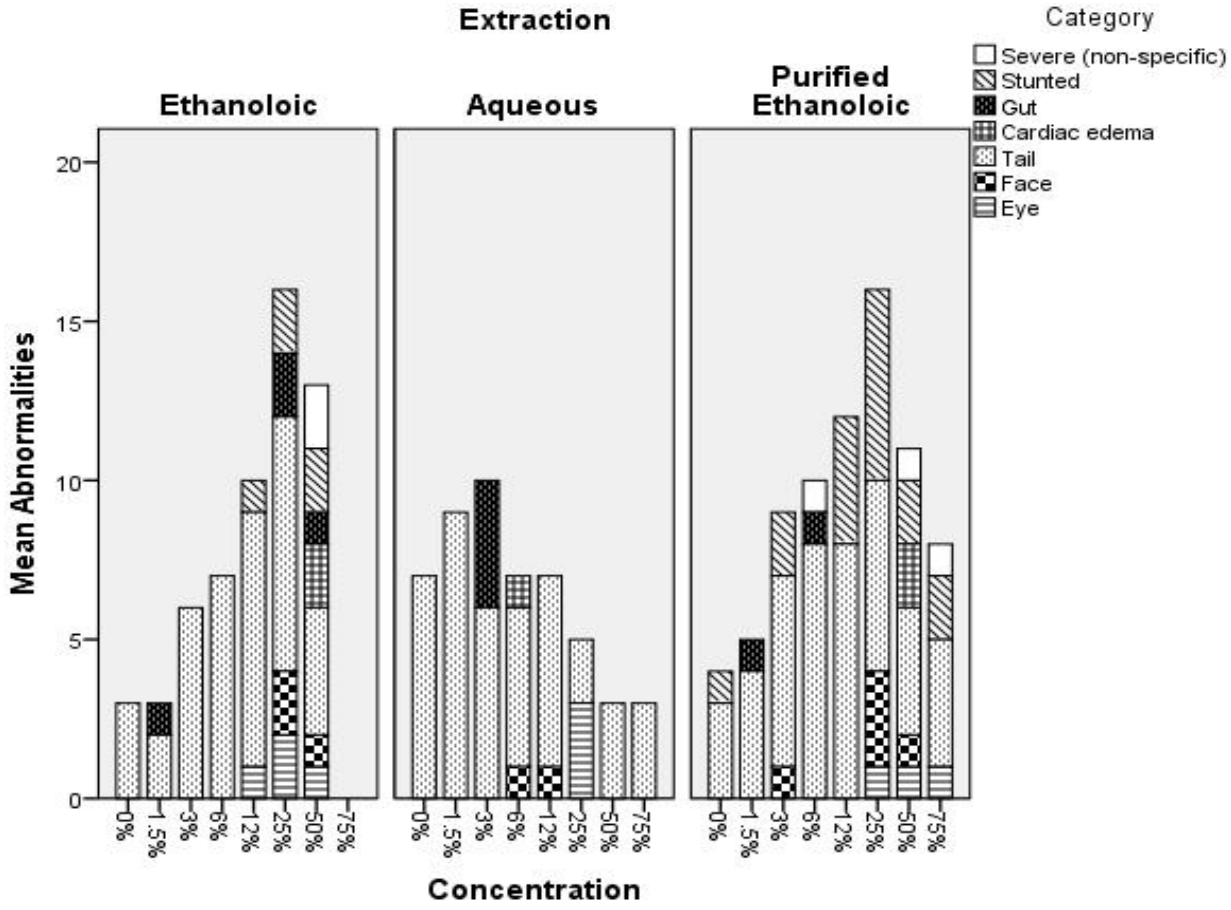


Fig 4. 32; Proportionate malformations of embryos exposed to different concentration of the three extracts (crude ethanolic, crude aqueous and purified ethanolic)

Figure *4.32* above describes the malformations noted after the 96-hour exposures of the frog embryos to the different extracts. The abnormalities were based on the live embryos noted after the 96hr as in CE extracted extracts.

With regards to crude ethanolic extracts, at 75% concentration, there were no laevis to be examined since all of them had died, while in water extracts, there were some few, though not compared to the purified version of ethanolic extracts.

The most common abnormality noted across all the extracts were the tail malformations, followed by stunted growth which also was geared up by the increases in the concentrations.

Table 4.12 below summarizes the EC_{50} in relation to the abnormalities.

Table 4. 12; UD EC_{50} determination

UD extract	chemical extractor	experimental condition	% EC50
Ethanolic	98% ethanol(crude)	96h EC_{50} (FETAX)	11.09%
Aqueous	distilled water(crude)	96h EC_{50} (FETAX)	1.45E+18
Purified ethanolic	98% ethanol (column purification)	96h EC_{50} (FETAX)	22.06%

From the above table, crude extracts distilled water had the highest percentage of EC50 followed purified ethanolic extracts. The lowest being crude ethanolic extracts

Teratogenic index (TI)

Teratogenic index (TI) with regards to ethanolic crude UD extracted extracts was (LC_{50}/EC_{50} 4.47/11.09) 0.403, while in water crude extracts was (LC_{50}/EC_{50} 9.93/1.45E+20)6.44E-20. Ethanolic purified extracts=27.29/22.05 = 1.24.

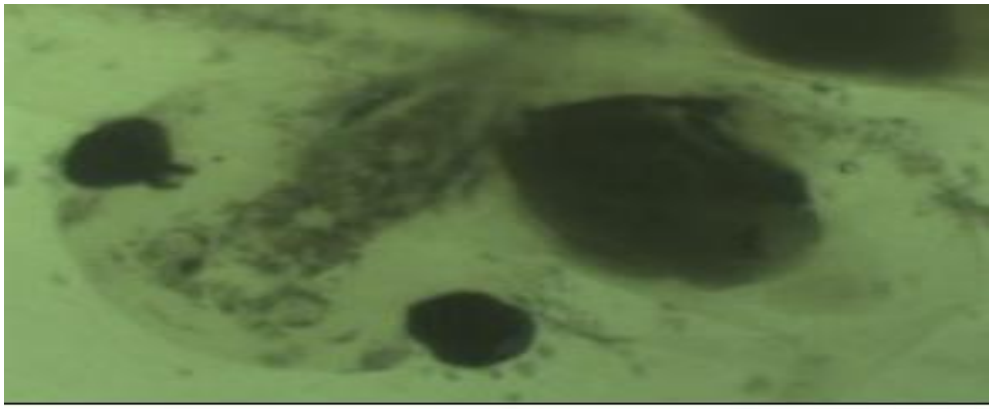
Some of the Malformations /abnormalities as noted from crude ethanolic, crude water and UD purified ethanolic

(a) Ethanolic

(i)

Fig 4. 33; UD ethanol ethanolic malformation

(b) Water (aqueous)

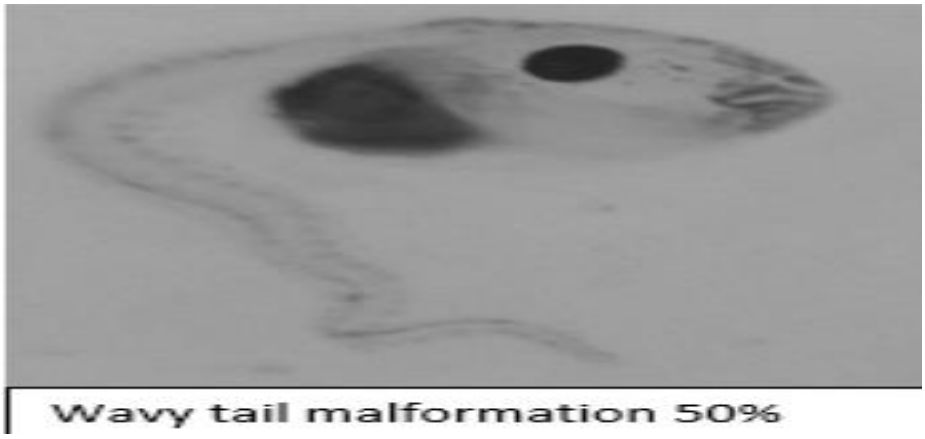


facial malformation with depigmentation at 12.5%

Fig 4. 34; UD aqueous malformations

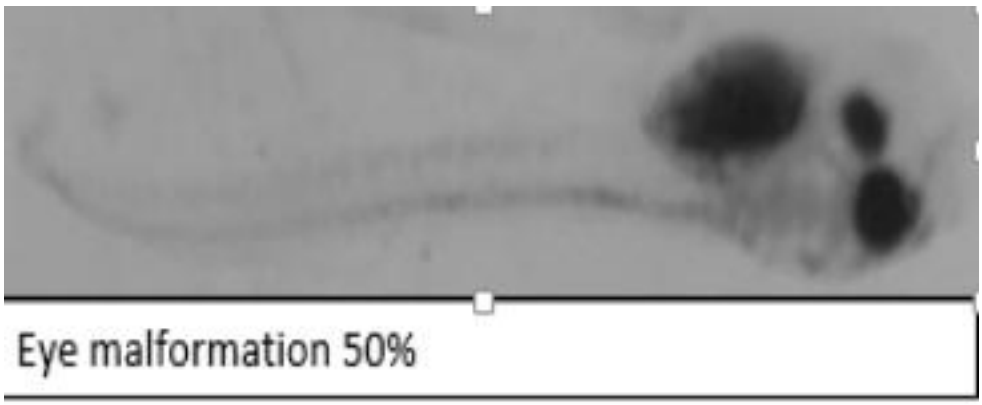
Purified UD

(i)



Wavy tail malformation 50%

(ii)



Eye malformation 50%

Fig 4. 35; Purified UD malformations

Growth in length (mm)

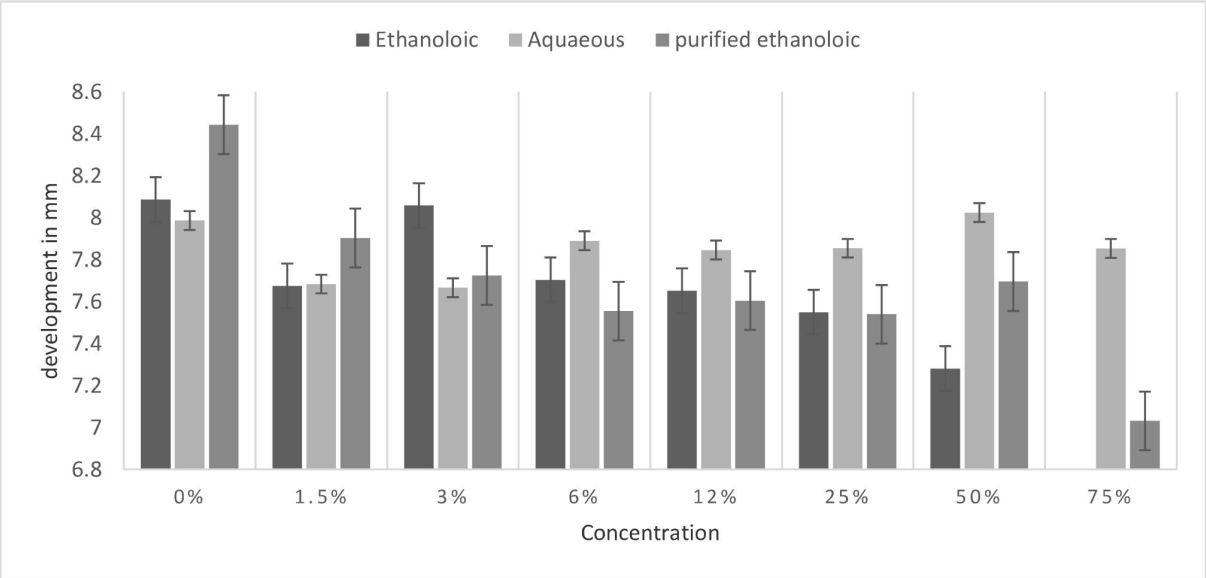


Fig 4. 36; Comparison of growth and development, with regards to the extracts UD extracts

Generally, controls had the highest length compared to cases. Comparing crude extracts cases (from 1.5% to 50% conc), water in general across all the concentrations, had a higher length compared to ethanolic extracts with exception of 3% concentration. The purified version of ethanolic extracts had a higher length between 1.5% and 3% compared to the crude version and vice versa between 6% and 50%.

In the cases, the lowest length was at a concentration of 3% at 7.666mm and highest at a concentration of 50% at 8.0434mm.

Mixtures of UD and CE ethanolic & aqueous and their purified ethanolic version (PUD+PCE)

Mortality/embryotoxicity

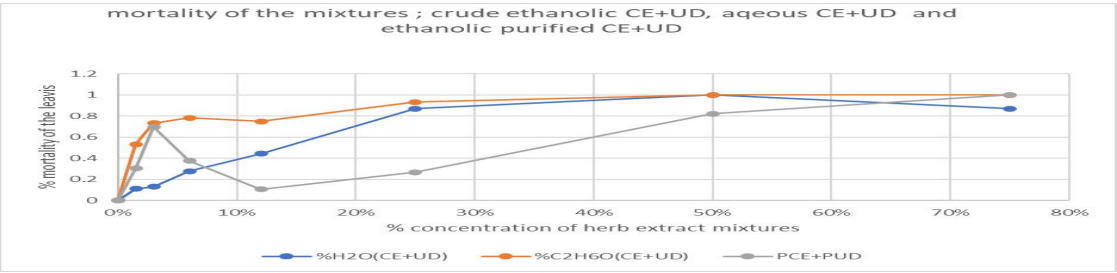


Fig 4. 37; Mean mortality seen in UD+ CE 50/50 water mixtures, UD + CE 50/50 ethanolic mixtures and purified version of ethanolic mixtures

From the figure 4.37, above lowest deaths were seen ethanolic purified mixture, followed by water mixtures. Highest deaths were seen at crude ethanolic mixtures.

The concentration that can kill 50 percent (LC_{50}) of the laevis exposed to ethanolic mixtures, aqueous mixtures and purified ethanolic mixtures was 4,686.2mg/kg, 2,127/kg and 17.56mg/kg, respectively.

Teratogenicity

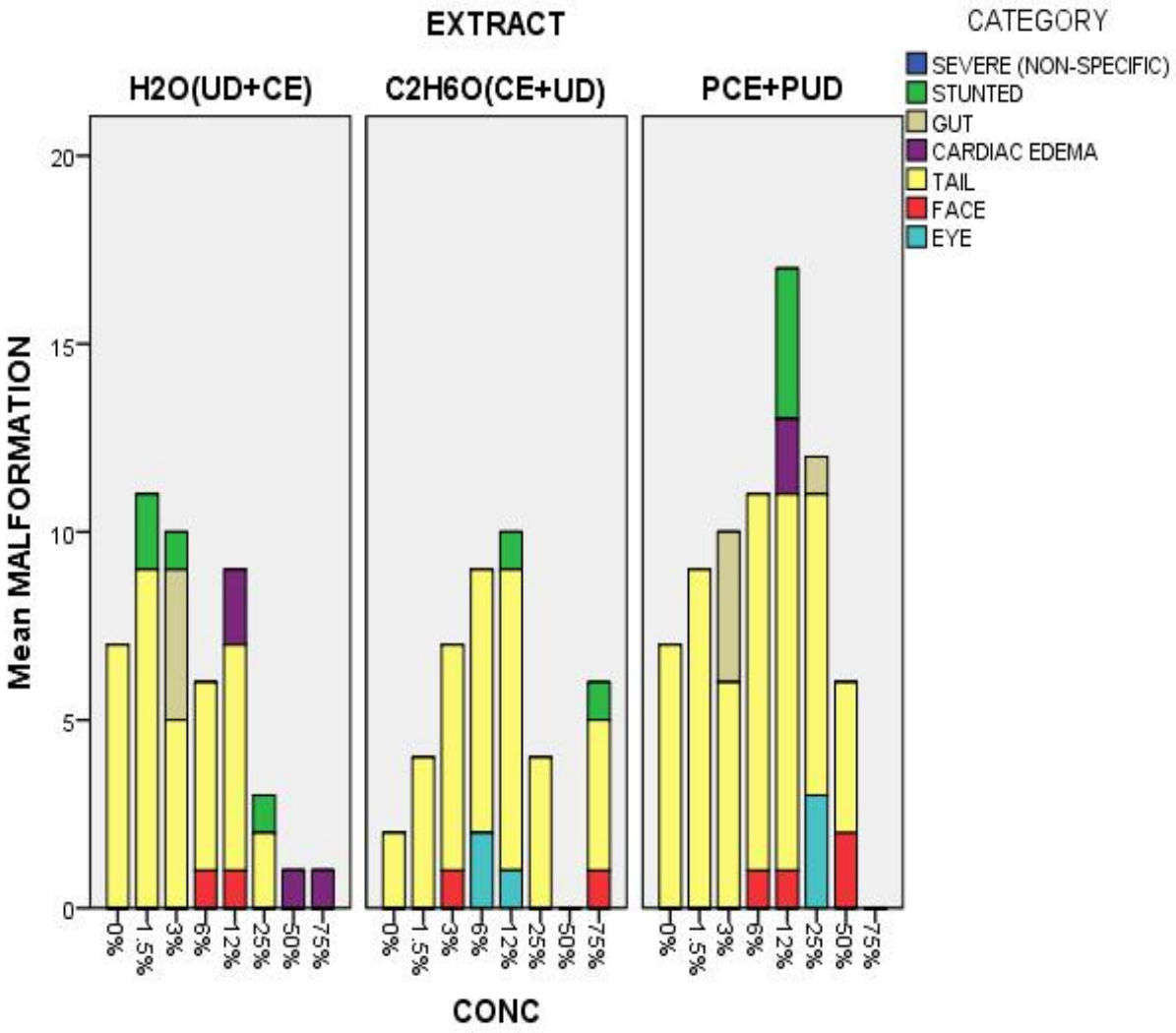


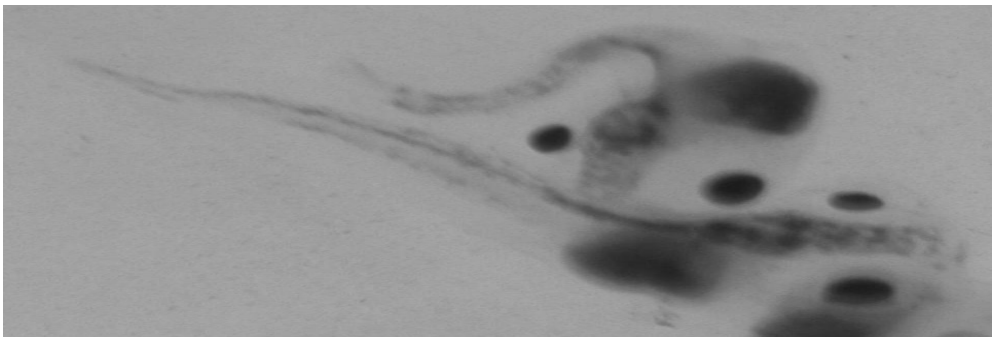
Fig 4. 38; Abnormalities seen in UD+ CE 50/50 water mixtures and UD + CE 50/50 ethanolic mixtures and purified ethanolic mixtures

The majority of the abnormalities noted with the mixture's extracts were in the tail. More malformations noted on purified ethanolic, compared to the crude extracts.

No live laevis was seen at 50% crude aqueous mixture concentration, hence the absence of recorded abnormalities to them.

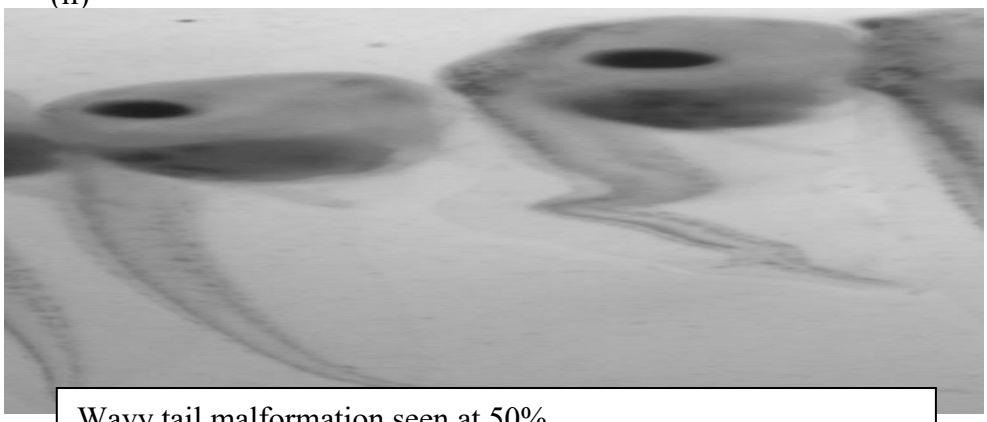
Selected Pictures of Abnormalities Exposed to The Mixture of CE, UD (Aqueous and Ethanolic) and Purified Mixtures of CE+UD

(i)



Complex Flexure of the tail end seen at 50%-crude ethanolic

(ii)



Wavy tail malformation seen at 50%

Fig 4. 39; Tail malformations 50% PCE+PUD

Growth in length

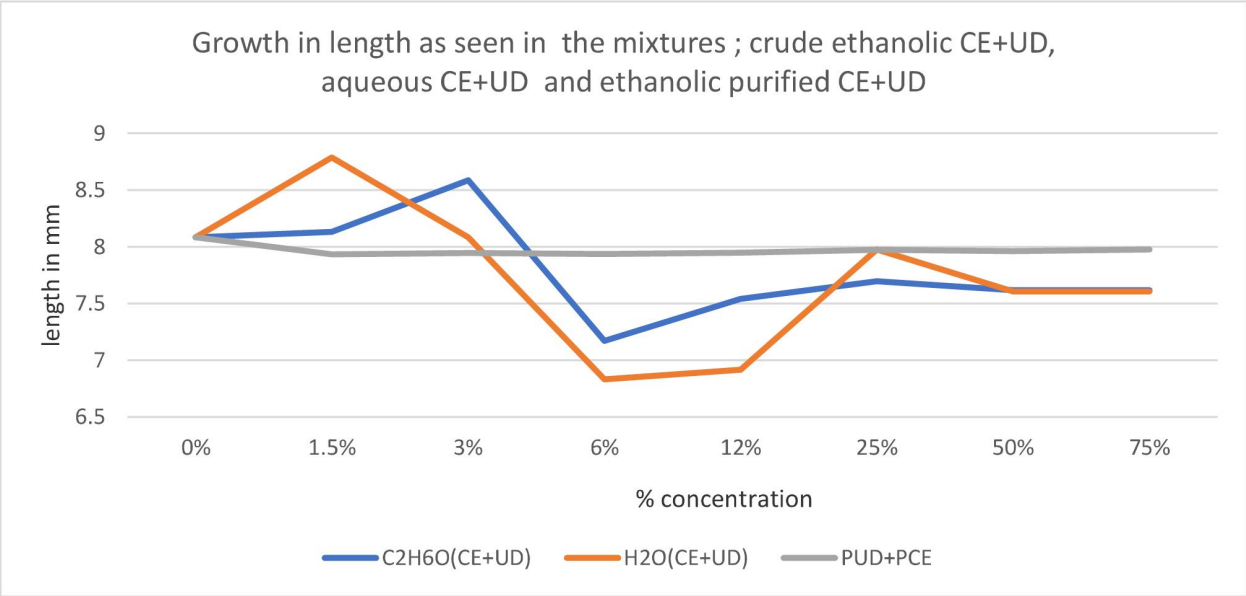


Fig 4. 40; Growth in length (mm) seen in UD+ CE 50/50 water mixtures and UD + CE 50/50 ethanolic mixtures and purified ethanolic mixtures

Looking at the figure 4.40 above, there is almost no changes in length in purified mixtures compared to the crude extracts. From concentration 3% onwards, crude extracts somehow experienced a downward trend.

Effective concentration (EC_{50}), calculation

The concentration that can cause 50 percent malformation on laevis exposed to crude ethanolic extracted extracts of CE+UD mixtures was 4.16%, in water extracted extracts mixtures was 6.2% and purified (PUD+CE) was 4.87%

Teratogenic index (TI)

TI of crude ethanolic and crude aqueous mixtures was at 1.56 and 2.43 respectively, while that of purified ethanolic mixtures was at 17.44.

4.3.3.4 Description selected malformations

Wavy tail

Though there was quite a number of tail malformations across all the extracts, one such prominent tail malformation was a wavy tail. An abnormality characterized by complex axial abnormality in which the distal portion of the tail is flexed and a slight dorsal flexure of the axis is present (Bantle, Finch, *et al.*, 1991). See the figure 4.47 below

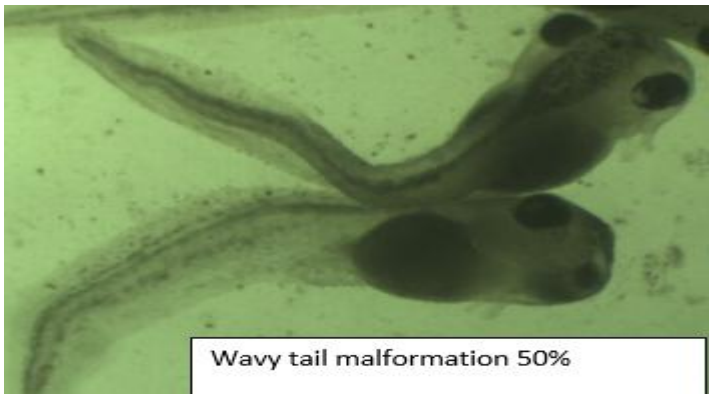
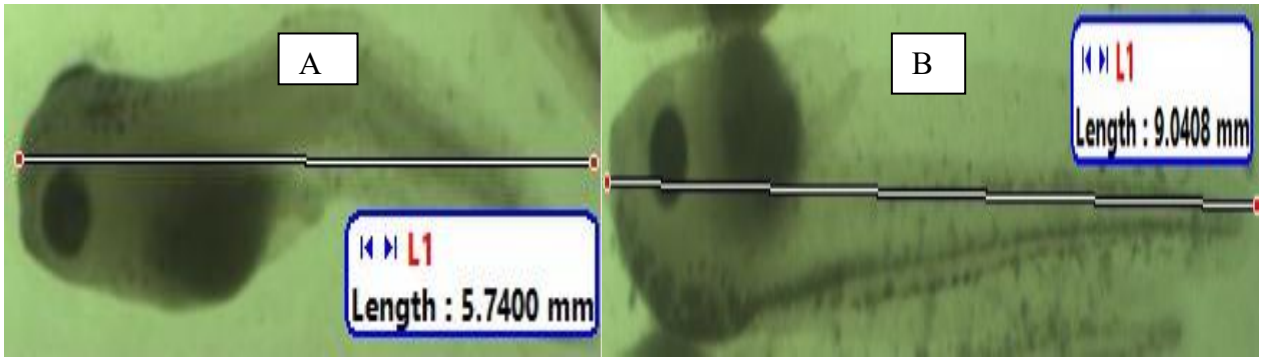


Fig 4. 41; UD ethanol purified Wavy tail seen at 50% concentration

Stunted growth

The other common noted abnormality was a stunted growth. Stunted growth is a disorder in which the tadpole is shorter compared to others on the same normal stage/age. **See figure 4.42** below; In the figure, two tadpoles were picked from 50% of ethanol crude extract. Toad pole A is shorter in length than toad pole, B. It therefore means toad pole A is stunted, compared to toad pole B.

Fig 4. 42; UD crude ethanol stunted growth



Severe abnormality -denotes to that type of malformations in which the tadpole contains abnormalities that cannot be placed among the other types of known abnormalities. Usually a range of multiple malformations. In **figure 4.43** below, the tadpole was obtained from 75% of the CE ethanol purified. The tadpole does not have defined eyes, stunted, no defined gut and also the no clear head features.

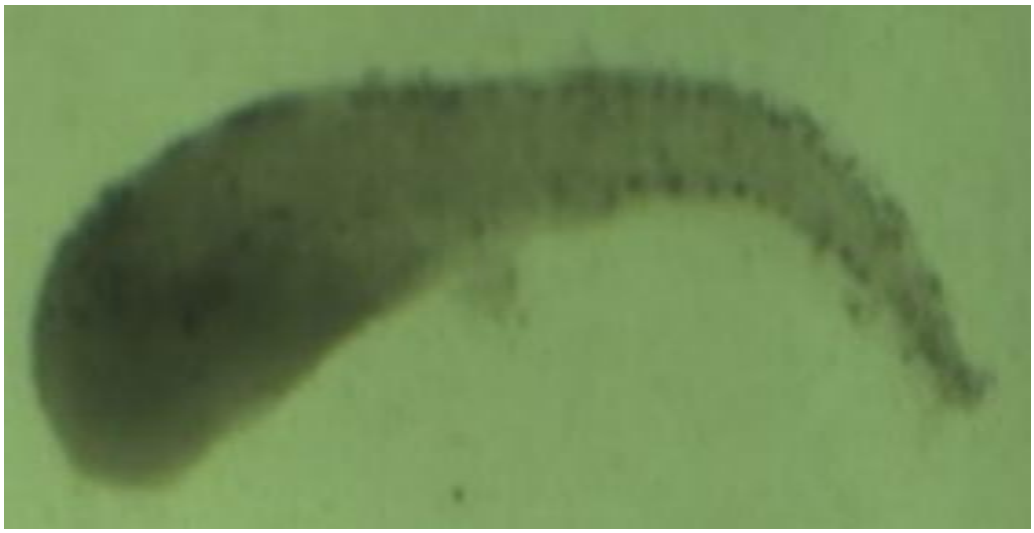


Fig 4. 43; CE ethanol purified severe edema at 75%

4.3.3.5 Summary of LC_{50}, LD_{50}, EC_{50} AND TI

	HERB	ETHANOLIC EXTRACTS						WATER/AQEOUS EXTRACTS				
	UNPURIFIED (CRUDE)											
		LC_{50} % CONC	LC_{50} in mg/l	LD_{50} in mg/kg	EC 50 % CONC	TI		LC_{50} % CONC	LC_{50} in mg/l	LD_{50} in mg/kg	EC_{50} % CONC	TI
1	CE	5.59	**4,322.7**	**4,322.67**	16.42	0.34	4	5.79	**14,433.3**	**14,433.3**	34.58	0.17
2	UD	4.53	**21,706.3**	**21,706.3**	11.09	0.41	5	9.33	**3,955**	**3,955**	1.45E +20	6.44 E-20
3	CE +UD	6.47	**4,686.2**	**4,686.2**	4.16	1.56	6	15.20	**2,127**	**2,127**	6.26	2.43
	PURIFIED ETHANOLIC EXTRACTS											
		LC_{50} % CONC	**LC_{50} in mg/l**	**LD_{50} in mg/kg**	EC $_{50}$ % CONC	TI						
7	CE	9.13	**1570**	**1,570**	13.77	0.66						
8	UD	27.79	**21**	**21**	22.05	1.26						
9	UD+CE	84.94	**1,71.5l**	**1, 71.56**	4.87	17.44						

Table 4. 13; LC_{50}/LD_{50}, EC_{50} and TI summaries

(Embryotoxicity) LD_{50}

From at table *4.13 above*; comparing UD ethanolic extracted extracts with CE extracted extracts, CE ethanolic extracted extracts are slightly toxic (LD_{50} of than 4,321.67mg/l, and 21,706.3 mg/l 4, respectively), A finding similar to crude aqueous extracts, (14,433.3mg/kg, and 36,916.7mg/kg respectively).

Generally, water extracted extracts are more toxic compared to ethanolic extracted extracts.

With regards to purified extracts, UD extracts are more toxic compared to UD (LD_{50} of 21mg/l, and 1570mg/l, respectively).

Purification of extracts generally increased the level of toxicity across the board.

Teratogenicity

Table 4. 14; Interpretation of teratogenic index of crude and purified extracts

Crude extracts			
		TI	Interpretation
Ethanolic	CE	0.34	No big worry on developmental hazard (not teratogenic)
	UD	0.41	No big worry on developmental hazard- (not teratogenic)
	CE&UD	1.56	Increasing developmental hazard
water	CE	0.17	No big worry on developmental hazard-not teratogenic
	UD	6.44E-20	No big worry on developmental hazard-not teratogenic
	CE&UD	2.43	Increasing developmental hazard
Purified			
Ethanolic	PCE	0.66	No big worry on developmental hazard-not teratogenic
	PUD	1.26	Increasing developmental hazard
	PCE+PUD	17.44	Of great concern

Key;

Non- Teratogenic
Increasing chances of a developmental hazard
Of great condemn-could highly likely to be teratogenic.

4.3.4 Efficacy/Antidiabetic Activity Results -Rats Protocol

The dosages used in efficacy studies was obtained from the analysis of FETAX studies LD 50 (see *3.6.11*).

The results were divided into;

(a) Crude samples efficacy (crude extracts of ethanolic and aqueous extracts of CE, UD and their mixtures) and

(b) Purified samples efficacy (purified samples of ethanolic CE, UD and their mixtures).

4.3.4.1 Crude samples efficacy studies

Introduction

The analysis was based on 35 rats that were randomly assigned to 7 treatment groups (CE Ethanol, CE Aqueous, UD Aqueous, UD Ethanol, UD & CE Aqueous, UD & CE Ethanol, and Metformin). The first 6 treatment groups were treated as cases and the last group, (the seven-given metformin) treated as control. Each treatment group had 5 rats allocated. At the end of the experiment 2 rats of the total 35 died. All the deaths occurred in the CE groups (One in ethanolic extracts extracted group and also one in aqueous extracts extracted group) No death witnessed in all the UD groups.

The weight of the rats was taken before the start of the experiment and after the experiment. The sugars level was taken 4times (at 0hr, fed with the drug then sugar measured at 1hr, 2, 3hrs) per animal for three weeks (each 4th day of the week). The rats were fed at 0.5mls/100g/rat [(Daradka *et al.*, 2014; Muralidharan, 2015] as shown in table 3.11 and the for controls, they were given , metformin orally at 100mg/kg as described by (Cheng *et al.*, 2006).

NOTE: Diabetic status in the rats was induced in all the groups using 140mg/kg of alloxan

Weight distribution

Table 4. 15; Rats weight distribution

	Treatment extracts	Mean (gms)	Median (IQR) (gms)	Minimum w (gms)	Maximum W (gms)
Cases	a. Ethanol CE Roots Extract	201.04(4.1)	200(197.2, 203)	197	208
	b. Aqueous UD Leaves Extract	208.98(4.2)	210.9(209, 211)	201	213
	c. Aqueous CE Roots Extract	201.48(2.8)	201(2007, 203.9)	196.8	205
	d. Ethanol UD Leaves Extract	180.60(3.3)	180.1(177.6, 183.7)	176.6	185
	e. Ethanol UD Leaves Extract & CE Roots Extract	171.40(2.6)	171.7(169.2, 173.7)	167.8	174.6
	f. Aqueous UD Leaves Extract & CE Roots Extract	189.00(3.1)	190.1(187.2, 191)	183.9	192.8
Controls	g. Metformin	210.94(3.0)	211(208, 213.7)	207.1	214.9

From Table 4.15 above, the weight range mean in cases was between 171.4 to 208.98g and for controls it was 210.94±3. There was a significant difference in average rats' weight between treatment groups ($p<0.001$) in all the groups, though with regards to CE, post-hoc test showed both the ethanolic and aqueous were statistically comparable in average weight. Nevertheless, the dosage that was used of 0.5mls per 100 grams of a rat in all the groups (control and cases) made the rats equal in terms of dosages. See the graphically the weight distribution box plots in figure 4.44 below

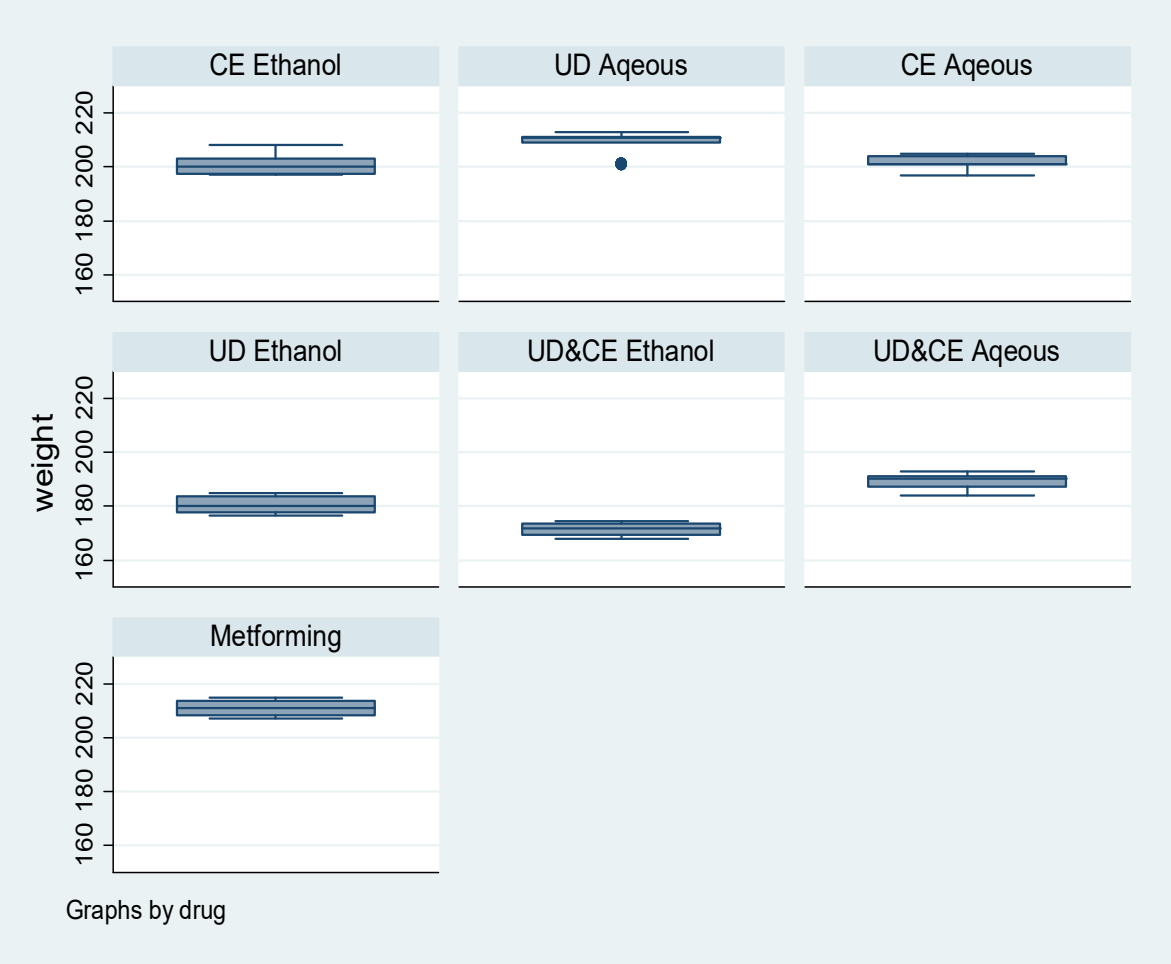
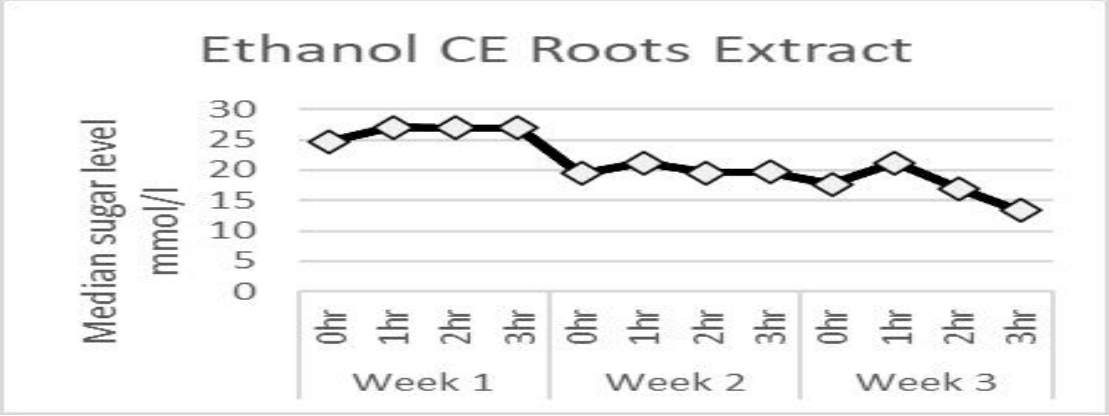


Fig 4. 44; Weight distribution box plots

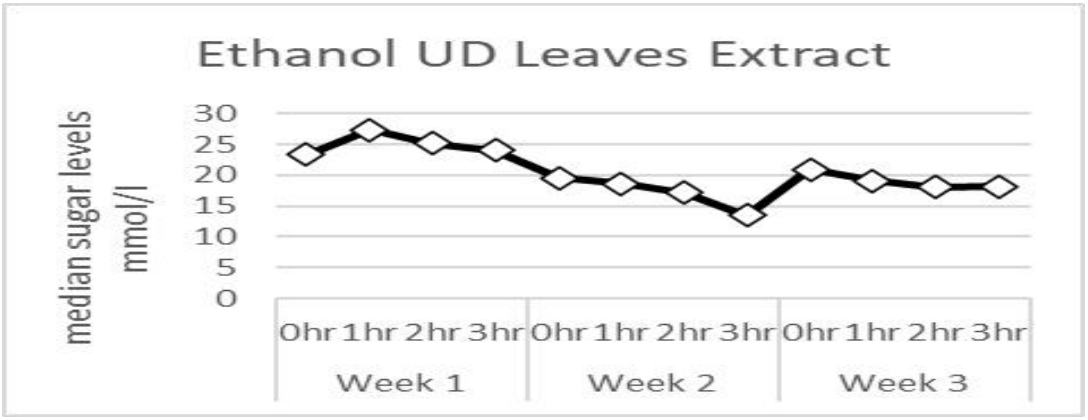
General sugar reduction trends in the 3 weeks in both the cases and controls

In general, the average blood sugar level trends over 3 weeks period were, as shown in Figure 4. 45 below, considering the experiment was done three time (3 weeks) and sugar levels measured 4 times in each period.

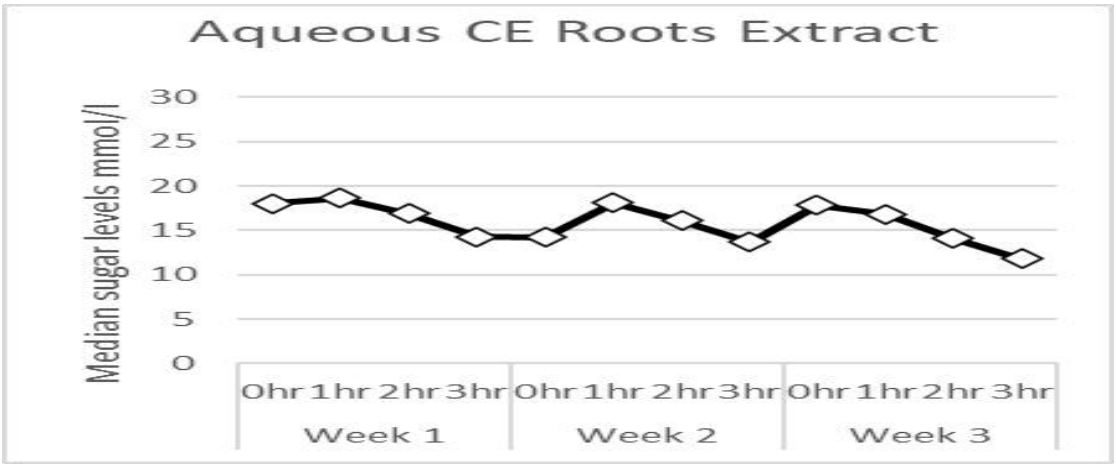
(i)



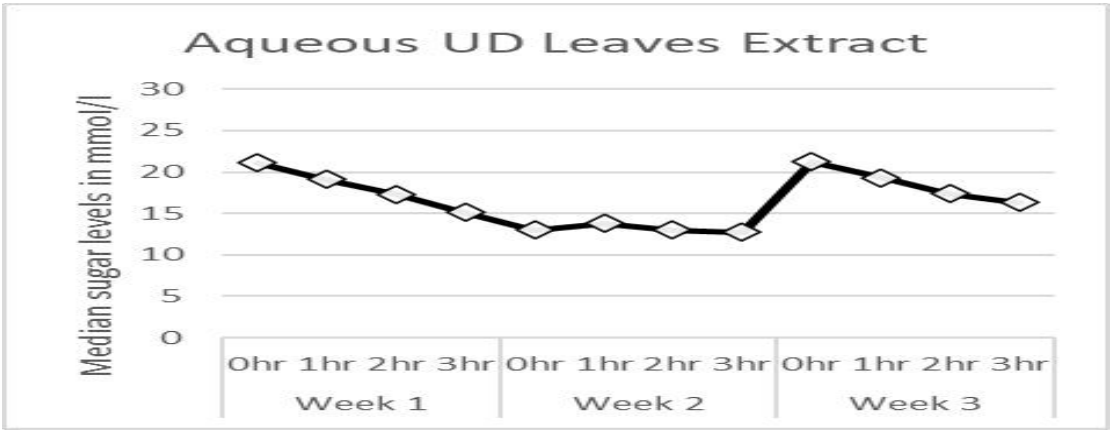
(ii)


Ethanol UD Leaves Extract
median sugar levels mmol/l
30
25
20
15
10
5
0
Ohr 1hr 2hr 3hr Ohr 1hr 2hr 3hr Ohr 1hr 2hr 3hr
Week 1
Week 2
Week 3

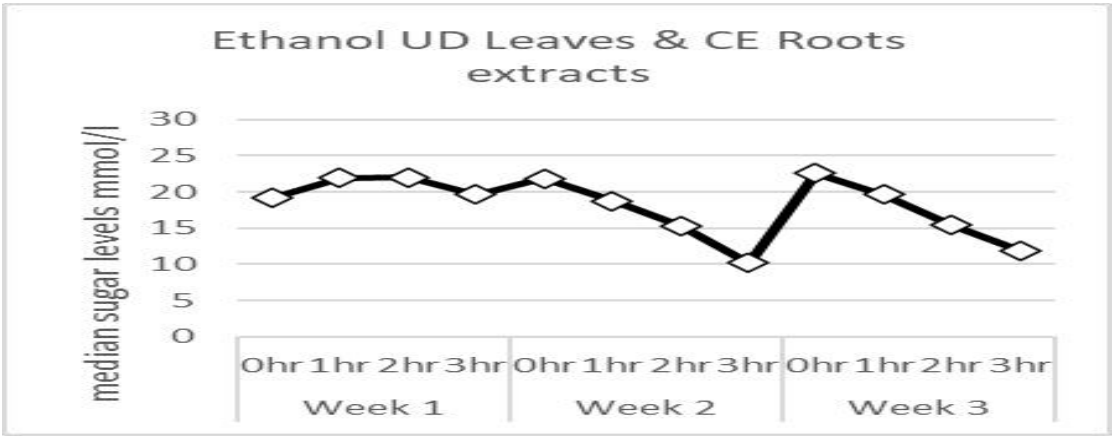
(iii)


Aqueous CE Roots Extract
Median sugar levels mmol/l
30
25
20
15
10
5
0
Ohr 1hr 2hr 3hr Ohr 1hr 2hr 3hr Ohr 1hr 2hr 3hr
Week 1
Week 2
Week 3

(iv)


Aqueous UD Leaves Extract
Median sugar levels in mmol/l
30
25
20
15
10
5
0
Ohr 1hr 2hr 3hr Ohr 1hr 2hr 3hr Ohr 1hr 2hr 3hr
Week 1
Week 2
Week 3

(v)



(vi)

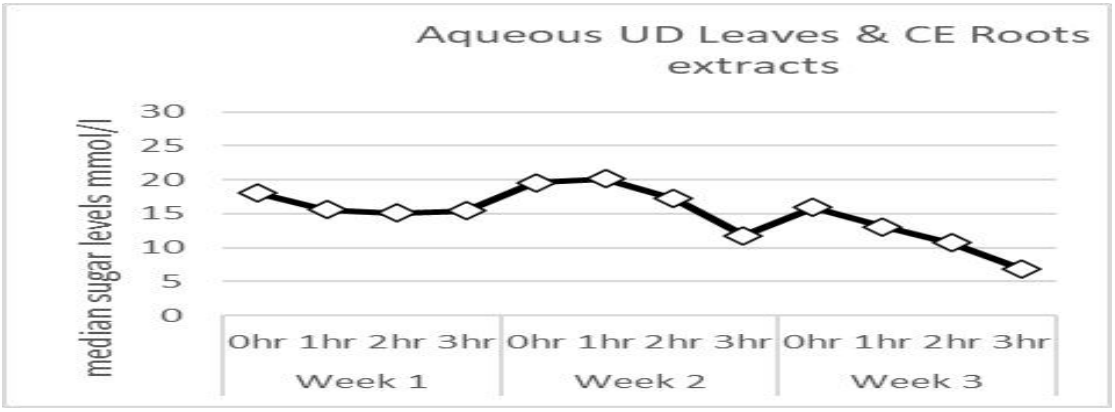


Fig 4. 45; Sugar reduction trends of; CE and UD ethanolic extracts, CE and UD aqueous extracts and their ethanolic and aqueous mixtures (cases)

(vii)

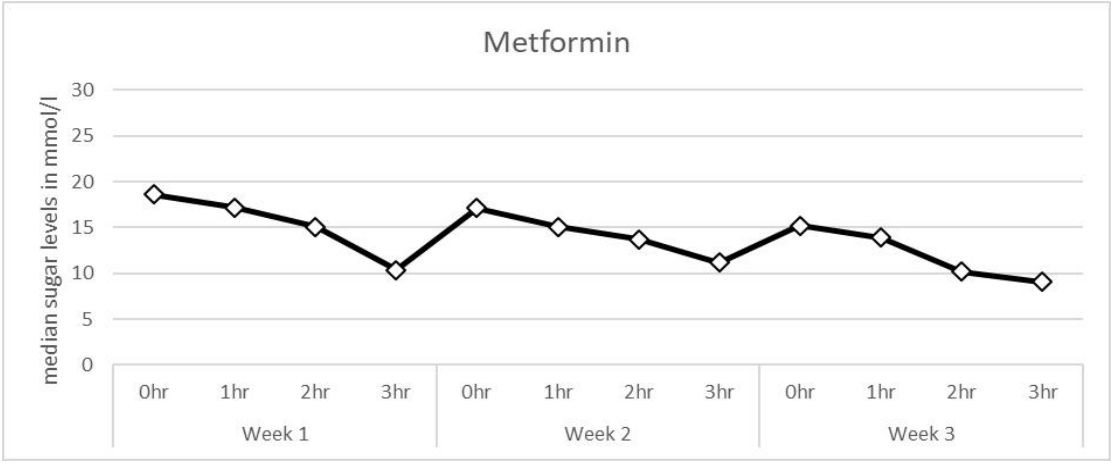


Fig 4. 46; Sugar levels trend for metformin treatment group (Controls)

By the end of the third week all drug regimens had achieved lower blood sugar level at an average of less than 20mmol/l compared to when the experiments started at day 0.

Mean average sugar reduction level in all the groups (case and controls) over the 3 weeks period in reference to ethanolic and aqueous extracts

As stated earlier, each week had 4 blood sugar levels taken (initial, (fed), after 1 hour, 2 hours, and 3 hours). Each group had 5 subjects (rats) thus making the n = 20 (number of rats multiply by the number of blood sugar level measurements [4]). For CE Ethanol and CE Aqueous groups the n of the 3rd week reduced to 16 due to the death of two. ANOVA/ Kruskal Wallis and Post Hoc tests were used to test the difference between the means/medians of the three periods.

Cases

I. CE

Table 4. 16; Average blood sugar levels distribution for CE roots treatment groups

Group	Period	N	Mean Mmol/l	Median (IQR) Mmol/l	Range Mmol/l	p-value
Ethanolic extracts	Week one	20	27.02(3.0)	26.9(24.9, 28.1)	20.6 – 36.0	<0.001
	Week two	20	22.35(6.3)	19.5(18.5, 23.4)	14.3 – 36.0	
	Week three	16	17.63(3.8)	17.7(14.2, 20.7)	11.0 – 23.9	
Aqueous extracts	Week one	20	18.56(4.1)	18.3(14.8, 22.4)	11.4 – 26.1	0.011
	Week two	20	15.52(3.4)	16.5(12.8, 18.2)	9.4 – 20.7	
	Week three	16	13.87(6.3)	15.3(10.5, 18.8)	2.3 – 21.6	

From table 4.16 above, there was a well-defined reduction in CE groups but resulted in mortality on the 3rd week both extracts.

All differences in average blood sugar levels were significant ($p<0.001$) for CE Ethanolic group but for CE Aqueous; only week one and week two were significant ($p<0.05$) but not in week 3 ($p>0.05$).

Ethanolic extracts compared to aqueous extracts were slightly more efficacious ($p<0.001$ compared $p<0.011$).

Nonetheless, there was a significant reduction in average blood sugar level from the 1st week to the 3rd week for both (aqueous and ethanolic) CE treatment groups.

II. UD

Table 4. 17; Average blood sugar levels distribution for UD leaves extracts treatment groups

	Period	**N**	**Mean Mmol/l**	**Median (IQR) Mmol/l**	**Range Mmol/l**	**p-value**
Ethanol	Week one	20	23.41(4.3)	25.1(18.7, 26.7)	14.9 – 29.7	<0.001
	Week two	20	16.86(4.4)	17.3(12.8, 19.6)	9.1 – 24.6	
	Week three	20	19.94(3.0)	19.2(17.9, 21.2)	16.2 – 26.2	
Aqueous	Week one	20	18.16(4.9)	18.4(14.5, 21.4)	10.9 – 27.7	0.026
	Week two	20	13.83(4.9)	13.0(10.6, 16.9)	7.0 – 23.6	
	Week three	20	17.41(5.8)	18.1(15.2, 21.2)	5.7 – 27.0	

From the above *table 4.17*, in both drugs the reduction in blood sugar levels was seen on week two and then the average blood sugar levels sprung back to average higher levels in week three. However, no mortality was seen in these two treatment groups. For ethanolic group, only in week two average blood sugar levels were not significantly different from each other (p-value=0.052). Week one and week 2 average blood sugar levels were significantly different from each other (p-value=0.035).

Just like it was in CE, ethanolic extracted extracts was slightly much more efficacious than aqueous extracted extracts (p-value of <0.001, compared of p-value of =0.026).

UD and CE Mixtures

Table 4. 18; Average blood sugar levels distribution for UD leaves & CE roots extracts treatment groups

	Period	N	Mean Mmol/l	Median Mmol/l (IQR)	Range Mmol/l	p-value
Ethanolic solvent extracted extracts	Week one	20	20.71(4.2)	19.75(17.9, 23.6)	14.0 – 29.8	0.052
	Week two	20	17.66(5.6)	17.45(13.1, 22.1)	9.2 – 28.1	
	Week three	20	16.94(5.2)	17.3(13.1, 20.1)	5.9 – 27.4	
Aqueous solvent Extracted extracts	Week one	20	16.72(8.1)	15.5(9.2, 24.1)	5.1 – 32.0	0.121
	Week two	20	18.35(5.9)	18.4(12.4, 23.0)	10.8 – 31.1	
	Week three	20	13.82(6.5)	12.4(9.2, 17.8)	4.9 – 27.9	

For ethanol group mixtures there was steady decline in average blood sugar levels over the period though the decline was not statistically different. However, in Aqueous group there was rise in average blood sugar levels in week two and then followed by a decline week 3 though none of the deference in ethanolic and aqueous mixtures was statistically significant (p-value=.0.052 and p-value= 0.121 respectively).

CONTROLS

I. Metformin fed rats (Given 100mg/kg)

Table 4. 19; Average blood sugar levels distribution for Metformin treatment group

Period	N	Mean (g)	Median (IQR)	Range	p-value
Week one	20	14.47(4.0)	15.25(10.5, 17.6)	6.5 – 21.6	0.145
Week two	20	14.58(5.4)	13.8(10.6, 18.3)	6.3 – 25.0	
Week three	20	12.18(2.9)	12.25(10.1, 14.4)	7.1 – 17.1	

For metformin group there was a steady decline in average blood sugar levels over the period though the decline was not statistically different (p=0.145).

Sugar reduction trends scatter graphs

The figures below show how the various tested extracts performed with regards to the sugar reduction, for the period of the study (21 days)

Cases

[i] CE ethanolic extracted extracts sugar reduction trends

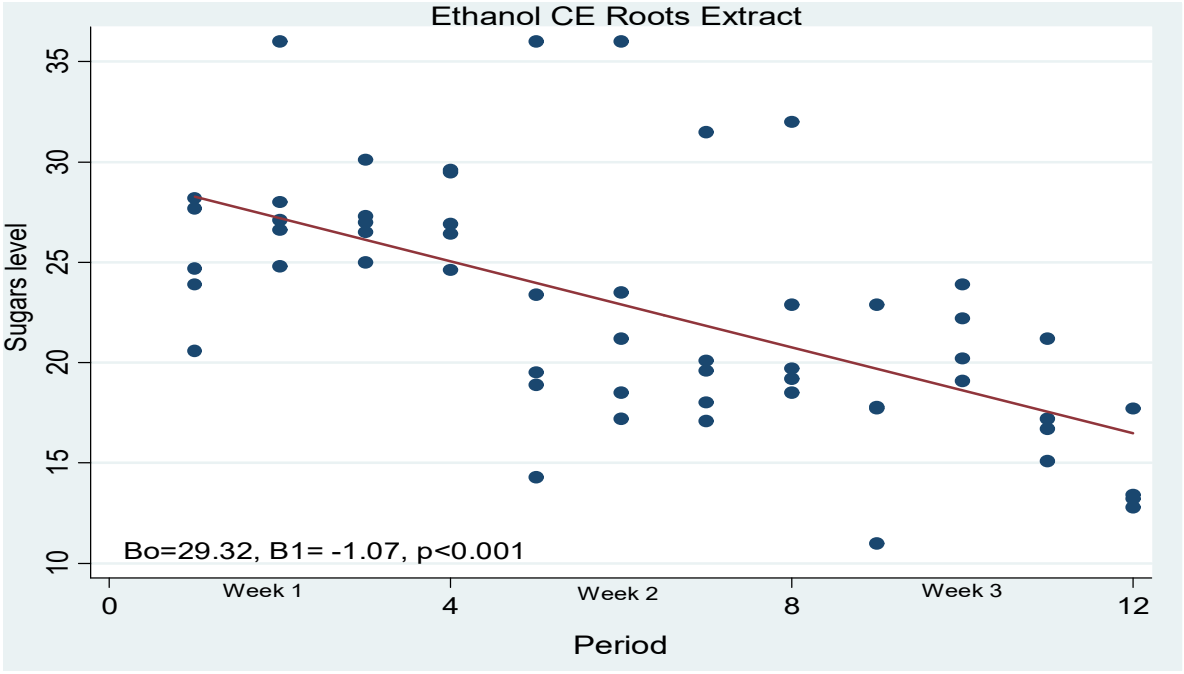


Fig 4. 47; Sugar levels trends for CE ethanolic roots extracts

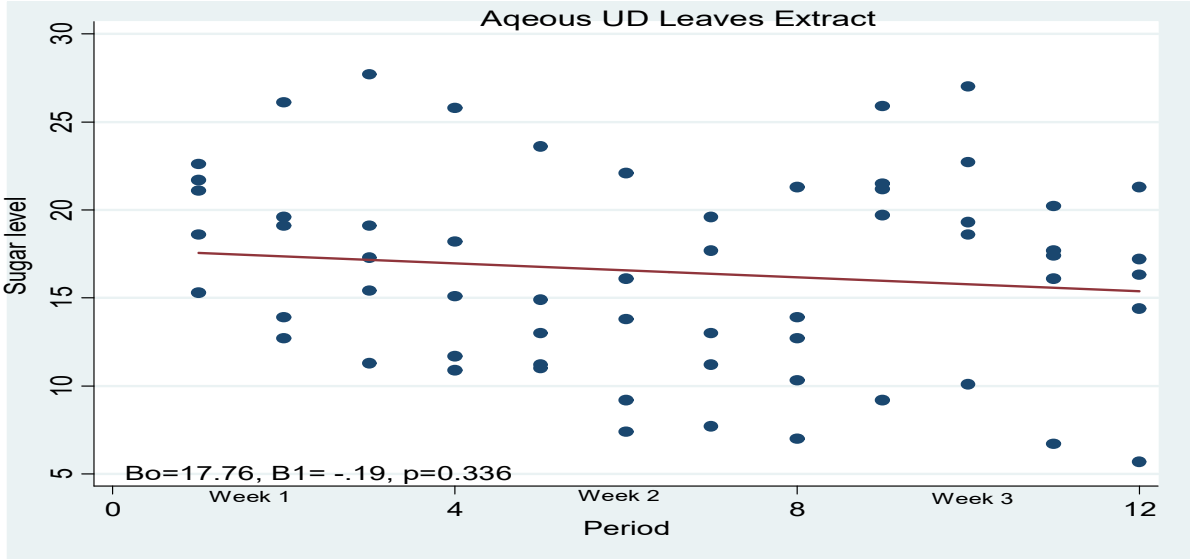


Fig 4. 48; Sugar level trend for UD aqueous leaves extracts

[iii] CE, Aqueous extracted extracts sugar reduction trend

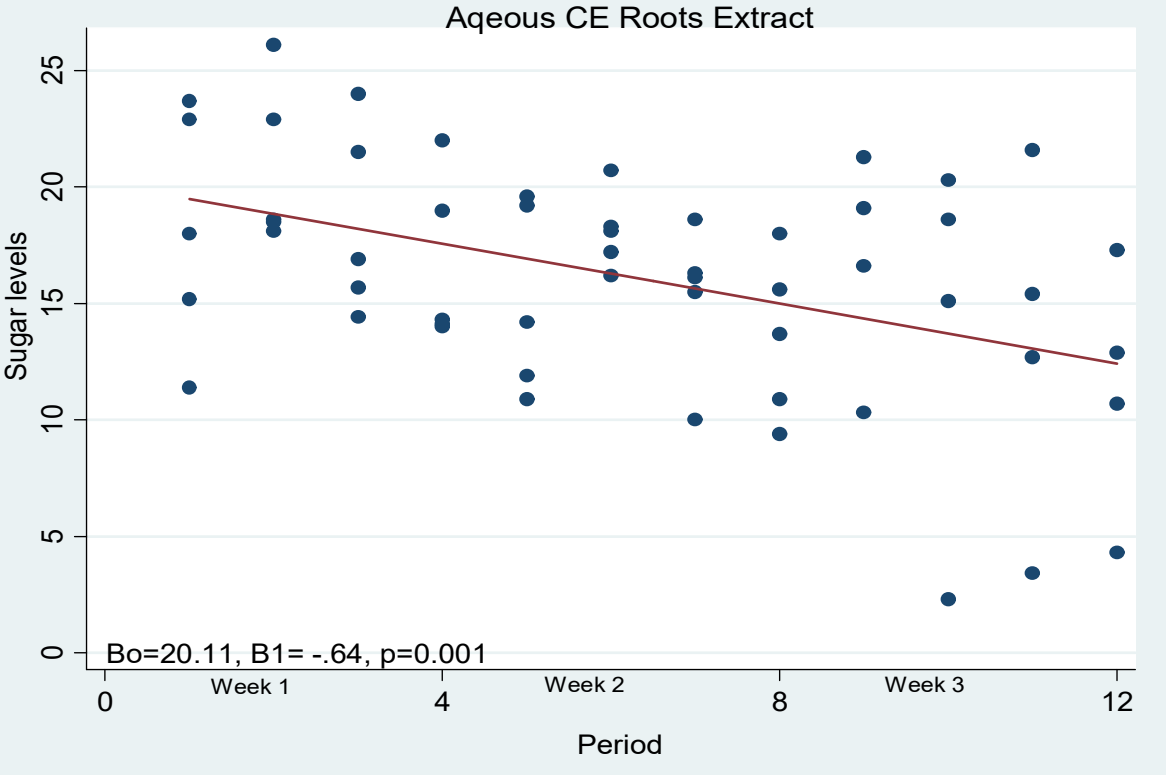


Fig 4. 49; Sugar levels trends for CE aqueous root extracts

[iv] UD Ethanolic extracted extracts, sugar reduction trend

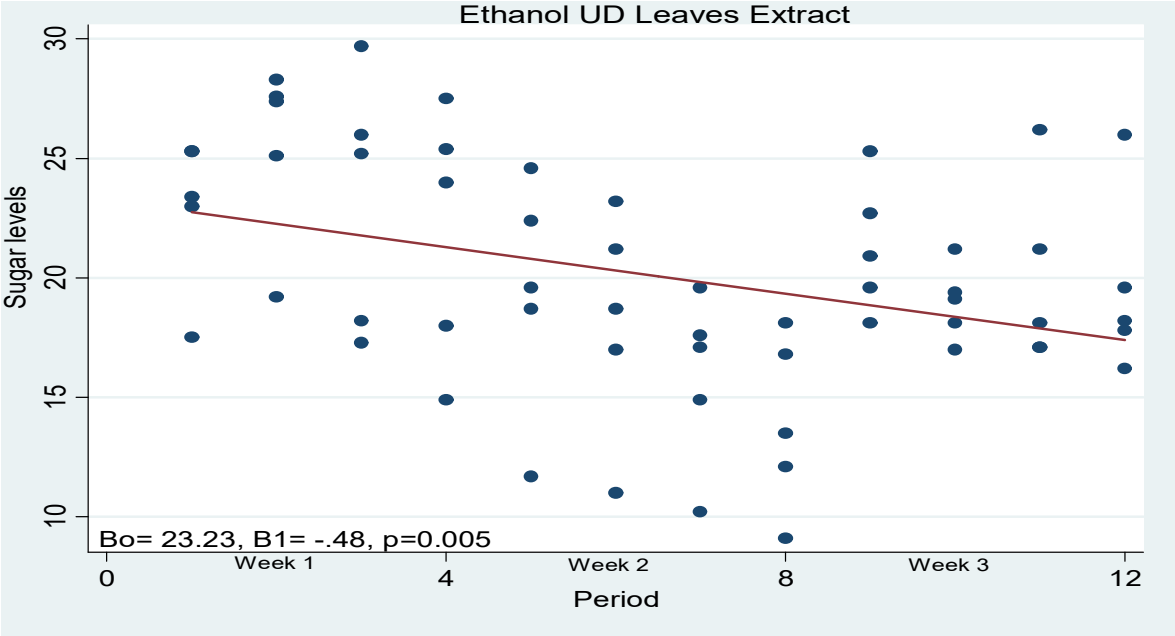


Fig 4. 50; Sugar level trends for UD ethanolic extracts

[v] UD&CE ethanolic extracted extracts in a ratio of 50:50 sugar reduction trend.

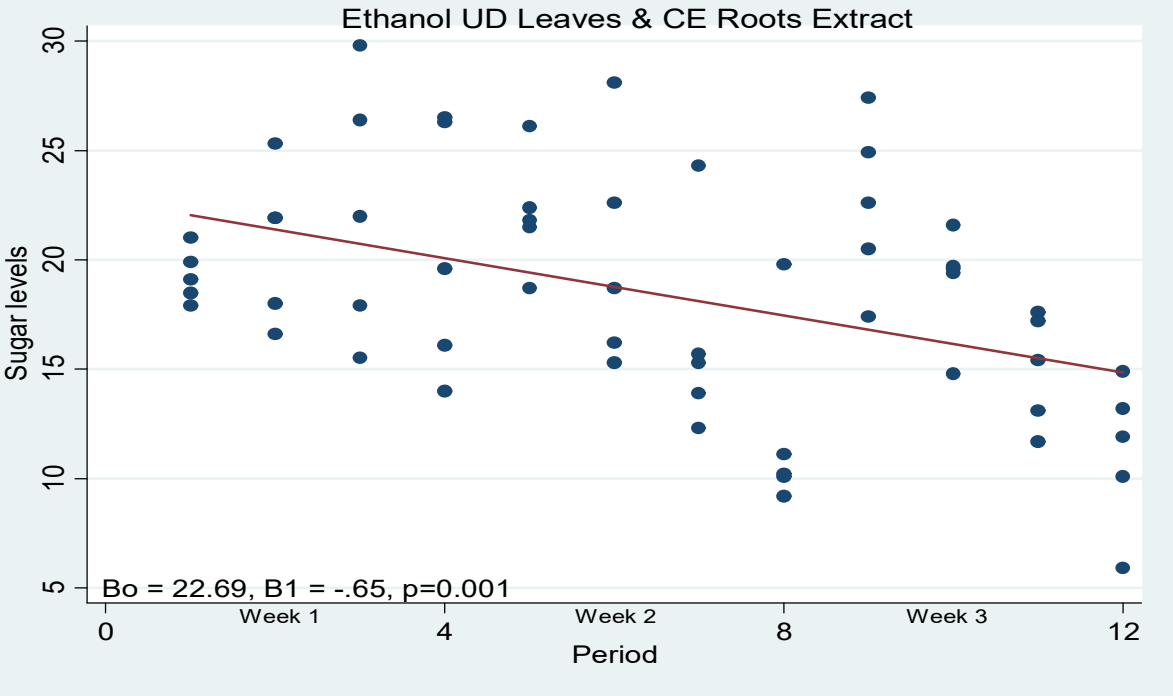


Fig 4. 51; Sugar trend levels for UD and CE ethanolic extracts mixtures

[vi] Aqueous mixtures of CE&UD extracted extracts, sugar reduction trend

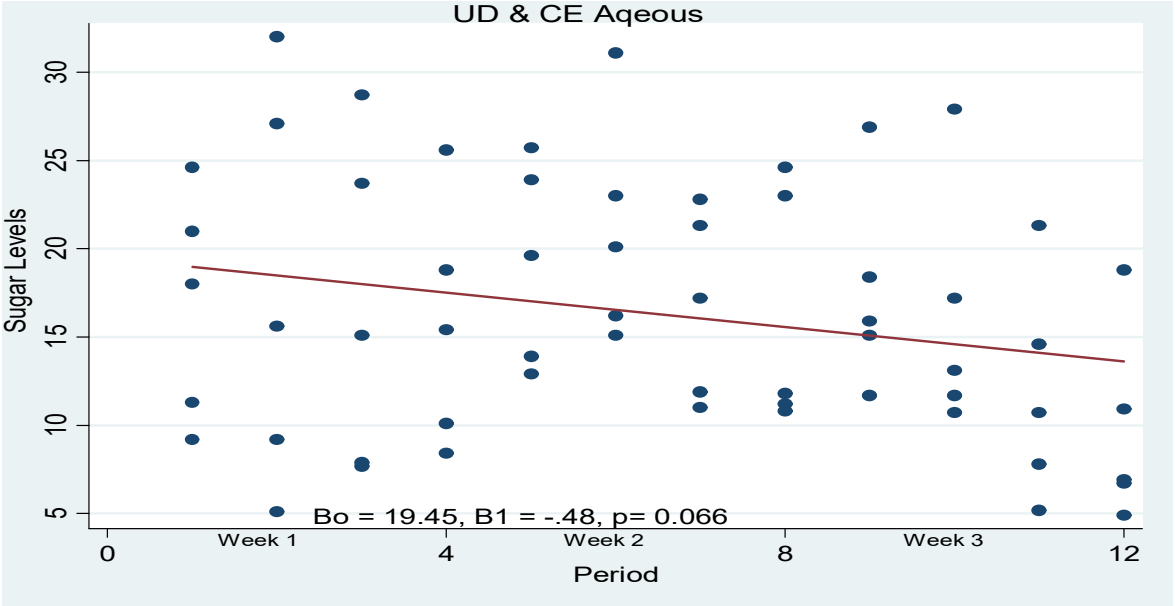


Fig 4. 52; Sugar trends for UD and CE aqueous mixtures extracts

Controls

[vii] Metformin sugar levels reduction trend

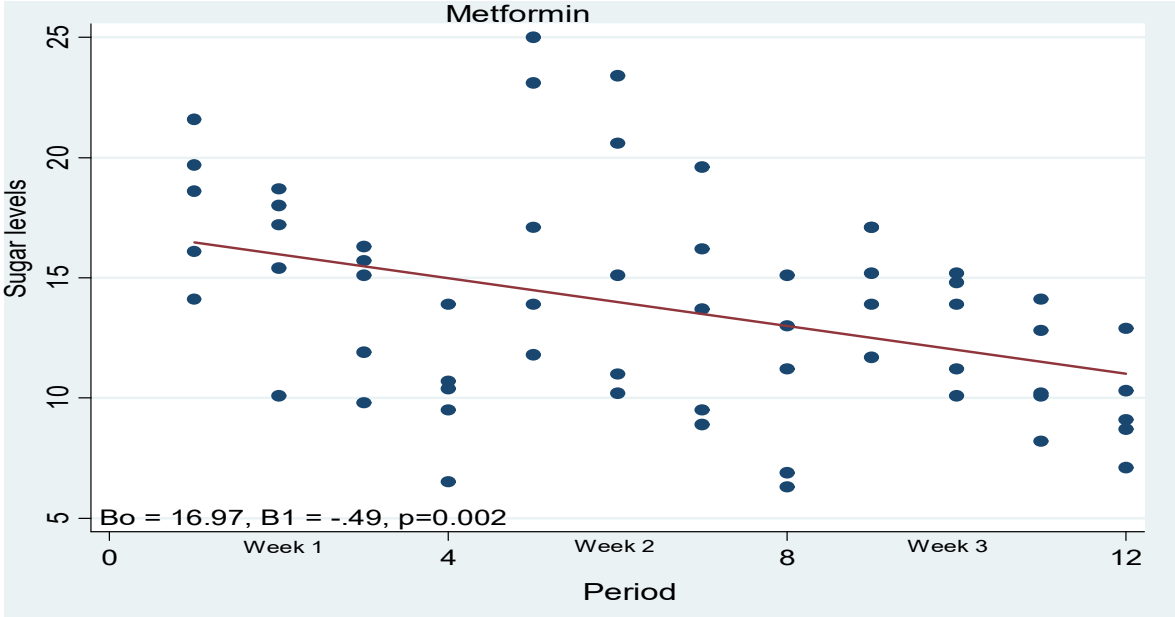


Fig 4. 53; Metformin sugar levels reduction trends

Summary of sugar reduction regression coefficients of efficacies in order (cases and controls)

Table 4. 20; Regression coefficients for different treatment groups

	Drug/herb	p-value
Cases	Ethanolic extracted extracts CE Roots	<0.001
	Ethanol extracted extracts UD Leaves & CE Roots	0.001
	Aqueous extracted extracts CE Roots	0.001
	Ethanol extracted extracts UD Leaves	0.005
	Aqueous extracted extracts UD Leaves & CE Roots	0.066
	Aqueous extracted extracts UD Leaves	0.336
Controls	Metformin	0.002

4.3.4.2. Purified samples efficacy studies

Introduction

The analysis was based on 18 rats that were randomly assigned to 3 treatment groups of the purified ethanolic extracted samples of CE and UD. [(CE6 [PCE]), UD6[PUD]

and a mixture of the two-CE6+UD6 [PCE+PUD] [50:50 mixture]). Dosages given see table 3.11.

Six rats were assigned to each group, however group 1 (CE6 [PCE]) lost two rats eventually (2/6), group 2 (UD6 [PUD]) lost 1 rat (1/6) while group 3 (CE6 and UD6 [PCE+PUD]) also lost 1 rat (1/6).

The weight of the rats was taken before the start of the experiment. The sugars levels were taken 4 times (at 0hr, fed with the drug then sugar measured at 1hr, 2, 3hrs) per animal for three weeks (each 4th day of the week). The feeds were given at 6pm previous night and in the morning initial sugars taken (Random blood sugars) then the drug given, and subsequently sugars measured in hour 1, 2 and 3.

Weight distribution

Table 4. 21; Wistar rats for purified compounds weight distribution

Treatment group	Mean (g)	Median (IQR)	Minimum	Maximum
Ethanol PCE roots Extract	238.7(50.6)	225(195, 282.5)	190	315
Ethanol PUD Leaves Extract	99.8(20.2)	94(86, 96)	84	139
UD6 & CE6 [PUD+PCE] mixture	89(7.2)	88(84, 92)	80	101

There was a significant different in average rats' weight between the treatment groups ($p<0.001$), though, a post-hoc test analysis showed only Ethanolic PCE Roots Extract and PCE+PUD mixture were statistically comparable in average weight. However, the dosage used of 0.5mls per 100 grams, was assumed that there would be an equal herb/drug reaction in the wistar male albino rats' bodies.

Sugar levels trend across all three treatment groups (CE6[PCE], UD6 [PUD] and CE6&UD6 [PCE+PUD] mixtures)

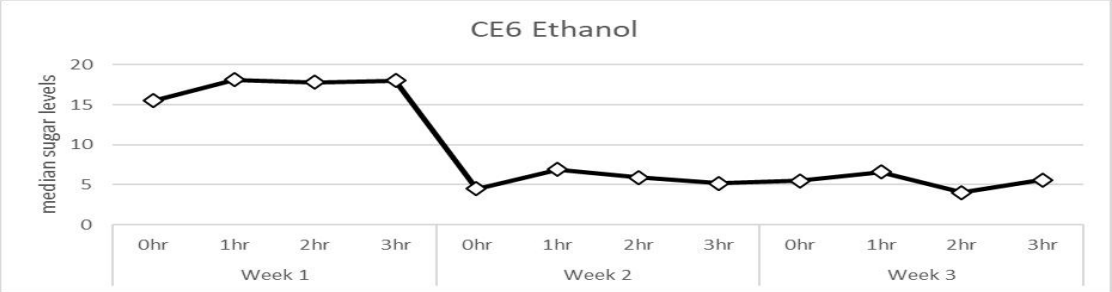


Fig 4. 54: CE6[PCE] ethanol sugar reduction trend

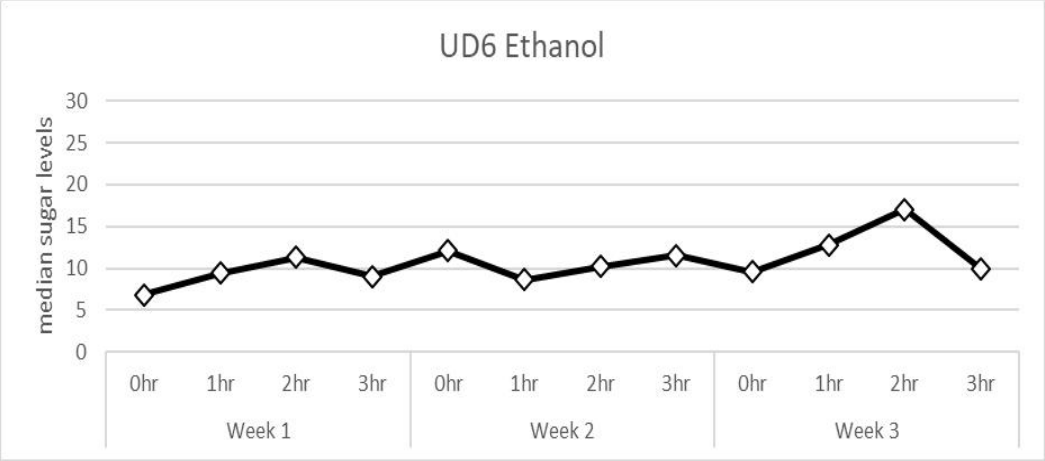


Fig 4. 55; UD6[PUD] ethanolic sugar reduction trend

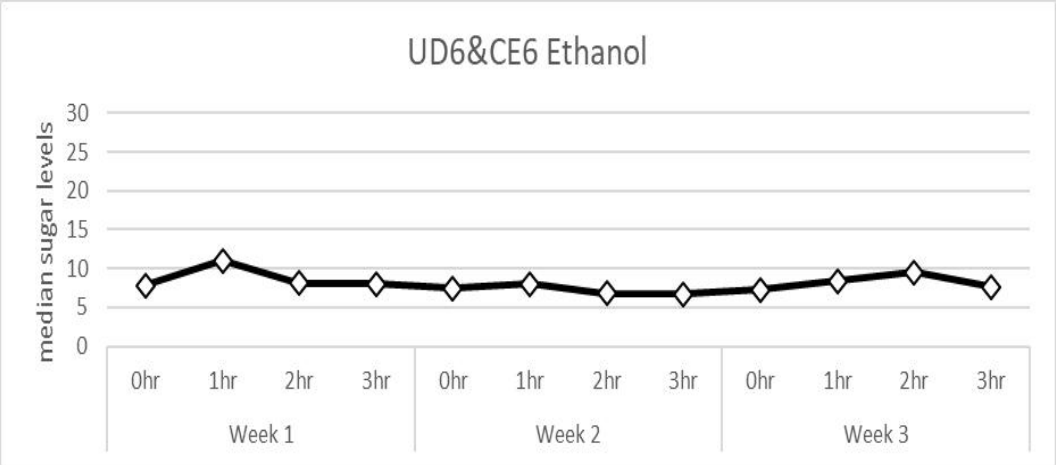


Fig 4. 56; UD6&CE6[PUD+PCE] mixtures ethanolic sugar reduction trend

From the above figures, PCE shows a sharp decline in average sugars levels after week one compared to PUD. There was no much change in sugar reduction with regards to their mixtures.

Sugar change trends –Post Hoc Tests

In each week 4 blood sugar reading levels were taken (initial, after 1 hour, 2 hours, and 3 hours). For Ethanolic PCE roots extract groups n in the 3rd week reduced to 16 because two rats died. ANOVA/ Kruskal Wallis and Post Hoc tests were used to test the difference between the means/medians of the three periods, where appropriate. **See the Table 4.22 below;**

Table 4. 22; Average blood sugar levels distribution for PCE, PUD and PCE+PUD Roots Extract treatment groups

Group	***Period***	***n***	***Mean***	***Range***	***p-value***
PCE	Week one	16	17.94(4.50)	11.8 – 30.8	<0.001
	Week two	16	5.81(1.67)	3.3 – 8.9	
	Week three	16	6.25 (2.69)	3.2 – 12.4	
PUD	Week one	20	10.13(6.36)	2.9 – 25.6	0.177
	Week two	20	14.04(8.94)	3.6 – 33.5	
	Week three	20	13.40(5.25)	5.9 – 22.1	
PCE+PUD	Week one	20	14.39(9.99)	5.4 – 33.5	0.401
	Week two	20	10.76(7.78)	4.1 – 28.6	
	Week three	20	11.85(8.07)	4.4 – 30	

The weekly average sugars level was significantly different only for PCE. Though the average sugar level between week two and week three were not statistically different. PUD did not to reduce sugar levels and a combination with PCE reduces the effect of PCE.

Mean/ median sugar reduction of the purified extracts

Table 4. 23; Sugar change average for three drugs/herbs

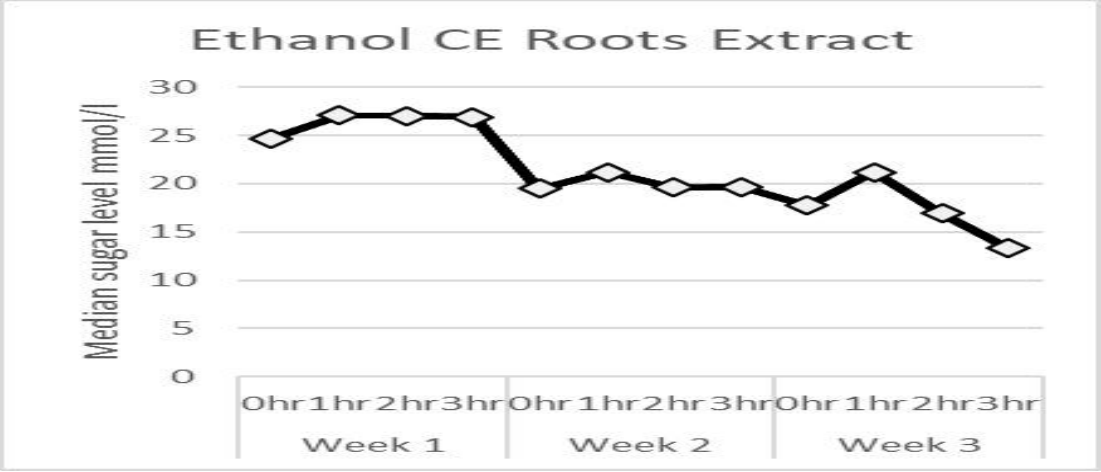
Group	*n*	*Mean*	*Median (IQR)*	*Range*
PCE	4	11.2(9.8)	7.9(4.5, 17.8)	3.7 – 25.3
PUD	5	4.8(9.7)	2.9(-0.4 – 11.6)	-7.4 – 17.3
Combined	5	-1.2(7.3)	-0.4(-0.6 – 2.7)	-13.4 – 5.9

The table above shows the change in sugar levels from first measurement before drugs were to the final day the sugar level were measured. The table shows very high variance between the differences in measurement for different animals within the treatment groups. The heterogeneity within treatment groups was very eminent. However, it is evident that PUD treatment drug had a higher drop in sugar levels on average compared to the rest of the treatment groups.

4.3.4.3 Comparisons between the crude and purified extracts

1. **CE ethanolic extracted extracts and CE6 (PCE)**

   (i)



(ii)

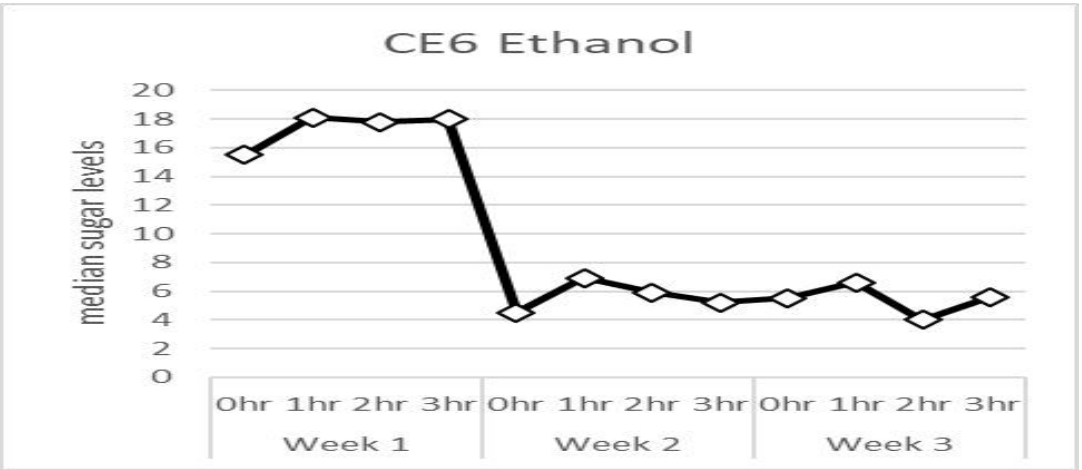
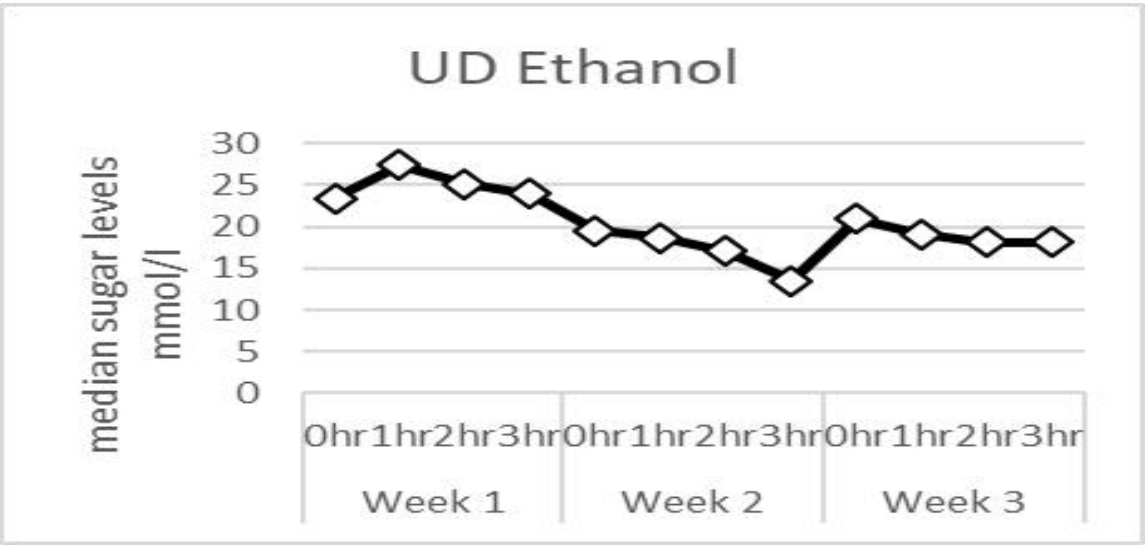


Fig 4. 57; Sugar levels trend comparing CE and CE6[PCE]

The two drugs had the same effect in reduction of sugar level in rats, though the reduction of sugars in the purified version of CE was much more

2. UD ethanolic extracted extracts and UD6(PUD)

(i)



(ii)

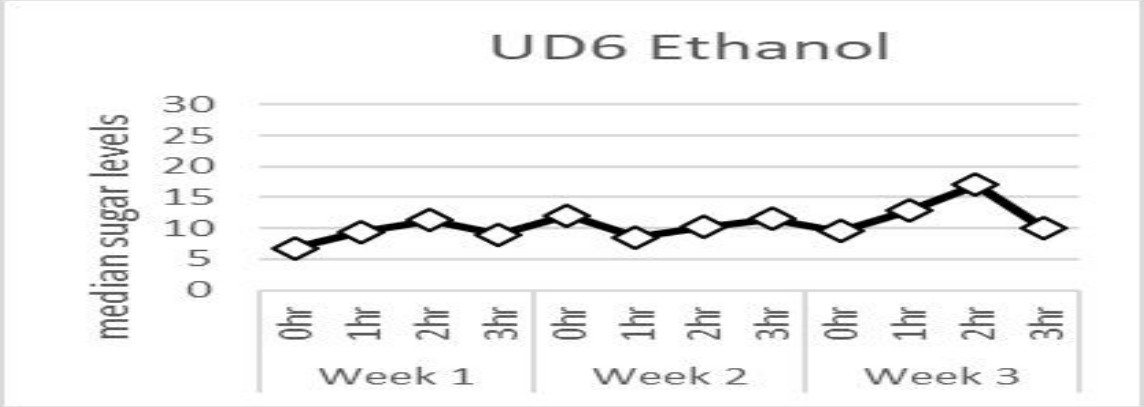
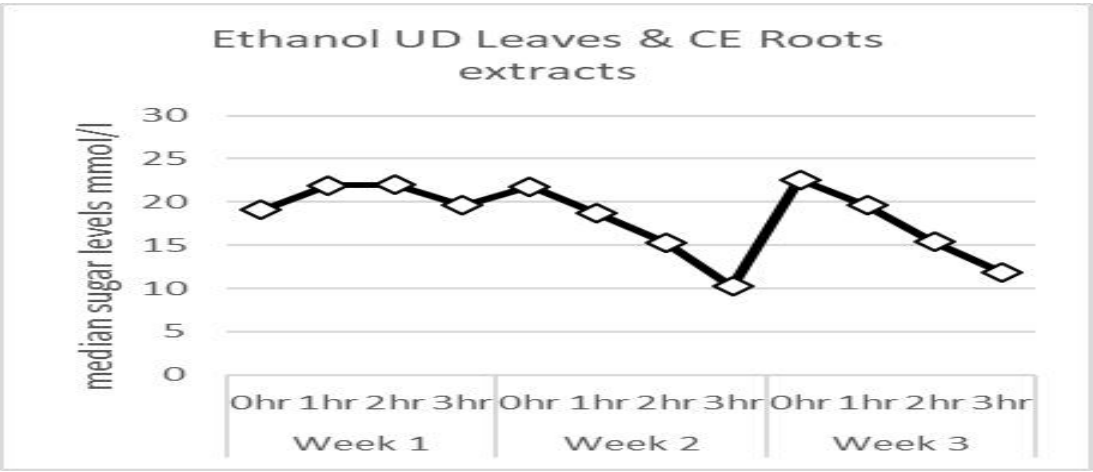


Fig 4. 58; Comparison of UD and UD6[PUD] ethanol sugar trend reduction

UD6 extracts does not work well compared to UD.

2. Ethanolic mixtures of CE & UD and the mixtures of (PCE) CE6 and UD6 (PUD)

(i)



(ii)

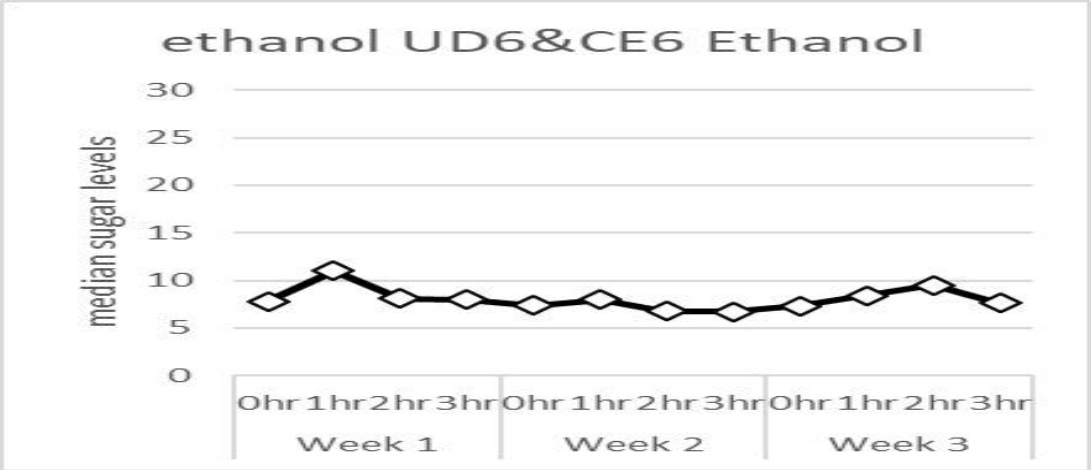


Fig 4. 59; Comparison of CE&UD crude mixtures and their purified mixture CE6&UD6 [PCE+PUD] sugar reduction trend

These two groups combination of UD and CE, does not to reduce sugars. The efficacy of CE is “eliminated” by UD.

4.3.4.4. Summary of the rats utilized in the antidiabetic test experiment

Table 4. 24; The number of rats utilized

		RATS RECRUITED	RATS AT THE END OF EXPERIMENT	PERCENTAGE DEATH/LOSS
		A	B	A-B =X, $^{X}/_{B}$*100
CRUDE EXTRACTS - Allox an Diabe tes induc ted	CE ethanolic extracted extracts	5	4	20%
	UD ethanolic extracted extracts	5	5	0
	CE aqueous extracted extracts	5	4	20%
	UD aqueous extracted extracts	5	5	0
	CE+UD ethanolic extracted extracts	5	5	0
	CE+UD aqueous extracted extracts	5	5	0
	Control groups	5	5	0
	TOTAL	***35***	***33***	***5.7%***
CRUDE EXTRACTS - Not induc ted Diabe	CE ethanolic extracted extracts	*2*	*2*	0
	UD ethanolic extracted extracts	*2*	*2*	0
	CE aqueous extracted extracts	*2*	*2*	0
	UD aqueous extracted extracts	*2*	*2*	0
	CE+UD ethanolic extracted extracts	*2*	*2*	0
	CE+UD aqueous extracted extracts	*2*	*2*	0

ticall y	Control groups	*2*	*2*	0
	Total	***14***	***14***	***0***
PURIFIED - INDUCTED WITH ALLOXAN	CE	6	4	33.3%
	UD	6	5	16.7%
	CE+UD	6	5	16.7%
	TOTAL	**18**	**14**	**22.2%**
GRAND TOTAL NUMBER OF RATS		**67**	**61**	**8.96%**

The table 4.24 above, indicates the total number of rats recruited and the final number of the rats which remained after the experiment.

With regards to crude extracts, other than CE ethanolic and aqueous extracted extracts, which reported a death each (20%), there were no deaths reported in all the other extracted extracts.

Total deaths recorded in rats fed with crude extracts were (2/35 [5.7%])

When CE and UD extracts were purified, the number of deaths increased across all the extracts, with CE still recording the highest numbers (4/18 [33.3%]).

In general, more deaths were seen in purified extracted extracts (22.2%) than crude extracted extracts (5.7%).

4.3.5 CE and UD Toxicity Studies-Histology (In-Vivo Studies)

4.3.5.1 Introduction

These results were based on 31 randomly selected rats that were fed with varied concentrations of both crude aqueous and ethanolic mixtures of CE, UD, metformin and water ad-libitum for the 21 days.

Seventeen (17) of the 31 rats, were part of the 35 rats that were fed with crude extracts of CE, UD (cases) and metformin (controls)- and the additional 14 rats, were new rats - not inducted but fed with CE, UD, aqueous and ethanolic mixtures extracted extracts and metformin.

Normal tissues samples

Since the major aim of this study was to compare normal vs abnormal histological presentation of the tissues, its imperative first to display how normal tissues look like first and later the abnormalities that accrued after inducing the rats and exposing them to the herbs. *See the Figures; fig 4.60 to fig 4.64, below*

Samples of normal tissues for comparison purposes (Belhadj *et al.*, 2018)

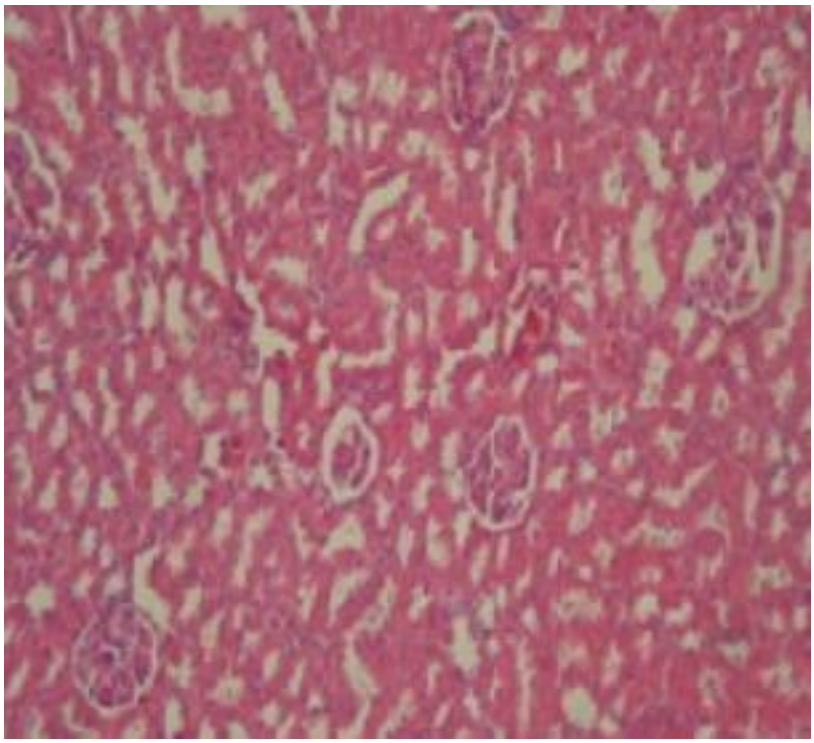


Fig 4. 60; Normal kidney tissue

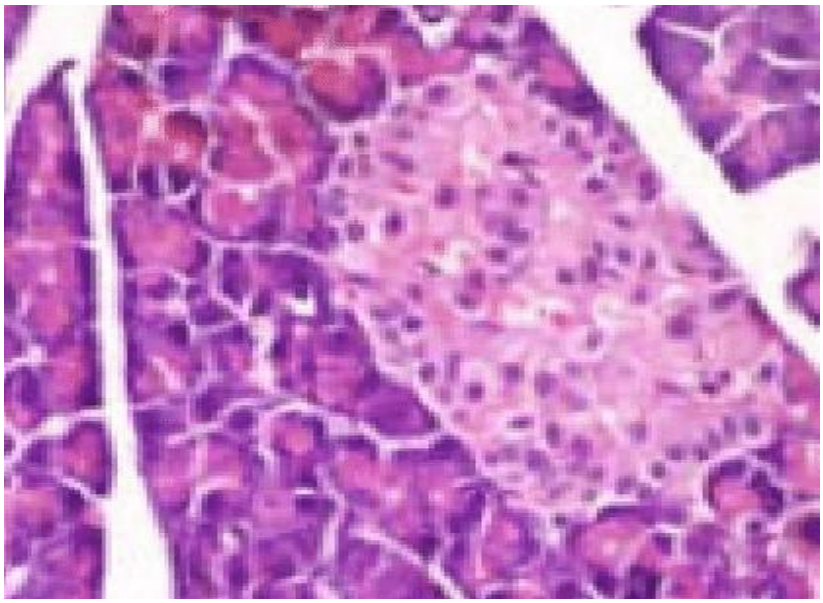


Fig 4. 61; Normal pancreatic tissue

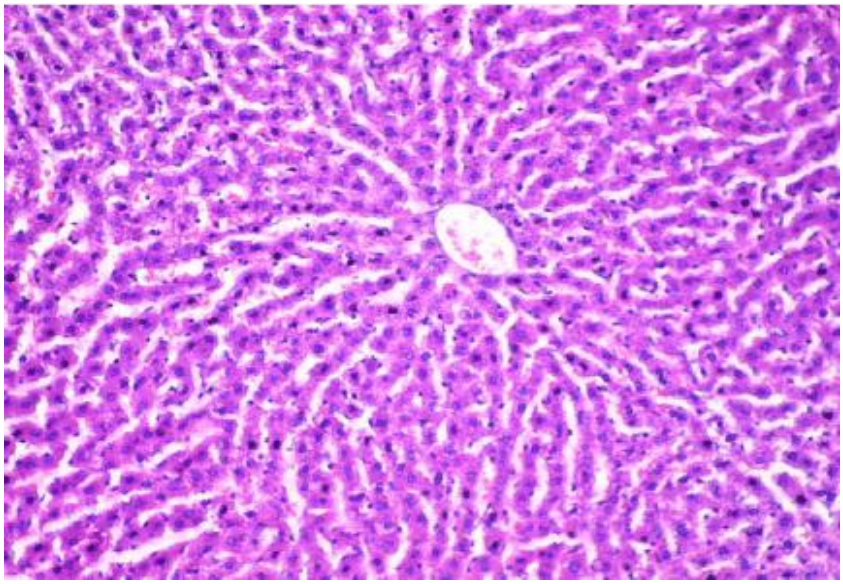


Fig 4. 62;Normal liver tissue

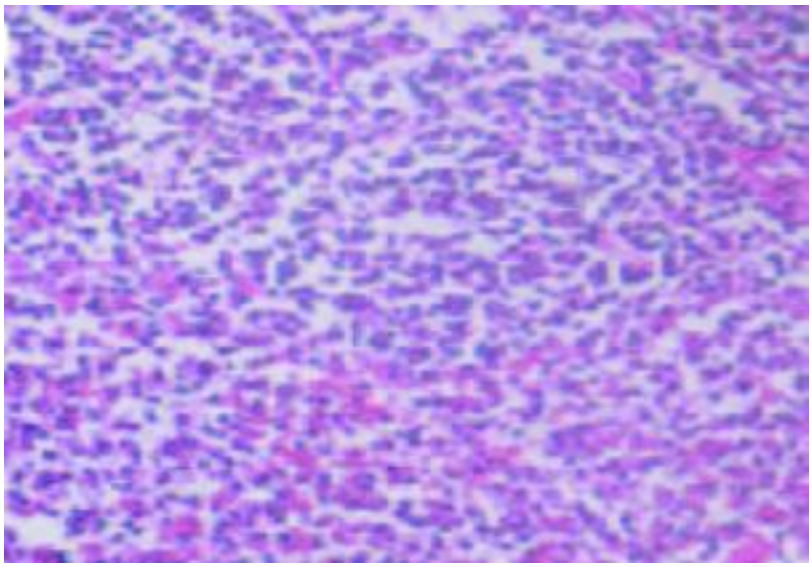


Fig 4. 63; Normal splenic tissue

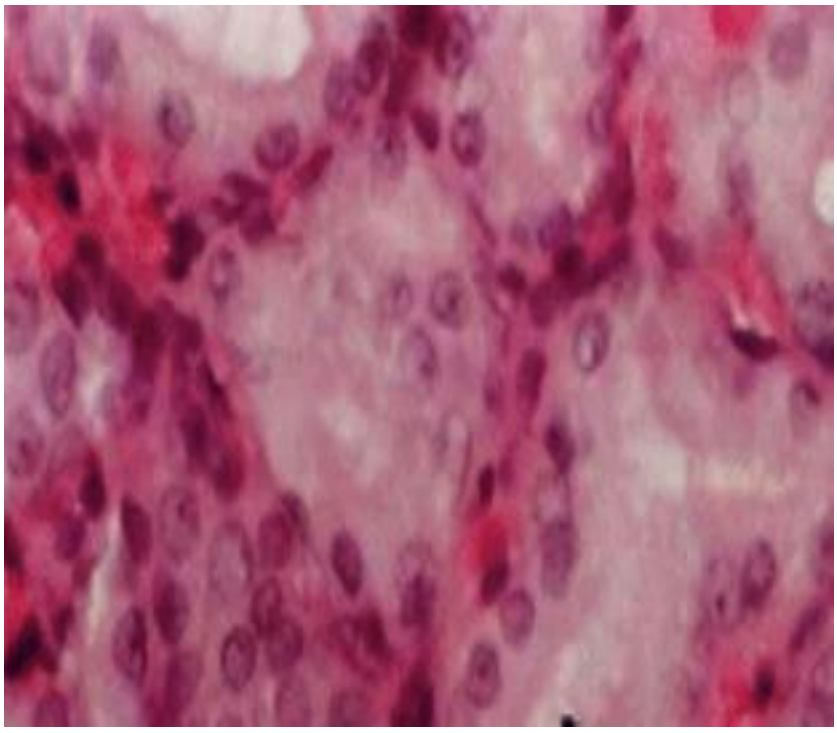


Fig 4. 64;Normal thyroid gland

4.3.5.3 Outcomes of CE Histology

After histological examination of the *kidney, liver, spleen, brain, pancreases and the thyroid* of each of the exposed rats, the findings are as summarized in the *table 4.25* below;

Table 4. 25; Carissa edulis histological changes results

5 Rats/Cage		*Treatment*	*Induction Status*	*Selected*	*Summarized histological changes after 21 days of exposure*
C1	CASES	CE ethanolic extracted extracts	Induction - 140mg/kg of alloxan	R1	**Kidney** – Distal Convoluted Tubule Vacuolization (DCTV) - All other tissues selected tissues were normal
				R5	**Kidney**- DCTV - other tissues were normal
C2		CE aqueous extracted extracts		R2	**Kidney**- Diffuse interstitial nephritis with vacuolated lumen of proximal convoluted Tubules **Liver**- Periportal accumulation of inflammatory cells
				R5	**Kidney**-: Diffuse DCTV, interstitial nephritis, Congested blood vessels, Hyper cellularity of many renal corpuscles. All other tissues were normal.
C3	Controls	Metformin		R1	**Kidney**- Diffuse interstitial nephritis, **Pancreas**- Cells with clear cytoplasm
				R2	**Kidney**- Diffuse interstitial nephritis.
C4		Water and food ad-libitum		R2	All tissues normal
				R3	**Kidney**-Diffuse interstitial nephritis, Other selected tissues were normal
				R5	**Kidney**-interstitial nephritis, All the other tissues were normal
C5		CE ethanolic	NO INDUCTION	R1, R3	All tissues normal
C6		CE aqueous		R3, R1	
C7		Fed with Metformin		R2, R4	
35 RATS				15	

key;

1. Not shaded: Cases- Inducted and fed orally with the herbal medication of CE.
2. Light blue colour shaded: Controls -Inducted. Same across the three study groups
3. Grey colour shaded: controls -Not inducted.
4. Yellow color -Cage 7-controls - same across the other 3 study groups

Selected tissues with histological changes

A. CASES

(i) CE ethanolic extracts

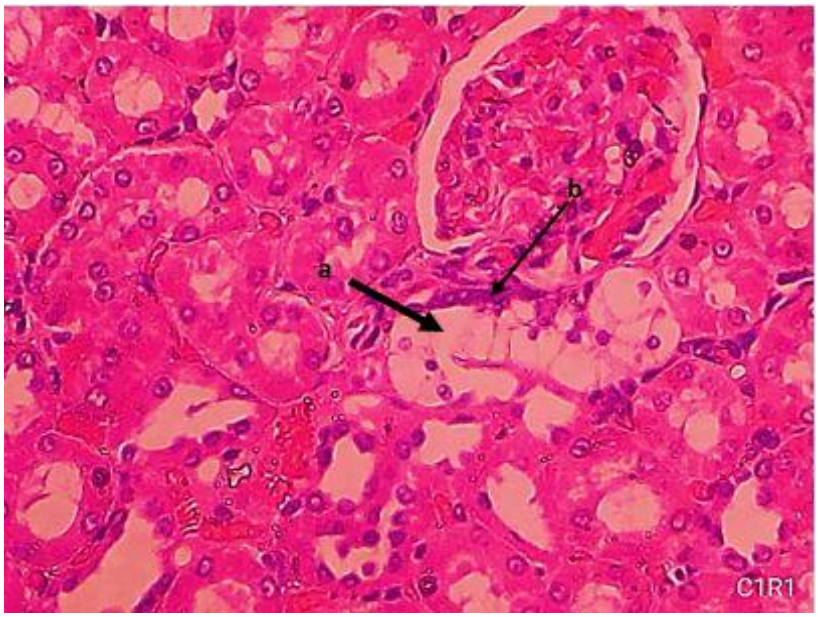


Fig 4. 65; C1R1, Kidney tissue

The above figure (4.65), depicts diffuse tubular vacuolization (as shown by arrow indicated by letter 'a') involving only distal convoluted tubule with normal architecture of the macula densa cells as indicated by arrow labelled 'b'.

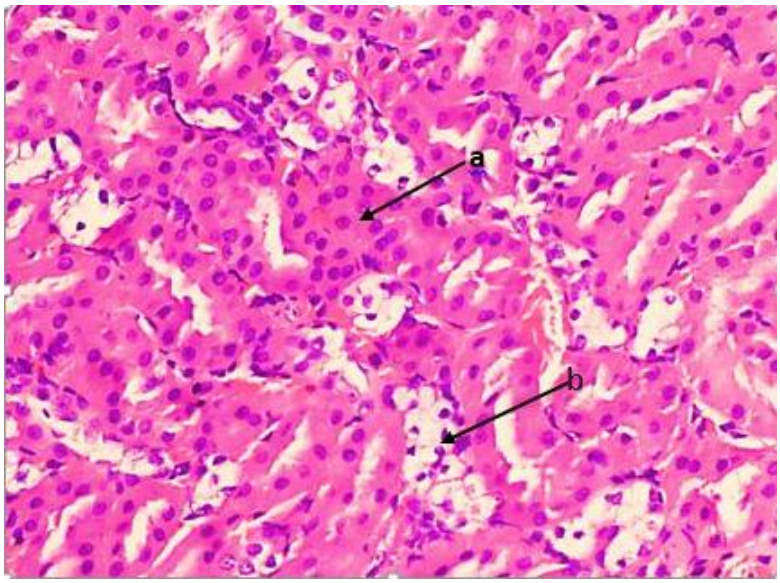


Fig 4. 66; C1R5, Kidney tissue

Figure 4.66, above shows, focal areas of interstitial nephritis (‘a’) and distal convoluted tubular vacuolization (‘b’) with disrupted luminal surface of proximal convoluted tubule.

CE aqueous extracts

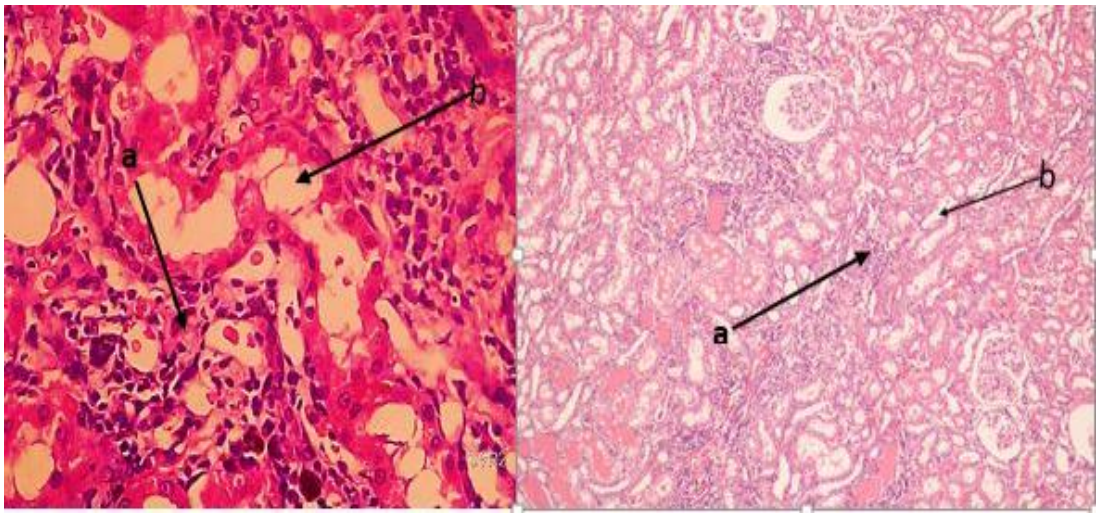


Fig 4. 67; C2R2, Kidney tissue: CE aqueous extracts

The figure 4.67 above shows, diffuse interstitial nephritis (‘a) with vacuolated lumen of Proximal Convoluted Tubules (PCT) (‘b) some distal convoluted tubules are filled with an amorphous mass

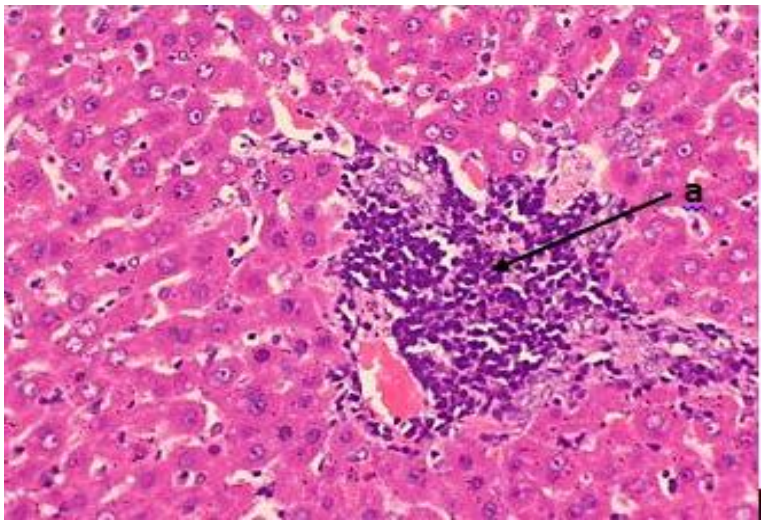


Fig 4. 68; C2R2, Liver tissue

Figure 4.68, shows, a periportal accumulation of inflammatory cells -see the direction shown by the arrow ‘a’

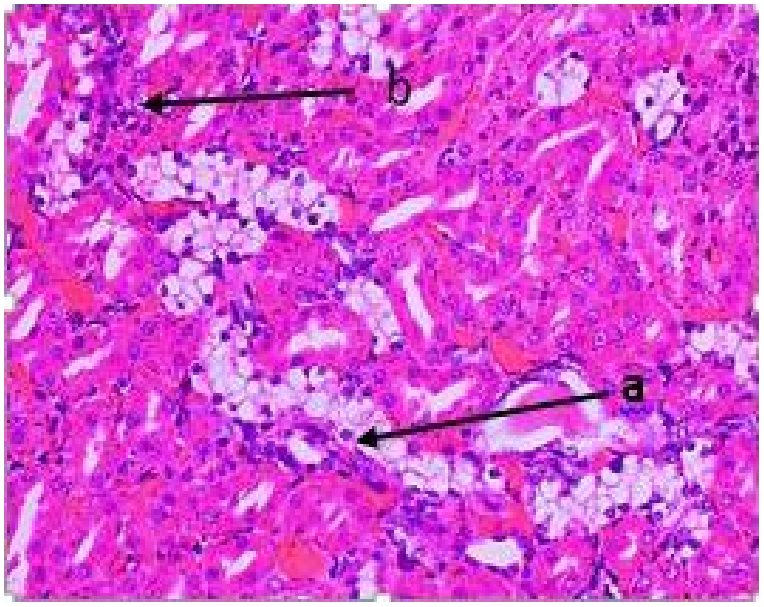


Fig 4. 69; C2R5, The Kidney tissue

The above figure indicates a diffuse DCTV (‘a’) and interstitial nephritis (‘b’),

B. Controls

(I) Diabetic induced rats

Fed with metformin

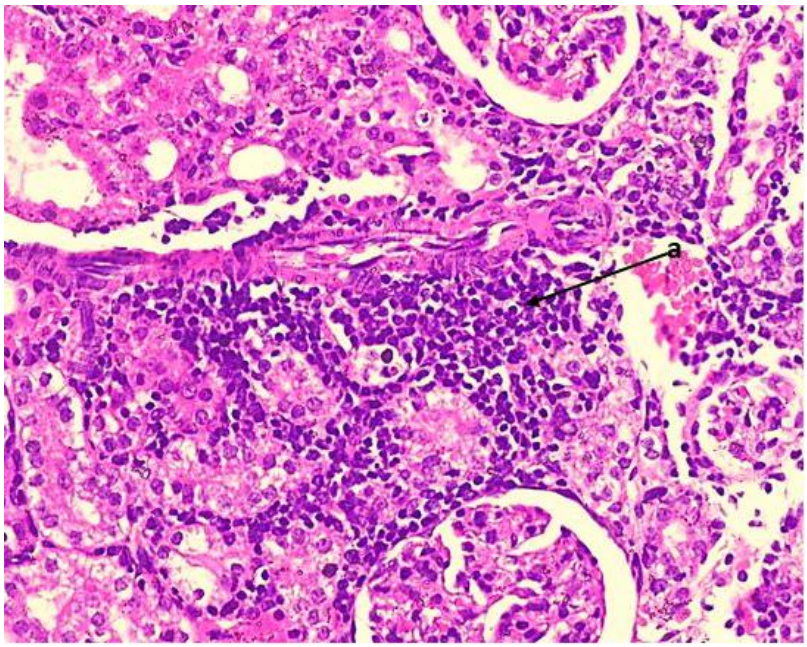


Fig 4. 70; C3R1 Kidney tissue

Figure 4.70 above shows a diffuse interstitial nephritis -shown by the arrow ‘a’ above

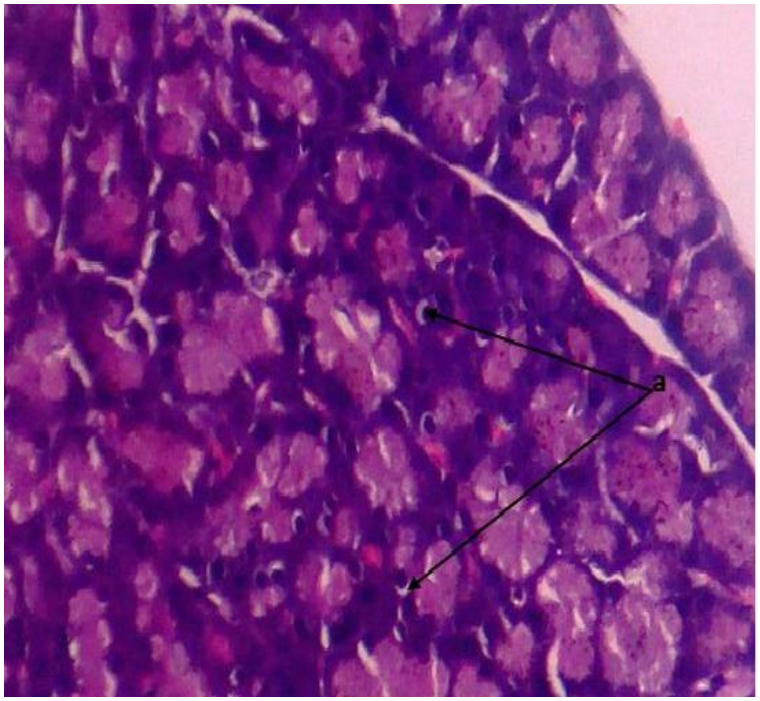


Fig 4. 71; C3R1 Pancreatic tissue

Above figure (4.71) indicates Cells with clear cytoplasm (depicted by letter ‘a’) in the pancreatic tissue

4.3.5.2 Outcomes of UD histology

Table 4. 26; Urtica dioica summary of histological changes

5 Rats/Cage		Treatment	Status	Selected	Histological outcomes
C1	CASES	UD aqueous	Induction - 140mg/kg of alloxan	R3	All tissues normal
				R1	**Kidney**- DCTV Other tissues were normal
C2		UD ethanol		R2	Kidney- Very few tubular vacuolization, PCT has web like projections into the lumen, other tissues were normal
				R3	Kidney-Diffuse interstitial nephritis DCTV. other tissues were normal
C3		metformin		R1	Kidney- Diffuse interstitial nephritis pancreas- Cells with clear cytoplasm other tissues OK
				R2	Kidney- Diffuse interstitial nephritis. Other tissues OK
C4		Water & food		R2	All tissues normal
				R3	Kidney -Diffuse interstitial nephritis, other tissues were normal
				R5	Kidney-interstitial nephritis. other tissues were normal
C5		UD ethanol	No induction	R3	Kidney- Hyper cellularity of the glomerular mesangium, other tissues were normal
				R4	Liver- Some focal lymphocytic infiltration, other tissues were normal
C6		UD aqueous		R4	Pancreas- Vacuolization of pancreatic acinar cells. Other tissues were normal
				R2	Kidney- Diffuse focal interstitial nephritis. Liver- Diffuse periportal accumulation of mononuclear inflammatory cells, other tissues OK
C7	Controls	metformin		R2 R4	All tissues normal
35 RATS				15	

Key:

1. Not shaded: Cases- Inducted and fed orally with the herbal medication of UD.
2. Light blue colour shaded: Controls -Inducted. Same across the three study groups
3. Grey colour shaded: controls -Not inducted
4. Yellow color -Cage 7-controls - same across the other 3 study groups

(a) Sampled pictures of noted histological changes

A. Cases

(i) **Aqueous extracts**

Figure 4.72, below shows a picture of kidney indicating periportal inflammation of cells

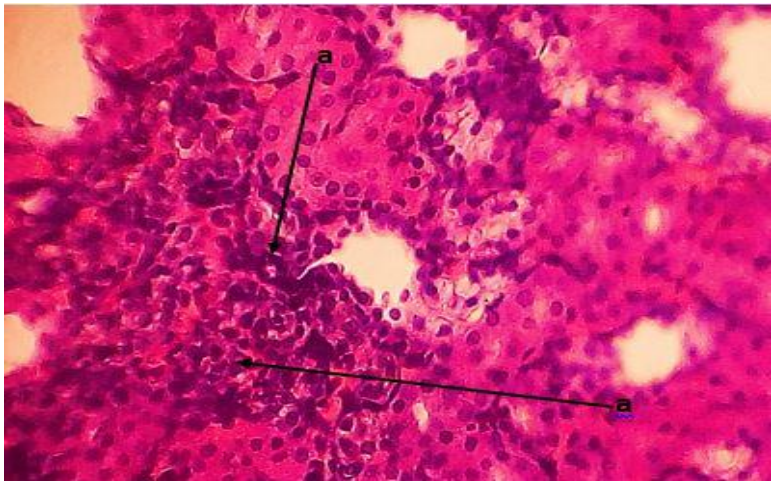


Fig 4. 72; C1R3, Kidney: Periportal accumulation of inflammatory cells 'a',

(ii) Ethanol extracts

Figure 4.73, below shows a kidney with diffuse interstitial nephritis

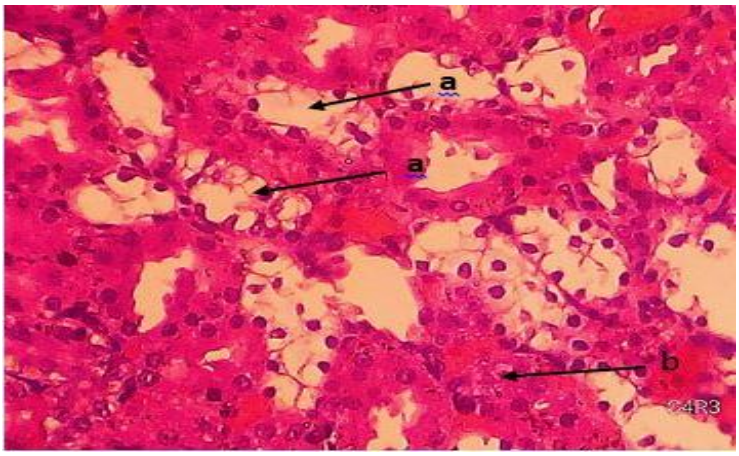


Fig 4. 73; C2R3 Kidney: Diffuse interstitial nephritis with DCTV ('a')

B. Controls

The picture (fig 4.74) below denotes a picture of a liver showing some focal lymphocytic infiltration

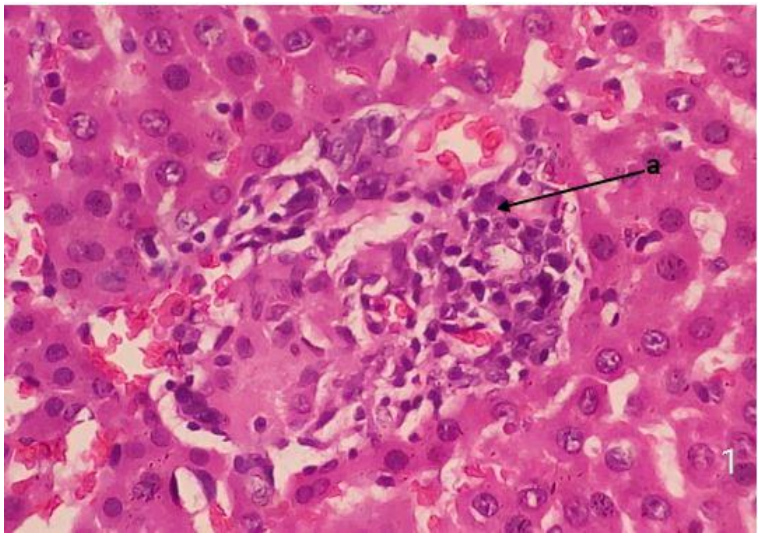


Fig 4. 74; C5R4- Liver: Liver: Some focal lymphocytic infiltration 'a'

Figure 4.75 below shows a kidney tissue, indicating diffuse focal nephritis

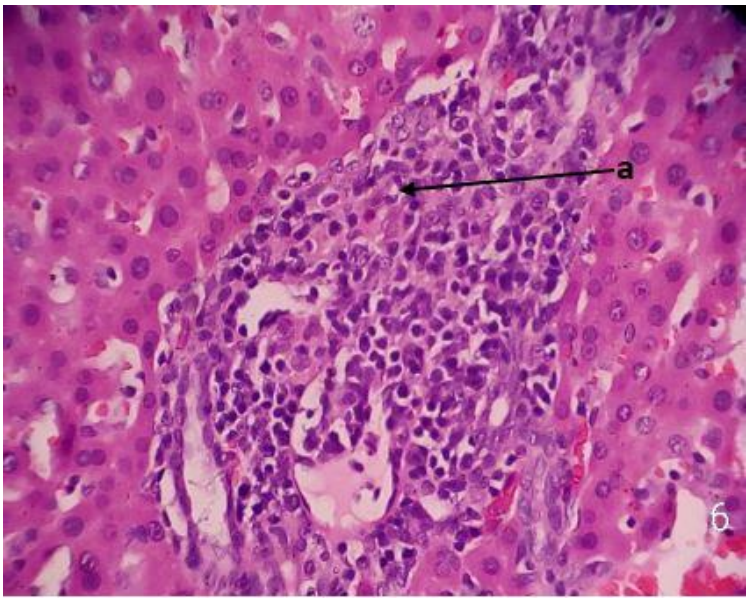


Fig 4. 75; C6R2 AQUEOUS. Kidney: Diffuse focal interstitial nephritis indicated by infiltration of mononuclear inflammatory cells between the tubules and around the Bowman's corpuscle. Glomerular areas are not affected

4.3.5.4 Mixtures of CE and UD histology outcomes.

Mixtures of CE +UD summaries

Table 4. 27; Mixtures of CE and UD (Both ethanolic and aqueous)

5 Rats/Cage		*Treatment*	*Induction Status*	*Selected*	*Histological outcomes*
C1	cases	CE and UD ethanol mixture	Induction -	R2	**Kidney** Focal Tubular vacuolization. **Pancreas**- Exocrine pancreatic cells showered some dark nucleus with clear cytoplasm
				R4	**Kidney**- some DCT showed DCTV **Pancreas**- Note clear cell cytoplasm of exocrine pancreas
C2		CE and UD aqueous mixture		R3	All normal
				R5	**Kidney**- Focal tubular vacuolization
C3		Metformin		R1	**Kidney**- Diffuse interstitial nephritis. Pancreas- Cells with clear cytoplasm
				R2	**Kidney**- Diffuse interstitial nephritis.
C4		Fed water and food ad-libitum		R2	All normal
				R3	**Kidney**-Diffuse interstitial nephritis
				R5	**Kidney**-interstitial nephritis
C5		CE & UD ethanol mixture	No induction	R2	All Normal
				R5	All Normal
C6		CE & UD Aqueous mixture		R3	**Kidney** –DCTV, **Liver**- Diffuse periportal accumulation of mononuclear inflammatory cells
				R4	All normal
C7	Controls	Metformin		R2	All Normal
				R4	
35 RATS				15	

Key:

1. Not shaded: Cases- Inducted and fed orally with the herbal medication of CE+UD mixtures.
2. Light blue colour shaded: Controls -Inducted. Same across the three study groups
3. Grey colour shaded: controls -Not inducted.
4. Yellow color -Cage 7-controls - same across the other 3 study groups

Sampled pictures

A. Cases

CE and UD ethanol mixture extracts

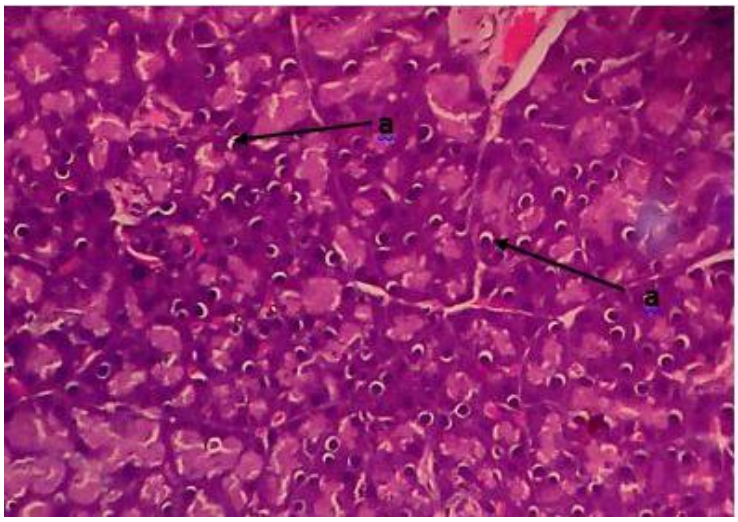


Fig 4. 76; C1R4 Pancreas: Note clear cell cytoplasm of exocrine pancreas.

B. Controls

C3R2 *Kidney*: Figure 4.77 shows, diffuse interstitial nephritis. Infiltration of inflammatory cells in the interstitial space. PCT have web like formation in the lamina. Glomerulus appears normal. Few areas show tubular vacuolization

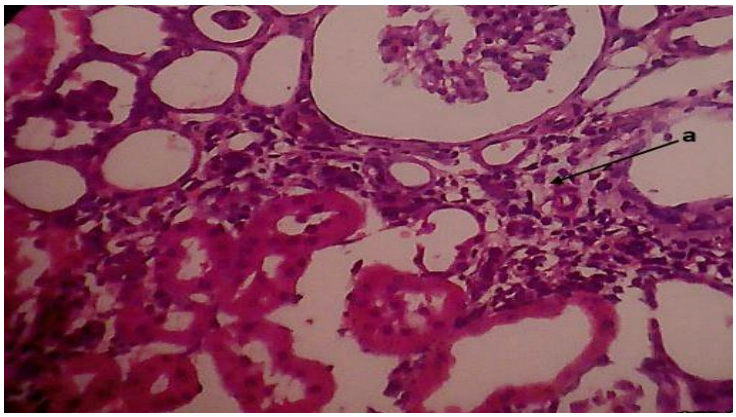


Fig 4. 77; C3R2 Kidney: Diffuse Interstitial Nephritis

4.3.8.6 Analysis of the results

At the end of the 21 days as shown in the tables and figures above, the following observations was made;

1. ***Carissa edulis*** **(CE, aqueous and ethanolic extracted extracts)**

   No histological changes were seen

2. ***Urtica dioica*** **(UD, aqueous and ethanolic extracted extracts)**

Histological changes were seen in both aqueous and ethanolic extracts of *Urtica dioica* (UD). The change seen was noted in the;

- kidneys; *hyper cellularity of the glomerular mesangium and diffuse focal interstitial nephritis.*
- The liver presented with some *focal lymphocytic infiltration and diffuse periportal accumulation of mononuclear inflammatory cells* while,
- the pancreas exhibited some *vacuolization in the pancreatic acinar cells*

4. Controls

No histological changes were seen.

5. Alloxan diabetically induced rats

Histological changes were seen in all the tissues exposed to.

CHAPTER FIVE
DISCUSSION

The study was aimed at finding out the major antidiabetic herbs used in Baringo County, biochemical properties of the identified herbs and the efficacy and safety of two commonly used herbs.

The safety of the two identified herbs was to be first determined using an ethically less demanding protocol and later [**using the findings, to obtain a lesser toxic dosages, which were to be used in examining -the identified herbs toxicity in rats]** in a more ethically demanding protocol **(use of rats protocol)** .This lesser demanding protocol was a FETAX assay which was originally developed to detect suspected teratogenic agents in mammals, but can nowadays be used in prediction of toxicity in a human and animal drugs (Herkovits & Perez-Coll, 2003; Perkins *et al.*, 2000).

Firstly, the major antidiabetic herbal medications, were identified in the study area - Baringo County. This identification utilized, a total of 39 diabetics and 12 herbalists. The 39 diabetics had an average ages similar to the findings of (Agyare *et al.*, 2009) .With regards to the herbalists age, they were found to be concurrent with the findings of (Abdelhalim *et al.*, 2017; Cheikhyoussef *et al.*, 2011; Frimpong & Nlooto, 2019; Mathibela *et al.*, 2015) contrary to studies by (Agyare *et al.*, 2009; Boadu & Asase, 2017; Kandula, 2017).

Comparing the level of education of diabetics and herbalists prescribing the antidiabetic herbal medicines, diabetics had a higher level of education relative to the herbalists with majority of them having had no formal education , a finding comparable to a study done by (Frimpong & Nlooto, 2019; Karanja *et al.*, 2017) and contrary to a study done by (Boudjelal *et al.*, 2013). Level of education therefore was found not to be a requirement for somebody to work as an herbalist, meaning that education behind diagnosing and prescription of these herbs could be as a result of apprenticeship by previous prescribing herbalists. Despite the lack of formal education, they had a good grasp of what diabetes is -a finding which was more or less similar to that of (Frimpong & Nlooto, 2019; Karanja *et al.*, 2017). Their knowledge on signs and symptoms was also fair-a finding, partly in agreement with that of (Frimpong & Nlooto, 2019) and (Akanbonga, 2015). Diabetics on the other hand, other than the signs and symptoms mentioned by the herbalists, they reported others like; '*dry mouth'* (69.2%) and '*frequent urination'* (56.4%). An understanding which was much

more precise as opposed to that of the herbalists and this could be attributed to their level of education and their inherent diabetic status.

The commonly used antidiabetics by the Tugen community have also been reported elsewhere in world by (Abate & Mengistu, 2018; Asadi-Samani *et al.*, 2017; El Haouari & Rosado, 2019; Gohari *et al.*, 2018; Kaunda & Zhang, 2017). These two herbal medications (CE and UD) were not only used to manage diabetes, but also other conditions like hypertension and abdominal related ailments. Their abundant occurrence in nature, could explain why these herbs are commonly in use. The other herbs reported include *Tamarinda indica, Zanthoxylum chalybeum, Eriobotrya japonica, Zingiber officinale*, *Sorghum bicolor, Tinospora cordofolia*, and *Cinnamomum verum* , which have also been reported elsewhere (Abate & Mengistu, 2018; Chen *et al.*, 2008; de Morais Cardoso *et al.*, 2017; Keter & Mutiso, 2012; Patel *et al.*, 2012; Shidfar *et al.*, 2015), though not so widely compared to the earlier two . Other than the ones mentioned, the other reported herbs included; *Hypoestes forskaolii, Mangifera foetida* and *Aloe tweedie species* which have seldom been reported as an antidiabetic, though plants in similar genera, *Aloe vera* and *Mangifera indica* have been reported, to be widely used (Moradi *et al.*, 2018).

Most of the above herbs were directly obtained from the forest by both the herbalists and diabetics, a finding that concurs with the works of (Chege *et al.*, 2015). Though, the source was in a forest, most of them, did not divulge to one another, which part of the forest they got their herbs from, and this could be because of the fear that; their source might be depleted or exposed. Because of these varied sources, so to the varied methods of preparation and dosaging. Most of the herbs were boiled in water, taken fresh without subjecting to any process or chewed , hence the difficult for one to clearly define the best method of preparation or quantify the dosage , a finding concurrent to a study done by (Karanja *et al.*, 2017).

Notwithstanding, the lack of standardized methods for preparation and dosaging, majority of the herbalists (66.67%) believed that the medications do not only stabilize blood sugars, (25%) but it also cures, a finding in congruence to that of (Karanja *et al.*, 2017), and contrary to that of (Frimpong & Nlooto, 2019) - "*Diabetes is not curable it can only be managed.".*

The use of these antidiabetics by diabetics was majorly as result of the influence by friends and relatives (84.6%) a finding that resonates with the studies done by

(Abdelhalim *et al.*, 2017; Alami *et al.*, 2015; Azizi-Fini *et al.*, 2016; Putthapiban *et al.*, 2017; Tulunay *et al.*, 2015) . They take this herbs because they believe that they are cheap (64.10%) helped in reducing their blood sugars when high (43.59%), easily available, accessible , (79.48%) and also because having used conventional drug for quite some time they had not felt better', a result that concurs with studies done by (Agyare *et al.*, 2009; Alhadramy, 2016; Junlapeeya *et al.*, 2018; Mekuria *et al.*, 2018; Tsabang *et al.*, 2016) .

With regards to their knowledge on conservation, both the herbalist and the diabetic's patients had an idea about what conservation was , a finding consistent with a study done by (Kariuki *et al.*, 2018; Negi *et al.*, 2018).

Unsustainable exploration of these important biological resources of social and economic value (such as diabetic herbal medications), may lead to loss of biodiversity, if not well regulated. Taking care of biodiversity and its services, therefore in the community, creates one of the reasons, why we should enhance and promote conservation and sustainable use of medicinal plants. (OECD, 2003)

On the study of phytochemicals (*biochemical components of the herbs utilized in Baringo county*) present in all the identified herbs found in Baringo County; the study used a qualitative analysis method, based on the visualization of the colour changes exposed to the herbal extracts. In general, almost all (above 8/14) the herbs in Baringo had saponnins, flavonoids and glycosides, most of which were in aqueous extracted extracts while flavonoids, glycosides, quinones, coumarins and alkaloids were found plenty in ethanolic extracted extracts.

Carissa Edulis (CE) and *Urtica Dioica (UD)* which were the most commonly used antidiabetics were rich on flavonoids, phenols and glycosides, phytochemicals which are known to poses antidiabetic activities (Gupta *et al.*, 2017).

Saponins were highly detected in the aqueous solvent because its hydrophilic in nature (water-soluble) (Wang *et al.*, 2008). They are known to activate adenosine monophosphate protein- kinase enzyme **(**AMPK), in calcium-independent channels, regulating gluconeogenesis and glucose uptake. They also reduce blood sugars by increasing; insulin uptake by the body tissues, plasma insulin levels, and induction of insulin release from the pancreas (El Barky *et al.*, 2017).

Ethanol extracted more alkaloids compounds than water in majority of the herbs, most of which had moderate (++) colour intensity and the least was phenols and tannins, with a low (+) colour intensity. Alkaloids increase insulin secretion to body tissues thus increasing glucose absorption (Kooti *et al.*, 2016).

Flavonoids were extracted moderately (++) in each herb, both aqueous and ethanol solvents, a finding similar to that of (Panche *et al.*, 2016). Flavonoids comprise of different compounds with different polarities, hence making it easy to be extracted by both aqueous and ethanolic solvents. Flavonoids are α-glucosidase inhibitors (Escandón-Rivera *et al.*, 2012; Y. Q. Li *et al.*, 2009), that prevent the digestion of carbohydrates and delay glucose absorption (Chen *et al.*, 2015; Yoshida *et al.*, 2008). They are also involved in protecting normal cell structure and function by maintaining redox homeostasis, quenching free radicals that are believed to play a major role in the pathogenesis of diabetes mellitus (Al-Numair *et al.*, 2015; Lukačínová *et al.*, 2008).

Phenols and tannins which were least extracted phytochemicals in ethanol compared to water, have been found to prevent oxidative damage of the cells and also control post-prandial hyperglycemia by inhibiting the digestive enzymes for example α-glucosidases (Ali Asgar, 2013; Goncalves & Romano, 2017).

Catechins were also least extracted in both the aqueous/water and ethanol extract with the approximate low (+) colour intensity in each herb. Catechins are effective in controlling high blood sugars by improving insulin sensitivity and also reducing the risk factors for Type 2 Diabetes Mellitus (DM type II), like oxidative stress, dyslipidemia, and obesity(Alipour *et al.*, 2018; Asbaghi *et al.*, 2019; Sasidharan *et al.*, 2011).

Most herbs yielded moderate (++) colour intensity in coumarins phytochemical test. Coumarins protect pancreatic beta cells from damage, improve abnormal insulin signaling, reduce oxidative stress/inflammation, activate AMP-activated protein kinase (AMPK), and inhibit a-glucosidases hence managing Diabetes (H. Li *et al.*, 2017).

Plant sterol was moderately extracted higher in water and slightly low in ethanol. Sterols, helps in dietary management strategy for hypercholesterolemia in persons with type 2 diabetes (Lau *et al.*, 2005).

Narrowing down to the most prescribed herbs, CE, UD, and HF. CE was found to possess 12 and 8 phytochemicals of water and ethanol extracts of the 14 screened compounds tested, respectively. Most of these phytochemicals detected showed moderate (++) colour intensity. The key detected antidiabetic phytochemicals found in CE were saponnins, alkaloids and flavonoids.

Both water and ethanol extracts of UD, had seven (7) phytochemicals each, among the fourteen (14) tested. In among the seven (7) detected, the key phytochemicals, were also Alkaloids, Flavonoids, and Saponnins (moderate [++] colour).

Hypoestes forskaolii in general recorded the least extracted phytochemicals in the top three most prescribed phytochemicals. Water extracted four (4), while ethanol extracted three (3). The key anti-diabetic phytochemicals present in HF were, phenols, saponnins, and tannins while in ethanol, the only detected relevant antidiabetic phytochemicals were terpenoids and xantho-proteins, though with low (+) or moderate (++) visualized colour intensities.

With regards to the least prescribed herbal medicine-*Solanum nigrum (SN), Aloe tweedie (AT),* and *Tinospora cordofolia (TC). Solanum nigrum* had five (5) and one phytochemical in water and ethanol extract respectively. SN water extracts had saponnins, tannins, and phenols. SN Ethanol extracts detected none of the key anti-diabetic phytochemicals, though it detected oxalates with low (+) colour intensity. This low detection of oxalates extracted in the anti-diabetic plants of Baringo County could be advantageous, because, excess intake of Oxalates leads to the development of kidney stones (Eisner *et al.*, 2010).

Flavonoids, Saponnins, and Coumarins were both detected in water and ethanol extracts of *AT* moderately *(++)*. Ethanol extract generally extracted eleven (11) of the key phytochemicals from *AT* compared to water with six (6). For *TC*, it detected nine (9) and seven (7) phytochemicals in ethanol and water respectively. Predominantly in TC, in both solvents, alkaloids, saponnins, and flavonoids were moderately [++] visualized.

Notwithstanding the biochemical components of the most utilized herbs in Baringo County, their safety component is also very important. These safety concerns may be either general or herb-specific, hence the need to study them. Tests for toxicity is

therefore very important for the development of drugs and to some equal extent, the protocols of doing the tests.

Use of animals like rats, mice and guinea pigs etc. in tests for toxicity has been there since time immemorial, but of late, *Animal rights advocates'*, see the use of these animals as unethical,' especially when a lot of them are killed in the process of doing studies. That is why , they have been advocating for the use of an alternatives or refining/ reducing the animals to a minimum, if there is no viable alternative (Schipani, 2015). It was on that, spirit that this study therefore utilized, frog embryo teratogenesis assay -Xenopus (FETAX) protocol (an in vitro study)- a protocol with fewer ethical issues, to predict the dosages that would not bring a lot of deaths or abnormalities to the animals used in the studies [the selected rats].

It was after exposing the frog embryos to the extracts of CE, UD, and their mixtures for 21 days, that an LD_{50} was calculated. Based on previous studies of CE and UD ethanolic extracts, given orally to rats and mice respectively, a LD_{50} of 3,807.9 mg/kg (Ngulde et al., 2013) and 17,213 mg/kg(Fatima *et al.*, 2018) was obtained respectively, a finding more or less same to that of this study. The dosages therefore given to the rats, were based on this finding. The least quantity of dosages that would produce minimal effects, were then determined and given to the rats. The endpoint result was that, the number of the rats that died, were few [(5.7% for crude extracts and 22.2% for purified extracts), though these deaths could have occurred due to the toxic nature of the drug used to induce the diabetic status on rats- the alloxan.

With regards to teratogenicity, in invitro studies, the teratogenic index of CE extracts, were all below 1.5. According to (Mouche *et al.*, 2017; Osano *et al.*, 2002) ,any TI value below 1.5 do not predict toxicity, signifying that CE extracts were not teratogenic, a finding that was confirmed on exposure of the same herb extracts to the male wistar albino rats-in vivo. This later finding is similar to the works of (Ya'u *et al.*, 2013), though in the case of Ya'u , *Carissa edulis* roots and bark was used as an hepatoprotective agent, to treat liver lesions brought out by drugs used to induce diabetes. In another similar study, done by (Razack *et al.*, 2017) on-CE leaves, exposed to Wistar albino rats for 90 days at a dosage were 31mg/kg for one group and another 125mg/kg for another group (Razack *et al.*, 2017), a mild histological change was seen in the kidneys –*glomerular nephritis*. A finding similar to that of (Osseni *et*

al., 2016) , but contrary to the findings of this study. The little discrepancies, found in these two studies could be brought about by the duration of exposure and the dosage of the drugs utilized i.e. Osseni exposed the mice for 90 days while this study exposed the rats for 21 days.

UD extracts on the other hand in in vitro studies, also showed a TI of less than 1.5 on frog embryos, but when the same herb was exposed to the male Wistar albino rats (in vivo), there was a manifestation of some level of toxicity. A finding similar to that of (Mukundi *et al.*, 2017), though the author used mice diabetically inducted with alloxan 186.9mg/kg and the dosage given was aqueous UD at dose of 1000mg/kg. This toxicity reported in UD, may be related to the mixtures of active compounds that they contain, including the high content of chlorophyl inherent in it (Rodriguez-Fragoso *et al.*, 2008).

With regards to efficacy, there is only one randomized control trial that has been done on CE (El-Fiky *et al.*, 1996) and it dealt, on the leaves part of CE and not the roots as opposed to this study. Nevertheless, the study reported a positive CE antidiabetic outcome in all its various extracts (aqueous, ethanolic and purified), a finding similar to this study.

With respect to UD antidiabetic properties , this study found UD to be efficacious, (UD) [(p-value <0.001 [ethanolic] & p-value =0.026 [aqueous])] a result that concurs with the findings of (Bnouham *et al.*, 2003; Gohari *et al.*, 2018; Ziaei *et al.*, 2019). UD is believed to inhibit the intestinal absorption of glucose and stimulate the islets of Langerhans to secrete more insulin, which in turn decreases high blood sugar (Boudjelal *et al.*, 2013; Mukundi *et al.*, 2017; Rashidi *et al.*, 2013; Said *et al.*, 2015).

The mixtures of CE and UD, in general, did not necessarily improve, sugar reduction potential. When CE and UD were used each singly (*whether crude ethanolic or aqueous; or ethanolic purified)* , a statistically significant sugar reduction result was elicited (p-value of ≤ 0.026) while when mixed, a non-statistical p-value was realized (a p- value of ≥ 0.052), a finding in congruence with that of (Njoroge *et al.*, 2016). Mixing of these two herbal compounds in either way produces an antagonistic interaction effect.

Comparing CE extracts (CE aqueous [crude], CE ethanol and PCE [purified]) sugar reduction capability, purified ethanolic extracts had an upper hand, followed by crude ethanolic and lastly aqueous extracts, a finding that was also seen with the extracts of *Urtica dioica*. This finding is similar to previous studies done on other hyperglycemic plants, (Abbas *et al.*, 2019; Abdullah & Kasim, 2017; Mourya *et al.*, 2017). Ethanolic extracted herbal extracts have an upper hand with regards to diabetic efficacy compared to aqueous extracted plants extracts.

In conclusion, most of the antidiabetics reported in Baringo county were common and had been reported elsewhere in the world with exception of Hypoestes *forskaolii*. The commonly used antidiabetic herbs were Carissa *edulis* and Urtica *dioica*, and they possess some of the key antidiabetic phytochemicals known to be antidiabetic as reported elsewhere, such as saponnins, alkaloids and flavonoids- that explains why they are in use and are efficacious. Ethanolic extracted extracts seem to have an upper hand in sugar reduction potential than aqueous extracted extracts. The use of FETAX invitro studies, firstly in assessing toxicity of herbal medication is imperative, in ensuring that less or no animals are lost during in vivo toxicity studies.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The commonly used herbal medications in the management of Diabetes Mellitus in Baringo County, were Urtica *dioica (UD),* Carissa *edulis (CE),* Tamarinda *indica (TI)* and Hypoestes *forskaolii (HF)*. Least reported used herbal medications were Sorghum *bicolor* seeds and Solanum *nigrum*.

2. Most of the commonly prescribed herbal medications [CE and UD] possess, some of the key known antidiabetic phytochemicals such as (saponins +++, alkaloids ++, and flavonoids ++).

3. On the dosages determined via in-vitro (FETAX) studies administered in the in-vivo (rat -protocol) studies, **Carissa *edulis*** is **safe and efficacious** while **Urtica *dioica* though efficacious** (ethanolic extracts), it is not safe- it portrayed **some significant levels of toxicity** (Objective 3&4).

6.2 Recommendations

6.2.1 To the policy makers/ diabetic herbal research scientists/ herbalists and diabetics

1. Most of the herbalists and diabetics, prescribed /used (preparation) the different herbal medications in different ways– though similar. There is need of standardization of these drugs preparation/use, to maximize their potential effects while minimizing their toxicities.

2. FETAX in vitro studies can be used as a precursor for in vivo studies [especially when evaluating toxicity of medicinal plants]. It can give a good indication of how toxic/safe a substance is (hence minimizing the use [*in terms of numbers and potential deaths*] of animals).

6.2.2 Suggestions for further studies

1. Though Urtica *dioica* is common and efficacious, the study reported to some extent, some levels of toxicity on its part (in vivo studies), hence the need of much more evaluative studies on the same (safety).

2. Though Baringo County most prescribed drugs are endowed with vital phytochemicals, there is still a scarcity of information on how most of these phytochemicals (phytochemicals in Baringo herbs) -actually help in the reduction of blood sugars, hence the need for further explorative studies.

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APPENDICES

Appendix I: Questionnaire and Interview Guide

PART I: DIABETIC PATIENTS USING HERBAL MEDICINES RESEARCH QUESTIONNAIRE'S (Researcher administered).

DEMOGRAPHY

1. Date of birth……. Age in years……
2. Gender (*Tick where appropriate)*

   Male □ Female □
3. Marital Status *(Tick where appropriate*)

   Married □ Single □

   Divorced □ Separated □ widowed □
4. Level of education *(Tick where appropriate)*

   Did not completed Primary □

   Completed Primary □

   Did not complete secondary □

   Completed Secondary □

   College □

   University □

   Not gone to school □
5. Occupation…….
6. Religion *(Tick where appropriate*)

   Christian □ Islamic □ Hindu □ Atheist □

   Others……

B. KNOWLEDGE OF DIABETES MELLITUS

7. When you were first diagnosed with diabetes...

   Or/for how long have been diabetic?

   ……

   ……
8. How did you know that you had

   diabetes…….?

   ……

   ……..

……

……..

……..

9. What do you know about this disease ……?

……

……

……

10. What medications/ remedies are you on …….

……

……

11. How do you use these treatments/remedies…….?

……

…….

12. Have you experienced any side effects with regards to the drugs that you are using?

Yes □ NO □ (*Tick where appropriate*)

If yes what are these side effects…….

13. Do you think the herbs you are using are of any help to you? Yes…….

No…. (Tick appropriately) if No

Explain……

……

14. Have ever considered other alternative remedies (if any) ……

15. If yes what are these remedies……

……

……

(C) DIABETIC HERBAL REMEDIES AND CONSERVATION

16. Have you heard about herbal medication for diabetes?

YES □ NO □ **(***Tick where appropriate*)

If YES

17. How did you come to know about this herbal drug of diabetes? Explain

……

……

……

18. Have you ever used this herbal medication? YES □ ……NO □……

(Tick where appropriate)

If YES

(i) When did you first use your herbal medication……?

(ii) What is/was the name of the herb you are using/used……

(iii) What is/was the dosage of the herb you are using/used.....?

(iv) For how long have been using it…….

(v) How often do you use it?.....

(vi) How is it prepared? (N/B if it has not yet been prepared) …….

(vii) How does it test?.....

(viii) How does it smell?.....

(ix) Have you ever experienced any side effects? YES □ …… NO □ …

If yes,

Explain ……
…

(x) Are you using the herb alone (with no other medication)? YES □ NO □ (*Tick where appropriate)*

If YES

What are those drugs? ……

How do you use them? Explain …….

Do you think being together has enhanced the treatment?.....

(xi) Do you think it has helped you? YES □ …… NO □**……**

(xii) Would you recommend diabetic herbal treatment to somebody YES □ …NO □**.**…….

19. Why these herb/s? explain *(answers objective 5)* ....

20. Where do get your herbal medication …… ……

21. Have you heard about medicinal plant conservation? If YES. describe……...

……

……

……

22. How would you conserve medicinal plants? (objective 6)

……

……

THANKS

PART II: HERBALIST PRESCRIBING HERBAL MEDICINES FACE TO FACE INTERVIEW GUIDELINE

Interview

Herbalist……. Registration No ……

(a) Age……sex……level of education…….

(b) For how long have been in this business?

(c) What conditions do you treat? ……. …….

(d) Have you heard about Diabetes……? …… Yes……NO (tick appropriately)

(e) If yes, what is Diabetes……. …….

(f) How is diabetes manifested……. ……

(g) Do you have herbal treatments for diabetes…….? …….. Yes …….NO (tick appropriately)

(h) If yes, what is the name of the herbal medicine…….

(i) How is it prepared …….

How does it function……

What is the dosage……

How often is it being used……?

For how long is being used …….

(j) Are there any clients of his/her that used the drugs? Yes □ NO□

If yes, what are their responses with regards to your treatment (explain by giving examples) ……

……

……

……

(k) Is it being used alone or with any other drugs ….....? Yes NO (tick appropriately)

(l) If yes what are these other medicines……

……

…….

(m) Have you had of any side effects/complains with regards to the herb from your clients?

Yes NO (tick appropriately)

If yes what are some of these complains……

…….

How do you respond to those complains……?

……

….

(n) Where do get your medications

……

……

(o) How often do you harvest your medications?

……

……..

(p) How long would you think it can last you if you continue harvesting?

……

…….

(q) Other than you, who else knows about your medications

……

…….

(r) What have done to ensure continuous and constant supply of your medications?

……

……

…….

(s) Have you heard about conservation?

……

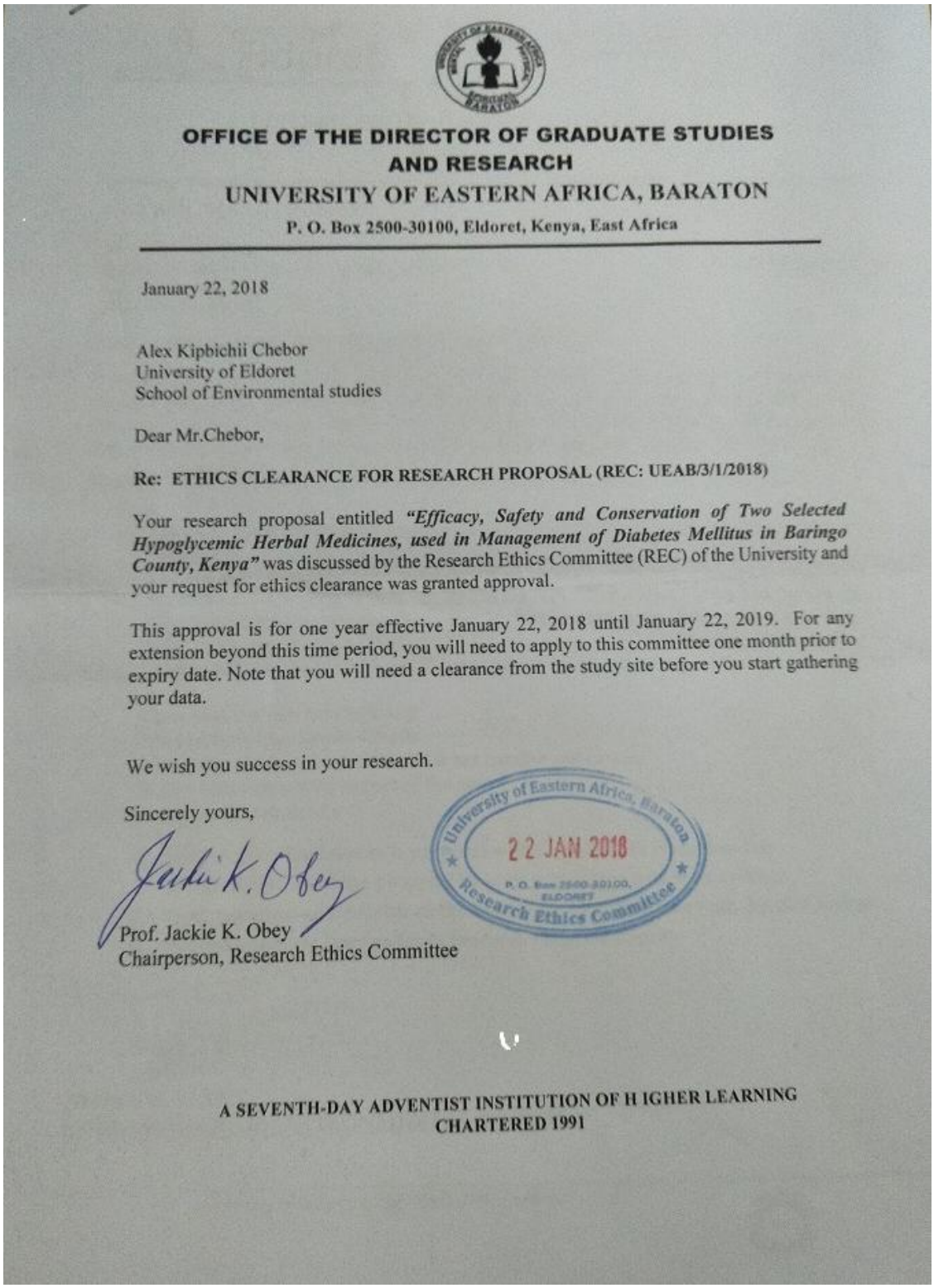
……..

(t) What do you think about conservation?

END

Appendix II: Ethical Clearance

Ethical clearance from UEAB-IREC



OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH

UNIVERSITY OF EASTERN AFRICA, BARATON

P. O. Box 2500-30100, Eldoret, Kenya, East Africa

January 22, 2018

Alex Kipbichii Chebor
University of Eldoret
School of Environmental studies

Dear Mr.Chebor,

Re: ETHICS CLEARANCE FOR RESEARCH PROPOSAL (REC: UEAB/3/1/2018)

Your research proposal entitled ***"Efficacy, Safety and Conservation of Two Selected Hypoglycemic Herbal Medicines, used in Management of Diabetes Mellitus in Baringo County, Kenya"*** was discussed by the Research Ethics Committee (REC) of the University and your request for ethics clearance was granted approval.

This approval is for one year effective January 22, 2018 until January 22, 2019. For any extension beyond this time period, you will need to apply to this committee one month prior to expiry date. Note that you will need a clearance from the study site before you start gathering your data.

We wish you success in your research.

Sincerely yours,

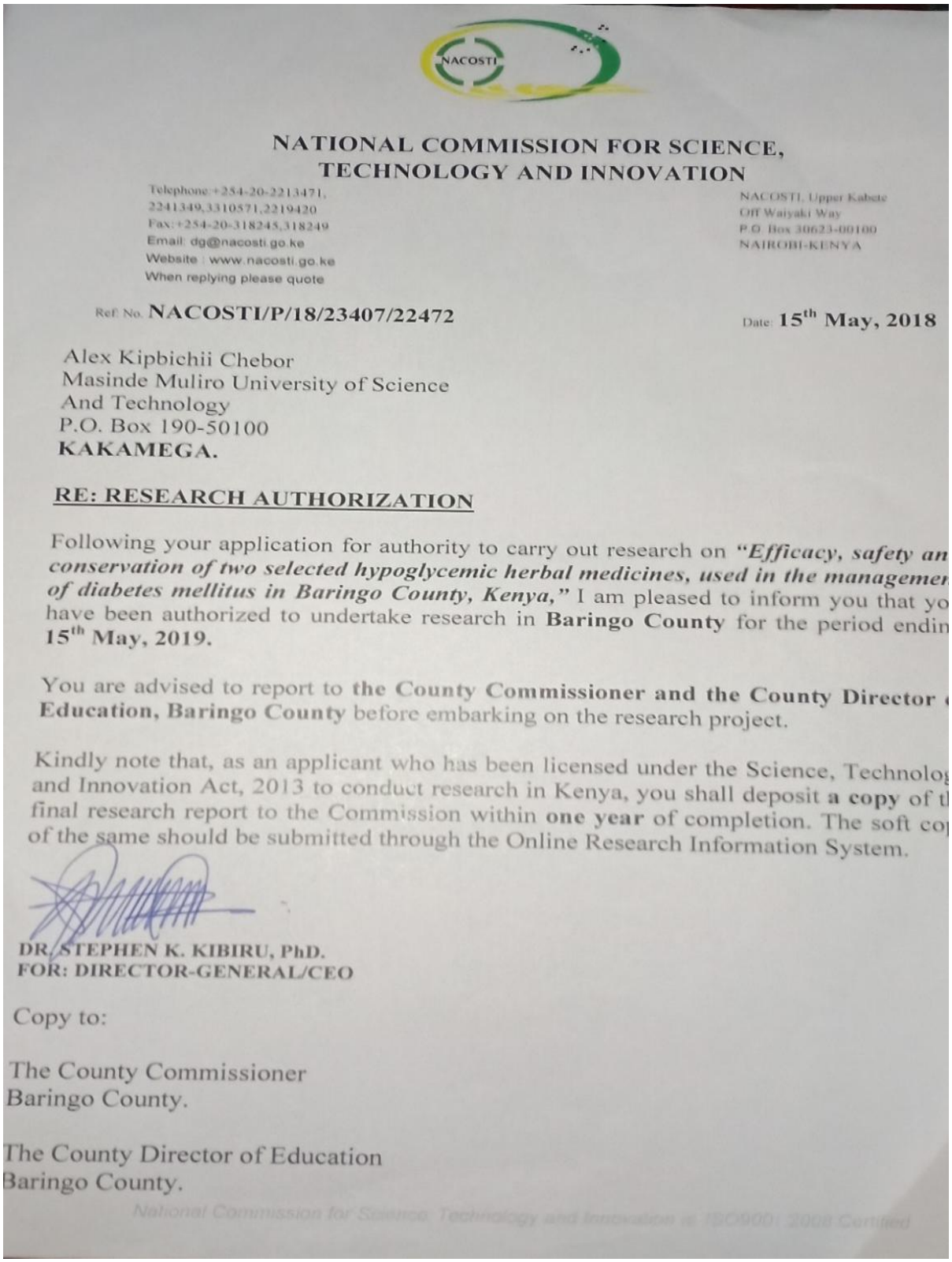
Prof. Jackie K. Obey
Chairperson, Research Ethics Committee

University of Eastern Africa, Baraton
22 JAN 2018
P. O. Box 2500-30100, ELDORET
Research Ethics Committee

A SEVENTH-DAY ADVENTIST INSTITUTION OF H IGHER LEARNING
CHARTERED 1991

National Commission for Science Technology and Innovation (NACOSTI)

Form A; Research Authorization from NACOSTI/P/18/23407/22472



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
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Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/18/23407/22472**

Date: **15th May, 2018**

Alex Kipbichii Chebor
Masinde Muliro University of Science
And Technology
P.O. Box 190-50100
KAKAMEGA.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Efficacy, safety an conservation of two selected hypoglycemic herbal medicines, used in the managemer of diabetes mellitus in Baringo County, Kenya,"*** I am pleased to inform you that yo have been authorized to undertake research in **Baringo County** for the period endin **15th May, 2019.**

You are advised to report to **the County Commissioner and the County Director Education, Baringo County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technolo and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of t final research report to the Commission within **one year** of completion. The soft co of the same should be submitted through the Online Research Information System.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

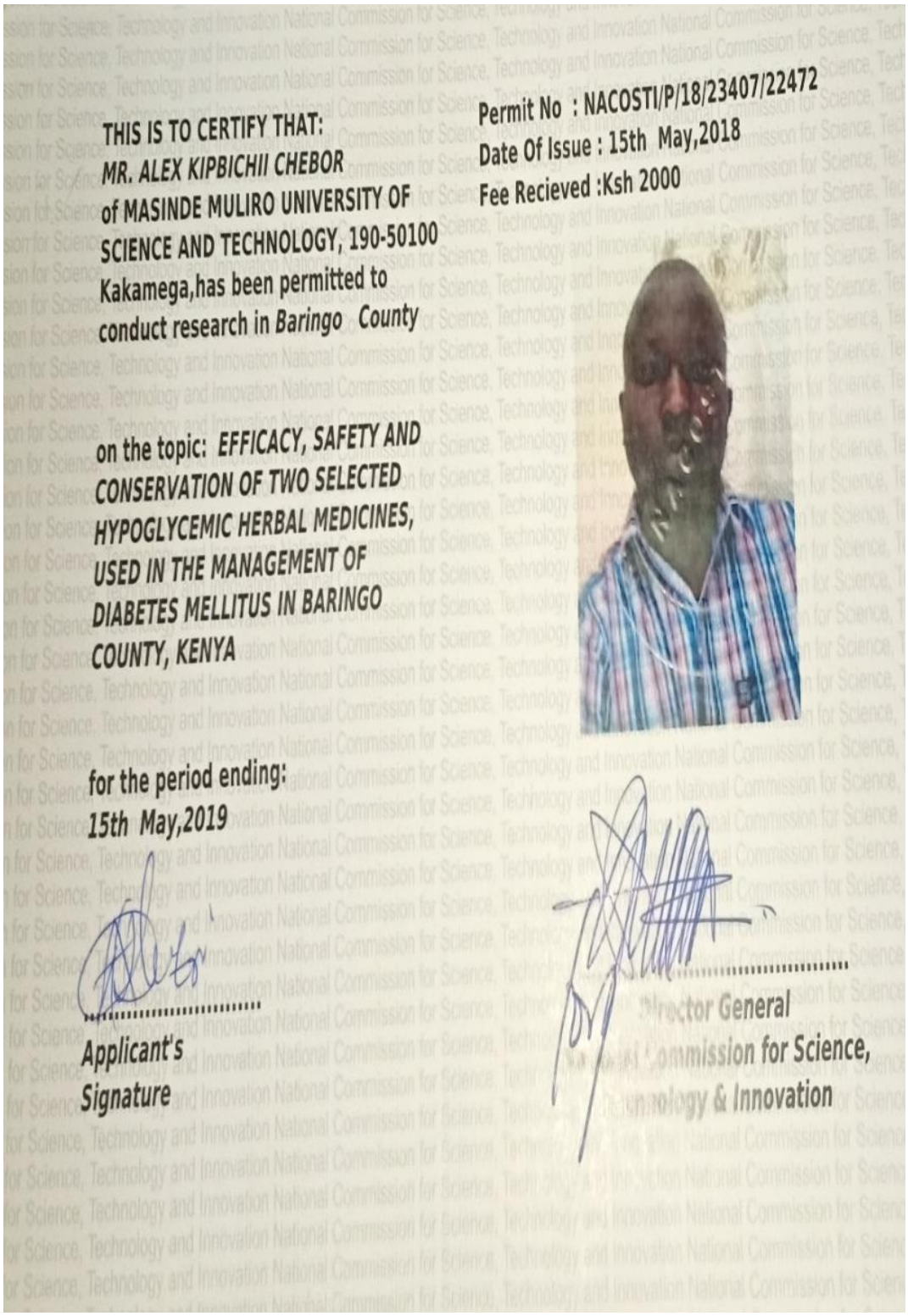
The County Commissioner
Baringo County.

The County Director of Education
Baringo County.

National Commission for Science, Technology and Innovation is ISO9001: 2008 Certified

Form B; Research clearance permit number -NACOSTI/P/18/23407/22472

(i) Front side



THIS IS TO CERTIFY THAT:
MR. ALEX KIPBICHII CHEBOR
of MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY, 190-50100 Kakamega,has been permitted to conduct research in *Baringo County*

on the topic: *EFFICACY, SAFETY AND CONSERVATION OF TWO SELECTED HYPOGLYCEMIC HERBAL MEDICINES, USED IN THE MANAGEMENT OF DIABETES MELLITUS IN BARINGO COUNTY, KENYA*

for the period ending:
15th May,2019

.....
Applicant's Signature

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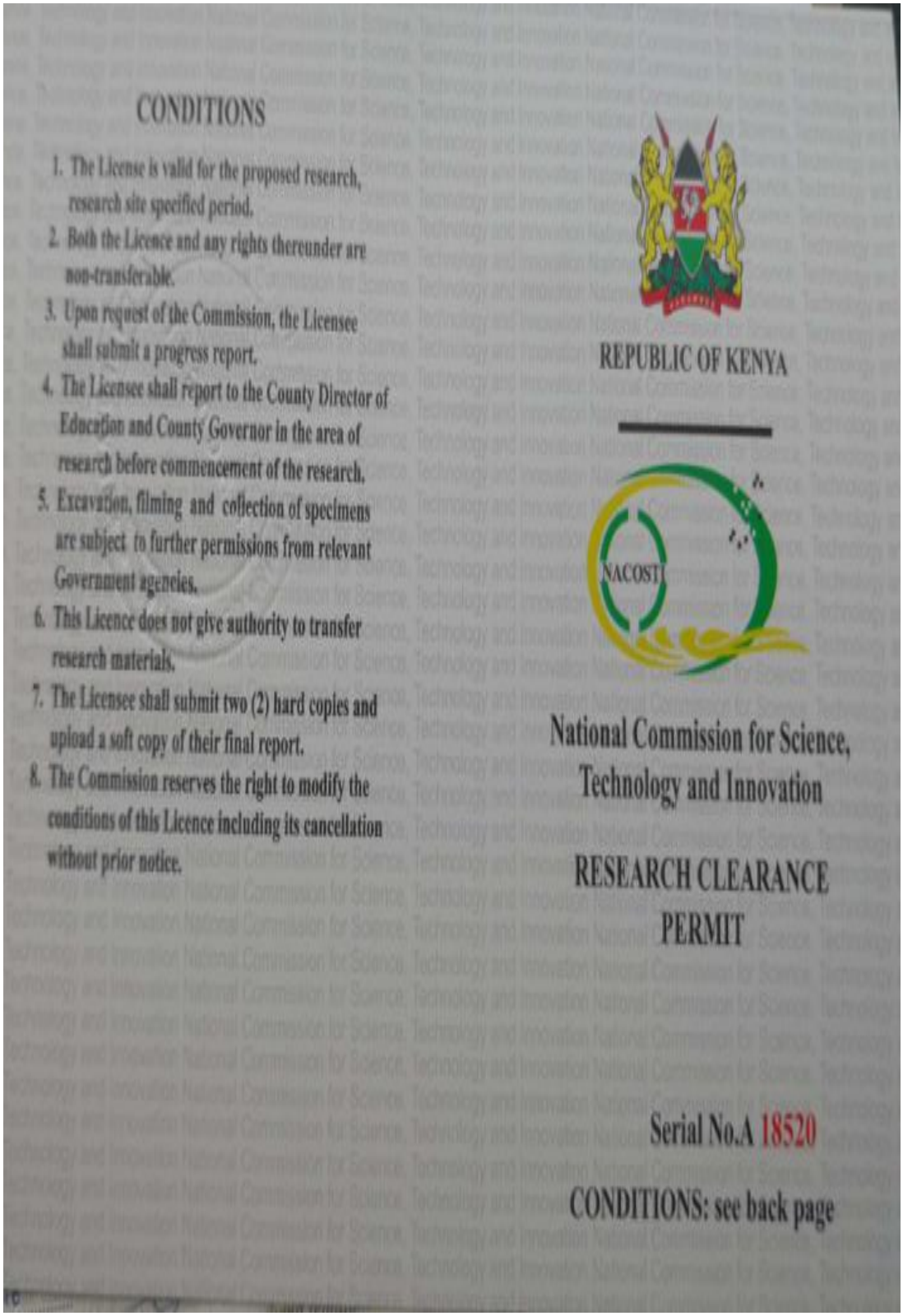

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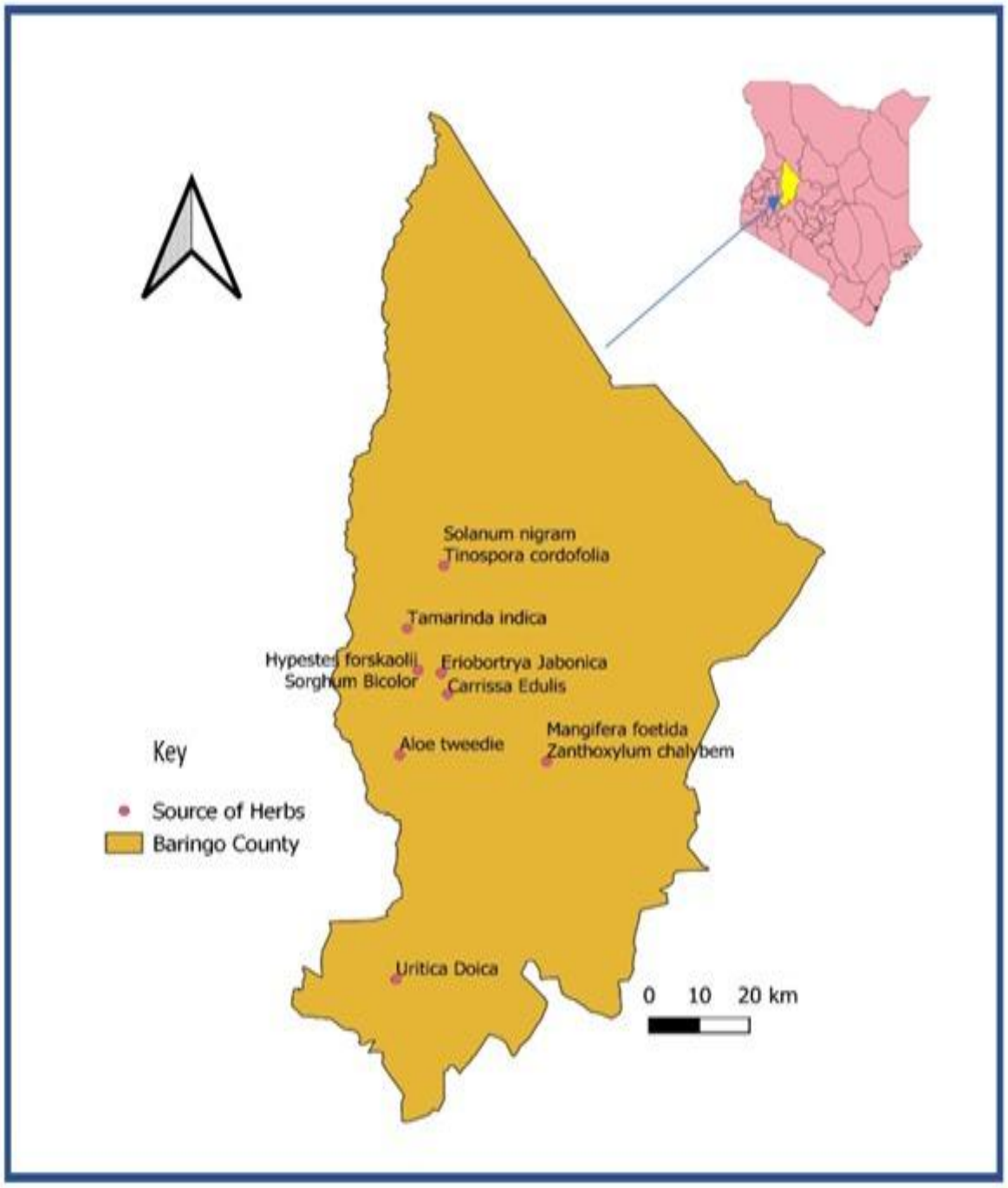
Appendix III: Map of Baringo County (source of the herbs)



Appendix IV- Selected Data and Protocols

A. Fetax Test- Data Ce Ethanol Exposure

CE ETHANOL															
	DAY 1-24 hr. ALIVE			DAY 2-48 hr. ALIVE			DAY 3-72hr. ALIVE			DAY 4-9 hr. ALIVE			ALIVE	DEAD	MEAN DEATH
conc	A	B	C	A	B	C	A	B	C	A	B	C			
0%	19	20	19	19	20	19	19	19	19	20	22	23	65	10	3.3
1.50%	20	19	19	20	19	19	18	16	17	17	16	17	50	25	8.3
3%	18	19	19	17	18	18	16	16	15	16	15	14	45	30	10
6%	20	18	18	20		17	13		16	12	0	14	26	49	16.3
12%	19	19	20	17	18	19	14	10	10	10	7	8	25	50	16.7
25%	19	19	20	15	17	20	14	10	18	4	5	3	12	63	21
50%	18	18	19	15	16	17	9	8	6	2	3	1	6	69	23
75%	15	19	17	13	18	17	0	2	1	0	0	0	0	75	25

B. Crude Extracts Efficacy Data

		Weight (g)	*day 1 sugars (*	*First week*				*Second week*				*Third week*			
				day 4 sugars				*day 4 sugars*				*day 4 sugars*			
				0hr	*1hr*	*2hr*	*3hr*	*0hr*	*1hr*	*2hr*	*3hr*	*0hr*	*1hr*	*2hr*	*3hr*
Cage 1 (CE ETHANOL)	r1	200	19.9	27.7	28	27.3	26.9	14.3	17.2	17.1	19.2	17.8	19.1	16.7	17.7
	r2	203	28.9	28.2	HI	30.1	26.4	18.9	21.2	18	19.7	17.7	20.2	15.1	13.2
	r3	197	25.2	23.9	26.6	26.5	24.6	23.4	23.5	19.6	18.5	22.9	23.9	17.2	12.8
	r4	208	HI	20.6	27.1	27	29.6	HI	HI	31.5	32	died			
	r5	197.2	30.8	24.7	24.8	25	29.5	19.5	18.5	20.1	22.9	11	22.2	21.2	13.4
Cage 2 (UD AQEOUS)	r1	213	19.8	21.7	26.1	27.7	25.8	13	7.4	17.7	21.3	25.9	22.7	20.2	21.3
	r2	201	13.7	18.6	19.1	17.3	15.1	11	13.8	11.2	10.3	21.2	27	16.1	17.2
	r3	211	19.6	21.1	13.9	15.4	11.7	23.6	22.1	19.6	13.9	19.7	18.6	17.4	16.3
	r4	210.9	32	22.6	19.6	19.1	18.2	14.9	16.1	13	12.7	21.5	19.3	17.7	14.4
	r5	209	27.9	15.3	12.7	11.3	10.9	11.2	9.2	7.7	7	9.2	10.1	6.7	5.7
Cage 3 (CE AQEOUS)	r1	201	14.1	15.2	18.5	15.7	14.1	10.9	20.7	10	15.6	died			
	r2	203.9	27	11.4	18.6	14.4	14	14.2	17.2	16.1	13.7	16.6	15.1	12.7	10.7
	r3	205	21.9	23.7	26.1	24	19	19.2	16.2	18.6	18	21.3	20.3	21.6	17.3
	r4	196.8	18.5	18	18.1	16.9	14.3	11.9	18.1	15.5	10.9	19.1	18.6	15.4	12.9
	r5	200.7	27.7	22.9	22.9	21.5	22	19.6	18.3	16.3	9.4	10.3	2.3	3.4	4.3
Cage 4 (UD ETHANOL)	r1	185	28.3	23	25.1	26	27.5	11.7	11	10.2	9.1	20.9	21.2	21.2	19.6
	r2	176.6	11.9	23.4	27.4	17.3	14.9	18.7	18.7	17.6	12.1	18.1	19.1	17.1	18.2
	r3	180.1	15.7	17.5	19.2	18.2	18	24.6	23.2	17.1	16.8	19.6	17	17.1	16.2
	r4	183.7	11.3	25.3	28.3	29.7	25.4	22.4	21.2	19.6	18.1	25.3	19.4	26.2	26
	r5	177.6	25.3	25.3	27.6	25.2	24	19.6	17	14.9	13.5	22.7	18.1	18.1	17.8
Cage 5 (UD&CE ETHANOL)	r1	167.8	17.3	18.5	18	17.9	16.1	18.7	15.3	12.3	9.2	17.4	14.8	13.1	10.1
	r2	171.7	24.6	21	21.9	22	19.6	22.4	18.7	15.3	10.2	22.6	19.7	17.2	13.2
	r3	169.2	12.7	19.1	16.6	15.5	14	21.5	16.2	13.9	10.1	20.5	21.6	17.6	14.9
	r4	173.7	18.4	19.9	25.3	26.4	26.3	21.8	22.6	15.7	11.1	27.4	19.4	11.7	5.9
	r5	174.6	17.5	17.9	21.9	29.8	26.5	26.1	28.1	24.3	19.8	24.9	19.6	15.4	11.9
Cage 6 (UD&CE AQEOUS)	r1	192.8	14.9	11.3	9.2	7.9	10.1	13.9	15.1	11	10.8	15.1	17.2	14.6	10.9
	r2	183.9	15.2	9.2	5.1	7.7	8.4	12.9	16.2	11.9	11.2	11.7	10.7	7.8	6.9
	r3	187.2	9.2	24.6	32	28.7	25.6	25.7	31.1	21.3	24.6	15.9	11.7	5.2	4.9
	r4	190.1	29.7	21	27.1	23.7	18.8	23.9	23	22.8	23	26.9	27.9	21.3	18.8
	r5	191	18.6	18	15.6	15.1	15.4	19.6	20.1	17.2	11.8	18.4	13.1	10.7	6.7
Cage 7 (METFORMIN)	r1	207.1	13.9	14.1	10.1	9.8	6.5	23.1	20.6	16.2	13	11.7	10.1	8.2	7.1
	r2	211	25.3	19.7	17.2	16.3	10.7	17.1	15.1	13.7	11.2	15.2	13.9	10.2	9.1
	r3	213.7	12.7	16.1	15.4	11.9	9.5	25	23.4	19.6	15.1	17.1	15.2	14.1	12.9
	r4	214.9	11.9	18.6	18.0	15.1	10.4	11.8	11	8.9	6.9	13.9	11.2	10.1	8.7
	r5	208	HI	21.6	18.7	15.7	13.9	13.9	10.2	9.5	6.3	17.1	14.8	12.8	10.3

C. EC_{50} and LC_{50} Calculations

1.Calculation of EC 50, CE ethanolic extracts- probit analysis

	X							Y	x		y		
CONC, C%	LOG(C%)	examined	abnormal	% abnormal	adj % alive	adj % dead	probit	probit +5	(X-mean X)	x2	Y- mean Y	y2	xy
0		65	2	0.030769	1	0							
1.5	0.176091	50	5	0.1	0.928571	0.071429	-1.46523	3.534766	-0.60561	0.366758	-0.83023	0.689274	0.502789
3	0.477121	45	7	0.155556	0.871252	0.128748	-1.13233	3.86767	-0.30458	0.092766	-0.49732	0.247329	0.151472
6	0.778151	26	8	0.307692	0.714286	0.285714	-0.56595	4.434051	-0.00355	1.26E-05	0.06906	0.004769	-0.00024
12	1.079181	25	9	0.36	0.660317	0.339683	-0.41333	4.58667	0.297484	0.088497	0.221679	0.049141	0.065946
25	1.39794	12	8	0.666667	0.343915	0.656085	0.401801	5.401801	0.616243	0.379755	1.036809	1.074973	0.638926
50	1.69897	6	6	1	0	1							
75	1.875061	0	0										
MEAN	0.781697							4.364992					
SUM										0.92779		2.065487	1.358889

Mean (X)=	0.781697
Mean (Y)=	4.364992
SUM(x^2) =	0.92779
SUM(y^2) =	2.065487
SUM(x*y) =	1.358889
y=ax+b	
Slope(a)=	1.464652
intercept(b)=	3.220078
when Y=5	
Then X=	1.215253
Concentration =	16.41545
r^2	0.963598

2. Calculation of LC 50, CE ethanolic- Probit analysis

	X						*Y*	*x*		*y*		
CONC, C%	*LOG (C %)*	*MEANDEAD*	*% dead*	*adj % alive*	*adj % dead*	*probit*	*probit +5*	*(X-mean X)*	*x2*	*Y- mean Y*	*y2*	xy
0		3.333333	0.133333	1	0							
1.5	0.176091	8.333333	0.333333	0.769231	0.230769	-0.73632	4.263684	-0.75848	0.575299	-0.99175	0.983568	0.752227
3	0.477121	10	0.4	0.692308	0.307692	-0.5024	4.497598	-0.45745	0.209265	-0.75784	0.574315	0.346676
6	0.778151	16.33333	0.653333	0.4	0.6	0.253347	5.253347	-0.15642	0.024469	-0.00209	4.35E-06	0.000326
12	1.079181	16.66667	0.666667	0.384615	0.615385	0.293381	5.293381	0.144605	0.020911	0.037947	0.00144	0.005487
25	1.39794	21	0.84	0.184615	0.815385	0.897915	5.897915	0.463364	0.214706	0.642481	0.412782	0.297703
50	1.69897	23	0.92	0.092308	0.907692	1.326678	6.326678	0.764394	0.584298	1.071244	1.147563	0.818852
75	1.875061	25	1	0	1							
MEAN	0.934576						5.255434					
SUM									1.628948		3.119673	2.221272

Mean (X)=	0.934576
Mean (Y)=	5.255434
SUM(x^2) =	1.628948
SUM(y^2) =	3.119673
SUM(x*y) =	2.221272
y=ax+b	
Slope(a)=	1.363624
intercept(b)=	3.981024
when Y=5	
Then X=	0.747256
Concentration =	**5.587994**
r^2	0.970928

D.**Unpurified Efficacy Tests Protocol**

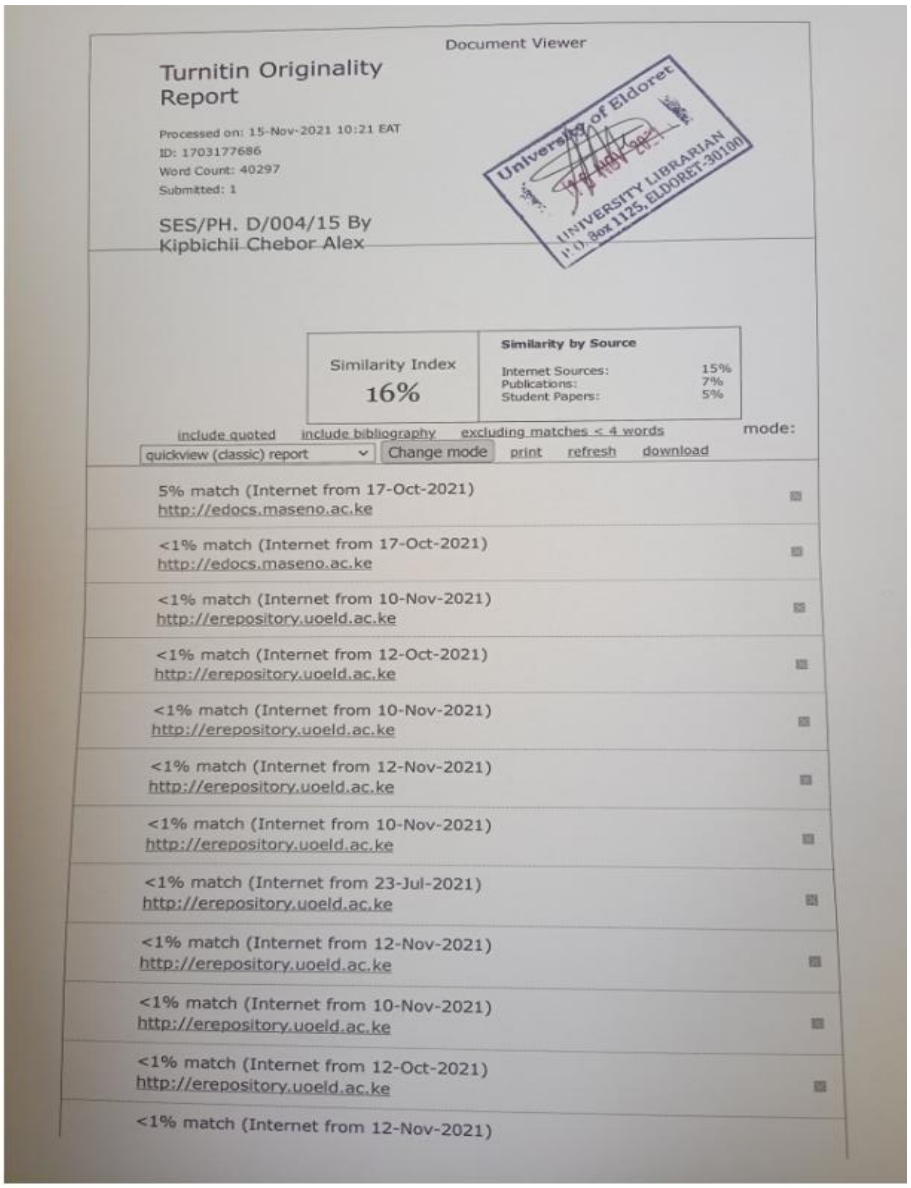
cages	Control/cases	rats	***Weight (g)***	***day 1 sugars (***	***First week***				***Second week***				***Third week***			
					day 4 sugars				***day 4 sugars***				***day 4 sugars***			
					0hr	***1hr***	***2hr***	***3hr***	***0hr***	***1hr***	***2hr***	***3hr***	***0hr***	***1hr***	***2hr***	***3hr***
Cage 1 Carrissa *edulis-CE* (Ethanolic extracts)	Cases	5														
Cage 2 Urtica *dioica* UD (aqueous Extracts)		5														
Cage 3 Carrissa *edulis-CE* Aqueous		5														
Cage 4 Urtica *dioica-UD* Ethanolic extracts		5														
Cage 5 Mixture of Carrissa *edulis* -CE and Urtica *dioica* Ethanolic extracts		5														

Cage 6 Mixture of Carrissa CE *edulis* and Urtica *dioica-UD* aqueous Extracts		5														
Cage 7 (METFORMIN)	Controls	5														

E. Efficacy of the Purified Test Protocol

		Weight (g)	***day 1 sugars (***	***First week***				***Second week***				***Third week***			
				day 4 sugars				***day 4 sugars***				***day 4 sugars***			
				0hr	***1hr***	***2hr***	***3hr***	***0hr***	***1hr***	***2hr***	***3hr***	***0hr***	***1hr***	***2hr***	***3hr***
Purified ethanolic extracted extracts of herb Carrissa *edulis-CE*	r1														
	r2														
	r3														
	r4														
	r5														
Purified ethanolic extracted extracts of Urtica *dioica-UD*	r1														
	r2														
	r3														
	r4														
	r5														
Purified mixtures of ethanolic extracted extracts of Carrissa *edulis-CE* and Urtica *dioica-UD*	r1														
	r2														
	r3														
	r4														
	r5														

Appendix V: Similarity Report



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