PHYSICAL-CHEMICAL AND BIOACTIVITY INVESTIGATIONS OF ANTI-DIABETIC TRADITIONAL DRUGS

BY

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DECLARATION

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

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DEDICATION

To my Family, Father, Mother and Uncles

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GLOSSARY

General Abbreviation

AIDS	Acquired Immune Deficiency Syndrome
AR	Analytical reagent
ATP	Adenosine triphosphate
BMI	Body mass index
b.w.	body weight
^{O}C	Degrees centigrade
$^{13}CNMR$	Carbon 13 nuclear magnetic resonance
CADE-A	Crude aqueous extract A
CADE-B	Crude aqueous extract B
CHR	Carbon hydrogen ratio
COLOC	Correlated spectroscopy via long range coupling
Conc	Concentration
COSY	Homonuclear correlation spectroscopy
DEPT	Distortionless enhancement by polarization transfer
DNA	Deoxyribonucleic acid
ε	Extinction coefficient
FAB-MS	Fast atom bombardment - mass spectrometry
GLC	Gas Liquid chromatography
GR	General Purpose reagent
G.T.T	Glucose Tolerance Test
HMBC	Heteronuclear multiple bond correlation
$^{1}HNMR$	Proton nuclear magnetic resonance
HPLC	High performance liquid chromatography
hRf	retention time relative to front times 100
IHD	Index of Hydrogen Deficiency
IR	Infra red
Kg	kilogram

LC-MS	Liquid chromatography – mass spectrometry
M	Molar
Me	Methyl
m.p.	melting point
NAPRALERT	Natural Products Alert
nm	nanometers
NOESY	Nuclear overhauser effect
PC	Paper chromatography
ppm	parts per million
RDA	Recommended dietary allowance
Rt	Room temperature
TLC	Thin layer chromatography
TM	Traditional medicine
UV/Vis	Ultra violet/visible
WHO	World health organization

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Water extract of *Podocarpus* sp (CADE-A), chloroform, methanol and a mixture of water extracts of *Rhamnus prinoides*, *Carissa edulis*, and *Toddalia asiatica* (CADE-B) have been used traditionally by Kenyan communities for the purpose of stimulating myometrial contractions, alleviating diabetes, polio, colics, malaria, bronchial pains, indigestion, remedy for paralysis and hypertension (Kokwaro, 1976). Solutions of 8ml CADE-A/kg b.w, 20mg/kg b.w. of chloroform and methanol extract of *Podocarpus* sp prepared in a solution containing 2.5g/kg b.w. of glucose improved glucose tolerance in group 1, 2 and 3 rabbits weighing mean 3.1 ± 0.90 kg. Absorption of sugar from the gut was improved by both CADE-A and chloroform extract where maximum absorption occurred at (140.70 ± 1.69 mg/100ml) and (165.32 ± 4.18 mg/100ml) at 60 and 90 minutes respectively. Glucose absorption was improved slightly by methanol extract where it occurred at (117.24 ± 2.14 mg/100ml) after 90 minutes. The end point blood glucose levels were significantly reduced in group 1 and 2 ($P<0.01$). The rate of absorption of glucose into the tissues in group 1, 2, and 3 were significantly reduced ($P<0.01$). During the oral glucose tolerance test, peak blood glucose levels in group 4 rabbits were 129.59± 2.05mg/100ml where it occurred at 120 minutes. The glucose tolerance curve of the rabbits over entire period of sampling was statistically significant ($P<0.01$).

Muscle contraction of CADE-A and CADE-B on isolated rabbits heart and rats' non-gravid myometrial strips show dose dependent inhibition of muscle contraction at physiological doses. The contraction in the heart is augmented by propranolol. Higher unphysiological levels cause concentration-dependent increase in tension in the intestinal musculature of the rabbit. CADE-A and CADE-B, both cause the reduction in amplitude of more than 20 % of the normal contraction of the heart. In addition each causes a slowing down of the heart rate by more than 20% of the normal and complete relaxation of the uterine musculature. Each extract increases the tension in the ileum by more than 70%. At very high doses, each extract causes concentration-dependent increases in tension and contractile response to these high doses of the extract is ionotropic - increasing the amplitude by about 35% of the normal. All these relaxations and increases in the tension of the isolated organs are statistically significant ($P< 0.01$).

The levels of essential elements in CADE-A were Copper 0.05 mg/L, Zinc 1.5mg/L, Chromium 0.034mg/L and Iron 1.4mg/L and likewise in CADE-B were Copper 0.60 mg/L, Zinc 0.030mg/L, Chromium 0.100mg/L and Iron 4.2mg/L. These low concentration

levels of the first row transition elements are essential for action of many enzymes in biologically important molecules in life.

Saponins and sapogenins in the water extract are most probably the biochemical basis on hypoglycemic and muscle contraction. The compounds identified, isolated, characterized in *Podocarpus* sp bark extract are compound **A,** a sapogenin, melting point (mp) 201-203 ^{O}C, UV/Vis absorption at λ_{max} 472nm, molecular ion peak $[M]^{+}$ at m/z= 538 amu determined by MS-FAB analysis corresponding to molecular formula $C_{32}H_{42}O_7$. Compound **B,** a saponin, melting point (mp) 184-187 ^{O}C, UV/Vis absorption at λ_{max} 478nm and molecular ion peak $[M]^{+}$ at m/z= 537.2 amu corresponding to the molecular formula $C_{31}H_{39}NO_7$.

CHAPTER ONE

1.0 INTRODUCTION

1.1 General historical overview

From the beginning of man's existence, he has exploited plants for many purposes such as food, shelter, clothing and medicine. The history of man's use of plants as medicines dates back over 5,000 years, to the Eqyptian ancient civilization and Indian medical system, the Ayurveda, which basically means the 'science of life'. This system recorded over 8,000 plant-based remedies. A thousand years later the Assyrians listed over 250 plants with medicinal uses and the ancient Sumerians were found to have recorded over 1000 medicinal plants. Hippocrates (approx. 460-377 B.C.) discussed and prescribed between 300 and 400 species for the alleviation of sickness (Scandalios, 1992, Farnesworth *et al,* 1985).

Every culture has a history of using medicinal plants to cure its ailments. Traditional societies have a wealth of such knowledge, which they have built up over several thousand years, and passed down through the generations. People believed that God put plants on this earth for human use, and that the shape of the plant was a clue as to what ailment it cured. For example, the similarity of walnuts to the human brain led people to believe that it was helpful in treating conditions of the brain (Marles *et al*, 1996). This idea was known as the doctrine of signatures. It was soon displaced by the science of hypothesis testing and the experimental approach in the seventeenth and eighteenth centuries. Throughout the centuries, scientists have used this knowledge by observing native peoples and listening to folklore, as in the case of William Withering in the late eighteenth century. He found a cure for 'dropsy', the term used then to describe oedema (a swelling of body parts due to fluid accumulation) from the Purple foxglove (*Digitalis purpurea*) after investigating a family cure. The drug isolated was named digitalis and is still used to this day in the treatment of heart conditions (Farnesworth *et al*, 1992).

Plants are part of what Mother Earth has to offer to humanity. As a result of deforestation to open up new lands for agricultural use, settlement, industrial development and timber production, we have abused that gift. Many of these plants are under threat of extinction. It had been estimated that if the trends continued, some 20,000 plants used in traditional

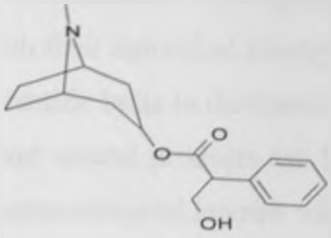
medicine as healing agents would have ceased to exist by the end of the last century (Scandalios, 1992).

Changing of our environment and lifestyles leads to loss of knowledge on indigenous medical systems. Species of medicinal plants which have been a source of traditional medicine and an ancient practice of our forefathers is also endangered. Until a national database intended for storage of historical knowledge is established, all the useful ancient know-how will persistently disappear as more and more traditional medical experts die.

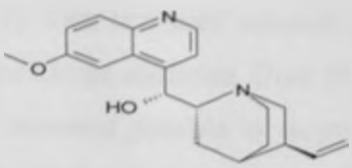
1.2 Plants as a source of traditional medical therapies

Many other reported medicinal plants were studied in the centuries that followed the advent of isolation techniques and other scientific methods. Plants' compounds were discovered after following ethnobotanical leads. Most of the drugs produced at that time were isolated from plant-derived extracts. But after a while scientists started to concentrate on finding the structure of these compounds and synthesizing them. The ability to synthesize these natural compounds has led to redundancy of these 'useful' plants, since scientists have been able to produce medicines that are superior to those originally produced by extraction of the plants in terms of bioactivity and the yield after production as well as they may have less side effects (Farnesworth, 1992).

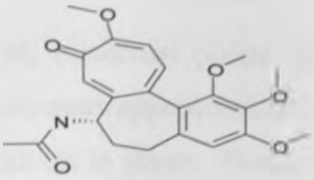
Since the advent of synthetic organic chemistry in the second and third quarters of last century, syntheses of compounds have been the most popular means of drug discovery (Farnesworth, 1994). This now consumes the major portion of research institutions' time, effort and of research funds. A number of essential drugs used in modern day medical practice are plant derived and includes atropine (anticholinergic), codeine (antitussis/analgesic), colchicine (antigout), digitoxin (cardiotonic), quinine (anti-malarial), reserpine (antihypertensive) as shown below. Saponin extracts that are chemically altered to produce sapogenins, necessary for the manufacture of steroidal drugs were, until recently, obtained from extracts of neotropical yams of *Dioscorea*. Cocaine derived from *Erythroxylon coca* provided the chemical structure for the synthesis of procaine and other related anaesthetics. Many more can be cited (Akerele, 1992).



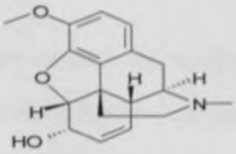
Atropine



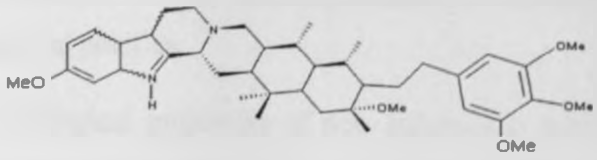
Quinine



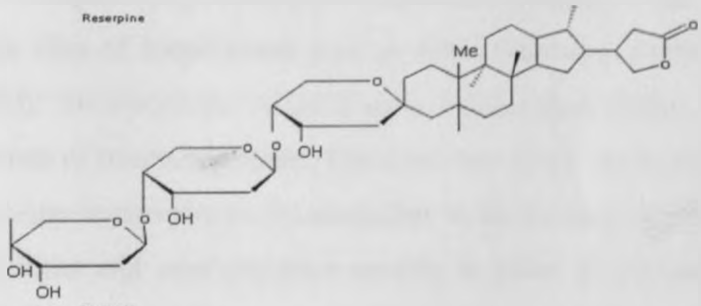
Colchicine



Codeine



Reserpine



Digitoxin

African countries still rely on imported synthetic chemical compounds and preparations for use in pest control especially mosquitoes and anthropods, that cause malaria and anthropod born-diseases respectively. One way of solving this problem is proper utilization of our natural resources in pursuit of developing cheap insecticidal agents – from locally available plants – that are effective in controlling pests in commercial cash crops which would in turn lead to increased food production (Abiy, 1997).

The numbers of natural products isolated from higher plants, since the beginning of the last century, are in the thousands. Chemists using increasingly more efficient and sophisticated equipment e.g. Liquid chromatography, ^{1}HNMR, ^{13}CNMR and Gas Chromatography and Mass spectrometry analytical techniques, have now established

their structures from reproducible results. It is then possible to correlate their structures with their individual biological activity. Two centuries' research in this field has given a scientific basis to the institutions of traditional medicine. Once the structures of bioactive plant natural products are known, it becomes possible to prepare synthetic products of pharmacological interest which are identical or analogous to the natural ones, or to mimic their activity with simplified structures.

In the past, medicinal plants were the main source of medicaments for humans. At present, two main approaches are followed to establish the presence of pharmacologically active products in plants. These include a systematic screening of plant extract samples, in a kind of roulette, which may sometimes give interesting results, besides the identification of the active principles of medicinal plants recognized by traditional medicinal practice.

The biological properties of new compounds may be attributed to their ability to bind onto particular receptors (e.g. CNS, and sympathetic system), or their ability to interfere with the active sites of biopolymers such as DNA (tumours, AIDS) and also on their ability to modify' the enzymatic action in cells, besides their ability to block the growth or the metabolism of micro-organisms. The structures of natural products with biological activity often cause excitement to the researcher in the developed world and stimulate in him/ her new ideas and new computer models in order to design new drugs. In the developing world, where research funds and equipment are scarce, finding a new active natural molecule can be equated to locating a needle in a haystack.

Traditional medicinal extracts may be an alternative to patent drugs for alleviating malignant diseases. There is, therefore, global continuous interest in the search for new bioactive compounds from higher plants. This provides possible medication for some particular diseases such as malaria, diabetes, tumors and even AIDS.

1.3 Rain forests as a source of diverse plant products

Equatorial and tropical rain forest support extremely diverse habitats and is home to a wide variety of medicinal plants. Diverse plants found in these habitats are an economic source of timber. Land for cattle ranches, human settlement along with other factors, is causing rapid disappearance of these medicinal plants and their habitats. One way of

protecting the land, plants and the people who live on it is to create extractive reserves. These are areas of forest set aside for continuous harvesting of renewable forest products such as rubber, nuts, fruits, sisal fibre, oils and medicinal plants. Other important reasons why we should prevent the destruction of the rain forests is their essential role in macro-ecological processes. These include the balance of atmospheric gases that affect global climate, and their role in the water cycle. In tropical rain forests, many extractive reserves are based solely on two products, rubber and wood. The indigenous people in the forest use plants for so many different things. Therefore the key to a successful extractive reserve is to make use of all possibilities within it in utilizing and managing these natural resources (Farnesworth, 1990).

If managed by international agreements aimed at protecting the intellectual property rights of ancient medical systems, the setting up of extractive reserves is an extremely viable idea that represents a socially just means of conservation. There are many other sustainable ways of preserving the rainforests. The National Park schemes and such NGOs as the Green Belt Movement in Kenya and organizations like the World Wide Fund for Nature are setting up reserves such as this one to protect and conserve sections of forests and their inhabitants.

1.4 Chemical compounds from higher plants

Until the dawn of last century, natural products had been accepted as the mainstay of all medicines world-wide. Although herbalism has declined in the west, it continues to thrive vigorously throughout the developing world especially in Kenya. Large sections of indigenous communities of the world still rely on herbal preparations for requirements in healthcare management: for example, in the Congo basin in Africa, the Amazon, China and India. In these regions conventional medicine is sometimes out of reach (Farnesworth, 1992).

The systematic drug development programs from natural sources are based on the bioassay-guided isolation of natural products, taking into consideration the folklore uses of local plants. A number of bioassays have been developed recently to direct the isolation work by research institutions especially Kenya Medical Research Institute (KEMRI). The author has adopted the above-mentioned strategy for identifying new lead

compounds from a variety of reputed medicinal plants. The examples given below and bioactive compounds reported by other researchers, exemplify the importance and the potential of bioactivity - directed phytochemical investigations.

Phytochemicals in fruits, vegetables, spices and traditional herbal medicinal plants have been found to play protective roles against many human chronic diseases including cancer and cardiovascular diseases (CVD). These diseases are associated with oxidative stresses caused by excess free radicals and other reactive oxygen species. Antioxidant phytochemicals exert their effect by acting as antioxidants: hence neutralizing reactive free radicals in our body systems. Among the tens of thousands of phytochemicals found in our diets or traditional medicines, polyphenols and carotenoids stand out as the two most important groups of natural antioxidants. Hundreds of carotenoids, curcubitacins and thousands of polyphenols have been identified so far from various plants. A single plant could contain highly complex profiles of these compounds, which sometimes are labile to heat, air and light, and they may exist at very low concentrations in the plants. This makes the separation and detection of these antioxidant phytochemicals a challenging task (Dagne, 1996).

Pharmaceutical companies, research institutions and research groups in various countries have developed great interest in the identification of herbal remedies found in the tropical rain forests. The use of a traditional ethnobotanist in naming of these herbal plants found in the tropical rainforests has been important in identification and classification of numerous medicinal plants (Farnesworth *et al*, 1985).

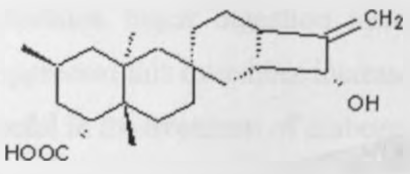
Given the rate at which the natural vegetation, especially in the tropics, is being seriously depleted and the tribal peoples acculturated, our ability to discover useful drugs and bring them into daily use is being compromised. Owing to the relatively small number of scientists in the tropical world capable of addressing this problem, it is clear that choices in the allocation of time and resources must be made.

1.4.1 Recent bioactive compounds

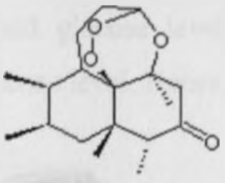
Marini Bettolo, 1992, focussed on an approach of screening plant extracts using different biological tests in order to determine the presence of particular properties such as (e.g.

anti-tumor, antiviral and immunostimulant) and the research for the active principles of plants claimed by indigenous traditions all over the world. With this approach the researcher reports new groups of molecules isolated from various Maytenus species, growing in South America, used in traditional medicine. The bark extract of Maytenus contains a substance named tingenone, which has a capacity to bind with the DNA. This is in fact in agreement with the use of Maytenus extracts in traditional medicine in the Amazon basin for the treatment of skin related tumors.

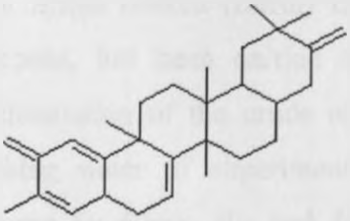
In recent years the chemical compound named taxol has raised a certain amount of interest. Taxol (Gueritte - Voegelein *et al,* 1991) is active against some types of tumours, astractiligenin is a blocking agent of phosphorylation while artemisinin (Jing - Ming *et al,* 1989) is useful in the treatment of malaria.



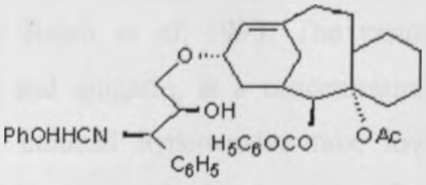
Tingenone



Artemisinin



Astractiligenin

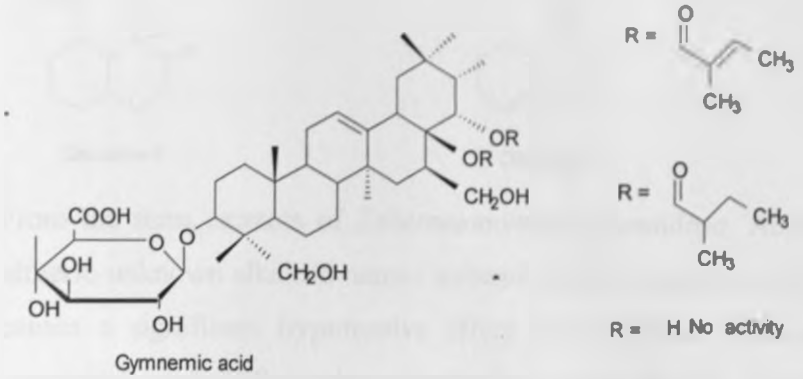


Taxol

The number of diabetics and overweight people has greatly increased in many countries. For the prevention of obesity and as a therapy of diabetes, it is necessary to limit sugar ingestion. Taste modifiers - that change taste sensation by modifying taste receptors - in addition to sweet proteins (used for preventing excessive sugar ingestion) can be used as harmless low calorie sweeteners.

All such substances come from tropical plants (Kurihara, 1992). Maeda *et al.* 1989 purified such a sweetener - gymnemic acid - using HPLC and determined its structure. Isolated from the leaves of *Gymnema sylvestre*, gymnemic acid is a glucoronide of

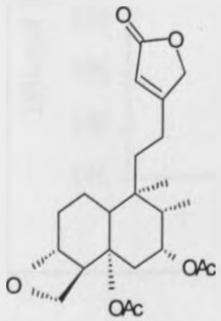
triterpene whose OH group at C-21 in the genin is esterified with tiglic acid or 2–methylbutyric acid.



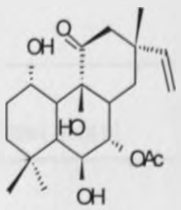
Gymnemic acid

The leaves have been used for therapy and prevention of diabetes in India. According Kurihara, 1992 gymnemic acid has the ability to suppress glucose absorption in the small intestines. Sugar ingestion increased the blood glucose level, while gymnemic acid suppressed this overshoot increase in blood glucose level. Hence gymnemic acid could be useful in the treatment of diabetes.

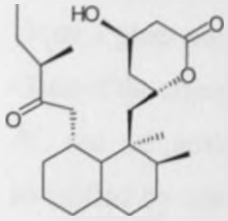
Studies of the anti-hypertensive properties of crude extracts of the East African medicinal plant *Ajuga remota* (Berth) Labiatae and its major component, ajugarin, a clerodane diterpene, has been carried out by Rajab *et al*, 1993. The results show that the administration of the crude extracts and ajugarin, at a concentration of 10mg/L into drinking water of experimentally - induced hypertensive rats, lowered their blood pressure by 40mm Hg and 50 mm Hg, respectively. A series of naturally occuring clerodanes-1, clerodane-2, clerodane-3, and clerodane-4 were isolated and their bioactivities determined (Rajab *et al* 1993).



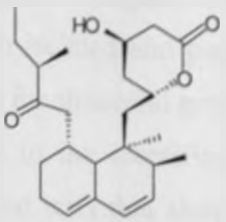
Clerodane-1



Clerodane-2

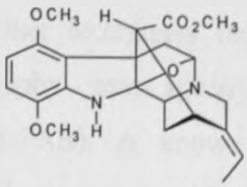


Clerodane-3



Clerodane -4

From the stem extracts of *Tebernaemontana glandulosa,* Achenbach, 1986, isolated a hitherto unknown alkaloid named tarbenulosine. In pharmacological tests, tarbernulosine causes a significant hypotensive effect when injected intravenously into genetically hypertensive rats. After a dose, the blood pressure falls by 25 mm Hg as shown in Figure 1.1.



Tarbenulosine

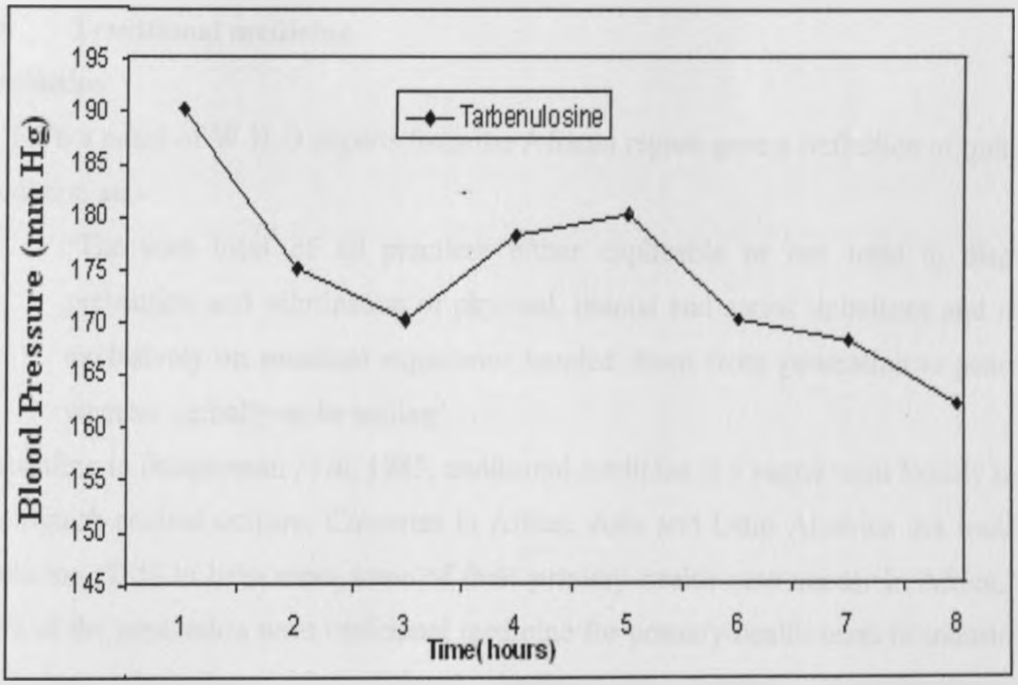


Figure 1.1 Effect of tarbernulosine on blood pressure of genetic hypertonic rats on administration of 18mg/kg b.w. intravenously

1.5.0 Methods used in identification of organic compounds.

Once a plant constituent has been isolated and purified, it is necessary to determine the class of the compound and search for structural groups present within that class. TLC and PC are the initial methods used in the identification. The class of the compound is identified by carrying out chemical tests that show appearance of characteristic colour, solubility tests and chromatographic properties e.g. hRf values (Harbone, 1973).

Complete identification within the class depends upon testing for other properties and comparing these data with those in the literature. This may include physical tests e.g. the determination of melting point [for solids], boiling point [for liquids] (Harbone, 1973). Important chemical properties of an isolated plant chemical compound are spectral characteristics including UV/Vis, IR spectroscopy and nuclear magnetic resonance (^{1}-HNMR and ^{13}CNMR, other techniques include HMBC, DEPT, NOESY, COLOC, COSY, liquid chromatography - mass spectrometry (LC-MS), Mass-spectrometry - fast atom bombardment (MS-FAB). A known plant compound usually is identified, characterized and chemical structure elucidated on the basis of the information acquired from the analytical techniques just mentioned.

1.6 Traditional medicine

Definition

In 1976 a panel of W.H.O experts from the African region gave a definition of traditional medicine as: -

> 'The sum total of all practices either explicable or not used in diagnosis, prevention and elimination of physical, mental and social imbalance and relying exclusively on practical experience handed down from generation to generation whether verbally or in writing'.

According to Bannerman *et al*, 1983, traditional medicine is a vague term loosely used to distinguish ancient culture. Countries in Africa, Asia and Latin America use traditional medicine (TM) to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. In industralized countries, adaptations of traditional medicine are termed "Complementary" or "Alternative" medicine (W.H.O, 2003).

1.6.1 Increasing use and reputation

The use of traditional drugs to screen biological activity and lowering of blood glucose causing increased utilization of the same by body cells in experimental animals closely parallels the pathological course of *Diabetes mellitus* and in this context fulfills correction of biological and physical imbalance. According to W.H.O fact sheet No. 134, traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in industrialized countries. In China, traditional herbal preparations account for 30%-50% of the total medicinal consumption, while in Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home (WHO, 2003).

1.6.2 The traditional healer

The traditional healer is a person who is recognized by the community in which he lives as competent to provide health care by using plant materials, animal and mineral substances as well as certain other methods. This is based on social, cultural and religious background as well as on knowledge, attitudes and the beliefs that are prevalent in the community regarding physical, mental and social well being and causation of disease and disability (Kokwaro, 1976).

1.6.3 Positive aspects of traditional medicine

Traditional medicine is currently being used to a large extent in India, China, Africa and Native America. Though it has not been accepted in medical health regulatory guidelines, below are some of the positive aspects of this system of health care.

a) In the face of the ever-increasing cost of modern drugs and the resistance to act on disease causing organism, the use of traditional medicine could mean that the treatment of certain diseases using this system might become more effective and cheaper.

b) People in many countries are known to have used traditional medicine from time immemorial and the very fact that it existed is one of its advantages. Thus in many localities that are still far from modern heathcare centres, the traditional healers are the only resource when health problems arise. A large number of diseases could be treated by people whom, while not formally qualified, are,

nevertheless, within reach: for example, the traditional healers. Furthermore, because of his/ their knowledge and social usefulness, his/their special relationship with those around him and his understanding of the social and cultural environment, including the patient's family millieu, he could become an active agent in the promotion of health.

c) Medicinal plants extracts in aqueous form contain thousands of polar active compounds that have synergistic bioactivity against many disease causing organisms hence may be better in terms of activity as an alternative therapy. High loads of conventional synthetic drugs, with continuous use, may accumulate in the body if there is shortage of water or electrolytes for excretion leading to renal failure or malfunctioning (British National Fomulary, 1971).

d) According to Native America Coyote medicine, the native people believe that all healing is ultimately spiritual healing, and that the integration of mind, body, and spirit is crucial to getting well. Many traditional healers grow up with an implicit understanding of this principle and through the course of life, have come to explicitly believe this as well. Understood within indigenous culture concepts of illness is an appreciation that it is a territory in which we find ourselves, and not an inseparable trait of the person who is ill. Rather than describe someone as a cancer patient, we would say that this person finds herself in the territory of cancer. Implicit also is the understanding that illness is a marker of imbalance. When balance and harmony are restored, illness can be transformed. The limits of this healing transformation are set by the divine and cannot be known by humans (Lewis, 2007). Finally, indigenous cultures know that sickness and death are not necessarily related. Illness does not invariably lead to death, and one does not have to be ill to die.

1.6.4 Negative aspects of traditional medicine

It has to be admitted that some aspects of traditional medicine call for corrective measures if it is to be incorporated in health services (Akerele, 1992). They include;

i) Safety, efficacy and efficiency

ii) The un-precise nature of diagnosis

iii) The lack of precision in dosage

iv) The possible misuse of its non-material aspects

v) The practices of sorcery and quackery

1.6.5 Safety and efficacy issues

Scientific evidence from randomized clinical trials only vindicates same uses of acupuncture, use of some herbal medicines and application of some manual procedures used in traditional management of disease. Further research is needed to ascertain the efficacy and safety of several other practices and medicinal plants. Unregulated or inappropriate self-administration of traditional medicine in terms of inappropriate dosage or administration of medicines by inexperienced practitioners can have negative or dangerous consequences. For instance, the herb "Ma Huang" (Ephedra) is traditionally used in China to treat respiratory congestion. In the United States, the herb was marketed as a dietary aid and according to WHO over dosage led to at least a dozen deaths, heart attacks and strokes.

The World Health Organization launched its first ever comprehensive strategy to promote safe, effective and affordable traditional medical practice in 2002.

The strategy is designed to assist countries to:

i) Develop national policies on the evaluation and regulation of TM/CAM practices;

ii) To improve safety, efficacy and quality of the TAM/CAM products and practices;

iii) Promote therapeutically sound use of TM/CAM by providers and consumers;

iv) Document traditional medicines and remedies.

1.6.6 Biodiversity and sustainability

In addition to patient safety issues, there is the risk that a growing herbal market and its great commercial benefit might pose a threat to biodiversity through the over harvesting of the raw materials for herbal medicines and other natural health care products. These practices, if not controlled, may lead to the extinction of endangered species and the destruction of natural habitats and resources. Another related issue is that at present, the

requirements for protection provided under international standards for patent law and by most national conventional patent laws are inadequate to protect traditional knowledge and biodiversity.

1.6.7 Priorities for promoting chemical research in traditional medicine

Over one-third of the population in developing countries lack access to essential medicines. The provision of safe and effective TM/CAM therapies could become a critical tool to increase access to health care. While China, the Democratic People's Republic of Korea, the Republic of Korea and Vietnam have fully integrated traditional medicine into their health care systems, many countries are yet to integrate this type of medical practice in their heathcare systems.

According to WHO, 70 countries have a national regulation on herbal medicines but the legislative control of medicinal plants has not evolved around a structured model. This is because medicinal products or herbs are defined differently in different countries and diverse approaches have been adopted with regard to licensing, dispensing, manufacturing and trading. The limited scientific evidence about TM/CAM's safety and efficacy as well as other considerations make it important for governments to:

i) Formulate national policy and regulation for the proper use of TM/CAM and its integration into national health care systems in line with the provisions of the WHO strategies on Traditional Medicines;

ii) Establish regulatory mechanisms to control the safety and quality of products and of TM/CAM practice;

iii) Create awareness about safe and effective TM/CAM therapies among the public and consumers;

iv) Cultivate and conserve medicinal plants to ensure their sustainable availability. It would be necessary, moreover, to make the traditional healers aware of the limits of their skills and encourage modern medical practitioners to show greater interest in the interaction between patients and traditional healers since this, more often than not, has a bearing on the management of health and disease in the society (Kokwaro, 1976).

1.7.0 Statement of the problem

1.7.1 Hypertension and *Diabetes mellitus*

Hypertension is one of the most common complex disorders, with genetic heritability averaging 30%. (Guyton *et al*, 2005). Data supporting this view emerged from animal studies as well as in population studies in humans. Most of these studies support the concept that elevated blood pressure is one of the condition phenotypic expressions. The risk of hypertension is 5 times higher in the obese as compared to those of normal weight and up to two-thirds of cases can be attributed to excess weight. More than 85% of cases occur in those with a BMI 25 (Makoto *et al* (2006).

Diabetes occurs when a malfunctioning pancreas is deficient in the production of insulin, leading to uncontrollable blood sugar levels. Insulin is a polypeptide hormone secreted by cells in the islet of langerhans, which are contained throughout the pancreas. Its main purpose is to regulate the levels of glucose in the body antagonistically with glucagon through negative feedback loops. Insulin also exhibits vasodilatory properties. In normotensive individuals, insulin may stimulate sympathetic activity without elevating mean arterial pressure. However, in more extreme conditions such as that of the metabolic syndrome, the increased sympathetic neural activity may over-ride the vasodilatory effects of insulin. Insulin resistance and/or hyperinsulinemia have been suggested as being responsible for the increased arterial pressure in some patients with diabetes and hypertension (Haslam *et al*, 2005). Diabetes and hypertension are related in a sense that they are leading causes of death by disease; diabetes is responsible for numerous amputations; it causes blindness and kidney failure and despite the advances in sciences, the disease has no cure.

Doctors and researchers attending the 16th Congress of International Federation on Diabetes in 1996 suggested that unless an artificial pancreas is developed, people worldwide will continue to suffer from the disease (Kenya Times, 1997). Insulin injectors developed 70 years ago are still the best development in search for the control of the disease. In management of diabetes, a balance of sugar and insulin is important. Food as it gets digested is broken down to sugar, which then enters the blood stream. The role of insulin is to help facilitate the sugar to be absorbed from the blood into the cells where

it can be burned down as energy fuel and utilized for biosynthesis. Thus diet is important in ensuring that the amount of sugar present in the blood is proportional to availability of insulin.

As a result of the rising cases of diabetes in old, malnourished and overweight people, it has become an alternative to use and compliment confentional antidiabetic drugs with traditional medicinal preparations. Cases of diabetes have been on the rise in the past years all over the world in old and malnourished people. A report from Kenya Diabetic Association shows that more than 5 million Kenyans are diabetic and that the incidence is ever increasing. It had also been predicted in 1997 that an estimated 12 million Kenyans would be diabetic by the year 2001, and diabetes then became the number seven killer ailment in Kenya signifying why this rising situation should be controlled (Kenya Times, 1997).

Approximately one third of the essential hypertensive and diabetic population is responsive to sodium and sugar intake respectively. This is due to the fact that increasing amounts of salt in a person's bloodstream causes cells to release water (due to osmotic pressure) to equilibrate concentration gradient of salt between the cells and the bloodstream; increasing the pressure on the blood vessel (Makoto *et al* (2006).

Prevention of hypertension only goes as far as the cause; one can adjust lifestyle related causes but genetics, race, age and gender are outside the realm of change. Modifiable factors include diet, weight, exercise levels and stress management. Low-sodium and low-fat diets can reduce cardiovascular risks, keep arteries clear of plaque and blood volume at normal levels.

Losing even 10% of body weight can have benefits towards health. Exercise maintains a healthy heart, thus healthy cardiac contractions and functions. The heart is a muscle too, working out the cardiac muscles makes the heart beat more efficiently, thus pumping blood around the body more effectively.

Stressors can negatively affect blood pressure by activating the sympathetic nervous system, thus fight or flight responses which increase heart rate and blood pressure. Chronic stress can lead to regular and frequent activation of the system and repeated high

blood pressure. Effective management of stress can reduce this particular risk (Makoto *et al*, 2006).

Data in support of an accurate assessment of the real value of usage of traditional anti-diabetic drugs in Kenya in health or in economic terms are difficult to come by, as detailed studies are costly and require scientific skills that are not adequately available in most African countries, Kenya included. Compounds with anti-diabetic bioactivity have not been exhausted and there is need to isolate these structures and systhesize analogues that can be used in large scale production of drugs. Therefore high cost of synthetic drugs has motivated research of new biologically active compounds from plants to alleviate *Diabetes mellitus* and hypertension.

There is a widespread trend today to turn back to natural products extracted from plants by exploiting indigenous traditional medicine systems of the world as a remedy for wide range of diseases like diabetes, hypertension and cancer.

It is in view of scarce data on the chemical constituents of traditional medicines used in the treatment of diabetes and hypertension that I set out my objectives to be to carry out physical, chemical and bioactivity investigations on some such traditional medicinal decoctions and also on the plant sources of these decoctions.

1.8 Objectives of the study

The main objective of the study was to carry out physical, chemical and bioactivity investigations of anti-diabetic traditional drugs prepared from the barks of Kenyan medicinal plants. However, the overall objective was broken down into: -

a) i) Isolating water fractions and pure compounds from *Podocarpus* sp and testing their bioactivities towards lowering of blood glucose, muscle contractions of isolated rabbits' heart, ileum and rat uterus.

ii) Isolating water fractions from *Rhamnus prinoides, Carrisa edulis Toddalia asiatica* and testing biological activities towards muscle contraction of isolated rabbits, heart and rat uterus.

b) Identifying/ characterizing compounds in the fractions responsible for biological activity.

c) Determining the levels of essential elements (copper, zinc, chromium and iron) in the isolated water fractions of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and *Toddalia asiatica.*

1.9 Hypothesis

H_O

1. Water extracts of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and *Toddalia asiatica* are administerd by traditional healers to patients to manage subjects suffering from *Diabetes mellitus* and hypertension. There are bioactive compounds in these extracts that are responsible for the biochemical basis for the reduction of blood glucose and blood pressure in diabetics.

2. Water extracts of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and Toddalia *asiatica* are administered as an alternative to conventional drugs with quality approval by the traditional healers. Quality parameters supporting approval are taste and visual colour tests.

3. Water extracts of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and Toddalia *asiatica* are administered by traditional healers as source of minerals to the ailing and malnourished patients. There are essential elements that occur in low concentrations in such people.

H_1

1. Water extracts of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and *Toddalia asiatica* are administerd by traditional healers to manage subject suffering from *Diabetes mellitus* and hypertension. The compounds responsible for activity in the fractions have not been identified, classified, isolated and characterized in order to elucidate the chemical structures.

2. Water extracts of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and Toddalia *asiatica* are administered as an alternative to conventional drugs with quality approval by the traditional healers. There is lack of reliable chemical tests to identify, assay and ascertain the quantity of the chemical compounds that constitute the proper dosage and the biological action required in the body.

Furthermore there is no guarantee for safety, efficacy and effectiveness for the amount of drug administered.

3. Water extracts of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and Toddalia *asiatica* are administered by traditional healers as source of minerals to the ailing and malnourished patients. The essential elements in the fractions have not been identified nor have they been analysed.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Approaches to drug discovery

Different strategies have been developed by various research groups to determine a sound and practical basis for the selection of plants for scientific examination. One of the strategies followed by different groups involved in drug discovery programs is to use bioassay-guided fractionation to isolate and identify substances that have medicinally desired activity. More recently combinatorial chemistry and high throughput robotic screening techniques have been employed as viable strategies for drug discovery programs in many countries (Farnesworth, 1994).

The selection of plant material for investigation by a research group is guided by the belief that the plants that have acquired the status of marketed commodities have already been screened by traditional methods. In most cases the plant materials and safety of several other practices and medicinal plants materials are extracted by aqueous methods and sold by the traditional herbalists· who come from indigenous communities. The experience with strategy has given us an exciting study of these important plants.

There is need to produce a solid body of knowledge that will assist countries in formulating national drug policies in which medicines of plant origin are used to their full potential for improving people's health now and in the future. Research in investigation of herbal and traditional medicine is no longer restricted to pharmacists and chemists. Even in the universities, chemistry for chemistry's sake is no longer very exciting. There was a time when plant chemical research invariably ended up in chemical taxonomy. Now it interconnects with biology, pharmacology and medicine.

To demonstrate a pharmacological effect, nothing can replace observation of animal models, but as they are expensive and often difficult to interpret, simpler tests are used. These tests require less effort and also make it possible for a better understanding of the mechanisms of action of substances being tested on diseases like diabetes and hypertension (Turner, 1996). Several reviews pertaining to approaches as candidates for drug discovery programs have been used (Verpoorte *et al*, 2000); however, most concern

screening plants for anticancer or antidiabetic activity. Examples from the literature are intended to be representative but not exhaustive; these include (Fabricant *et al*, 2001).

2.1.1 Random selection followed by chemical screening

These so-called phytochemical screening approaches have been used in the past and are currently pursued mainly in the developing countries. The tests are simple to perform, but false-positive and false-negative tests often render results difficult to assess (Verpoorte *et al*, 2000). More important, it is usually impossible to relate one class of phytochemicals to specific biological targets; for example, the alkaloids or flavonoids produce a vast array of biological effects that are usually not predictable in advance.

2.1.2 Random selection followed by one or more biological assays

In the past, plant extracts were evaluated mainly in experimental animals, primarily mice and rats. Approximately 2,000 plant species were evaluated for several biological activities, including antibacterial, antidiabetic, antifertility, antifungal, antihypercholesteremic, anti-inflammatory, antitumor, cardiovascular, central nervous-system depressant, cytotoxicity, diuretic, and others that have not been named (Dhar *et al*, 1966).

2.1.3 Follow-up of biological activity reports

These reports showed that the plant extracts had interesting biological activity, but the extracts were not studied for their active principles. The literature from the 1930s through the 1970s contains these types of reports.

2.1.4 Follow-up of ethnomedical (traditional medicine) uses of plants

Several types of ethnomedical information are available:

a) Plants used in organized traditional medical systems.

Ayurveda, Unani, Kempo, and traditional Chinese medicine have flourished as systems of medicine in use for thousands of years. Their individual arrangements all emphasize education based on an established, frequently revised body of

written knowledge and theory. These systems are still in place today because of their organizational strengths, and they focus primarily on multicomponent mixtures (Bannerman *et al*, 1983).

b) Herbalism, folklore, and shamanism.

The center on an apprenticeship system of information is passed to the next generation through a shaman, curandero, traditional healer, or herbalist. The plants that are used are often kept secret by the practitioner, so little information about them is recorded; thus there is less dependence on scientific evidence as in systems of traditional medicine that can be subject to scrutiny. The shaman or herbalist combines the roles of pharmacist and medical doctor with the cultural/spiritual/religious beliefs of a region or people, which are often regarded as magic or mysticism. This approach is widely practiced in Africa and South America (Rastogi *et al*, 1982).

c) Use of databases.

The NAPRALERT database (Natarajan *et al*, 1999) currently contains information on 43,879 species of higher plants covering ethnomedical, chemical, and pharmacological (including clinical studies) uses. Of these, 13,599 species contain ethnomedical data, distributed among 3,607 genera and 273 plant families. Thus it is possible to correlate ethnomedical use with experimental biochemical or pharmacological activities (*in vitro*, *in vivo*, or in humans) to identify plants having both types of activity for a given effect e.g., anticancer, antidiabetic, antimalarial (Natarajan *et al*, 2000).

2.2 *Diabetes Mellitus*

Diabetes mellitus is defined simply as a chronic state of high blood glucose levels as a result of overproduction of glucose by the liver, defective utilization by the tissues such as muscles or due to absolute or relative lack of insulin. This leads to abnormalities of the metabolism of carbohydrates, proteins and fats.

The blood sugar is not necessarily high at all times; it may be high after a meal or during glucose tolerance tests (G.T.T.). When blood glucose is not utilized in the functioning of the muscle cells and the brain then there is an increase in blood sugar.

Administration of drugs in the management of *Diabetes mellitus*, whether insulin dependent or non-insulin dependent, is to lower the blood glucose levels hopefully by causing increased utilization of the same by body cells.

2.2.1 Carbohydrate metabolism

All tissues in the body utilize glucose and its entry into the muscle and adipose tissue is controlled by insulin. One function of the liver is to control the level of glucose. When the level is elevated, as is the case after a meal, the liver takes glucose and converts it mostly to glycogen. Little of this glucose is utilized for the energy needs of the liver, which consumes mostly fatty acids. When the level of glucose is low, as for instance during starvation/fasting, the liver releases a large amount of glucose to the blood for the benefit of the brain, erythrocytes and other tissues. This glucose is provided by the breakdown of glycogen in the liver. Soskin has emphasized that the concentration of glucose in the blood is the primary stimulus that elicits glucose uptake or glucose output by the liver - and compared this homeostatic control of glucose by the liver to a thermostat system in a furnace. The hepatic threshold of glucose is defined as glucose concentration at which the liver converts from an organ of glucose output to an organ of glucose uptake. This threshold corresponds to the level of glycaemia that any animal usually maintains and which varies according to hormonal conditions (Soskin, 1940). Figure 2.1 shows a brief summary of glucose metabolism.

Glucose occupies a unique position in intermediary metabolism for two reasons: -

a) It is the substrate of glycolysis, which is the only pathway to produce adenosine triphosphate (ATP) in anaerobic life. Although anaerobiosis is an exceptional condition for mammals, it is important to mention that erythrocytes, by virtue of being deprived of mitochondria, are entirely dependent upon glycolysis for their supply of ATP.

b) It is a major substrate of brain metabolism. Indeed fatty acids are bound to albumin and cannot penetrate the blood brain barrier. The brain utilizes

ketone bodies but their concentration in blood is normally very low and only increases after fasting. A significant decrease in the level of blood glucose could therefore cause major brain damage. This is a justification for the rather elaborate and sometimes expansive mechanism of control that the liver has developed to maintain a constant level of blood glucose.

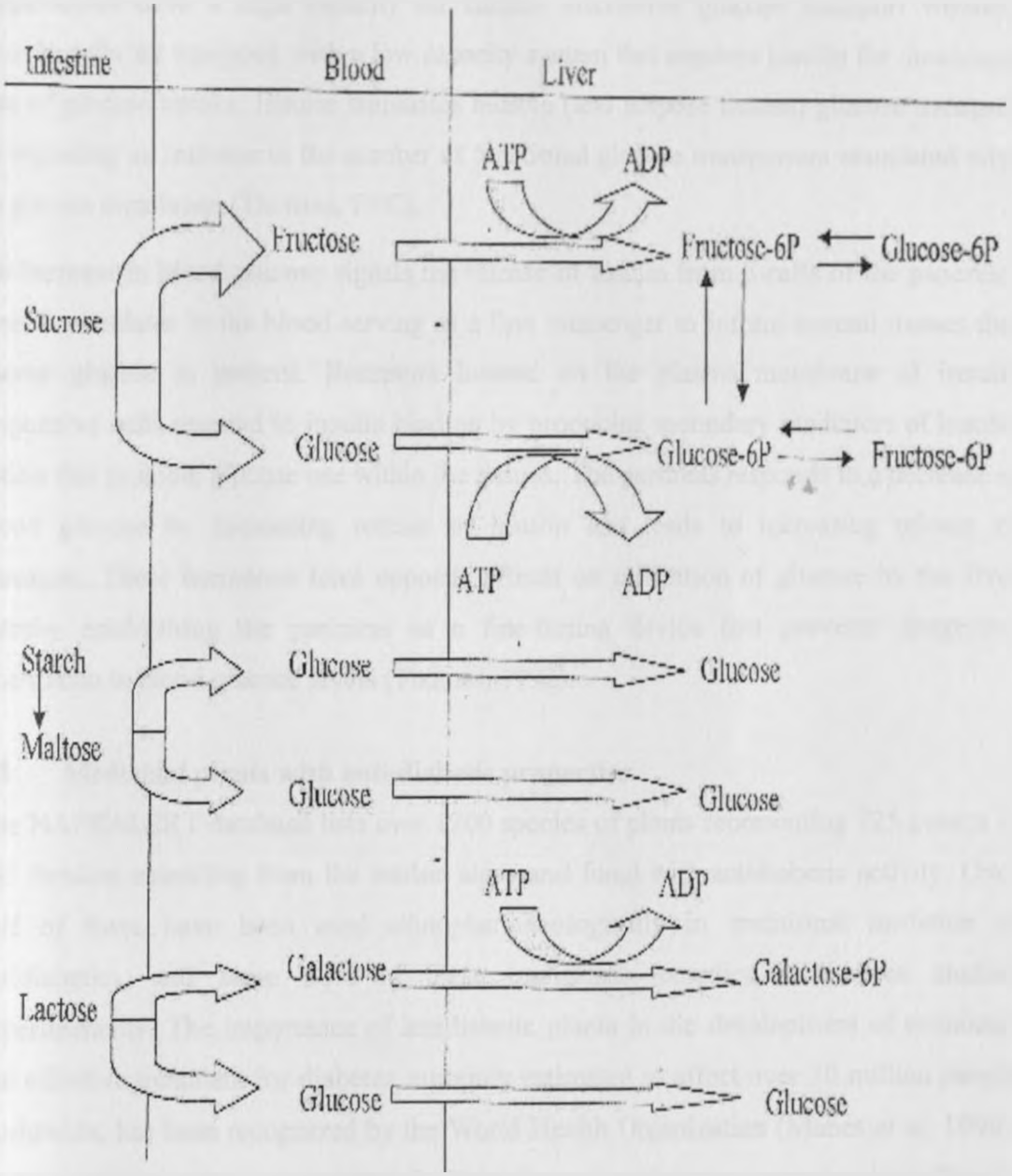


Figure 2.1 A brief summary of glucose metabolism (Thomas, 1992)

2.2.2 The role of insulin in glycogen synthesis and glucose metabolism in the liver and in the muscle

Insulin increases glucose metabolism and utilization rates partly by promoting glycogenesis and inhibiting glycogenolysis in the muscle and liver. Insulin stimulation of glucose transport at the plasma membrane is important for these effects in the muscle. Hepatocytes have a high capacity for insulin insensitive glucose transport whereas muscle cells are equipped with a low capacity system that requires insulin for maximum rate of glucose uptake. Insulin stimulates muscle (and adipose tissues) glucose transport by signaling an increase in the number of functional glucose transporters associated with its plasma membrane (Thomas, 1992).

An increase in blood glucose signals the release of insulin from β-cells of the pancreas. Insulin circulates in the blood serving as a first messenger to inform several tissues that excess glucose is present. Receptors located on the plasma membrane of insulin responsive cells respond to insulin binding by producing secondary mediators of insulin action that promote glucose use within the tissues. The pancreas responds to a decrease in blood glucose by decreasing release of insulin and leads to increasing release of glucagon. These hormones have opposite effects on utilization of glucose by the liver thereby establishing the pancreas as a fine-tuning device that prevents dangerous fluctuation in blood glucose levels (Thomas, 1992).

2.3 Medicinal plants with anti-diabetic properties

The NAPRALERT database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with antidiabetic activity. Over half of these have been used ethnopharmacologically in traditional medicine as antidiabetics, and some 50% of these traditional remedies have been studied experimentally. The importance of antidiabetic plants in the development of economic and effective treatment for diabetes, currently estimated to affect over 30 million people worldwide, has been recognized by the World Health Organization (Manes *et al*, 1996). Assay methods used to screen plants for hypoglycemic activity are varied and not directly comparable.

In vivo techniques include animals with normoglycemia or induced hyperglycemia (alloxan, streptozotocin, various hormones, or surgery) as well as diabetic human subjects. Natural compounds with antidiabetic activity considered in descending frequency of occurrence, include complex carbohydrates, alkaloids, glycopeptides, terpenoids, peptides and amines, steroids, flavonoids, lipids, coumarins, saponins and sapogenins, sulfur compounds, inorganic ions and others.

The antidiabetic mechanisms involved in hypoglycemic activity are numerous and they include direct competitive antagonism with insulin, stimulation of insulin secretion, and stimulation of glycogenesis and hepatic glycolysis, adrenomimeticism, pancreatic beta cell potassium channel blockers, cAMP (2nd messenger) stimulation, and modulation of glucose absorption from the gut, among others (Manes *et al*, 1996).

In Kenya, herbal clinics that attend and administer concoctions and aqueous extracts to diabetic patients have mushroomed. Plants with antidiabetic activity have often been used by practitioners of herbal medicine in treating individuals with non-insulin-dependent (type 2) diabetes. The patient's response is carefully monitored to see whether any significant benefit can be gained from such therapies. However, the use of such herbs by type 1 (insulin-dependent) diabetics is dangerous. The patient must therefore monitor their blood sugar to prevent hypoglycemic and hyperglycemic comas.

Consultation with the prescribing physician is necessary and an integrative management of the case by conventional and herbal practitioners working together would be preferred. The shared goal would be to regulate the dosage of both types of medication and enable a smooth transition to lower dependence on insulin in cases where such is desirable and attainable.

While hypoglycemic herbs may offer promise in the treatment of diabetes in their combined effect with insulin, treatment is inherently disruptive and extreme caution must be exercised in order to promote a smooth transition, maintain suitable blood sugar levels and avoid insulin shock (WHO, 2003). Some Kenyan medicinal plants identified to have some antidiabetic activity are shown below.

Table 2.1 Some plants identified to have anti-diabetic activity (Liaquat *et al*, 1992, Iwu, 1992, Kumar, 1992, Akerele, 1992).

Plant Family	Active principle
Coccinia indica	Coccinine
Musa sapientum	Serpentine
Momordica charantia	Momordicoside
Allium satium	Allicin
Figus senegalensis	Leucocyanidin
Podocarpus sp	Podocarpine
Dioscorea dumetorum	Dioscoretine
Harpagophytum procumbens	
Centela asiatica	
Rumex usambarensis	
Warbugia sulcata	

Three of the plants have been discussed in the following subsection.

2.3.1 *Coccinia indica* (Cucurbitaceae)

The various parts of *Coccinia indica* Wight and Am (Curcubitaceae), which grows abundantly in India, have been widely used in the traditional treatment of diabetes. Chopra *et al*, 1972 and Murkherjee *et al*, 1972 showed that the aqueous and methanol extracts of *Coccinia Indica* leaves possessed hypoglycemic activity on rats. The effects of leaf preparation of *Coccinia Indica* were examined and it was found that the blood glucose levels were significantly lowered after long duration of administration (Khan *et al*, 1980). The alkaloids isolated by Khuda *et al*, 1965 on the other hand did not have any hypoglycemic activity. The oral administration of the pectin isolated from the plants fruit at a dose of 20mg / kg body weight per day showed significant hypoglycemic activity on normal rats. The pectin administration resulted in significant reduction in blood glucose and an increase in liver glycogen (Kumar *et al*, 1992). The reduction may be due to increase in absorption of glucose from the blood and a higher rate of glycogenesis as envisaged by the higher activity of hexokinase (one of the key enzymes of glycogenesis).

Enhanced rate of glycogenesis is evidenced by the higher amount of liver glycogen present in the pectin administered groups (Kumar *et al*, 1992).

2.3.2 *Dioscorea dumetorum*

Extracts of tubers of *Dioscorea dumetorum* (Dioscoraceae) have been used in African traditional medicine for the treatment of *Diabetes mellitus* (Iwu *et al*, 1992). According to Kokwaro, 1976, the roots of *D. dumetorum* and those of *Cadaba kirkii* are dried, ground and the powder mixed with water.

Previous investigations have revealed that the crude extracts of *D. dumetorum* showed hypoglycemic effects in normal rats and rabbits and checked the effects of alloxan poisoning (Undie *et al*, 1986). Iwu *et al*, 1992 isolated dioscoretine from the aqueous fraction of ethanol extract of *D. dumetorum* tubers. Dioscoretine, when administered intraperitoneally to normal and alloxan diabetic rabbits (Figure 2.2 and 2.3), produced significant hypoglycemic effects at a dose of 20mg / kg b.w. Corley *et al*, 1985 had previously reported the isolation of a new alkaloid dumetorine from the methanol extract of *D. dumetorum*. Recent findings indicate that the reported hypoglycemic effect of *D. dumetorum* could be due to presence of dioscoretine in the aqueous fraction of the alcoholic extract (Iwu *et al*, 1992).

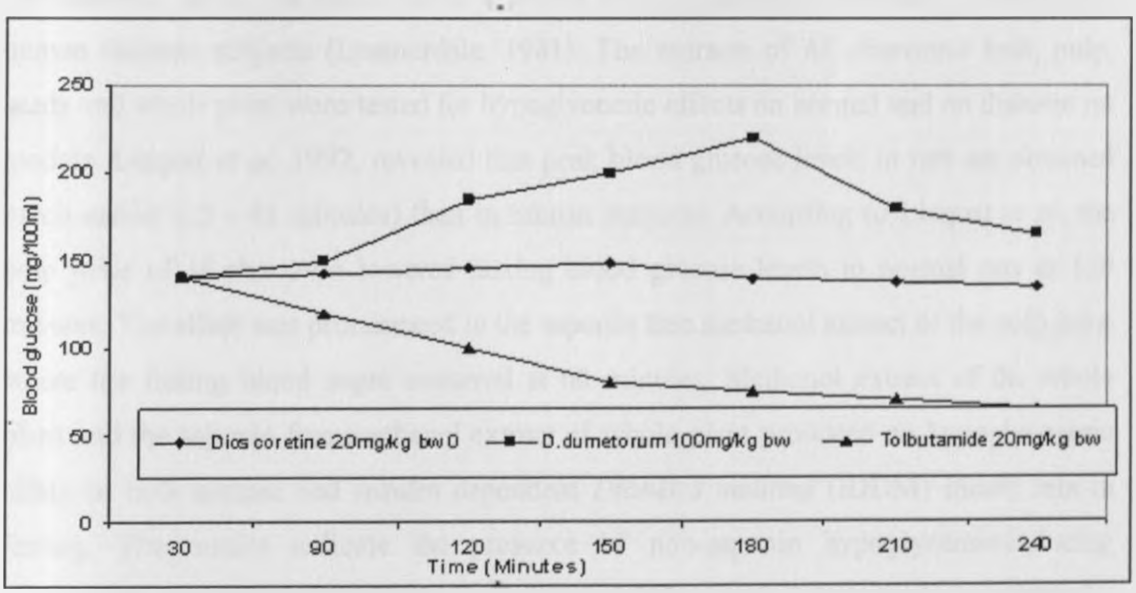


Figure 2.2 Blood glucose activity of dioscoretine (20mg/kg), *D. dumetorum* 100mg/kg) and tolbutamide (20mg/kg) (Iwu *et al*)

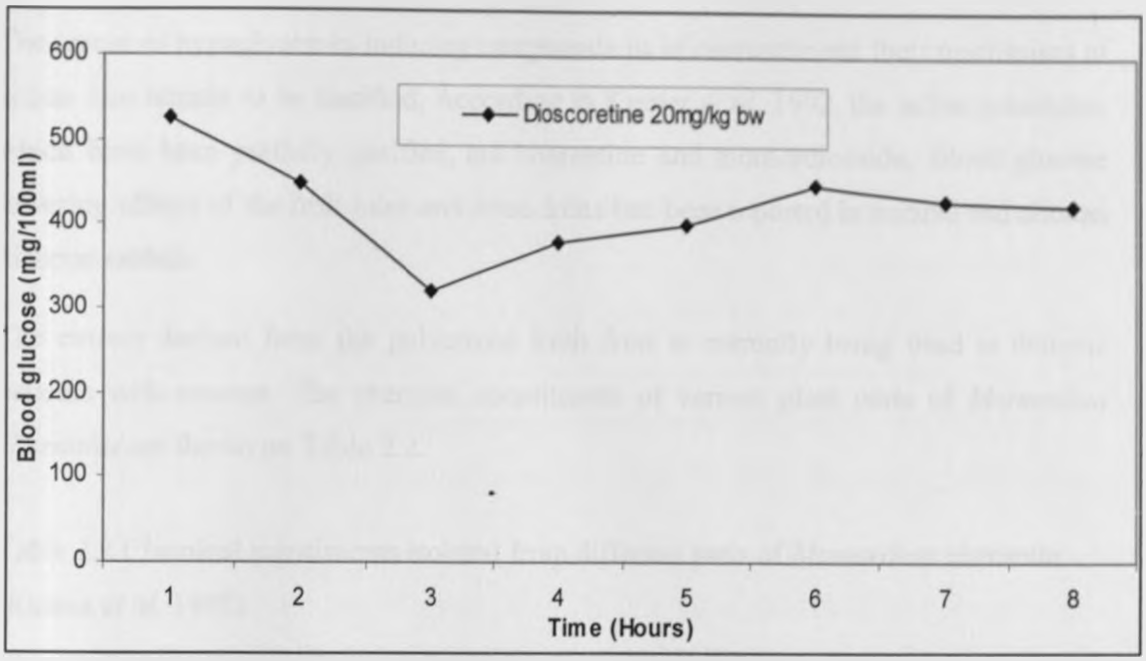


Figure 2.3 Effect of dioscoretine (20mg)/ kg b.w on blood glucose in alloxan diabetic rabbits (Iwu *et al*, 1992)

2.3.3 *Mormordica charantia*

Mormordica charantia (Cucurbitaceae), a plant widely used in traditional medicine as an anti-diabetic agent, has been shown to lower blood glucose in laboratory animals and human diabetic subjects (Leatherdale, 1981). The extracts of *M. charantia* fruit, pulp, seeds and whole plant were tested for hypoglycemic effects on normal and on diabetic rat models. Liaquat *et al,* 1992, revealed that peak blood glucose levels in rats are obtained much earlier (15 - 45 minutes) than in human subjects. According to Liaquat *et al*, the pulp juice of *M charantia* lowered fasting blood glucose levels in normal rats at 120 minutes. The effect was pronounced in the saponin free methanol extract of the pulp juice where the fasting blood sugar occurred at 60 minutes. Methanol extract of the whole plant and the saponin free methanol extract of whole plant produced no hypoglycoemic effect in both normal and insulin dependent *Diabetes mellitus* (IDDM) model rats in fasting. The results indicate the presence of non-saponin hypoglycemic-inducing compounds in *M charantia* fruit pulp and the activity could be interpreted as either an improvement in the insulin secretory capacity of the β-cells or as being synergistic to the action of insulin.

The nature of hypoglycemia-inducing compounds in *M charantia* and their mechanism of action also remain to be clarified. According to Kumar *et al*, 1992, the active principles, which have been partially purified, are charantine and momordicoside. Blood glucose lowering effects of the fruit juice and dried fruits has been reported in normal and alloxan diabetic rabbits.

The extract derived from the pulverized fresh fruit is currently being used in diabetic patients with success. The chemical constituents of various plant parts of *Mormodica charantia* are shown on Table 2.2.

Table 2.2 Chemical constituents isolated from different parts of *Momordica charantia* (Kumar *et al*, 1992)

Part of plant	**Chemical group**	**Chemical constituent**
Fruits	Glycosides	Momordicosides
Seeds	Pectin, Cytokinins	Momordin, agglutinin
	Triterpene glycosides	Zeatin ribosides
Fruit tissue	polypeptides	Polypeptides

2.3.4 Oral conventional anti-diabetic drugs

The aim of drug administration in the management of diabetes mellitus - whether insulin dependent or non-insulin dependent is to lower blood glucose levels hopefully by causing increased utilization of the same by body cells. Non-insulin dependent diabetes is treated using synthetic drugs or drugs of plant origin (Mugo *et al*, 2005).

There are many hypogylcoemea inducing plants known in folklore, but their introduction into modern therapy, should be based on animal test systems that closely parallel the pathological course of *Diabetes mellitus* in humans. However such introductions have not been carried out following this procedure. For example, tea made from Queens Anne lace (*Daucus Carota*) or periwinkle - *Catharanthus roseus* - has allegedly been used successfully to maintain low blood sugar levels in humans, yet they have no effects on diabetes artificially induced animals. Tea from *Catharanthus roseus* has been used in

South Africa, South Vietnam, Nepal and Philipines and the proprietary products covinca and vinculin are still marketed as oral insulin substitutes (Lewis *et al.* 1977).

The hormone insulin is essential for life. This is because the hormone has direct or indirect effect on practically all organs and metabolic processes in the body. The most obvious actions and the most thoroughly studied are those on fatty tissues, muscle and liver. All oral anti-diabetic drugs are prepared synthetically. The sulphonylureas, which are derivatives of sulphanilamides, stimulate the pancreas to produce insulin and affect hepatic enzymes so that glycogen deposition is increased (Mugo *et al*, 2005). Moreover, the diguanidines increase glucose utilization by the tissues and tissue glycolysis and decrease hepatic glucose output.

All this leads to greater uptake of glucose from the blood and into the tissues. Insulin itself is prepared commercially from extract beef and swine pancreases. This means that antigenicity is a major problem with commercial insulin and synthetic antidiabetic drugs are themselves very expensive. The search for both hypoglycemia- causing compounds from plants and even chemically insulin - like compounds from plant sources is then worthwile (Renold *et al*, 1965).

There are many hypoglycemia causing plants that are known to indigenous folklore but not all these can be used for the purpose of reducing sugar in the blood through translocation of the same into the body cells. This is one of the two hallmarks in the treatment of *Diabetes mellitus,* the other one being prevention of entry of excessive sugar into the blood stream. For example, hypoglycemia- causing cyclopropanoid amino acids and hypoglycins A and B, derived from unripe fruit of West Indian tree *Blighia sapida* have action that differs from that of insulin in that they appear to act as antimetabolites capable of blocking the pathway of oxidation of fatty acids. The depletion of liver glycogen that follows their action subsequently induces hypoglycemia (Lewis *et al*, 1988).

2.4 Terpenoid compounds

A wide variety of plant derived substances are covered by the word 'terpenoids' which indicates all substances having a common biosynthetic pathway. Thus terpenoids are all

biosynthetically based on the isoprene molecule and their carbon skeleton is built up from the union of two more isoprene units. They are classified according whether they contain two (C_{10}), three (C_{15}), four, (C_{20}), five, (C_{30}), six (C_{35}), or (C_{40}) such units. They range from essential oils components, the volatile mono and sesquitertepenes (C_{10} and (C_{15}) through the less volatile diterpenes (C_{20}) to the volatile triterpenoids and sterols (C_{30}) and carotenoids pigments (C_{35}) (Manguro, 1994).

2.4.1 Saponins and sapogenins

Saponins are glycosides with a distinctive foaming characteristic. They are found in many plants, but get their name from the soapwort plant Saponaria (Latin name sapo for soap, the root of which was used historically as soap agent). They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached via C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and the water-soluble side chain. Saponins are bitter and reduce the palatability of livestock feeds. However if they have a triterpenoid aglycone they may instead have a licorice taste, as glucuronic acid replacing sugar in triterpenoids. Some saponins reduce the feed intake and growth rate of nonruminant animals while others are not very harmful. For example, the saponins found in oats and spinach increase and accelerate the body's ability to absorb calcium and silicon, thus assisting in digestion. Certain pasture weeds contain substantial quantities of dangerous saponins and result in life threatening toxicities for certain animal species (Edward *et al*, 1996).

2.4.2 Saponin glycosides

Saponin glycosides are separated into two kinds based on the chemical structure of their aglycones (sapogenins). Saponins on hydrolysis yield an aglycone known as "sapogenin" and a glycone (sugar). Neutral saponins are known to be derivatives of steroids with spiroketal side chains while the acid saponins possess triterpenoid structures. The main mechanistic pathway leading to both types of sapogenins is similar and involves the head-to-tail combination of acetate through mevalonate units. However, a subdivision occurs, after the formation of the triterpenoid hydrocarbon, squalene, that leads to

steroids (for example *Diosgenin*) in one direction and to cyclic triterpenoids in the other (Kato *et al*, 1995, Pompei *et al*, 1980).

All types of triterpenoids are separated by very similar procedures based mainly on TLC and GLC. Identification and structural characterization is confirmed by melting point, optical rotation, LC-MS, MS-FAB, IR and ^{1}H and $^{13}CNMR$ spectroscopy. Analytical TLC is carried out on precoated silica gel treated with $AgNO_3$ as a means for separating triterpenoids according to the number of isolated double bonds present in the molecule.

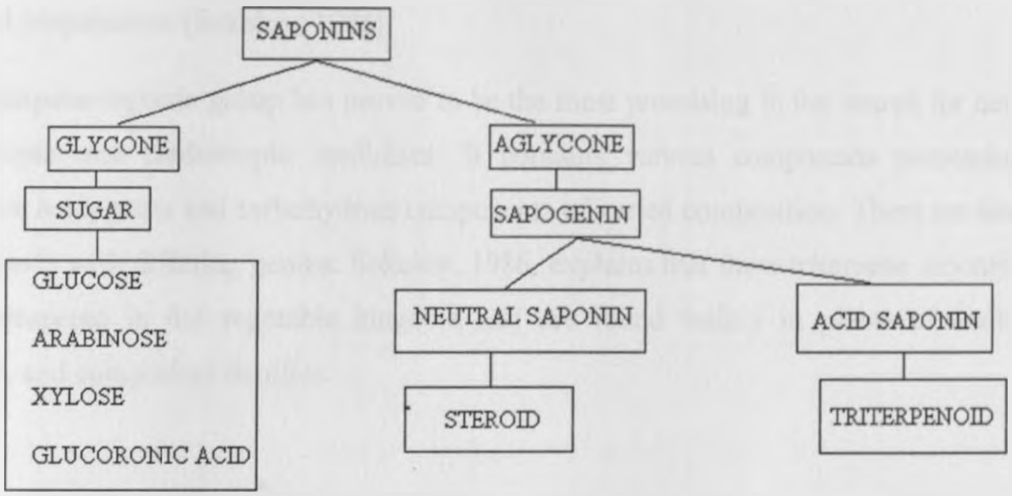


Figure 2.4 Classification of saponins

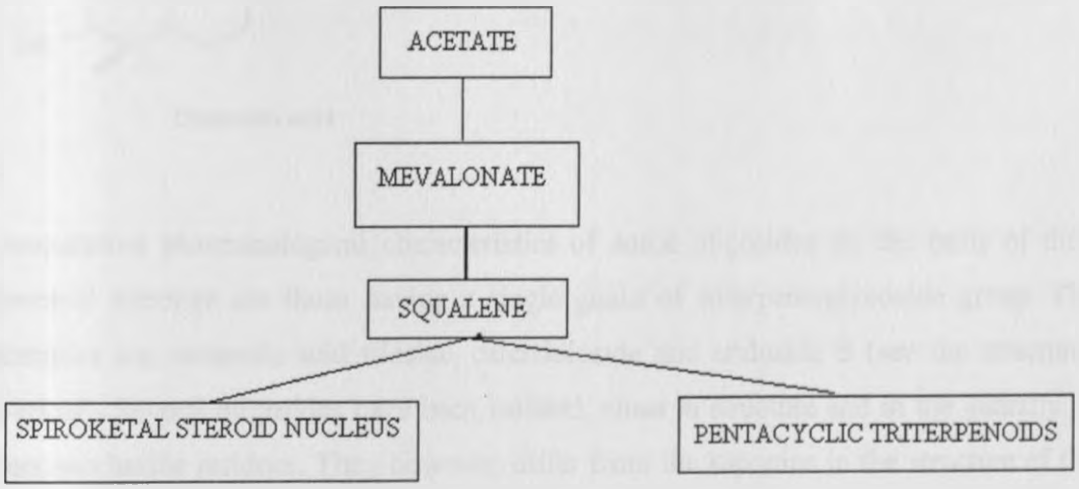
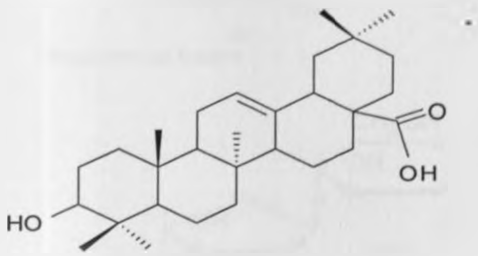


Figure 2.5 Mechanistic pathways for formation of triterpenoids and sterols

Saponins are more polar than sapogenins and more easily separated using PC and TLC on cellulose. However TLC on silica gel is also successful in solvents such as butanol saturated with water or chloroform:methanol (Harbone, 1970). Both chemists and pharmacologists have recently started conducting intensive research on saponins that are widespread in nature. As a result, it has been found that amongst the triterpene saponins, there are compounds characterized by low haemolytic activity when ingested. Furthermore, they can be regarded as being practically harmless to the host and they are, therefore, very interesting from the standpoint of research and development of new medical preparations (Sokolov, 1986).

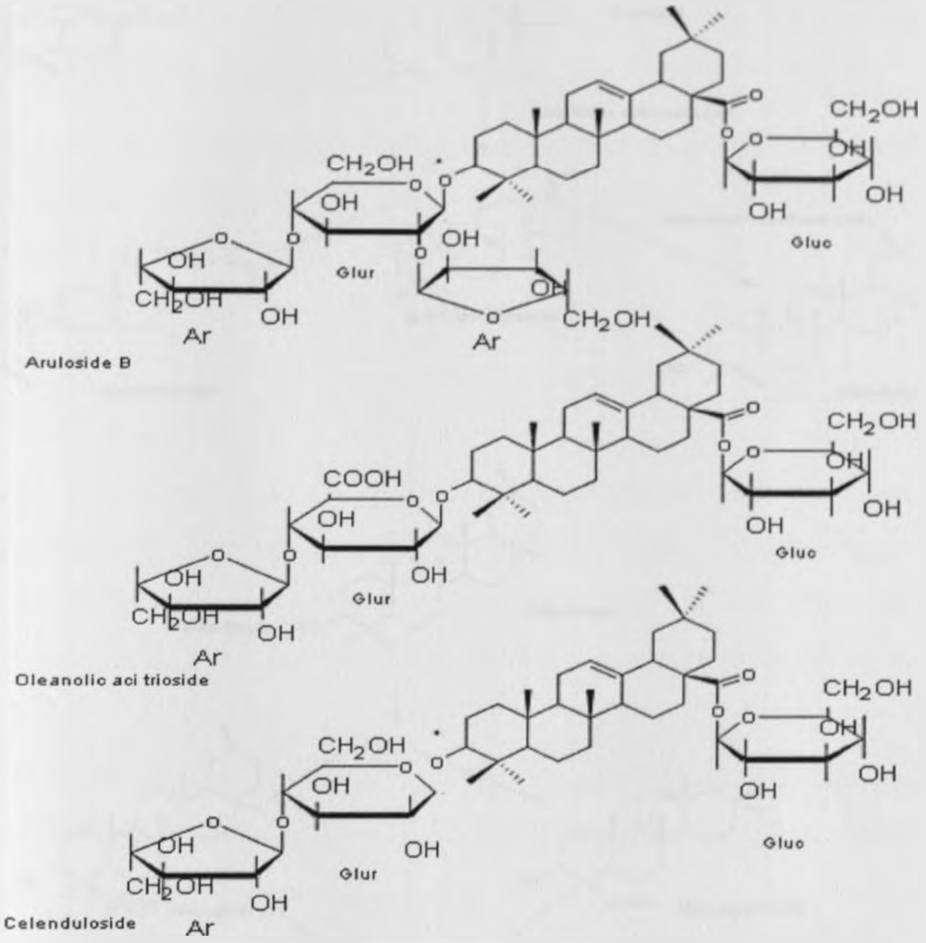
The triterpene-saponin group has proved to be the most promising in the search for new neurotropic and cardiotropic medicines. It contains various compounds possessing oleanolic acid, genin and carbohydrate components of varied composition. There are also compounds with differing genins. Sokolov, 1986, explains that these triterpene saponins are widespread in the vegetable kingdom and are found mainly in plants of aralia, valerian and compositae families.



Oleanolic acid

Comparative pharmacological characteristics of some oligosides on the basis of their chemical structure are those having a single genin of triterpene-glycoside group. The examples are, oleanolic acid trioside, calenduloside and aruloside B (see the structures overleaf). Several oligosides have been isolated, close in structure and in the quantity of monosaccharide residues. They however, differ from the saponins in the structure of the genin as in the case of gypsogenin – gypsoside – which is a nonaoside (Sokolov, 1986). These saponins are known to have haemolytic properties.

The study of the general pharmacology of the above triterpene saponins has shown that their level of toxicity and haemolytic activity are relatively low. Internal and hypodermic administration of the studied oligosides in the experimental doses (of 1, 10, 50mg/kg) did not cause any toxic side effects (Sokolov, 1986).



The biosynthesis of these compounds beyond the initial triterpene cyclization step is not understood as seen in Figure 2.6. (Huhman *et al*, 2005) has taken a functional genomics approach to identify and characterize various cytochrome P_{450} and glycosyltransferase (GT) enzymes which are involved in saponin biosynthesis. The strategy used DNA micro- and macro-array expression analysis to provide candidate genes for further functional characterization by expression in yeast (P_{450s}) or *E. coli* (GTs) (Suzuki *et al*, 2002). These are the new methods in biosynthesis of natural bioactive compounds.

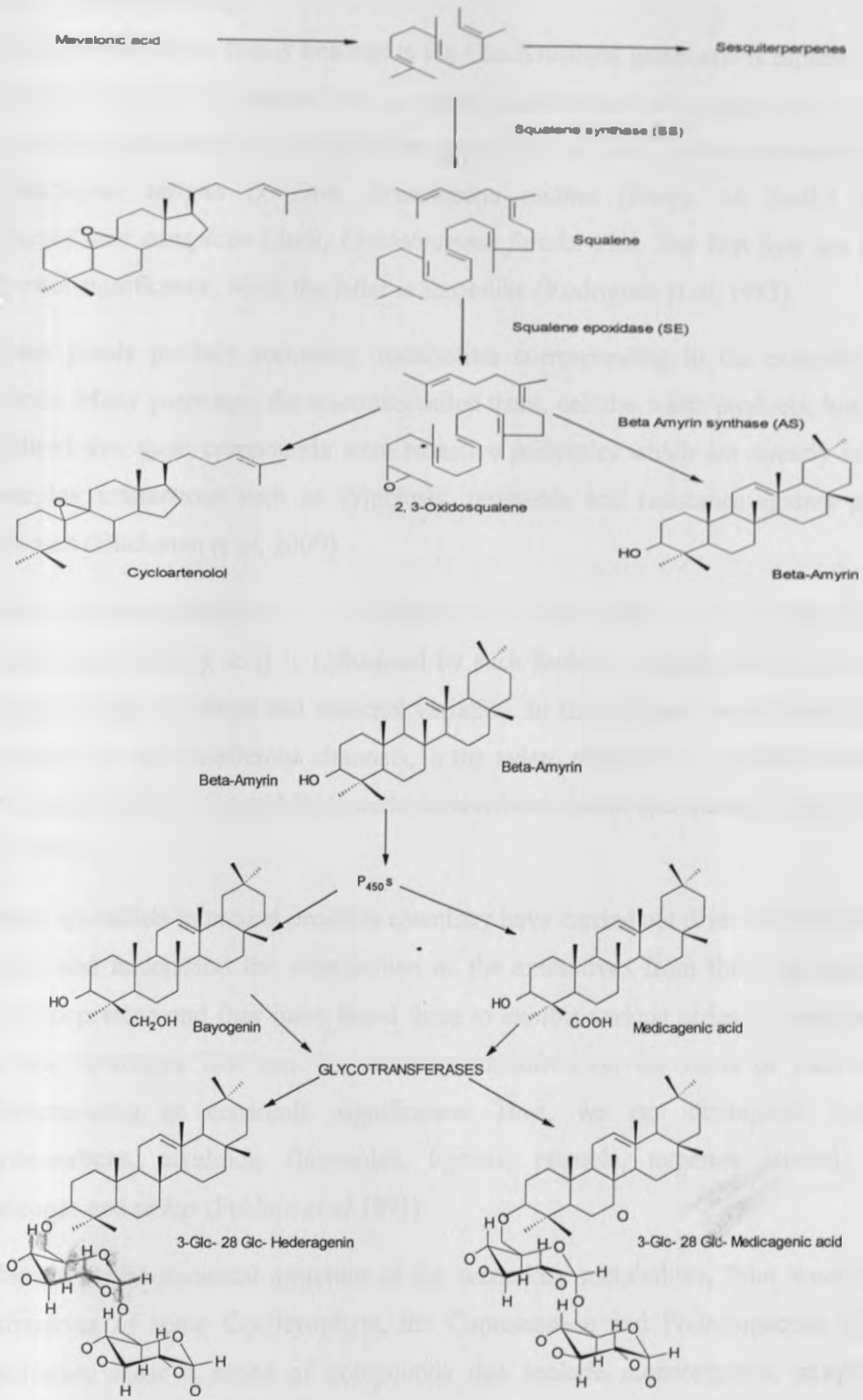


Figure 2.6 Triterpene saponin biosynthetic pathways in *Medicago Truncatula*

2.5 *Podocarpus* sp

The Podocarpaceae family belongs to the Coniferophyta group and is represented by 17 genera and about 125 species that are mainly distributed in the subtropical mountains of the Southern Hemisphere. In Chile five native species grow: *Podocarpus nubigena* Lind, *Podocarpus saligna D. Don, Prumnopitys andina* (Poepp. ex Endl.) de Laub., *Saxegothaea conspicua* Lindl, *Lipidothamnus fonckii* Phil. The first four are trees with forestal significance, while the latter is shrub-like (Rodriguex *et al*, 1983).

These plants produce secondary metabolites corresponding to the extractives of the woods. Many years ago, the scientists called them, cellular waste products, but later they realised that these compounds were bioactive principles which are directly involved in complex interactions such as symbiosis, resistance and resistance against plagues or diseases (Buchanan *et al*, 2000).

The chemical composition of the extractives of the plant varies dramatically in both quantity and variety as it is influenced by such factors as geographical area, the tree's age, growing conditions and seasonal variation. In the bark and wood these compounds are found in the resiniferous channels, in the xylem rays and at a cellular level they can be found as part of the middle lamella, intercellular spaces and tracheid walls (Cambie et *al*, 1985).

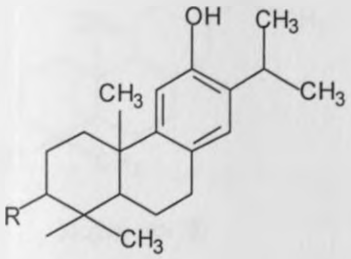
Many specialists in natural products chemistry have carried out diverse studies in order to know and understand the composition of the extractives from the Angiospermae and Gymnospermae and they have found these to exhibit various series of compounds with diverse structures that can be variously classified on the basis of their structural characteristics or economic significance. Thus, we can distinguish fatty acids, hydrocarbons, alkaloids, flavonoids, lignans, phenols, terpenes, steroids, tannins, quinones and resins (Poblete *et al* 1991).

Studies on the chemical structure of the secondary metabolites, from wood and bark extractives of some Coniferophyta, the Cupressaceae and Podocarpaceae families in particular, show a series of compounds that include monoterpenes, sesquiterpenes, sesquiterpenlactones, diterpenelactones, diterpenes, triterpenes, biflavones and lignans. In some cases those compounds would be responsible for the resistance of woods to the

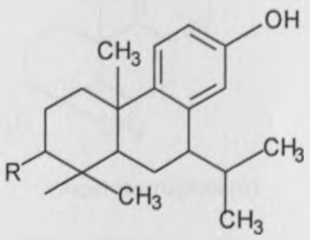
action of insects, fungi and bacteria. Thus, these compounds could have applications as pesticides in wood preservation (Kubo *et al*, 1992).

The species of the Podocarpaceae family have been studied particularly in Australia, Brazil, Japan, New Zealand and Chile. In Chile the ones that have been studied include *Prumnopitys andina, Podocarpus nubigena* and *P. saligna*. These species have been shown to contain diterpenes, triterpenes, diterpenelactones, norditerpenedilactones, bisnorditerpendilactones, steroids and moulting hormones. Some of those compounds exhibit a significant biological activity as herbicide, insecticide, cytotoxins, and they also have antitumoral activity. The resistance of the wood of *Podocarpus* species to termites is due to the presence of lactones (Keith *et al*, 1974).

From the bark and wood of the Chilean Podocarpaceae species: *Podocarpus nubigena, P. saligna, Prumnopitys andina* and *Saxegothaea conspicua*, ten diterpenes were isolated and identified and they include: ferruginol (la), hinokiol (ib), hinokione (ic), totarol (2a), totarodiol (2b), totarolone (2c), abietatriene (3), 6,7 dehydroferruginol (4), isopimarol (5), acetylferruginol (6) (Pompei, 1980, Prazeres, 1982) (see chemical structures below).



Ferruginol (1a), R = H
Hinokiol (1b), R = OH
Hinokione (1c) R = O

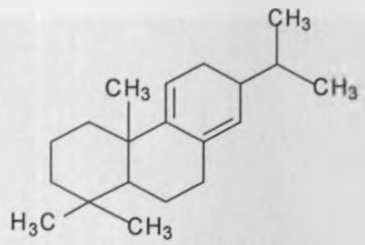


Totarol (2a), R = H,
Totarodiol(2b), R = OH,
Totarolone (2c), R = O

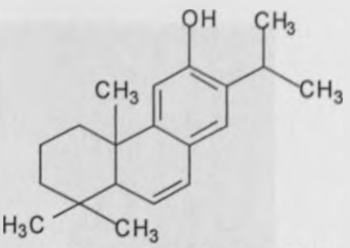
A comparative analysis of the diterpenes present in the wood and bark of the four species studied was carried out using GLC/EM analytical technique. *P. nubigena P. saligna* showed five diterpenes common in wood and two in the bark. Totarol was the major compound in both species, followed by totarodiol in wood. Totarol and totarodiol are also present in *S. conspicua*, but the main compound in the wood of this plant is ferruginol followed by hinokiol in the former and totarol in the latter.

A different composition of diterpenes was found in the wood and bark of *Prumnopitys andina*, since totarol and its derivatives were not found. However just as in *S. conspicua* ferruginol and hinokiol in the wood and ferruginol in the bark were found.

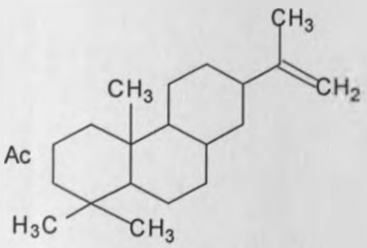
It is important to note that six of these diterpenes studied exhibit activity against *S. aureus* and *Pseudomonas* sp. The diterpenes with the highest activity are totarol, ferruginol, dehydroferruginol and acetylferruginol (see chemical structures below). The research work done by Kubo *et al.*, shows that the antimicrobial activity of totarol against gram positive and gram negative bacteria was confirmed. Similar activity is observed in other diterpene derivatives e.g ferruginol, 6,7,di-hydroferruginol, and acetylferruginol.



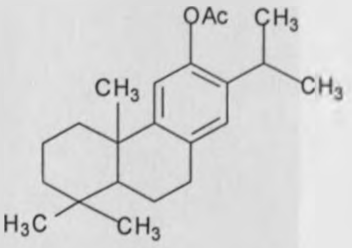
Abietatriene (3)



6,7 dehydroferruginol (4)



Isopimarol (5)



Acetylferruginol (6)

Those seven diterpenes were tested for activity against *Aspergillus* sp., *Fusarium fujikuroi, F. ciliatum*, *Mucor miehei*, *Nematospora coiyli*, *Penicillium notatum* and *Paecilomyces variotii* and six of them exhibited antimicrobial activities. Amongst these diterpenes, totarol and ferruginol are the most active.

These research activities suggest that the wood resistance to fungi and bacteria of Chilean Podocarpaceae is due to the high concentration of phenolic diterpenes to be found in these plants. These results also agree with the observations made by Cambie *et al* 1984 on the resistance of the Fijian Podocarpaceae woods to fungal attack.

2.6 *Toddalia asiatica* (Rutaceae)

Toddalia asiatica (**Rutaceae**) is a woody liana that can reach a height of 10m in forests; It uses other trees for support. The corky stems are covered with knobby thorns and are yellow when cut. The attractive shiny trifoliate leaves are light to dark green and are extremely aromatic, smelling of lemon when crushed. The twigs are covered in small, recurved thorns. The small, greenish-yellow flowers appear in spring and the plant continues flowering until the beginning of autumn. The berry-like fruits are borne right through summer. The fruit, 5-7mm in diameter, are orange in colour when ripe and taste like the skin of an orange (Watt *et al,* 1962).

2.6.1 Distribution and habitat

Toddalia asiatica always occurs in forests near rivers or streams. It grows fairly well in clay soils in Maasailand. In South Africa, its natural distribution is on the southern slopes of the Soutpansberg and south to Swaziland.

This liana occurs also further north in tropical Africa, Asia and Madagascar. It flourishes in frost-free areas with a fairly high annual rainfall (Watt *et al,* 1962).

2.6.2 Uses and cultural aspects

Toddalia asiatica is used medicinally by Venda herbalists. The fruit is used by the Maasai as a cough remedy and the roots in the treatment of indigestion and influenza. The leaves are used for lung diseases and rheumatism. In Madagascar the root and its bark have been used as a remedy for fever, malaria, cholera, diarrhoea and rheumatism. Usher 1974 reports that in India a yellow dye is extracted from the roots (called Lopez Root) and the root bark is used medicinally as a tonic and for stomach ailments.

Toddalia asiatica could be used very successfully in larger gardens where the glossy light green leaves attract attention. This plant tends to grow as a large shrub, not a liana if planted in full sun. Because it is extremely thorny, it could also be used as a security fence. Fortunately, *Toddalia asiatica* probably does not have many natural enemies or pests as the whole plant is covered in numerous glandular dots which contain acidic boils (Usher, 1974).

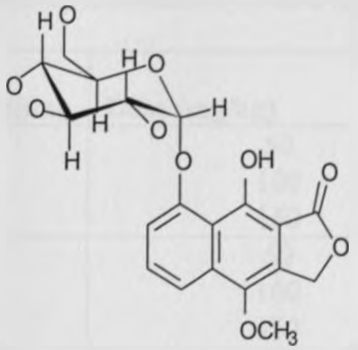
In recent years, a number of reports have shown the anticancer activity of plant extracts and phytoalkaloids. Hinorori *et al* have evaluated the cytotoxicity profiles of 157 extracts prepared from dietary or medical plants growing in the Okinawa Island, using 10 different cell lines. In vitro cytotoxicity screening indicated the presence of a highly selective cytotoxic compound in the extract of *Toddalia asiatica* Lam. The known alkaloid (1,3)-benzodioxolo-(5,6-c)-phenanthridine, 12,13-dihydro-2,3-dimethoxy-12-ethyl-(dihydronitidine) was identified as an active material from this plant. This alkaloid had highly specific cytotoxicity to human lung adenocarcinoma cells (Hinorori *et al*, 2007).

2.7 *Rhamnus prinoides* (Rhamnaceae)

R. purshiana and *R. frangula* (Rhamnaceae) are well known sources of the commercial drugs popularly known as Cascara and Frangula. These species do not occur in Africa. The only two *Rhamnus* species that occur in Africa are *R. staddo* and *R. prinoides. R. prinoides* L'herit is a very interesting plant widespread in many parts of eastern and

central Africa. In South Africa it is known as dogwood and is referred to as a drought and frost resistant plant. In Ethiopia it is a widely cultivated multipurpose plant. The fruits are used for the treatment of ringworm infections (Abegaz, *et al,* 1998). The leaves and stems are used as a bittering principle in the preparation of domestic beverages. Recent studies have shown that it can serve as a commercial hopping agent in the brewery industries.

Abegaz *et al,* 1999 studied the above ground parts of this plant and have isolated and characterized 20 compounds consisting of seven glycosides of emodin anthrone, flavonoids and three naphthalenic derivatives. Organoleptically the most important substance is the naphthalenic glucoside, geshoidin, which is responsible for the characteristic bitter flavour of the beverages derived from this plant.



Geshoidin

2.8. *Carissa edulis*

Carissa edulis Vahl (Apocynaceae) grows in tropical African region and its parts are used in folk medicine for wide varieties of remedies, such as fever, sickle cell anaemia and hernia. The plant parts are reported to be used for treatment of oedema, toothache, cough, ulcer, warm infestation and it is also used as source of dye (Omino *et al*, 1993).

The chemical compositions of the plant have extensively been reported. The extract yield benzenoids, phenylpropanoid, lignans, sesquiterpenes and coumarins steroids, terpenes, tannins, flavonoids and cardiac glycosides, (Ibrahim, 1997). The ethnomedical uses of the plant and the need to establish its biological activity have prompted research interest of the plant. The comparative analgesic properties of the various parts of the plant have been studied by Ibrahim *et al,* 2007.

The analgesic activity of the water extracts (50,100 and150 mg/Kg body weight) of the root bark, stem bark, leaves, fruits and seeds of *Carissa edulis* were evaluated in mice using the mechanical method (tail-chip method) and chemical method (acetic acid induced writhing). The plant was found to have analgesic activity, with the fruits having the highest activity, followed by the leaves, seeds, root bark and stem bark respectively using metamizol as standard with mechanical method as seen in Table 2.3. There was slight variation with chemical method where the seeds were found to be most effective followed by fruits, leaves, root bark and stem bark respectively using acetylsalicylic acid as standard (Ibrahim *et al,* 2007).

Table 2.3 Analgesic activities of the root bark, leaves, and fruit stem bark and seed water extracts of *Carissa edulis* by mechanical method (Ibrahim *et al,* 2007)

Extracts Mean of writhing	**Dose (mg/kg)**	**Mean of writhing**
RWE	50	14±0.12
	100	12±0.7
	150	9±0.27
LWE	50	12±0.22
	100	9±0.11
	150	9±0.24
FWE	50	12±0.07
	100	9±0.53
	150	7±0.24
SWE	50	9±0.13
	100	10±0.23
	150	5±0.12
SBWE	50	14±0.17
	100	13±0.24
	150	10±0.47
Control	0.2 ml	23±0.15
Standard	23±0.15	10±0.36

RWE = Root Water Extract, LWE = Leaves Water Extract, FEW = Fruit water Extract, SWE = Seed Water Extract, SBWE = Stem bar Water Extract, Control = Normal saline and Standard = acetylsalicylic acid (Aspirin®).

The analgesic activity compared well with metamizol and aspirin which were used as standard, the chromatographic analysis indicate the presences of salicylates as a seen in Table 2.4. The activity was found to be significant at $P < 0.001$ and $P < 0.0001$ (mechanical method) and $P < 0.037$ (chemical method). These result justified the use of the plant in the treatment of toothache, lumbago, oedema and chest complaints by the traditional medical practitioners (Ibrahim *et al*, 2007).

Table 2.4 Chromatographic analysis of the fruits water extracts (Ibrahim *et al*, 2007).

Sample	PC		TLC	
	hRf %	Colour	hRf %	Colour ($FeCl_3$)
Unhydrolysed Water extract	98.0 95.1	Purple Violet	52.8 73.6 86.0 94.3	Violet Violet Violet Violet
Hydrolysed water extract	95.0	Violet	72.0 56.0	Violet
Salicylic acid (standard)	94.0	Violet	88.7	Violet
Methylsalicylate (standard)	97.1	Purple	94.3	Violet

2.9 Essential trace elements in Biology and Medicine

Essential trace elements form part of structural component of tissues, cofactors and enzymes and participate in transport processes. In this research work, it is suggested that these trace elements do play a part in health and disease conditions of plants and animals. To play this role, they cater for the nutritional well being of organisms, while the availability or non-availability of trace elements can lead to diseases such as diabetes, anemia and brain impairment.

The absorption process of the trace essential elements determines their availability for biological processes in any organism. Once absorbed, the trace elements undergo transformations before they can participate in any biological functions. This may involve the change of oxidation state or coordination sphere which results in specific binding to

transport substances that direct organization of the trace elements into the chemical environment of their site of action (Wallach, 1990). The oxidation state of these elements determines what role they play in vivo.

An element is considered essential to a living organism if the organism cannot persist or function properly unless the element is supplied in sufficient quantities. Thus an essential element has a role to play in the organisms' metabolism and its biological functions. In addition, an essential element cannot be totally replaced by another element (Wallach, 1991).

2.10 Trace minerals as dietary supplements

Until recently, the widely accepted practice of supplementing diet with trace minerals was based on empirical knowledge with little or no scientifically proven justification. The practice was identified with survival and instinctive behavior in both humans and animals. In past few years a scientific rationale for the use of many trace minerals has been established. Maitai, 1988, reviews the importance of some of the trace elements and argues that deficiency of trace minerals in humans may be a contributory factor in many idiopathic disease conditions. However, our understanding of the role of these trace elements is still incomplete. They should not be regarded as a panacea for all human ailments, particularly since they may be toxic when used in large quantities or for long periods.

People who take mineral supplements do so to optimize their health. But if one is taking a mineral supplement it should be understood that nutrition professionals do not clearly understand the effects of self prescription nor that of intake of too many supplements. Over supplementing may lead to a mineral imbalance or toxicity.

The minerals commonly included in such preparations are Mg, Fe, Zn, Cu, Co, Mn, Mo, V, Cr, PO_4, S, and Se. Apparently it is assumed that Na, K, Ca, Cl, are readily available from the diet and therefore no supplementation is necessary. According to the promoters of these mineral products, the main indication for use is as dietary supplements in the elderly, children, pregnant women, breastfeeding women, patients on dietary restrictions or those in convalescence.

2.10.1 Zinc

Zinc is present in every tissue throughout the body and is an important component in energy metabolism (using food for energy). Zinc is essential for making protein, which means it helps grow muscles and other tissues. It can also aid in injury healing and immunity against diseases. Zinc deficiency leads to rough, dry skin and loss of appetite and ability to taste foods. Even mild zinc deficiencies can impair the immune system by as much as 20 to 30 percent (Bock, 1990).

The recommended dietary allowance (RDA) for zinc is 12 mg per day for women and 15mg for men. This is the amount commonly added to multivitamin and mineral supplements. Zinc from animal meat, seafood, and egg is far better absorbed than the zinc from plant sources. Food rich in high fibre may leads to reduced zinc absorption. In fact, zinc from beef (no fibre) is absorbed four times more effectively than zinc from a high fibre breakfast cereal (Clarkson *et al*, 1994).

Most studies on zinc supplements show no benefits to exercise performance. But studies do suggest that zinc supplements between 50mg to 100mg might have adverse consequences, such as altered copper and iron status, impaired immune function, and a decline in 'good' cholesterol (Fosmire, 1990). A good rule is to avoid supplements with zinc only because the intake can be too high (Zheng *et al*, 1993).

2.10.2 Iron

Iron is essential for the production of haemoglobin, which is the red pigment in the blood that carries oxygen from lungs to the body cells. When iron supplies are deficient the body cannot manufacture sufficient quantities of new-blood cells, resulting in anemia. Such symptoms of anaemia like fatigue and lack of energy reflect the body's inability to supply enough oxygen to the cells. Women are at a greater risk of anemia than men especially during pregnancy when the developing fetus 'borrows' iron supplies from the mother (Clarkson, 1994).

Iron is a part of red blood cells and helps transport oxygen to body cells. So, if one has low levels of iron and become anemic, muscles receives less oxygen and therefore produce more lactic acid. A buildup of lactic acid results in premature fatigue when one

works out. Anemia is more common among women than men because women lose iron through menstrual bleeding.

The RDA for iron is 10mg for men and 15mg for women. The iron derived from animal foods (heme iron) is absorbed better than the iron in plant foods (non-heme iron). The best way to boost iron intake is to eat lean meats, dried beans, wheat germ, whole grains, and fortified breakfast cereals. Side effect of iron supplements can be constipation (Michele *et al* 2001).

2.10.3 Selenium

Selenium is a key anti-oxidant that may exert anti-cancer properties, especially against stomach and esophageal cancer. Antioxidants are compounds that protect one from oxygen radicals that cause cell damage and in turn can lead to cancer. Very large doses may impair the immune response and cause loss of hair and nails. The body requires trace amounts of this mineral which is mostly obtained from cereals.

Selenium works together with vitamin E. It is also essential to the functioning of some enzymes; hence catalyse specific biological systems at levels of less than 0.1% mass in human body.

2.10.4 Chromium

Chromium (Cr.) is a member of Group VIB. Other group members include molybdenum, tungsten and uranium. It has four isotopes of atomic masses 50, 52, 53, 54 a.m.u, external electronic configuration 4s' 3d, common oxidation states of +2, +3, +6, covalent and ionic radii of 1.17A° and 0.52 A°, respectively, and first ionization potential of 6.77 Kj/mol (Block et at, 1974).

Chromium deficiency in animals is associated with impaired glucose tolerance, fasting hyperglycemia, hyperinsulinaemia and hypercholesterolemia. It can as well lead to aortic plaques, corneal lesions, decreased fertility and low sperm count (WHO, 1996, Davidson *et al*, 1968; Schroeder, 1968, Wallach, 1990). Chromium supplementation has been shown to improve or normalize the impaired glucose tolerance of some diabetics, old people and malnourished children.

Chromium is necessary for optimal growth of animals but toxic if used in large quantities (Block *et al*, 1974). It is destructive to tissues whether applied topically or administered orally and is known to cause glycosuria and nephritis when taken internally, while persons exposed to chromate dust develop deep skin and nasal mucosa ulcers that heal very slowly (Block *et al*, 1974).

Chromium is an essential mineral that one needs in small amounts to maintain normal blood sugar balance. The daily intake for chromium is 120 micrograms per day for men and women. There is limited knowledge about the dietary needs for chromium, the amount of chromium found in foods, and the chromium status of athletes and the general public (Morris *et al*, 1992). Among athletes, chromium has been touted as a way to build muscle and decrease body fat, but scientific research shows no fat-burning or muscle-building benefits (Hallmark *et al,* 1996).

2.10.5 Copper

Though copper has not been studied extensively, early results suggest that it too has a considerable effect on immunity. There is no recommended dietary allowance. Nutritionists recommend 2 to 3 mg up to a maximum of 5 mg daily. Deficiencies in copper have been shown to impair resistance to diseases and other immune functions. Copper and zinc absorption is closely related, and although copper is also needed in relatively small amounts, some discussions are under way on the optimum need of this mineral. If large amounts of copper are present, then zinc and vitamin C is reduced in the body, and vice versa (Clarkson *et al*, 1994).

In addition to above essential elements, there are approximately 20 known essential elements to human life that contribute to the proper functioning of living cells and enzyme catalysis of biomolecules necessary for life. They are taken as supplements in form of recommended dietary allowances (Zumdahl, 1992).

2.11 Importance of inorganic constituents in medicinal plant extracts.

The importance of organic constituents in medicinal plant extracts and herbal medicines has often been acknowledged. There is also increasing evidence implicating inorganic constituents, particularly trace metals in the etiology of diseases. The role of common

electrolytes like sodium, potassium, magnesium and calcium in these preparations has been investigated. Little attention, if any, has been paid to the role of the trace elements present in these materials, especially in cases where the preparation is essentially inorganic, such as those from ashed materials. The role of trace elements in the pathology and management of diseases is of particular interest especially in the treatment of epilepsy where the use of anticonvulsant drugs have shown effects of altering the levels of trace metals in both human and experimental animals (Kinyua, 1997).

The argument on trace element bioavailability and possible interactions with a medication - an interaction which more probably than not, affects the efficacy of a particular antidiabetic therapy - is briefly presented by way of highlighting known metabolic functional interactions, synergism and antagonism and limiting mobility of a given essential element (Zumdahl, 1992).

CHAPTER THREE

EXPERIMENTAL PART

3.0 MATERIALS AND METHODS

3.1 Instrumentation

Atomic absorption spectrophotometer Model Bechmann 332 at Nairobi City Council Quality Water Analysis laboratory was used to analyze for chromium, iron, zinc and copper. UV/Vis spectra of **Compounds A** and **B** were done/recorded on UVIVisible spectrophotometer model and Pye -Unicam SP 8-15, at the Department of Chemistry in University of Nairobi.

The NMR spectra of compounds **A** and **B** were done/recorded on 250 MHz, ^{1}HNMR and 100 MHz, ^{13}CNMR spectrophotometer, model XL-400, LC-MS and MS-FAB spectra of the compounds and the extracts were acquired on LC–MS spectrometer model Macintosh HD:LC 100 repectively at Schering Plough Research Institute, USA. pH was determined on Model Syntronic MK 1V meter.

3.2 Chemicals and Solvents

3.2.1 Analytical grade reagents

Methanol, Ethanol, T.L.C plates Polygram 254. Silica gel 60 (0.04-0.06mm,1230-400 mesh), Oxytocin, Propranolol, Isoprenaline, Adrenaline, Acetylcholine, Atropine, Glucose, Solution A; 0.2 M phosphate buffer at p.H 7 containing 40 mg peroxidase and 2.5mg glucose oxidase per ml.

3.2.2 General purpose reagents

Acetone, Chloroform, Dichloromethane, Methanol, Ethanol, Potassium dichromate, Copper Nitrate, Zinc Sulphate, Iron Nitrate, Sodium chloride, Hydrogen peroxide, Hydrochloric acid, Glacial acetic acid, Sulphuric acid, Aluminium Chloride, Magnesium turnings, T.L.C plates Polygram 254 , Hexane.

3.2.3 Equipment and apparatus

Havard Devices recorder, Oscillograph (Washington 400MD), Mixture of 95% 0_2 and 5%, CO_2 (East African Oxygen Ltd), Lagendorf apparatus, Thermal regulated water

jacketed organ bath, Glass tissue bath, Kymograph Cat 1020, Ventilator cat No. 5056 (Scientific and Research Instruments Ltd, England), Syringes and needles.

Table 3.1: Physiological solutions

Solution	Chemicals and solvents in 1 Litre						
	NaCl	KCl	$CaCl_2$	$NaHCO_3$	Glucose	$MgCl_2$	NaH_2PO_4
Lock's	0.42	0.42	0.24	0.2	1.0	-	-
De Jalon's	0.42	0.42	0.06	0.5	0.5	-	-
Tyrode's	48.0	0.2	1	-	0.1	0.5	

3.3 Plant materials and extracts

Several plants believed to have healing properties were collected by the author with the assistance of traditional healers from Kajiado and Laikipia. The plants were identified from their growing environments and authenticated in the Department of Botany. The plants were *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis* and *Toddalia asiatica*. A labelled specimen has been deposited in Department of Botany.

3.3.1 *Podocarpus* sp

The barks of *Podocarpus* sp Podocarpaceae also known as (Maasai red medicine) was collected by the author assisted by a traditional healer in September 1997 from dry areas of Kajiado. The plants grow in dry areas and have a thick red bark with a bitter taste. These were identified from their growing environments. A plant taxonomist in the Department of Botany Herbarium of the University of Nairobi authenticated the plant. An aqueous/water extract of the bark of *Podocarpus* sp was also supplied by the traditional healer to the author in the Department of Chemistry.

3.3.2 *Rhamnus prinoides, Carissa edulis, Toddalia asiatica*

The bark and stems of *Rhamnus prinoides* Rhamnaceae, *Carissa edulis* Apocyanaceae, *Toddalia asiatica* Rutaceae were collected by the author with the assistance of traditional healers from the dry areas of Laikipia. A plant taxonomist in the Department of Botany Herbarium of the University of Nairobi also authenticated the plant in September 1997. A labelled specimen has been deposited in Department of Botany.

3.3.3 Water Extract of *Podocarpus* sp bark – CADE-A

The water extract of *Podocarpus* sp was prepared by boiling the red bark in 5 litres of distilled water in an aluminum pot for six hours. The water fraction was stored in a clean, labelled polyethylene container. The extract was filtered through a sieve and re-concentrated by evaporation using vacuum evaporator to red brown syrup. The water extract was labeled CADE-A. The following physical parameters were tested:

a) **pH**

A volume measuring 50ml in a beaker was temperature stabilized at 20°C. The pH meter was calibrated with pH 4.00 and pH 7.00 standard solutions. The samples were then read using the calibrated pH meter.

b) **Taste**

A 5ml solution was measured into a 10ml test tube and using a dropper a little amount was dropped on the tongue and the taste rated as bitter, salty, sweet or acidic.

c) **Colour and physical appearance**

A solution was measured into a test tube and the colour observed visually and rated as brownish, reddish, dark, yellowish and greenish

d) **Stability**

A 100ml solution was kept at ambient conditions and the appearance, decomposition, change in colour and production of foul smell were monitored at intervals of one month for the entire period of nine months.

e) **Percentage non-volatile matter**

A 10ml solution was weighed into a dry crucible and heated at 105°C for 1 $^1/_2$ hours. The sample was removed, desiccated, weighed again and the percentage non-volatile matter calculated.

f) **Specific gravity**

A 25ml solution was weighed into a pycnometer and the weight recorded. Distilled water measuring 25ml was also weighed. The specific gravity was then calculated by dividing the weight of the CADE-A by the weight of distilled water.

3.3.4 Chloroform extract of CADE-A

5.0 litres of the water extract - CADE-A were hydrolyzed with 10% HCl by warming for 30 minutes to hydrolyse acid insoluble aglycones, filtered and the resultant filtrate divided into two equal portions. Each portion was then partitioned on three consecutive times with 500ml of analytical reagent chloroform. The resulting 1.5 litres of chloroform extract was concentrated using a vacuum evaporator. This residue was used to prepare aqueous solutions of various concentrations for use in the determination of the effect of *Podocarpus* sp chloroform extract on glucose metabolism, isolated rabbits' organs and for testing for secondary metabolites.

3.3.5 Methanol extract of *Podocarpus* sp bark

Dry stem bark weighing 300g of *Podocarpus* sp was ground to a fine powder and soaked in 1,500 ml of methanol for two days. The resultant extract was filtered using a sunction pump and concentrated using a vacuum evaporator. This solid residue was then used to prepare aqueous solutions of different concentrations for use in the determination of the effects of *Podocarpus* sp methanol extract on glucose metabolism, isolated rabbits' organs and testing for secondary metabolites.

3.3.6 Water Extract of *Carissa edulis, Rhamnus prinoides* and *Toddalia asiatica* plant stems (CADE-B)

Dry stems of *Rhamnus prinoides, Toddalia asiatica* and *Carissa edulis* each weighing 200g were boiled with 3000ml of distilled water for about six hours in an aluminum container while at least maintaining the volume of water lost during evaporation. The resultant 3000ml water extract was concentrated in vacuum for 3 hours. This extract was used for physiological muscular contraction experiments on the isolated rabbit's heart, ileum and the uterus at University of Nairobi Pharmacy Department. The water extract was named CADE-B tested for physical properties as in section (3.3.3 a-f).

3.4 Testing for secondary metabolites

Qualitative identification tests were carried out to determine triterpenoids and sterols, flavonoids, saponins and sapogenins in the methanol and chloroform extracts of *Podocarpus* sp.

3.4.1 Testing for presence of triterpenoids and sterols

Two grams of the crude methanol and chloroform extracts were re-extracted with hexane to remove non-polar compounds. The organic layer containing polar compounds of each was then discarded. The residue was then extracted with 40 ml of $CHCl_3$ and to 0.5 ml portions of the chloroform extract; 0.5ml of acetic acid was added followed by two drops of concentrated sulphuric acid. A slight green colour observed indicates presence of sterols and triterpenoids.

3.4.2 Testing for presence of flavonoids in bark extracts

Two samples weighing 1g of the chloroform and methanol extracts respectively were dissolved in hexane to remove any fatty/polar material and each of the residues dissolved in 80% ethanol and following experiments were performed.

a) To 3ml of ethanol solution, 4ml of aluminum chloride in the methanol solution was added. Pale color observed indicates presence of flavonoids.

b) To about 2ml of the ethanol solution, 0.5ml of concentrated HCl was added in a tube and a few magnesium turnings were added. A red color observed indicate presence of anthraquinones.

3.4.3 Testing for presence of saponins and sapogenins

i). Aqueous methanol extract and chloroform extracts were each shaken in a test tube while observing formation of foam above the liquid. Appearance of persistent foam is a positive identification of saponins.

ii). To test for haemolytic effect of the methanol and chloroform extracts, the following tests were carried out:

a) Blood gelatin solution was prepared by adding 100ml of 0.9% sodium chloride to 45g of gelatin powder. This was left standing for 30 minutes followed by heating the gelatin mass to 80°C on a water bath with stirring. 50ml of blood was added and mixed with the gelatin sodium chloride mixture to form red blood gelatin suspension.

b) The red blood gelatin was then poured immediately onto a thin layer chromatogram glass where the chloroform and methanol extract had been spotted.

c) After 1 hour, the colour of red blood gelatin was checked to find out if transparent. The change in colour is a positive test for haemolysis.

3.4.4 Testing for the presence of anthraquinones

About 0.5g dry powdered bark and chloroform extract was reacted with 5ml of 0.5M KOH. Into the mixture, lml of 3% w/v solutions of hydrogen peroxide was added. The mixture was boiled for 5 minutes, cooled, filtered and to 5ml of the solution, acetic acid was added and extracted with benzene. To the benzene layer, some 5ml of ammonia solution was added. A reddish colour indicated absence of anthraquinones in the bark and in chloroform extract.

3.5 Separation and isolation of saponins and sapogenins in chloroform and methanol extracts

Separation and isolation of the predominant class of compounds in the extract was carried out using simple chromatographic techniques such as thin layer chromatography (TLC) and column chromatography (GC,) techniques.

3.5.1 Thin layer chromatography

Analytical TLC was performed using Polygram 254 nm silica gel in separation of both chloroform and methanol extracts. Two extracts weighing 0.5 grams of chloroform and methanol extracts were dissolved in 20ml of chloroform and methanol respectively. The solutions were spotted on 4xl0cm strips of TLC plates. Elution was done by a mixture of hexane:methanol:chloroform in varying proportions/increasing polarities of eluting solvent). The best solvent combination that produced ideal separations was hexane:methanol:chloroform (6:2:2). The plates were viewed under UV light before exposing to fumes of concentrated ammonia in order to identify and evaluate spots of saponins. The spots were identified by spraying the TLC plates with a reagent containing 5ml acetic anhydride and 5ml concentrated sulphuric acid in 50ml absolute ethanol and

heated at 100 °C for 10 minutes. The fluorescing spots were observed under UV light λ_{max} 254nm.

3.5.2 Column chromatography

Column chromatography was performed using Silica gel 60 (0.04-0.06mm, 230-400 mesh). A 40 cm long and 30mm diameter glass tube column was packed with 80g silica gel 60: hexane slurry by pouring into the column slowly. Hexane solvent was then run into the packed column at a suitable rate to satisfactorily pack the column. On completion of packing of the column, 5 g of the chloroform extract was made into a paste and placed on the top of the column. Elution was then carried out using increasing polarities of methanol: hexane:ethylacetate (30:15:55) and analytical chloroform: methanol: water (60: 30:10) mixture. The solvents flow rates were set at 10ml per minute. Preparative TLC was then used to identify compounds in the eluted solvents. The collected elutes were separated and combined in 250 ml conical flasks and kept for future concentration using a rotary vacuum evaporator.

The isolated compounds were recrystalised in methanol and ethanol and their melting points, UV/ visible, ^{1}HNMR, and MS spectra determined. A spectrum of LC-MS and MS-FAB was run on both the methanol extract and chloroform extract and likewise to the isolated compounds respectively using Macintosh HD.LC-100 operation files with a spectra acquisition time of 10 minutes 58 seconds.

3.6 Determination of Chromium, Iron, Zinc and Copper

Samples of crude aqueous drug extract-A and B were analyzed for the presence of chromium, copper, iron and zinc using AAS spectrometer model Bechmann 332.

3.6.1 Sample digestion and analysis

Four, 100ml of CADE-A and CADE-B, previously stored in acid washed and water rinsed polyethylene bottles were digested by adding 5ml of concentrated Nitric acid, and heating on a hot plate to boiling. The contents in 125 ml flasks were evaporated slowly adding de-ionized water as necessary until digestion was complete. A 7ml volume of 30% hydrogen peroxide was added and slow heating was done until they were clear. The

samples were not allowed to dry during digestion. This was accomplished by constant washing of the sides of the flasks with de-ionized water.

A blank was also prepared at the same time when the sample solutions were being prepared. Finally the sample solutions were stored in 100ml water rinsed polyethylene bottles.

The standard stock solutions of Cr, Cu, Zn, and Fe (essential elements) that were prepared from their salts to 1000 ppm were diluted to 100 ppm and further to 1 ppm with deionised water. The resultant solutions were pippetted to make six volumetric solutions ranging from 1-6 ppm in 50ml erlenmeyer flasks. Absorbance readings of the solutions were obtained first by running the standard samples followed by sample solutions. The results were tabulated and the calibration curve of the standard solutions drawn. The concentrations of the ions in the samples were obtained from the standard solutions curves and the results tabulated.

3.7 Antidiabetic bioassays

Crude aqueous drug extract-A (CADE-A), methanol extract (ME) and chloroform extracts (CE) were used to determine their effects on blood glucose concentration on 48 hour fasted rabbits.

3.7.1 Experimental animals and drug administration

Six local and fourteen-White New Zealand mature rabbits, weighing mean (3.10 ± 0.9 kg), were used in this study. The animals were maintained on a normal diet of rabbit pellets and foliage and an unlimited amount of water supply. They were deprived food and water for 48 hours prior to intubation for the Glucose Tolerance Test.

From a total of 20 rabbits, ten were randomized and fed on different amounts of glucose ranging from 1 to 4.0g/kg body weight to determine the amount of oral glucose that resulted in the highest concentration of blood glucose.

Similarly fifteen experimental animals were randomized into three groups of five animals each. To group 1, 2 and 3 various dosages of the crude aqueous drug extract, methanol drug extract and chloroform extract dissolved in distilled water were administered

respectively. This was in order to determine the highest dosage that would produce the greatest effect on blood glucose levels without causing undue distress in the animal. This meant randomly assigning a different dose of the drug to be administered in conjunction with 2.5 g/kg body weight of glucose to each of the animals in the group and evaluating the baseline blood glucose level obtained at 30 minutes and at 150 minutes from the time of the administration of the drug.

Hypoactivity accompanied by lack of alertness within an hour after the administration of the drug was regarded as the initial signs of incipient distress. The process was repeated using a higher dose of the drug, at ten-day intervals, the interval setting was done to eliminate and allow the effects of the drug to wear off completely before the next dosage was administered to the animal.

For Glucose Tolerance Test, rabbits were deprived food and water for 48 hours before the administrations of the drugs while the controls, which had also been deprived food for a similar period of time, were given an equal volume of distilled water instead of the drug.

The experimental animals were randomized into three groups of five animals each. The first and second groups were dosed with 2.50g/kg body weight of glucose dissolved in 8ml of aqueous drug extract and 2.50g/kg body weight of glucose dissolved in 8ml of distilled water containing 20 mg/kg of the chloroform extract respectively. The third group was dosed with 2.50g/kg body weight of glucose dissolved in 8 ml of distilled water containing 20 mg/kg of the methanol extract. The last of the five animals was used as the control and each of these received only 2.50g/kg body weight of glucose dissolved in 8 ml of distilled water. The process was repeated three times for the three groups of sample testing and one group of the control.

The randomization process was repeated on two further occasions in the case of the three groups of experimental animals. This was for the purpose of repeating the Glucose Tolerance Test, always allowing the minimum of 15 days in between these tests to allow the animals to recover fully from the effects of the anaesthetic ether used for the intubation purposes and also to recover from the effects of the intubation itself which

may have led to some internal injuries and also to allow the animal to heal the wounds on its ear caused by the syringe needle.

3.7.2 Determination of blood glucose

Blood glucose measurements were performed by glucose oxidase method. Blood samples of 1ml was withdrawn from the marginal ear vein and deproteinized with 4.5ml of 3% perchloric acid. Aliquots of 0.2ml of the supernatant were pippeted into the test tube and 3ml of glucose oxidase reagent (prepared by mixing 40mg of peroxidase and 2.5mg oxidase per ml and 1ml of 1% o-anisidine in ethanol in 0.2M solution of phosphate buffer at pH 7.00) shaken and kept in the dark for 35 minutes for orange colour development. Likewise the standard's concentrations containing 10, 25, 50, 100, 150 and 200mg/100ml in dilute solution of benzoic acid were prepared and treated in the same away as the samples.

Glucose oxidase catalyses the reaction as shown below

1. β-D Glucose H_2O + O_2 $\rightleftharpoons$ D-Glucose

   The D-gluconolactone which is first formed is hydrolysed to D-gluconic acid. The hydrogen peroxide is decomposed in the indicator reaction and glucose oxidase catalyses the reaction.

2. By peroxidase, the oxygen liberated oxidizes a hydrogen donor DH_2 (in this case o-anisidine) the amount of dye formed is a measure of the amount of glucose oxidized. Optical density is measured at 436nm using Novaspec II spectrophotometer, Cambrigde, England against water blank.

3.8 Antihypertensive bioassays

Physiological tests were carried out on the isolated ileum, uterus and the myocardium using the plant extracts (CADE-A and CADE-B). The stimulation/inhibition on the contractile activity of muscle was observed on the organs after various doses of the extracts and conventional drugs were administered.

3.8.1 Myocardium experimentation

Four female rabbits mean (3.5 kg ± 0.65) kg were used for these experiments. A blow on the head killed the rabbits and the chest wall and rib cage quickly opened. The heart was then removed ensuring that at least 1 cm of aorta was left intact on the heart. This heart

was then freed of all extraneous tissue material and then immediately attached through the aorta onto the base of the Lagendoff apparatus containing Lock's solution at 32°C ensuring that air bubbles were not trapped within the heart chambers. This arrangement allowed the perfusion fluid to enter into the aorta thereby leading to the aortic valve and therefore for the fluid to flow into the coronary circulation and into the right auricle before escaping from the heart via the vena cava.

When the heart was beating satisfactorily the apex was hooked onto a thread of a pulley system attached to a Howard Device recorder. The crude water aqueous drug extract-A (CADE-A) was administered through the rubber cup, above the cannula, at increasing concentrations and the effects on the contractility of the myocardium compared to those seen on the administration of propranolol, atropine and isoprenaline.

A combination of *Podocarpus* sp extract with propranolol and another one with atropine and lastly one with isoprenaline were administered to the heart preparation to record the effects of such combinations on the contractility of the myocardium. The process was repeated using crude water extract-B (CADE-B) in the same way as CADE-A.

3.8.2 Uterine muscle experimentation

For uterine muscle contractions, eight female white New Zealand rats were used for the purpose. These rats had a mean 125g (range 112-162g). Twenty-four hours prior to killing of the rat, it was injected with 0.1 mg/kg body weight of oestradiol. This was to increase the sensitivity of the uterus' sarcoplasmic stimulation. A blow on the back of the head of the rats was done, the uterus dissected and the uterine horns located in the killed animal. The horns were then separated at the bifurcation to yield two separate muscle preparations which were then placed in a dish of de Jalon's Ringer solution.

One of the pieces was then placed in the thermally regulated glass tissue bath containing de Jalon's Ringer solution at 32°C attaching one end of it by a thread to the organ bath while the other end was connected to a force transducer for tension recording of the muscle. A recording of the normal contractions of the uterine muscle was taken using a kymograph with a device recorder prior to the administration of any drug. Oxytocin was injected into the organ bath containing the uterine muscle. Recordings of the muscle

contractions were taken for four minutes and muscle rinsed in fresh de Jalon Ringer solution and the perfusion fluid in the tissue bath replaced with a thermally regulated fresh solution whose temperature had been maintained at 32°C. This process was repeated using the water extracts instead of oxytocin when 0.5 ml of the extract was injected into the tissue bath followed by a similar dose every four minutes until the total water extract that was added to the organ bath was 3ml.

3.8.3 Small Intestine experimentation

From each of the rabbits used for the myocardial experimentation a piece of ileum was taken out soon after removing the heart and placed in an organ bath containing Tyrode's solution at 37 °C in the thermally regulated water jacketed glass tissue bath (Scientific and Research Instruments, Ltd England). The ileum (approximately 2-3 cm in length) preparation was then set up by fixing one end of it with a thread attached to the organ bath while the other end was attached to the force transducer which was attached to a Device recorder. The recording of contractions was done on a kymograph recorder (Scientific and Research Instruments Ltd, England).

The magnitude of contractions of the ileum was then recorded and on stabilizing of the recordings, the drug extract was then added into the organ bath in gradually increasing amounts and a record of the effect of the drug on the contractions of the ileum recorded on the kymograph paper. The organ bath was also rinsed twice with Tyrode's solution before the whole process was repeated with the next dose of the extract.

3.8.4 Statistical analysis of results

The results were analysed using statistical random variables and normal probability distributions. The random variable takes different values as a result of the outcome of a random experiment.

The mean of random variable is the sum of values weighted by the probability that the random variable will take. The mean and the expected value of a random variable X is

$$\bar{X} = \sum_{i=1}^{n} (X_i)\, pr\,(X_i)$$

$$E(x) = \sum_{i=1}^{n} (X_i)\ pr\ (X_i)$$

$$X = E(X)$$

The variance and the standard deviation measure how the values of random variable are dispersed about the mean. The variance of the random variable is defined as the sum of the squared deviations of the values of random variables from the expected value weighted by their probability. Variance is therefore denoted;

$$Var\ (X) = \sigma_X^2$$

$$\sigma_X^2 = E(X) = \sum_{i=1}^{n} [(X_i - E(X)].\ (X_i)\ .\ pr\ .\ (X_i)$$

Alternatively, it can as well be written;

$$Var\ (X) = \sigma_X^2$$

$$Var\ (x) = \sigma_X^2 = \sum_{i=1}^{n} [X_i.\ pr\ .\ (X_i) - [E(X)]$$

Where

E (X) is the expected value of the random variable

Xi is the value of random variable

Pr (Xi) is the probability of the ith value

3.8.4.1 Significance of test, standard error of mean and standard deviation

Standard error of mean was determined as follows

$$SE_x = \frac{\text{Standard deviation}}{\sqrt{n}}$$

Standard error of standard deviation was determined as follows

$$SEx = \frac{\text{Standard deviation}}{\sqrt{2n}}$$

The significance ratio X, symbolically described as Z, t is often calculated by dividing the difference between a parameter and a statistic, where

$$t = \frac{|x-\mu|}{SE_x}$$

Critical ''t'' is obtained from tables of level of significance. The **t** value is worked out using the following expression,

$$t = \frac{|x-\mu|}{\sigma_x}$$

Where

σ_x = Standard deviation of distribution

Z = number of standard deviation from x to the mean distribution

X = the value of the random variable under consideration

μ = mean of the distribution of random variable

When the calculated ''t'' value is less than the value that is in the Table of critical values of T for n values then the difference is insignificant. Otherwise it is significant when the value is higher.

The mean of the distribution of variable at 5% and 1% significance level is given by

$$\mu = X \pm SE_{x\,(t=0.05}$$

and

$$\mu = X \pm SE_{x\,(t=0.01)}$$

respectively. The confidence limits is used in estimating un-certainity of a particular determination and is given by

$$\text{Confidence limit} = \pm \frac{ts}{\sqrt{n}}$$

Where

t = a weighting factor based on statistical analysis

s = the standard deviation

n = the number of experiments carried out

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary remarks

The range and number of discrete molecular structures produced by plants is large and so also is the accelerating rate of advancement of our knowledge of them. Hence the major problem in today phytochemical research is the collation of existing data on each particular compound. In order to solve this problem research study in ethnobotany, pharmacology and chemistry is carried out.

The studys' ultimate goal led to an ethnopharmacological direction rather than-a-pure chemistry direction. Ethnopharmacology according to (Rivier *et al*, 1979) is a multidisciplinary area of research concerned with the observation, the description and the experimental investigation of indigenous drugs and their biological activities. (Bruhn *et al*, 1981) then have modified the definition as 'the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man.

The 'observation' part of this description is most often carried out by the etthnobotanist in the field and less often based on reports in the scientific literature by medical doctors, biologists, pharmacognosists, pharmacologists, chemists and a variety of other interested scientists, or by lay people.

There is interest all over the world to set up research programs meant to lead to discovering cures for cancer and AIDS. The focus is on new natural products that would have bioactivity against causative agents of a wide variety of diseases. The strategy for the studies on these natural materials is to work on organic and aqueous extracts.

4.2 Plant Extracts

The water extracts of *Podocarpus* sp CADE-A and the mixture of water extracts of *Carissa edulis, Rhamnus prinoides, Toddalia asiatica* CADE-B is safe during the first three and eight months respectively. The extracts are bitter in nature, foam when shaken. The compounds present in the extracts were identified as polar. These tests carried out provide a basis for the parameters that can be used to develop effective test methods and set quality standards for plant extracts.

4.2.1 Physical tests

Table 4.1 and 4.2 show the results of the physical tests carried out on the water extract of *Podocarpus* sp (CADE-A) and the water extract of the stems of *Carissa edulis, Rhamnus prinoides* and *Toddalia asiatica* (CADE-B). The pH of the extracts had acidic values. Acidity of CADE-A was higher than CADE-B by approximately two units.

Table 4.1 Physical tests of *Podocarpus* sp water extract (CADE-A)

TEST	**OBSERVATION**
Description	Brownish green foamy liquid with a bitter taste
pH	4.36 at 20 ^{O}C
Non-volatile matter	2.32 at 105 ^{O}C using 10ml sample
Specific gravity	2.32 using 25ml pycnometer
Stability	Stable for a period of 8 months at room temperature

Table 4.2 Physical tests of a mixture of *Carissa edulis, Rhamnus prinoides* and *Toddalia asiatica* water extracts

TEST	**OBSERVATION**
Description	Dark green foamy liquid with a bitter taste
pH	6.21 at 20 ^{O}C
Non-volatile matter	3.61 at 105 ^{O}C using 10ml sample
Specific gravity	1.263 using 25ml pycnometer
Stability	Stable for a period of 8 months at room temperature

Table 4.3 Percentage of yields of chloroform and methanol extracts

Solvent	**Volume of water extract (Litres) / Weight of *Podocarpus* sp bark (grams)**	**Weight of extract (g)**	**Yield (%)**
Chloroform	5 Litres	8.4	0.164
Methanol	300 Grams	12	0.40

Table 4.4 Identification tests of various secondary metabolites in chloroform and methanol extracts

TEST	Positive	Negative
Triterpenoids and sterols	Yes	
Saponins and sapogenins	Yes	
Anthraquinones		Yes
Flavonoids		Yes

The stability of the extracts over a period of time is unsatisfactory. CADE-A and CADE-B started decomposing after a period of 8 months and 3 months respectively. The non-volatile matter was high in CADE-B than CADE-A.

Table 4.3 shows the yields of chloroform extract of *Podocarpus* sp (CADE-A) that was hydrolyzed with 10% HCl to obtain the aglycone and glycosides. 8.4 g of crude solid extract was obtained on evaporation of the chloroform extract, indicating a 0.168 % yield. A higher yield is obtained by increasing the number of partitions during solvent extraction.

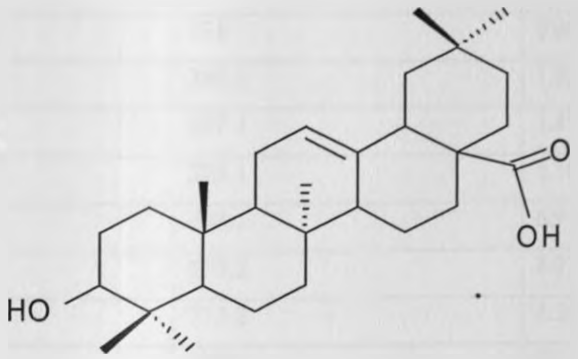
The yield for the methanol extract was 4.0 % as seen in Table 4.3. This indicates that the compounds in *Podocarpus* sp are more soluble in methanol. Saponins are polar compounds that dissolve very easily in water or in any polar solvent such as methanol. The rotor evaporation of the methanol extract was not efficient due to the foamy nature of the concentrated resinous materials.

4.2.2 Chemical tests

The chemical identification in both the chloroform and methanol extracts is positive for, triterpenoids, sterols, sapogenins and saponins. The physical description test results closely tally with the chemical test results in that when a compound has the ability to foam when shaken and during the extraction process, it indicates the presence of surface-active agents. These agents are bitter on tasting and are primarily saponins. The red blood gelatin poured on a thin layer chromatogram glass where chloroform and methanol extract samples had been spotted became transparent after one hour indicating a positive test for haemolysis.

4.3 Separation of saponins and sapogenins

Generally, sapogenins and saponins are compounds which contain a triterpenoid basic structure and, normally, there are glucose linkages. The tests for the presence of anthraquinones and flavonoids were negative. Triterpenoids are compounds with carbon skeleton based on six isoprene units and which are derived biosynthetically from the acyclic C_{30} hydrocarbon squalene. They have crystalline structure and often have high melting points and belong to triterpenoid's group of compounds having the basic parent polycyclic structure oleanolic acid as shown below. Sugar moities are attached at the ά-OH group position by esterification forming a genin oleanolic acid.



Oleanolic acid

4.3.1 Thin layer chromatography

Analytical TLC of the methanol extract of *Podocarpus* sp on a 4 x 10 cm precoated polygram 254 TLC plates and were developed with various proportions of chloroform: methanol: water (60:35:5) mixture.

Analytical TLC of the chloroform extract from *Podorcapus* sp CADE-A on a 4 x 10 cm polygram 254 plates were developed with various proportions of chloroform: methanol: water (60:25:15) mixture. They showed coloured spots under UV light at a wavelength 254nm, the predominant with hRf value 50. The Ethylacetate hexane (50:50) showed three spots with hRf values 80, 62, and 44. Detection of the spots on the plate was facilitated by a spray of antimony chloride in chloroform.

4.3.2 Column chromatography

Separation of 8.4g of chloroform extract of *Podorcapus* sp (CADE-A) in Silica gel 60 (0.04-0.06mm, 230-400) mesh packed column using increasing solvent polarities of hexane:ethylacetate (30:15:55) and chloroform: methanol 60: 30:10) led to separation of compounds **A** (40mg), **D** (120mg), **E** (22mg), **F** (16mg), and **G** (20mg). Compound **A** was recrystallized in ethanol to pure red granular crystals.

Table 4.5 LC-MS molecular ions (m/z), retention time (min) and relative intensity (%) separation profile of the compounds in chloroform extract of *Podocarpus* sp water extract (CADE-A)

Compound	Molecular ion (m/z amu)	Retention time(min)	Intensity (%)
1	171	0.80	90
2	340.1	1.55	73
3	437.1	3.81	100
4	523.1	5.16	20
4	585.1	6.91	42
6	679.2	8.01	45
7	775.2	8.56	10
8	932.3	9.01	11
9	1057.3	9.77	9
10	1118.3	-	9

Spectral acquisition of the methanol extract and the chloroform extracts of *Podocarpus* sp (CADE-A) and the bark was carried out using LC-MS spectrometer, ran in HD: Macintosh operation files. Figure 4.5 shows ten compounds separated from the methanol extract of *Podocarpus* sp bark using LC-MS detection system.

Column chromatography solvent elution with various proportions of chloroform: methanol: water (60:35:5) in increasing polarities of hexane:ethylacetate:chloroform (30:15:55) led to separation of Compound B, 50mg from 12g crude methanol extract of *Podorcapus* sp bark. Compound B was recrystallized in methanol to pure red crystals.

Table 4.6 shows LC-MS molecular ions (m/z), retention time (min) and intensity (%) separation profile of the compounds in methanol extract of *Podocarpus* sp bark

Compound	Molecularion (m/z a.m.u)	Retention time (min)	Intensity (%)
1	205.1	0.90	100
.2	287.1	1.65	85
3	381.1	2.30	57
4	452.1	3.76	50
4	579.1	4.26	100
6	699.2	5.76	15
7	784.2	6.96	20
8	851.2	8.01	35
9	962.3	8.81	26
10	1139.3	-	24

Table 4.6 shows ten compounds separated from the methanol extract of *Podocarpus* sp bark using LC-MS detection system. The last compound with molecular ion m/z 1139 was detected with the longest retention time with a relative intensity of 24.

4.4 Isolation, spectral analysis and characterization

4.4.1 Compound A

Compound **A** was recrystallized in ethanol to pure red granular crystals, melting point 201-203 ^{O}C, UV/Vis spectra recorded at λ_{max} (EtOH) 472nm, with extinction coefficient $\varepsilon = 1.31 \times 10^{4}$ mol/g in concentration of 1: 150,000 in methanol. The MS-FAB showed a molecular ion peak at $[M]^{+}$ at m/z 538 amu.

The proton ^{1}HNMR values for some types of protons in different magnetic environments present in compound **A** are shown in Table 4.7. The primary methyl group proton absorb at δ = 0.85-0.95. The carbonyl proton absorb at δ =8.5 and the vinyllic protons δ = 5.5-7.2. The C-OH of glucopyranoside absorption occurs at δ = 3.3-5.1 and deshielded protons at δ = 6.0-7.4 results from the saturated cyclic system (more than one C=C on extending along the molecule).

Table 4.7 ^{1}HNMR type of proton in the functional groups and chemical shift (δ ppm) for compound **A**

Proton No	^{1}HNMR absorption (δppm)	Proton No	^{1}HNMR absorption(δppm)
1	1.39	17	1.74
2	1.74	18	2.25
3	3.39	19	6.47
4	2.24	20	6.86
5	1.98	21	7.41
6	6.34	22	1.65
7	6.78	23	7.81
8	6.32	24	5.87
9	7.08	25	2.55
10	2.35	26	0.75
11	2.15	27	3.44
12	6.30	28	0.97
13	7.77	29	0.99
14	7.15	30	3.63
15	2.95	31	1.05
16	3.10	32	2.54

Type of proton	**Functional group**	**Chemicalshift (ppm)**
R-CH_3	Primary Alyphatic	0.9-1.2
R_2CH_2	Secondary Alyphatic	1.2-1.6
R_3CH	TertiaryAlyphatic	1.4-2
C=C-CH_3	Olefinic methylene	2.5-3.0
-CH_2-CH_2-	Aliphatic methylene	0.95-1.2
C=C-C=CH	Conjugated polyene	5.8-6.5
HC=O	Carbonyl	7.5-10.0
CH_2-OH	Hydroxyl	3.21-5.2
C-O- CH_3	Ether	3.5-4.7

Table 4.8 MS-FAB molecular ion (m/z amu), formula $[M]^+$, relative abundance (%), and index of hydrogen deficiency (IHD) of fragments of Compound A

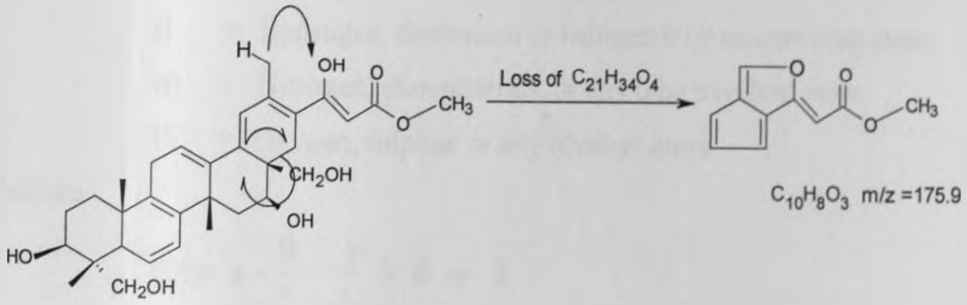
Peak	Molecular ion (m/z a.m.u)	Formula $[M]^+$	Relative abundance (%)	IHD
1	175.9	$C_{10}H_8O_3$	15	3
2	218.8	$C_{14}H_{18}O_2$	23	6
3	240.2	$C_{16}H_{16}O_2$	30	9
4	259.0	$C_{17}H_{23}O_2$	20	6
5	281.7	$C_{19}H_{21}O_2$	45	9
6	282.7	$C_{19}H_{22}O_2$	71	8
7	291.8	$C_{16}H_{19}O_2$	65	7
8	311.7	$C_{20}H_{23}O_3$	100	9
9	337.0	$C_{19}H_{29}O_5$	10	5
10	349.8	$C_{20}H_{30}O_5$	12	6
11	385.1	$C_{25}H_{38}O_5$	30	8
12	425	$C_{24}H_{36}O_4$	15	7
13	434.1	$C_{28}H_{35}O_4$	15	11
14	462.2	$C_{29}H_{35}O_5$	13	13
15	493.2	$C_{31}H_{38}O_5$	10	13
16	504.9	$C_{31}H_{35}O_6$	12	14
17	538.0	$C_{32}H_{42}O_7$	10	12

The MS-FAB analysis shows weak molecular ion peak $[M]^+$ at m/z= 504.9 corresponding to the molecular weight $C_{31}H_{35}O_6$. The less intense peak $[M]^+$ at m/z= 493.2 corresponding to molecular weight $C_{31}H_{38}O_5$ indicates retroloss of carboxylate ion $[M]^+$ at m/z = 45 (CH_1O_2) therefore confirms that the compound has a carboxylic acid moiety.

This is also explained by the ^{1}HNMR signal at δ = 7.5-8.2 and is most likely a carbonyl group. The most significant peak showed $[M]^+$ at m/z = 425 corresponding to the molecular weight $C_{24}H_{36}O_4$. The MS-FAB shows $[M]^+$ at m/z = 434.1 corresponding to loss of a cyclic system with IHD of 11. The difference between the $[M]^+$ at m/z 538 and

434.1 is 103.9 a fragment which later breaks down to m/z=62 and m/z=41 via Mc Lafferty rearrangement.

The peak at m/z=175.9, represents the loss of $C_{22}H_{34}O_4$ leading to formation of $C_{10}H_8O_3$ through cleavage of C_{18} with C_{21} carbon.



The base peak $[M]^+$ at m/z 311.7 has 100% relative abundance. The following molecular formulas fit the data for molecular ion peak and the M+1 peak.

Formula	M+1	M+2
$C_{20}H_{23}O_3$	22.0	3.02
$C_{19}H_{35}O_3$	20.9	2.78
$C_{18}H_{26}O_4$	19.8	2.58

The M+2 is 13.72 % of the parent peak and this best fits $C_{20}H_{23}O_3$, which designate the molecular formula. The relative intensity of the molecular ion increases with increase in unsaturation (IDE of 9) and number of rings. Therefore the higher the IDE and the lower the CHR for $[M]^+$ 311.7, the more intense is the molecular peak .

As seen from Table 4.8, the weak intensity of the molecular ion peaks indicates a highly branched and hyperconjugated compound. The molecular ion peak $[M]^+$ at m/z=538 is the molecular weight of the compound. The compound has a molecular formula $C_{32}H_{42}O_7$ on the basis of elemental analysis. The index of hydrogen deficiency (IDE) indicates the number of sites (or degrees) of unsaturation, multiple and cyclic in nature can be

estimated using the molecular formula. IDE is then the sum of the number of rings, the number of double bonds and twice the number of triple bonds. IDE is calculated for compounds containing carbon, hydrogen, nitrogen, halogen, oxygen and sulfur from the generalized formula $I_\alpha\ II_\beta\ III_\gamma\ IV_\delta$

$$IDE = Carbons - \frac{hydrogens}{2} - \frac{halogens}{2} + \frac{nitrogens}{2} + 1$$

For a compound with the formula $I_\alpha\ II_\beta\ III_\gamma\ IV_\delta$,

Where,

I = Carbon, Si or any other trivalent atom

II = Hydrogen, deuterium or halogen 9 i.e monovalent atom

III = Nitrogen, phosphorous, or any othe trivalent atom

IV = Oxygen, sulphur or any bivalent atom

Therefore

$$IDE = \alpha - \frac{\beta}{2} - \frac{\gamma}{2} + \delta + 1$$

Alternatively the index of hydrogen deficiency (the sum of multiple bonds and ring systems) for the formula, $C_{32}H_{42}O_7$ is calculated by substituting —CH_2-, for each oxygen in the structure. Thus, $C_{32}H_{42}O_7$ gives $C_{39}H_{54}$, an alkane with 39 carbon atoms and which would have the formula $C_{39}H_{80}$. The difference between the number of hydrogen atoms in $C_{39}H_{54}$ and $C_{39}H_{80}$ is 24 hydrogen atoms; therefore the index of hydrogen deficiency is 12. The number indicates the sum of double bonds, ring residues and ring systems forming part of the structure of compound **A.** This cyclic system contains 5 ring systems and 7 double bonds which partly accounts for the index of hydrogen deficiency of 12.

Compound A has UV/Vis absorption at the longest wavelength of visible light (λmax 472 nm, $\varepsilon=1.31\times10^4$, using ethanol as a solvent) as shown in Figure 4.1. The Woodward-Feiser and Woodward-Feiser-Khun rules for calculation on the UV/Vis absorption wavelength in conjugated dienes and trienes states that; diene absorption is in fact influenced by the molecular structure (Sharma *et al*, 1988). The parent structure is α,β-unsaturated carbonyl in conjugation with cyclic enone groups and the absorptions of endocyclic/exocyclic rings, ring residues, exocylic bonds, polar groupings and double

bonds extending conjugation are substractive or additive to afford the calculated UV/Vis of absorption (Woodward, 1974, Feiser, 1959).

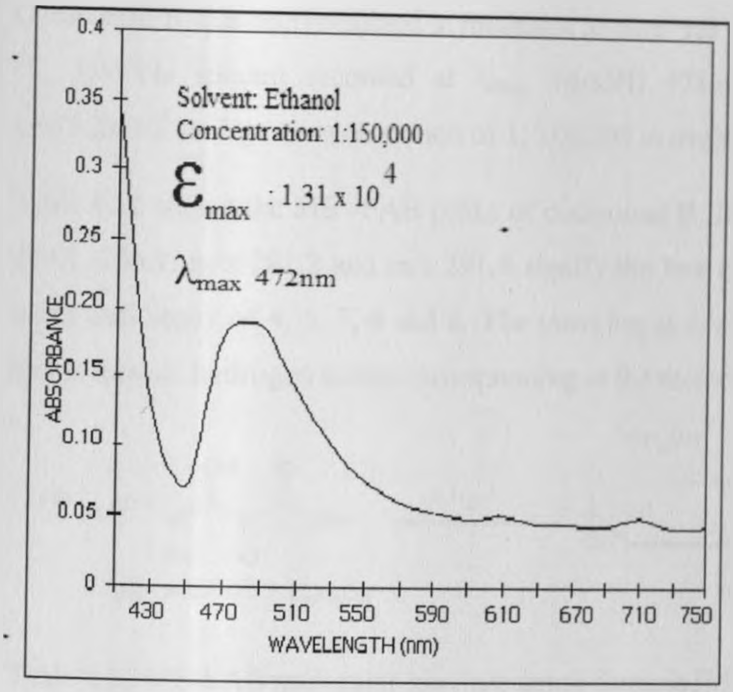


Figure 4.1 UV/Vis absorption spectrum of Compound A

Table 4.9 Calculated λ_{max} of absorption of compound A

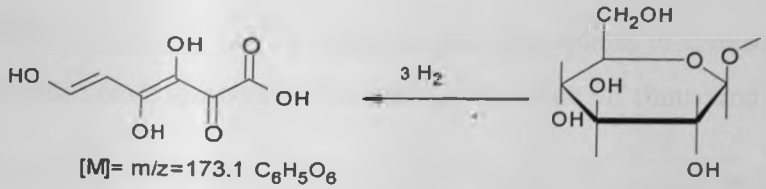
Group	Additions for substituents	Assignments (nm)
Parent chromophore	α,β-unsaturated carbonyl	+195
Ring residue	5 x 18	+90
Extended conjugation	4 x 30	+120
Polar grouping	1x30	+30
Homodiene component	39	39
No rings with endocyclic double bonds	0	0
No rings with exocyclic double bonds	-2x10	-20
Total calculated	-	464

The calculated value absorption of Compound A is λ_{max} 464nm

4.4.2 Compound B

Compound **B**, 50mg was isolated from 12g of methanol extract of *Podorcapus* sp bark. Compound **B** was recrystallized in methanol to pure red crystals, melting point 184-187 ^{o}C, UV/Vis spectra recorded at λ_{max} (MeOH) 478nm, with extinction coefficient $\varepsilon = 17.2x\ 10^{4}$ mol/g in concentration of 1: 100,000 in methanol.

Table 4.10 shows the MS –FAB peaks of compound **B**. The molecular ions at m/z 173.1, 214.1, 256.1, m/z 281.2 and m/z 291.8 signify the loss of molecular ion with hydrogen index deficiency of 4, 5, 7, 6 and 8. The mass ion at m/z 173.1 shows a loss of D-sugar that is less six hydrogen atoms corresponding to the molecular formula $C_6H_5O_6$



[M]= m/z=173.1 $C_6H_5O_6$

Table 4.10 MS-FAB molecular ion (m/z amu), formula [M^+], relative abundance (%), and index of hydrogen deficiency (IHD) of fragments of Compound B

Peak	Molecular ion (m/z a.m.u)	Formula $[M]^+$	Relative abundance (%)	IHD
1	173.1	$C_6H_5O_6$	45	4
2	214.1	$C_{13}H_{18}NO$	28	5
3	256.1	$C_{17}H_{22}NO$	53	7
4	281.2	$C_{14}H_{17}O_6$	15	6
5	291.8	$C_{15}H_{16}O_6$	75	8
6	311.7	$C_{22}H_{17}NO$	100	13
7	390.2	$C_{22}H_{28}NO$	40	9
8	477.0	$C_{29}H_{33}O_6$	62	13
9	501.2	$C_{31}H_{33}O_6$	33	15
10	537.3	$C_{31}H_{39}O_7N$	80	11

The less intense peak shows $[M]^+$ at m/z = 390.2 corresponding to molecular formula ($C_{22}H_{28}NO$).

The base peak $[M]^+$ at m/z 311.8 has 100% relative abundance. The following molecular formulas fit the data for molecular ion peak and the M+1 peak.

Formula	M+1	M+2
$C_{22}H_{17}NO$	24.2	3.48
$C_{16}H_{24}O_6$	17.6	2.74
$C_{22}H_{15}O_2$	24.2	1.94
$C_{21}H_{30}NO$	23.1	3.22

The M+2 is 14.38% of the parent peak and this best fits $C_{22}H_{17}NO$, which designate the molecular formula of the abundant peak..

Tables 4.11 shows the ^{13}C chemical shift absorptions of diverse carbon atoms in different magnetic environments in the chemical structure for compound B.

Table 4.11 ^{13}CNMR chemical shift (δ ppm) for compound **B**

Proton No	^{13}CNMR absorption (δppm)	Proton No	^{13}CNMR absorption (δppm)
1	26.81	17	26.42
2	32.13	18	44.60
3	68.60	19	135.53
4	137.75	20	130.62
5	144.51	21	158.48
6	127.58	22	19.80
7	136.21	23	19.10
8	148.12	24	21.32
9	147.88	25	46.30
10	46.17		
11	135.61	1	101.81
12	144.73	2	71.21
13	143.70	3	73.22
14	144.85	4	67.90
15	50.57	5	75.80
16	27.11	6	60.11

Compound B has 25 carbons in the aglycone(a cyclic triterpenoid structure) and 6 carbons forming a glucopyranoside as seen in Table 4.11.

Compound **B** has UV/Vis absorption at the longest wavelength of visible light (λ_{max} 478 nm, $\varepsilon=17.2x10^4$, using methanol as a solvent) as shown in Figure 4.2.

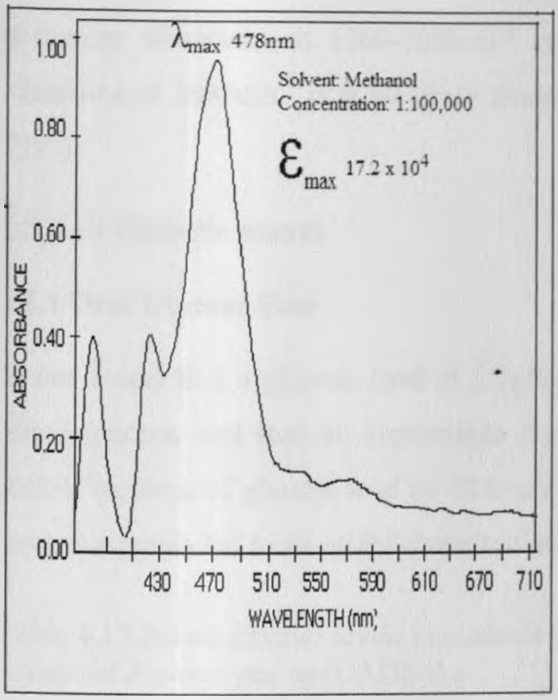


Figure 4.2 UV/Vis absorption spectrum of Compound **B**

Table 4.12 Infra red characteristic group absorption frequencies for compound B

Group	υ stretching (cm^{-1})
CH_3-C-	2832-2815
CH_3-N-	2820-2780
C=CH	3085, 3060, 3030
C-C	1600, 1495, 1455
C-N	1600-1430
C-O	1260-1000
OH	3300-2500
C=O	1730
-CH	3080-3100

The general appearance of the infra-red spectrum as seen in Table 4.12 concurs with UV/Vis absorption spectrum λ_{max}. This suggest degree of unsaturation as seen from C=C stretch at 1645cm^{-1}. The C=O absorption at 1640cm^{-1} and therefore at longer wavelength is due to resonance effect. The position of absorption depends upon the same structural environmental factors as the carbonyl absorption of other compounds. The strong C-O stretching vibrations at 1260-1050cm^{-1} are most likely vibrations for alcohols. The vibrations at 3085cm^{-1} is most likely from cyclic olefinic CH stretching (Morrill *et al*, 1981).

4.5 Anti-Diabetic assays

4.5.1 Oral Glucose Test

It was found that a glucose load of 2.5g/kg body weight produced the highest level of blood glucose and that no appreciable rise in these levels could be achieved by any further increase of glucose load on 48 hours fasted rabbits. Glucose Tolerance Test in the control animals led to an establishment of standard curve as shown in Figure 4.3.

Table 4.13 Blood glucose levels in controls (GTT), methanol, chloroform, and water extract of *Podocarpus* sp (CADE-A).

Time (minutes)	0	30	60	90	120	150	180
Methanol extract (15) Mean SD	51.27 ± 2.08	80.78 ± 1.76	91.61 ± 1.80	117.24 ± 2.14	113.61 ± 2.06	102.10 ± 2.08	
Chloroform (15) Mean SD	50.91 ± 1.13	119.51 ± 2.17	140.70 ± 1.69	110.36 ± 1.82	102.19 ± 1.88	98.64 ± 1.73	
Water (15) Mean SD	50.82 ± 1.01	100.13 ± 1.52	114.37 ± 2.09	165.32 ± 4.18	92.49 ± 1.56	86.90 ± 1.29	
Glucose control (15) Mean SD	49.42 ± 3.60	53.29 ± 2.33	60.63 ± 3.27	71.20 ± 3.05	129.59 ± 2.05	124.98 ± 4.06	122.42 ± 3.28

* The Figure in parenthesis denotes number of subjects

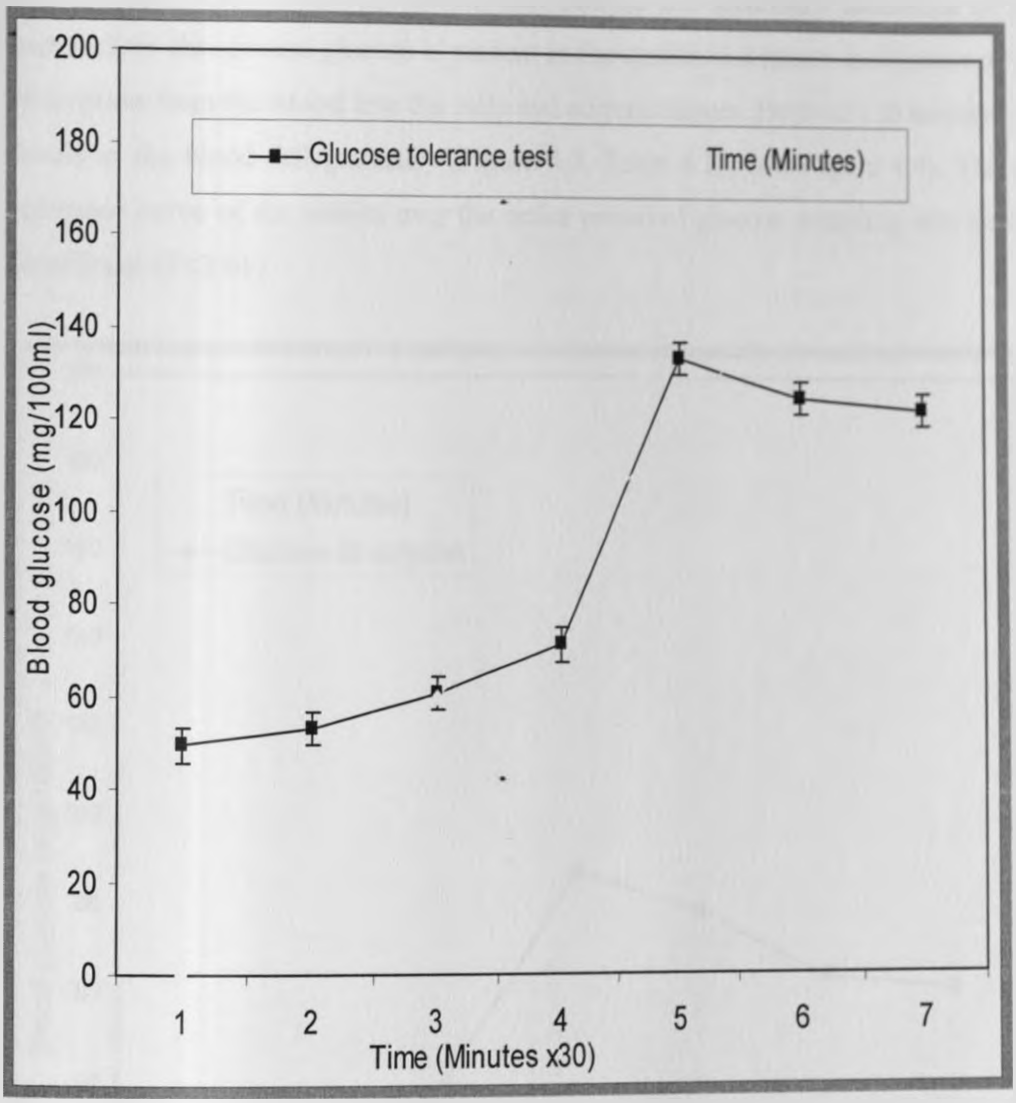


Figure 4.3 Glucose Tolerance Curve

Hypoactivity accompanied by lack of alertness within an hour after administration of the drugs was regarded as the initial signs of incipient distress in the rabbit. The absorption of glucose from the gut into the blood increased experimentally giving rise to an exponential glucose tolerance curve with the maximum absorption of study after two hours. The rate of absorption of glucose into tissues from the blood started to exceed the rate of absorption of glucose from the gut into the blood after 120 minutes. This was level of maximum absorption where it occurred at 129.59± 2.05mg/100ml.

The process is facilitated by insulin that informs the secondary mediators of glucose metabolism that excess glucose is present in the system and hence facilitation of glucose absorption from the blood into the cells and adipose tissues. Beyond 120 minutes glucose levels in the blood fell gradually (Figure 4.3, Table 4.12, and Figure 4.4). The glucose tolerance curve of the rabbits over the entire period of glucose sampling was statistically significant ($P<0.01$).

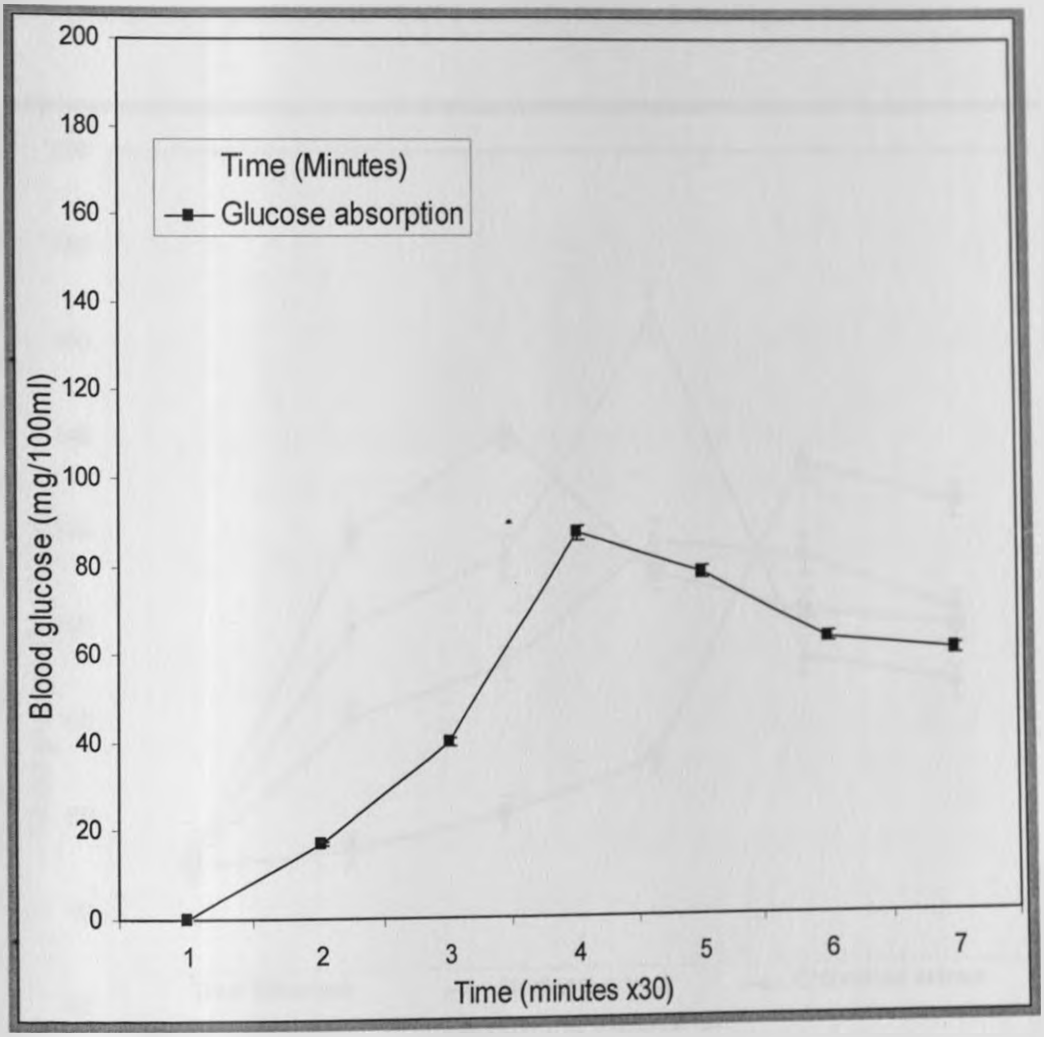


Figure 4.4 Absorption of glucose from the gut and blood

The fasting blood sugar was reduced to 98.64 ± 1.73mg/100ml by the water extract while it was reduced to 86.90 ± 1.29mg/100ml by the chloroform extract and to 102.10 ± 2.08mg/100ml by the methanol extract after 150 minutes. The assessment of the

influence of *Podocarpus* sp chemical compounds on blood glucose levels in fifteen rabbits show that these compounds lower blood glucose. There was a 33% improvement in the lowering of blood glucose as one compares the end time level of blood glucose in the animals dosed with the crude water extract with that in the controls.

Chloroform extract causes a 13% improvement while the methanol extract caused a 6% improvement of the same. All these improvements in end time blood glucose lowering are statistically significant ($P<0.05$.) as it can be seen from Figure 4.5.

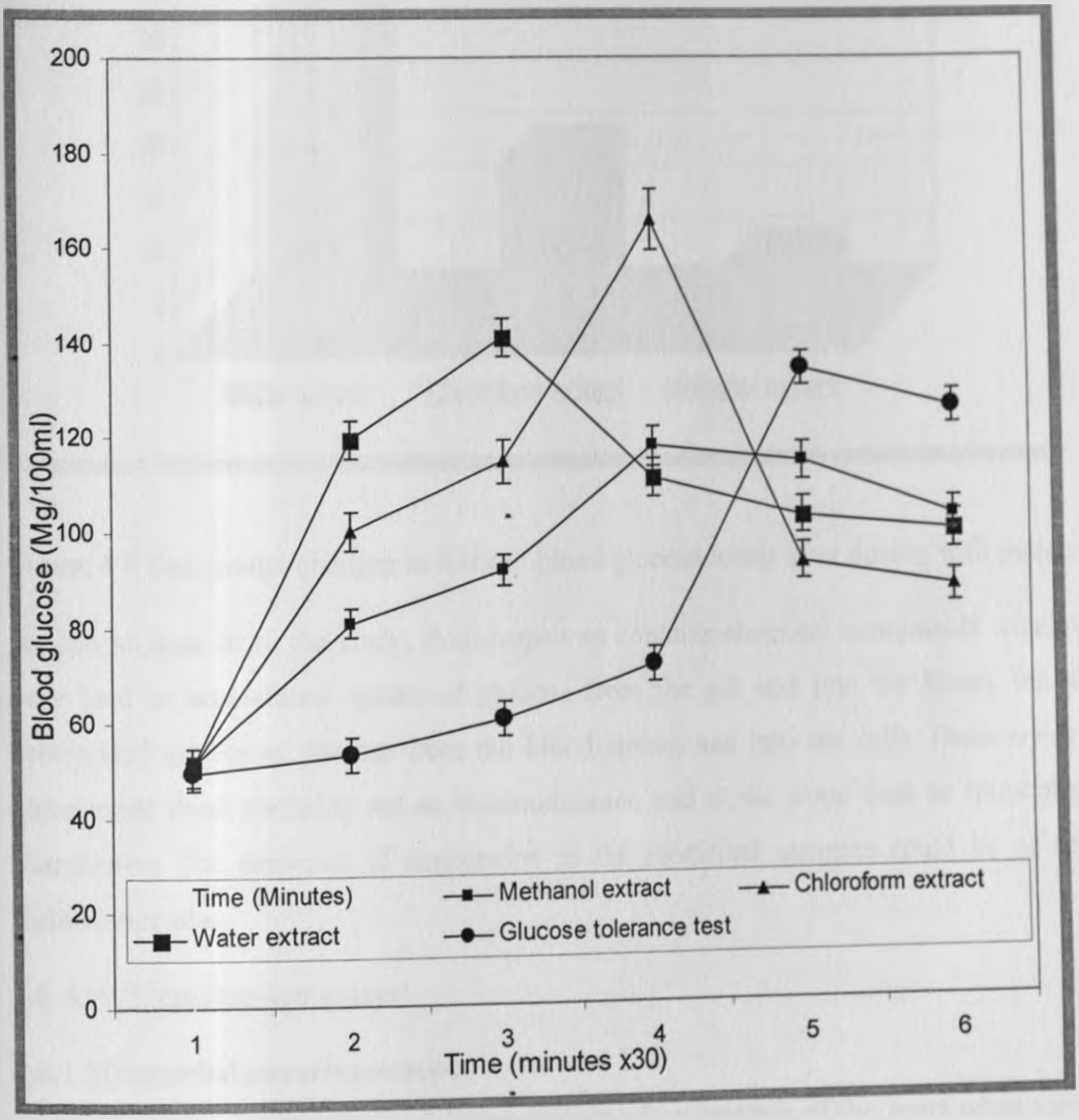


Figure 4.5 Effect on absorption of glucose by the water, methanol, and chloroform extracts of *Podocarpus* sp

The fact that blood sugar levels start to fall at 60 minutes from the baseline in animals dosed with both the crude water extract and the chloroform extracts and at 90 minutes in rabbits dosed with methanol extract, while it is not observed to happen in the control animals until the 120th minute is also very significant ($P< 0.01$) as observed in Figure 4.5.

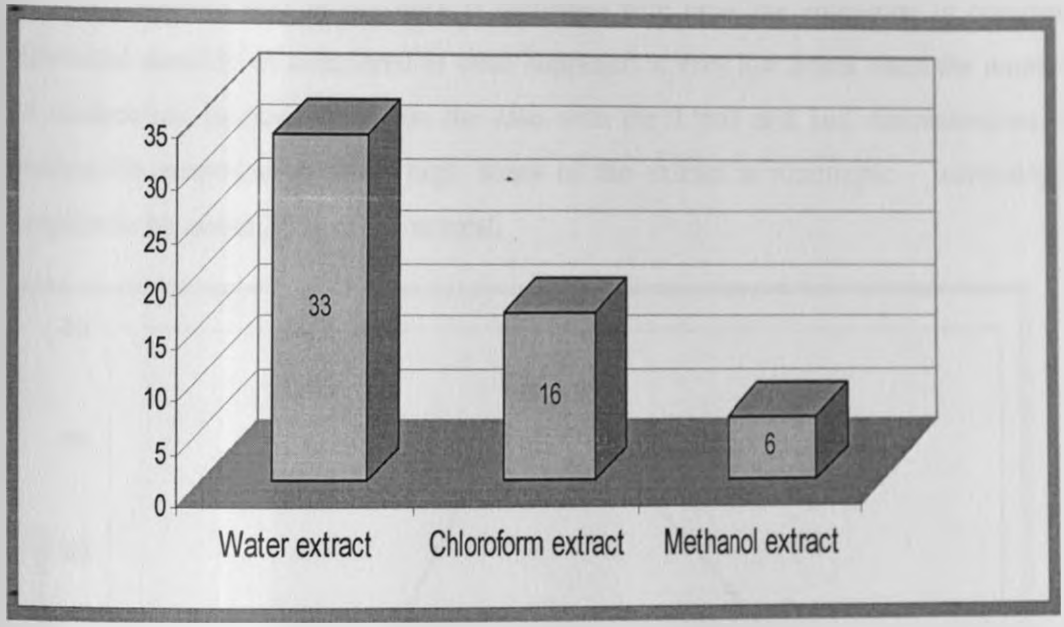


Figure 4.6 Percentage changes in rabbits' blood glucose levels after dosing with extracts

As can be seen from the study, *Podocarpus* sp contains chemical compounds which not only lead to accelerated uptake of glucose from the gut and into the blood, but also accelerated uptake of glucose from the blood stream and into the cells. *Podocarpus* sp compounds most probably act as transmembrane and at the same time as transcellular ionophores. The presence of sapogenins in the medicinal samples could be of some significance also.

4.6 Anti-Hypertensive assays

4.6.1 Myocardial experimentation

Figure 4.7 shows comparison of the magnitude of contraction of the heart when various quantities of water extract of *Podocarpus* sp were injected into the heart via the rubber cup of the Lagendorf apparatus. As it can be seen from the graphs, CADE-A of

Podocarpus sp causes a reduction in amplitude of contraction. At physiological levels the CADE-A causes concentration-dependent decrease in tension and the contractile response to these doses of the extract are ionotropic, reducing the amplitude by about 20% of the normal. The dose that causes the highest reduction of contraction of the heart is 0.2 ml.

It was observed that as the dose is increased with time, the amplitude of contraction increased steadily as compared to what happened at very low doses when the amplitude of contraction decreased as was the case with the 0.5ml and 1ml concentrations. The contractile response to these high doses of the extract is ionotropic - increasing the amplitude by about 30% of the normal.

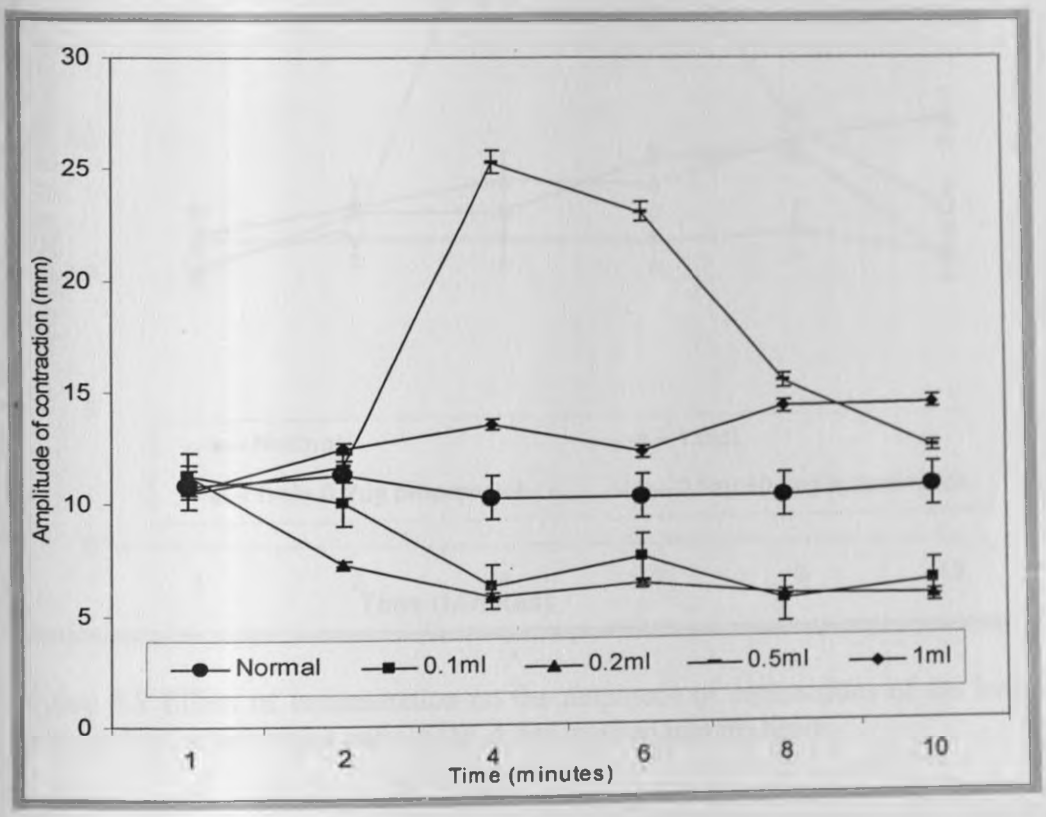


Figure 4.7 Effect of concentration on the amplitude of contractions when the isolated heart is dosed with various volumes of CADE-A

Figure 4.8 shows the magnitudes of contraction of the heart when isoprenaline, propranolol and CADE-A were injected into the heart via the rubber cup. Before injecting the drugs, the heart was washed with the physiological solution and made sure that the

heart contraction had normalized. 1 ml of the extract when injected caused a reduction in the heart's contraction. 0.1mg of isoprenaline caused an increase in contraction by more than 50%. CADE-A and propranolol combination augmented amplitude of contraction whereas CADE-A alone reduced the amplitude of contraction with time.

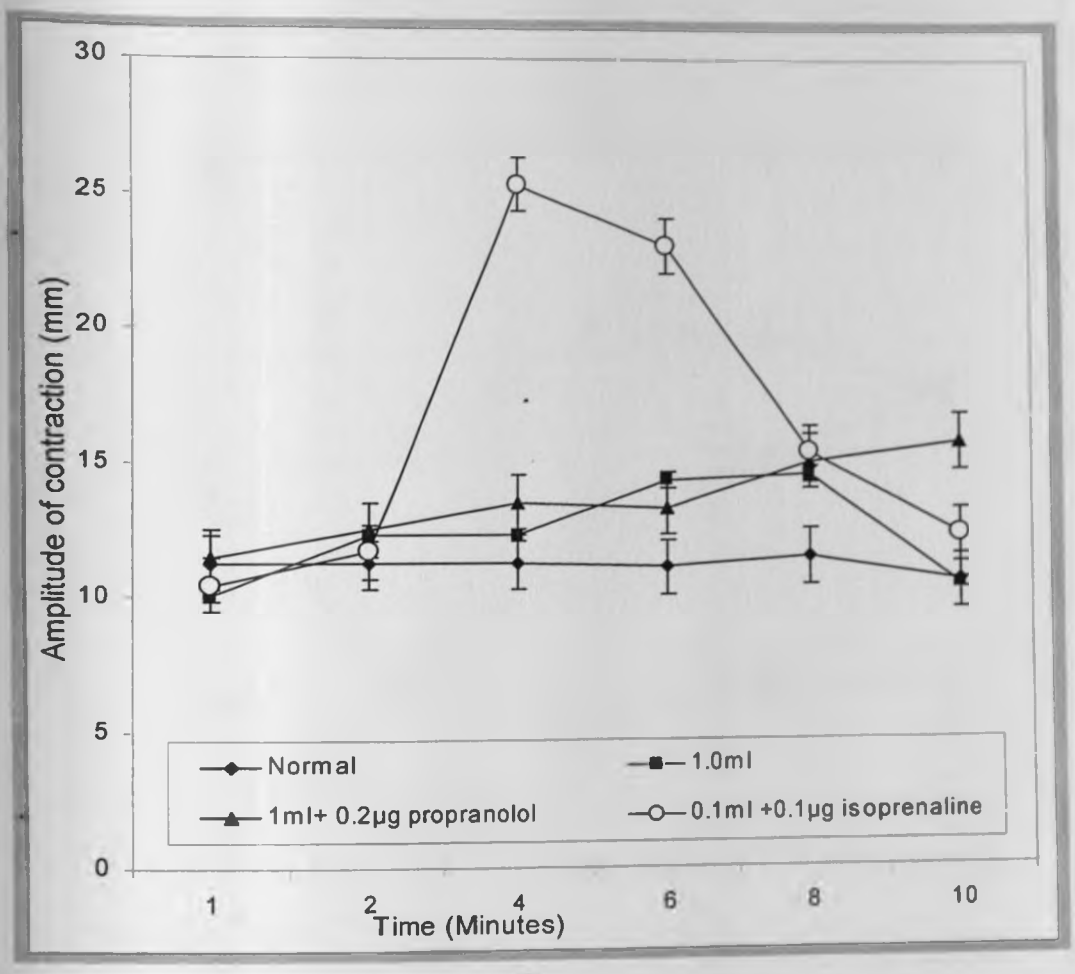


Figure 4.8 Effect of concentration on the amplitude of contractions of the heart, when isoprenaline, propranolol and CADE-A are injected into the heart

CADE-B causes a reduced contraction of the heart. The reduction in the contraction is directly proportional to the volume of the extract added. When 1 ml of the extract was added to the organ bath reduction of about 33% of the normal was observed as in Figure 4.9. A 2ml volume of the extract caused about 66% reduction of the normal contraction while 3ml caused 75% reduction of the normal. The organ was washed with the physiological solution but no stabilization of the muscle contraction to normal amplitude

was observed. Stabilization to normal was achieved by injection of adrenaline, which increased the contraction to about 80% as seen in Figure 4.9. The CADE-B causes a concentration-dependent reduction in the contraction of the heart.

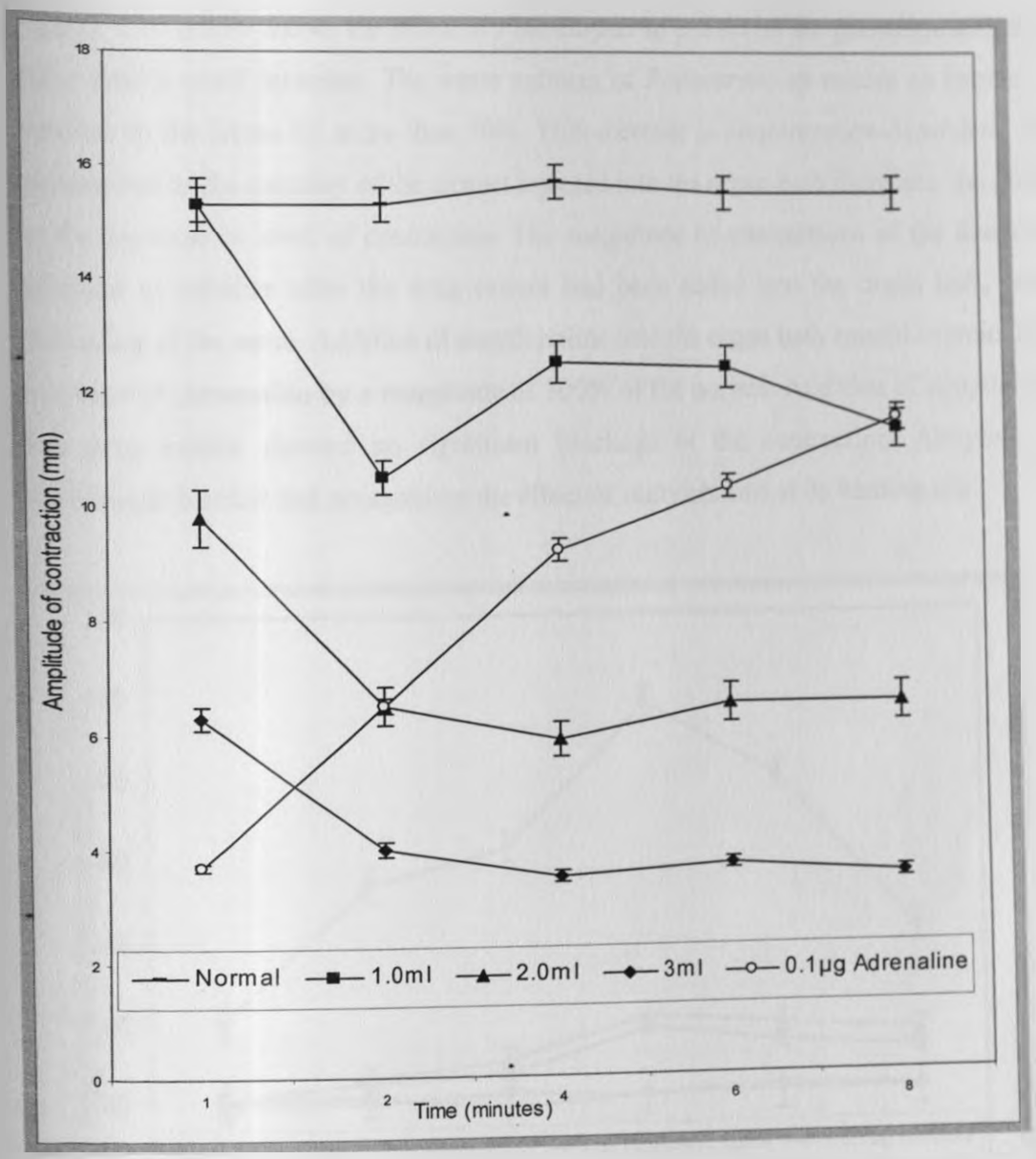


Figure 4.9 Effect of concentration on contraction of the heart when treated with various concentrations of CADE-B

From the experimentation, it was observed that increase of the quantity of CADE-B led to correspondingly increased reduction of both the rate and amplitude of the heart's contractile activity and eventually led to complete elimination of all contractile activity of

the organ. It is only after addition of 0.1mg of adrenaline that contraction of the muscle resumed as seen in Figure 4.9.

4.6.2 Small intestine experimentation

Figure 4.10 below shows the effect of *Podocarpus* sp extract on the peristaltic activity of the rabbit's small intestine. The water extracts of *Podocarpus* sp causes an increase in tension in the ileum by more than 70%. This increase is concentration-dependent. This means that as the quantity of the extract injected into the organ bath increases, the greater is the increase in level of contraction. The magnitude of contractions of the ileum was allowed to stabilize after the drug extract had been added into the organ bath, before recording of the same. Addition of aceytlcholine into the organ bath caused a tremendous increase of contraction by a magnitude of 300% of the normal. Addition of atropine with the drug extract showed no significant blockage of the contraction. Atropine is a cholinergic blocker and antagonizes the effect of acetylcholine at its binding site.

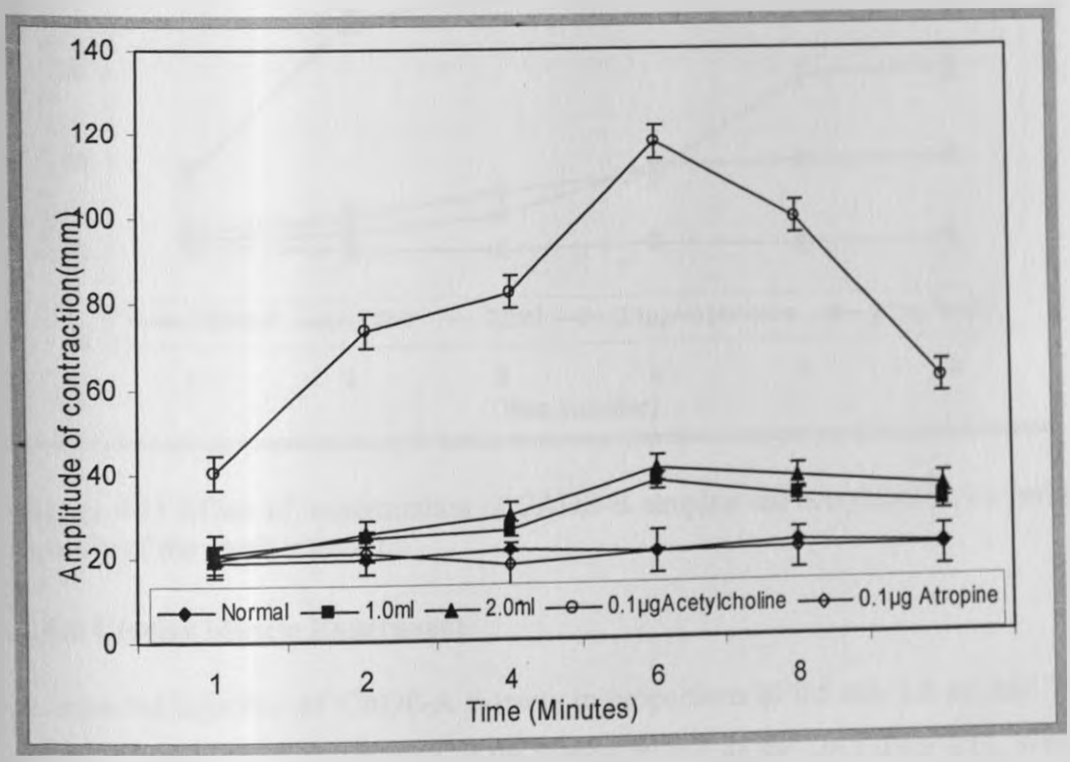


Figure 4.10 Effect of concentration of CADE-A, atropine and acetylcholine on the contraction of the small intestinal musculature

Figure 4.11 shows the effect of the CADE-B on the contraction of the ileum. CADE-B acts in the same way as the extract of *Podocarpus* sp. It causes concentration-dependent increased contraction of the ileum. A contraction of about 100% of the normal was observed after 10 minutes of injection of the 2ml of CADE-B.

CADE-B, however, is less effective in causing increased contractions of the ileum as compared to acetylcholine. The drugs are not antagonized by atropine, when both the extracts and the blocker are injected simultaneously. Acetylcholine increases the tone and the rhythmic activity of the smooth muscle of the gastrointestinal tract and enhances peristalsis.

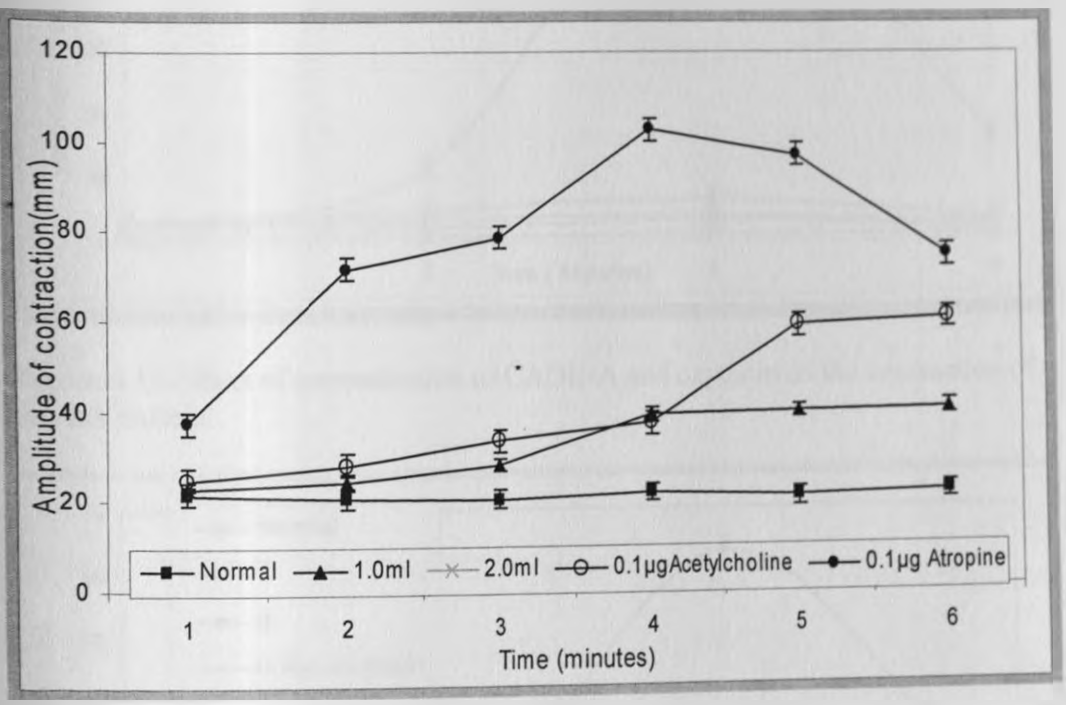


Figure 4.11 Effect of concentration of CADE-B atropine and acetylcholine on peristaltic activity of the small intestines

4.6.3 Uterine Muscle Experiment

A repeated injection of CADE-A extracts in proportions of 0.5 ml, 1.0 ml, and 2.0 ml caused no significant contraction of the uterine muscle as seen in Figure 4.12. When the organ bath was injected with 0.5 mg/ml of oxytocin there was a marked increase in contraction of the gravid myometrium. CADE-B in volumes of 1 ml on repeated injection

causes no significant contraction of the uterine musculature. 2ml of CADE-B causes slight increase at unphysiological doses as seen in Figure 4.13.

Muscle contraction of CADE-A and CADE-B on isolated rats' non-gravid myometrial strips show dose dependent inhibition of muscle contraction at physiological doses.

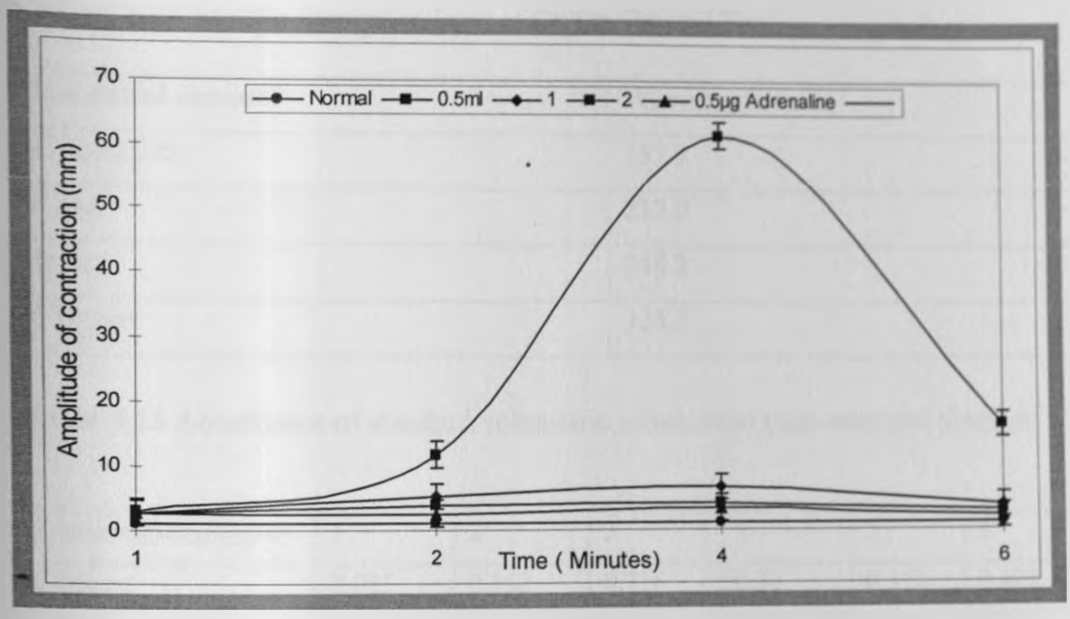


Figure 4.12 Effect of concentration of CADE-A and oxytocin on the contraction of the uterine muscle

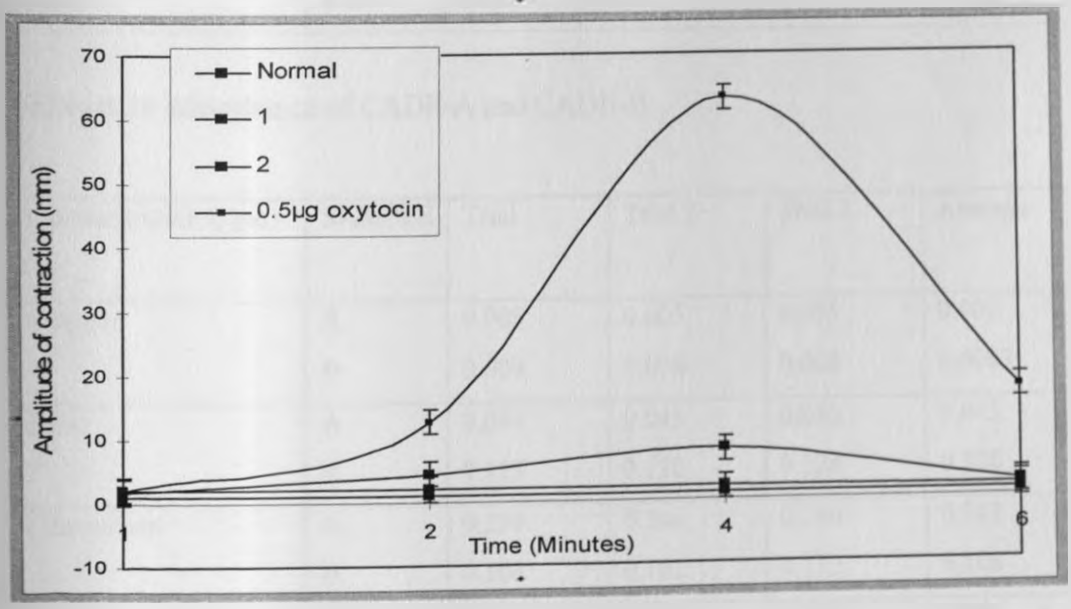


Figure 4.13 Effect of concentration of CADE-B and oxytocin on the contraction of the uterine muscle

4.7 Trace essential elements Cr, Fe, Cu, Zn

The standard absorbance readings of chromium, copper, zinc and iron, are shown in Table 4.15. The absorbance of the essential elements (chromium, copper, zinc and iron) in CADE-A and CADE-B are shown in Table 4.16.

Table 4.14 Atomic absorption lines of Cr, Cu, Zn, and Fe

Essential element	Wavelength (nm)
Chromium	357.9
Zinc	213.9
Iron	248.3
Copper	324.3

Table 4.15 Absorbance of standard volumetric solutions of trace essential element

Concentration (ppm)	1	2	3	4	5	6
Copper	0.085	0.152	0.216	0.32	0.372	0.408
Zinc	0.176	0.295	0.406	0.509	0.591	0.661
Chromium	0.01	0.02	0.035	0.041	0.059	0.066
Iron	0.034	0.064	0.093	0.124	0.151	0.181

Table 4.16 Absorbance of CADE-A and CADE-B

Concentration (ppm)	SAMPLE	Trial	Trial 2	Trial 3	Average
Copper	A	0.005	0.005	0.005	0.005
	B	0.009	0.008	0.008	0.0083
Zinc	A	0.044	0.045	0.046	0.045
	B	0.128	0.130	0.128	0.128
Chromium	A	0.230	0.246	0.246	0.242
	B	0.106	0.102	0.112	0.106
Iron	A	0.000	0.000	0.000	0.000
	B	0.070	0.014	0.035	0.003

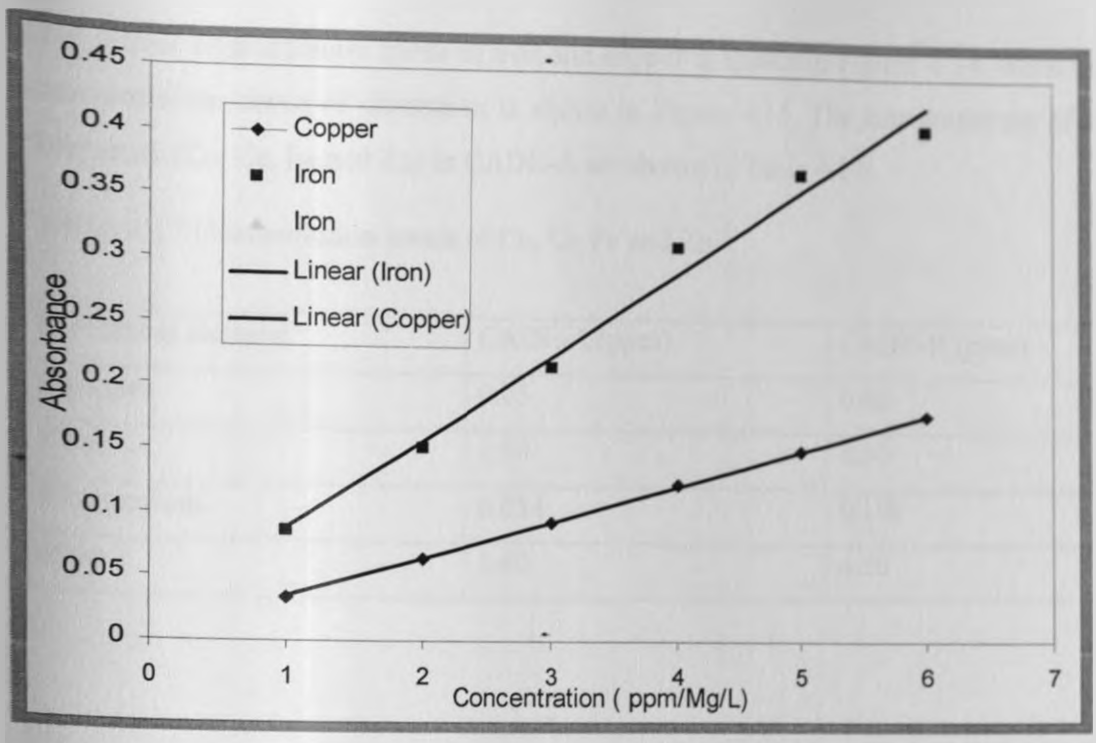


Figure 4.14 Standard concentration curve of Iron and Copper

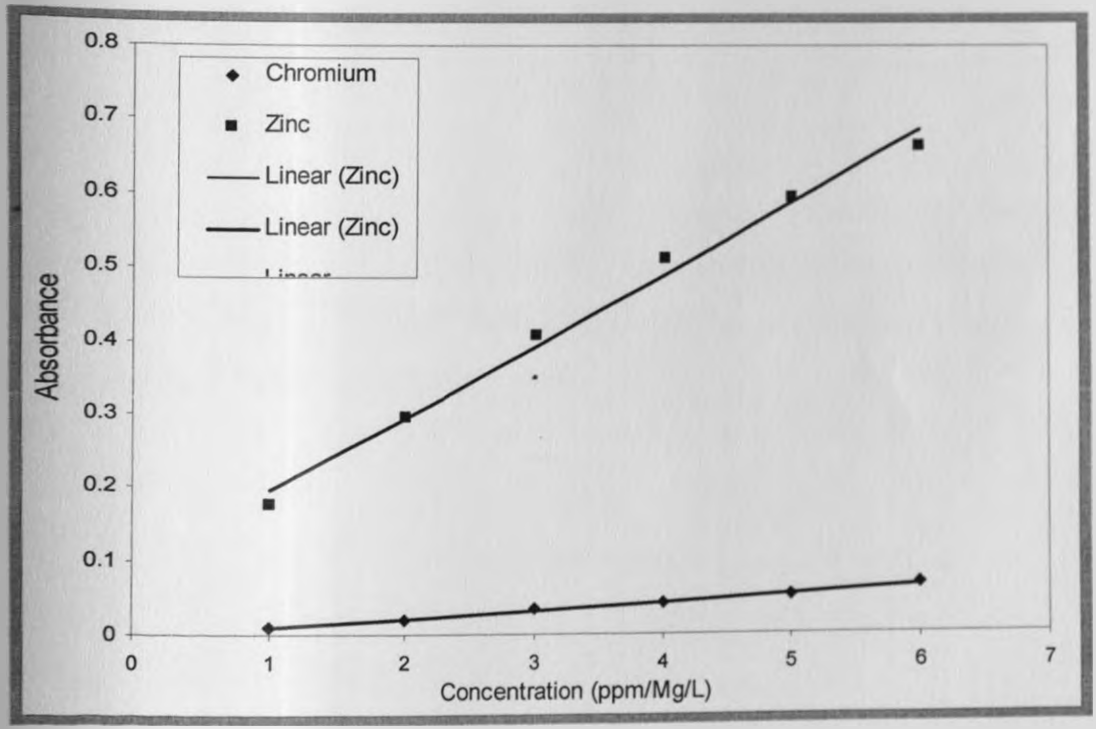


Figure 4.15 Standard concentration curve of Chromium and Zinc

The linear concentration curve of iron and copper is shown in Figure. 4.14. while linear concentration curve of chromium is shown in Figure 4.15. The concentrations of trace elements (Cu, Cr, Fe and Zn) in CADE-A are shown in Table 4.17.

Table 4.17 Concentration levels of Cu, Cr, Fe and Zn

Essential element	CADE-A (ppm)	CADE-B (ppm)
Copper	0.05	0.60
Zinc	1.50	0.30
Chromium	0.034	0.100
Iron	1.40	4.20

CHAPTER FIVE

5.0 DISCUSSION

The main objective of the study was to carry out physical, chemical and bioactivity investigations of antidiabetic traditional drugs prepared from the barks of Kenyan medicinal plants.

However, the overall objective was broken down to:

a) i) Isolating water fractions and pure compounds from *Podocarpus* sp and testing their bioactivities towards lowering of blood glucose, muscle contractions of isolated rabbits' heart, ileum and rat uterus.

ii) Isolating water fractions from *Rhamnus prinoides, Carrisa edulis Toddalia asiatica* and testing biological activities towards muscle contraction of isolated rabbits, heart and rat uterus.

b) Identifying/ characterizing compounds in the fractions responsible for biological activity.

c) Determining the levels of essential elements (copper, zinc, chromium and iron) in the isolated water fractions of *Podocarpus* sp, *Rhamnus prinoides, Carissa edulis,* and *Toddalia asiatica.*

The identification tests carried out confirmed the presence of saponins and sapogenins. The foamy nature on shaking of the solution, the haemolytic effects on defibrinated blood and the presence of the bitter taste suggest positive identification of saponins and sapogenins. Sapogenins containing heterocyclic nitrogen group have been known to be bitter because they also belong to the class of alkaloids as confirmed by the structure of compound B.

CADE-A was better in terms of stability than CADE-B. This also indicates that the compounds in CADE-B are labile in aqueous solution and may disintegrate faster with time than those in CADE-A.

The concentration of non-volatile matter was higher in extract CADE-B than CADE-A. The non-volatile matter indicates the total chemical mass, is polar nature and dissolves in water during extraction. The total chemical mass indicates the content of the chemical

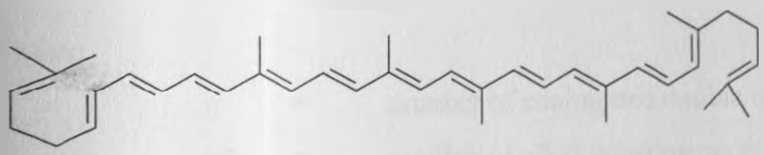
compounds that make up the solid material in the solution and which could be estimated in milligrams per ml.

The physical tests especially foamy nature, pH, bitter taste, stability and non-volatile matter are important in the assessment of quality of the plant extracts. This informs on a possible basis for assurance and verification of safety, efficacy and potency/dose of active ingredients. In this way regulation of national quality standards could probably be established in traditional medicine.

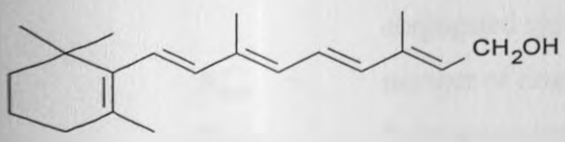
5.1 Structural elucidation of compound A and B

UV/Vis absorption of compound A is at the longest wavelength of visible light (λ_{max}472 nm, ε=1.31x10^4) as shown in Figure 4.1. This means that the energy required for transition of π to π^* electrons is less than that required by simple conjugated systems like ethene or 1, 3-butadiene which absorbs at max 171 nm and 277 nm respectively. The more the number of conjugated double bonds in a compound the longer the wavelength of absorption of light. This is seen when one compares the UV/Vis absorption data for Vitamin A_1 and A_2. Since the later has additional double bonds in conjugation, the position of absorption is at a longer wavelength.

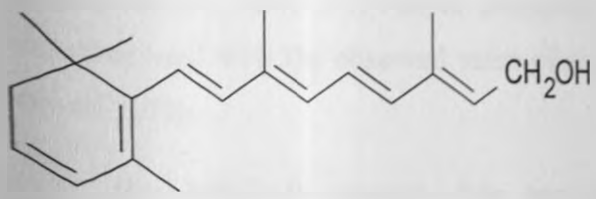
Sufficient conjugation shifts absorption to the visible region of the spectrum. Compound A has such conjugation and it is red in color. Lycophene, the compound that is responsible for red color in tomatoes, has thirteen extended conjugated bonds (Sharma *et al*, 1988).



Lycophene λ_{max} 504nm



Vit A_1 (λ_{max} 325nm, ε =51,000)



Vit A_2 (λ_{max} 351nm, ε=41,000)

The author has modified Woodward-Feiser-Khun rules to include a factor of 30nm addition resulting from an increment of polar hydroxyl group, an increment of 39nm as a result of presence of homodiene. These rules are used to calculate and confirm the wavelength of absorption for Compound **A**. The parent structure is α,β-unsaturated carbonyl in conjugation with cyclic enone groups and the absorptions of endocyclic double bonds (-16.5nm), exocyclic double bonds (-10nm), ring residues (18nm), and double bonds extending conjugation are additive to afford the calculated UV/Vis of λ_{max} absorption (Woodward, 1974, Feiser, 1959).

The calculated wavelength of absorption using the modified Woodward-Feiser-Khun estimation rules is given by;

$$\lambda_{max\ (EtOH)} = 195 + P_{group} + H_{md} + 18M_{enone} + n\ (48\text{-}1.7n) - 16.5R_{endo} - 10R_{exo}$$

Therefore calculated,

$$\lambda_{max\ (Ethanol)} = 195 + 30+39+10+ 6x18 + 4\ (48\text{-}1.7x4) - 16.5R_{endo} - 10R_{exo}$$

$$= 195+ 30+39 + 90+ 164\text{-}20\text{-}33$$

$$= 465nm$$

Where

n	=	number of conjugated double bonds
M	=	number of alkyl substituents on the conjugated system
R_{endo}	=	number of rings in the endocyclic double bonds in conjugated system
R_{exo}	=	number of rings with exocyclic double bonds
P_{group}	=	Polar group increment
H_{md}	=	Homodiene component

The calculated value UV/Visible absorption of Compound A is λ_{max} 465nm which compares well with the observed value of absorption λ_{max} 472nm with an of estimation error of 1.7%.

From the MS-FAB spectral data especially the mass and elemental analysis, characterization and elucidation were done. The first step in translating the MS-FAB spectra into molecular structure is to establish the correct molecular formula.

The molecular ion peak at $[M]^+$ is at m/z =538 amu. This is an even number molecular weight and therefore it is permitted that either there is no nitrogen atoms or even number of them. If C, H, O and N are present, the approximate expected % (M+1) and % (M+2) data can be calculated by use of the following formula;

$$\% (M+1) = 100 \left\{ \frac{[M+1]}{[M]} \right\}$$

$$= 1.1 \text{ x number of carbon atoms} + 0.36 \text{ x number of N atoms}$$

Likewise,

$$\% (M+2) = 100 \left\{ \frac{[M+2]}{[M]} \right\}$$

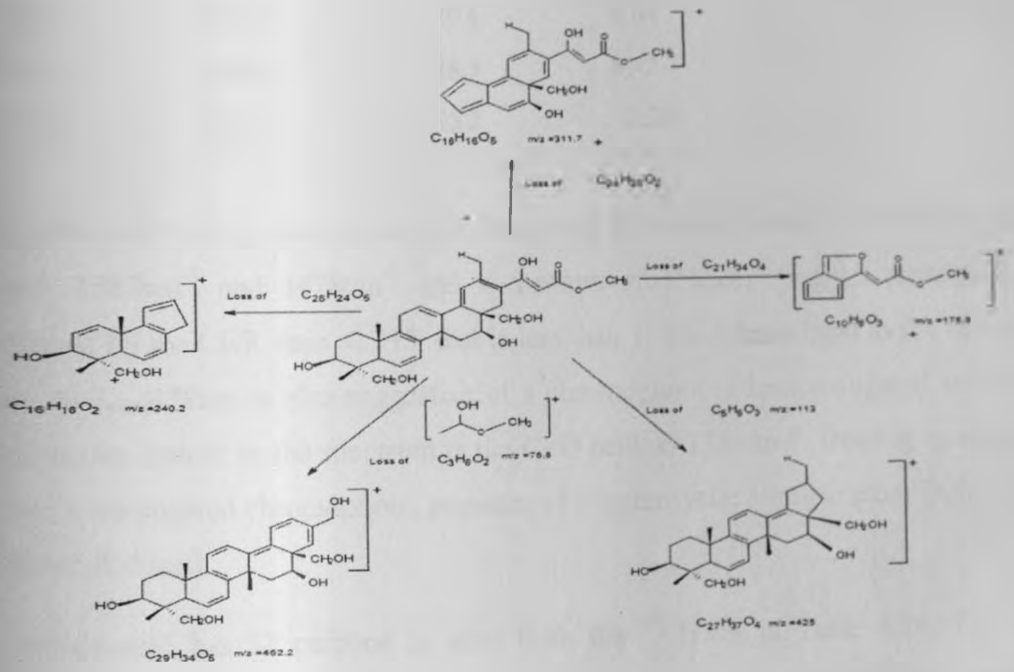
$$= 1.1 \text{ x } \frac{(\text{number of carbon atoms})2}{200} + 0.20 \text{ x number of O atoms}$$

The above equations are useful for cases in which one has preconceived notion about the molecular formula of the compound of interest. There are six formulas of molecular weight 538 amu containing only C, H, N and O (Morrill *et al*, 1981).

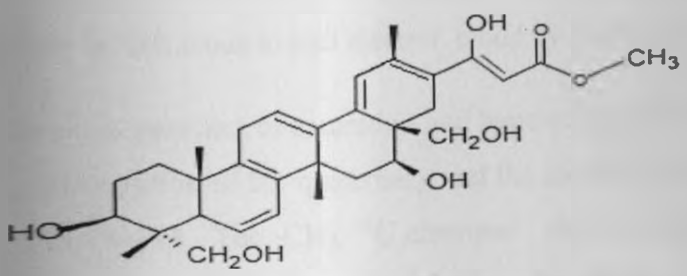
Formula	CHR	M+1	M+2
$C_{31}H_{38}O_8$	0.816	34.1	7.41
$C_{32}H_{42}O_7$	0.762	35.2	7.56
$C_{31}H_{40}O_7N$	0.775	34.1	6.21
$C_{35}H_{38}O_5$	0.921	38.5	8.41
$C_{36}H_{42}O_4$	0.857	39.6	8.60
$C_{34}H_{34}O_6$	1.00	37.4	8.68

One formula is eliminated because it contains an odd number of nitrogen atom. The (M+1) is 23.75% of the parent peak. This best fits $C_{32}H_{42}O_7$ as the deduced molecular formula with carbon hydrogen ratio (CHR) of 0.762 implicating unsaturation in the chemical structure of the compound.

The carbonyl carbon chemical shift is at upfield position at δ = 170.47 therefore the carbonyl carbon could be bonded to $-O-CH_3$ group. ^1H chemical shifts at δ =2.55 supports dishielding of methyl protons by oxygen in the methoxy group. Therefore the most likely moiety is an ester (acetyl group).



Based on the MS-FAB, UV/Vis, ^{1}HNMR, and ^{13}CNMR spectral analysis the structure of compound A has been deduced.



Compound A $[M]^+$ m/z =538 amu

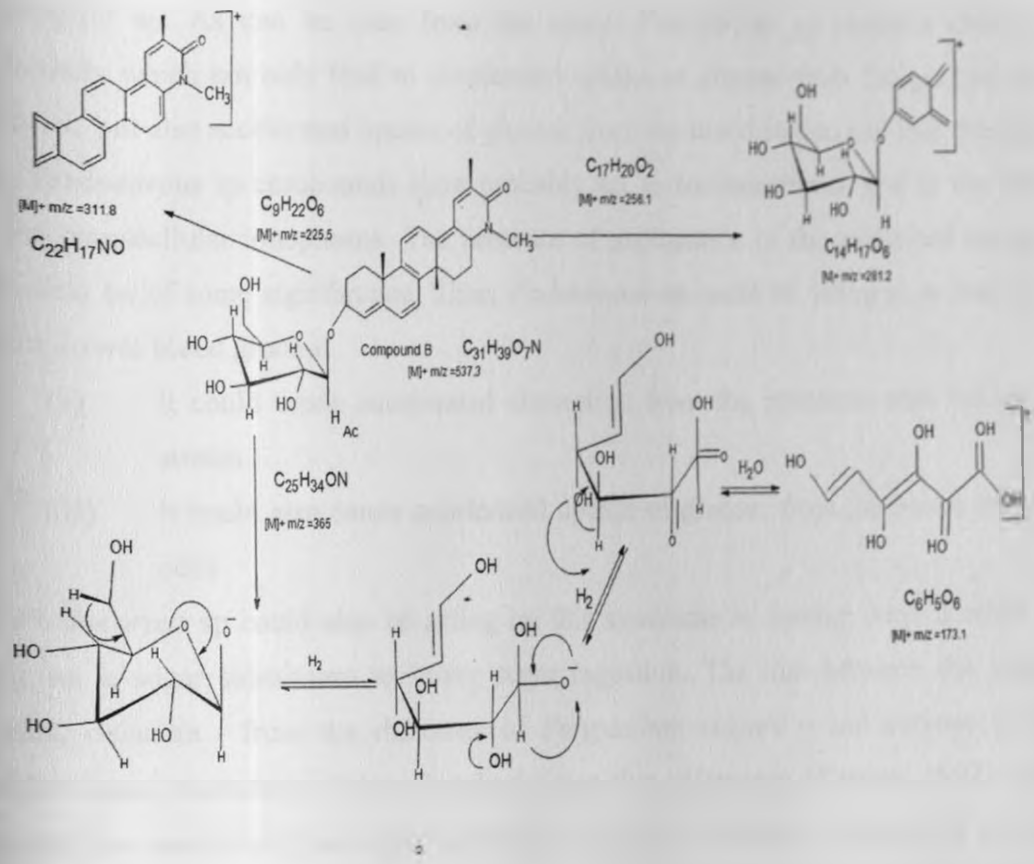
The following six formulas fit the data for molecular ion peak and M+1 peak for compound **B.** The high resolution molecular weight was found to be 537.3 amu, and pinpoints $C_{31}H_{39}O_7N$ as the molecular formula of choice. Likewise the (M+1) is 22.25% of the parent peak.

Formula	CHR	M+1	M+2
$C_{31}H_{39}O_7N$	0.795	34.1	7.57
$C_{32}H_{41}O_7$	0.780	35.2	7.59
$C_{36}H_{57}O_3$	0.632	39.6	8.44
$C_{36}H_{41}O_4$	0.878	39.6	8.64
$C_{35}H_{37}O_5$	0.946	38.5	8.42
$C_{32}H_{57}O_6$	0.561	35.2	7.20

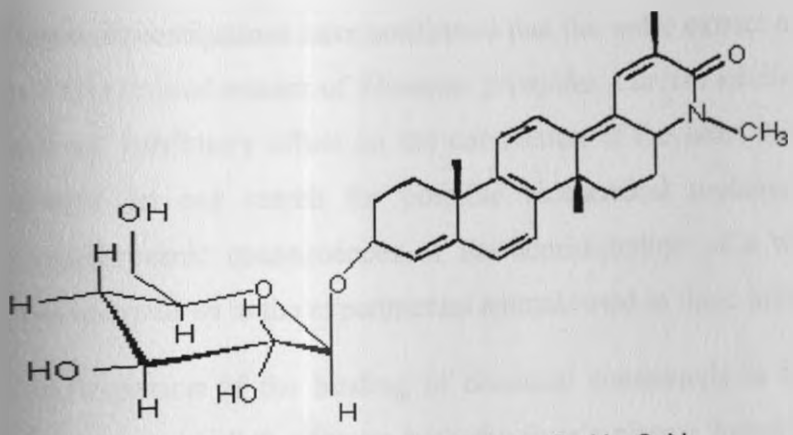
It is noted that strong absorptions for compound **B** beyond 800cm^{-1}, two strong peaks beyond 1587cm^{-1} and 1478cm^{-1} and a medium at 3106cm^{-1} suggest aromaticity as explicated by the CHR ratio 0.795 that is less than 1. The intense band in the ultraviolet region at λ_{max}478nm is also suggestive of a chromophore in hperconjugated scheme. A conspicuous feature in the spectrum is the C=O peak at 1730cm-1. Bearing in mind this may be a conjugated chromophore, presence of a heterocyclic nitrogen atom in the ring is therefore deduced.

The compound has 31 carbons as seen from the ^{13}CNMR in Table 4.11. The -CH_3 ^{13}CNMR chemical shift absorption at δ = 46.30 indicates that the methyl carbon is bonded to nitrogen atom. The carbonyl carbon chemical shift is at upfield position at δ = 156.48 which suggest, therefore, the carbonyl carbon could be bonded to a nitrogen atom which tends to pull electron cloud by resonance.

There is presence of saturation and hyperconjugation within the cyclic rings as evidenced by absorption of the quaternary and the methyne carbon of the olefinic chromophores at δ = 114-144. The -CH_2. ^{13}C chemical shifts are upfield at δ 21-29 and is distinctively upfied from the position of aliphatic unsaturated and hence indicates that the compound has six membered rings and possibly in C_{30} triterpenoid structure.



Supported by the MS-FAB, UV/Vis, ^{1}HNMR, and ^{13}CNMR spectral analysis the structure of compound **B** has been deduced.



Compound B [M]+ m/z =537.3 $C_{31}H_{39}O_7N$

5.2 Biochemical basis of *Diabetes mellitus* and hypertension

The plant whose water extract was examined for its perceived antidiabetic activities was *Podocarpus* sp. As can be seen from the study, *Podocarpus* sp contains chemical compounds which not only lead to accelerated uptake of glucose from the gut and into the blood, but also accelerated uptake of glucose from the blood stream and into the cells. Some *Podocarpus* sp compounds most probably act as transmembrane and at the same time as transcellular ionophores. The presence of sapogenins in the medicinal samples could also be of some significance. Thus, *Podocarpus* sp could be acting in at least two ways to lower blood glucose.

(i) It could cause accelerated absorption from the intestines into the blood stream

(ii) It could also cause accelerated uptake of glucose from the blood into the cells

Yet, *Podocarpus* sp could also be acting on this syndrome in another way. It could be acting as a sugar substituent to lower sugar ingestion. The link between the steroid saponin, oslandin - from the rhizomes of *Polypodium vulgare* - and sucrose is that oslandin has a sweetness of three hundred times that of sucrose (Harries, 1992). Such materials are used widely as sugar substitutes in diet to reduce the amount of glucose ingestion. That way they act as hypoglycaemic agents and it is of interest that the study of the general pharmacology of these triterpene saponins has shown that their level of toxicity is relatively low.

These investigations have confirmed that the water extract of the bark of *Podocarpus* sp and the mixed extract of *Rhamnus prinoides, Carissa edulis* and *Toddalia asiatica*, each had an inhibitory effect on the contraction of the heart muscle. This fact was an eye-opener in our search for possible biochemical processes underlying the observed hypoglycemic consequences of the administration of a water extract of the bark of *Podocarpus* sp to the experimental animals used in these investigations.

Confirmation of the binding of chemical compounds in the extract onto the α or β-adrenergic binding sites on both the liver's plasma membrane and on the muscle cell plasma membrane could explain the biochemical basis of the inhibition of the muscle

contraction leading to lowering of blood pressure at the same time suggesting a possible explanation for the lowering of blood glucose levels through inhibition of release of glucose from the liver. This is so because we could then invoke the inhibition of glycogenolysis in the liver of these experimental animals as a possible explanation of the observed hypoglycemic consequences of the administration of this extract. Glycogenolysis is the main in vivo mechanism for increasing blood glucose other than ingestion of the same. This process involves catecholamines, whether these are bound onto α or β-adrenergic receptocytes on the liver plasma membrane. The consequences of the binding of the catecholamine at the α-receptor binding site is the release of Ca^{++} which then activates phosphorylase kinase, which in turn activates glycogen phosphorylase, which in turn, then, catalyses the release of Glucose-1- phosphate from glycogen. This then is dephosphorylated through the process of hydrolysis to release glucose, which enters into the blood. If, by the same token, it could be shown that compounds from extract of *Podocarpus* sp bind onto the α or β-adrenergic binding site on the muscle fibre's plasma membrane and if it could also be shown that such binding was competing against the binding of the catecholamines, this observation would confirm the eventual release of Ca^{++} following such binding. Such Ca^{++} would activate the muscle protein actin leading eventually to muscle contraction.

The traditional healer had informed us that the extract is not only used for treating *Diabetes mellitus*, but also for the treatment of high blood pressure. This means that we had an extract with chemical principles that not only inhibited glycogenolysis, but also had both inhibitory and excitatory effects on the contractility of the smooth musculature. Therefore it also meant that it was most probable that these chemical principles of the *Podocarpus* sp extract were bound onto the catecholamine binding sites on the plasma membrane of both the liver and the muscle fibres. To test these hypotheses experiments were carried out to establish the contractile effects of the combination of the water extract and propranolol on the heart muscle. Propranolol is a non-selective α-adrenergic receptor blocker that causes marked inhibitory effects on myocardial contractions. Figures 4.7, 4.8 and 4.9 show the difference on the inhibition of contraction caused by the combination of the drugs as compared to the inhibition caused by the extracts alone. This suggests that the *Podocarpus* sp inhibitory principles and propranolol compete for the same binding

sites. This then means that *Podocarpus* sp contains chemical compounds that bind onto the α and β-adrenergic receptocytes in the body.

The physiological effects of epinephrine include stimulation of the heart so as to increase the heart rate and also the blood pressure, relax the musculature of the bronchi, the intestine and the uterus. At physiological concentrations, it relaxes the musculature of the arterioles of the splanchnic and skeletal muscles (Ahlquist, 1948). The extract relaxes the myocardium, relaxes the myometrium and causes increased contraction of the intestinal muscles. This means that as far as the myacordium is concerned, the extract chemicals act as antagonists much in the same way as they antagonize the activities of the catecholamines in the intestine. These chemical compounds only act as catecholamine agonists in the uterine muscle. This makes these compounds β-blockers with minimal intrinsic sympathomimetic activity.

Blockade of beta-adrenergic receptors has been associated with hypoglycemia in both diabetics and non-diabetics probably due to reduction of the beta-2-receptor mediated glycogenolysis. This activity of the extract could then possibly account, to some extent, for the hypoglycemic effects observed experimentally.

When the properties of the first beta-adrenergic blocker, pronethalol and subsequent congener for the group propranolol were carefully evaluated, the available evidence led to the conclusion that the beta-adrenergic receptor blocking drugs might be of value in the treatment of cardiac ailments. Besides other activities, these compounds decreased blood pressure even in resting subjects (Black *et al*, 1965). The α-adrenergic receptor blockers are also known to be vasodilators. The antihypertensive activity of the extract of *Podocarpus* sp and *Toddalia asiatica, Carissa edulis* and *Rhamnus prinoides* is associated with the presence of α, β-adrenergic receptor blocker kind of compounds.

Besides causation of accelerated absorption of glucose from the intestines and into the blood stream, in addition to causing accelerated uptake of glucose from the blood and into the cells of the body, we have now seen that the other mechanisms through which compounds of *Podocarpus* sp could be involved in lowering blood glucose are:

iii) Lowering ingestion; and

iv) Inhibition of glycogenolysis which means the breaking down of glycogen into constituent molecules, all of which are glucose molecules.

Assuming, then, that the four biochemical processes leading to the observed hypoglycemia involve different compounds of the *Podocarpus* sp, the desired hypoglycemic effect could be brought about through the efficacy of a consortium of these compounds at relatively low concentrations of each of them. The cumulative effect of the contribution of the individual compounds towards the lowering of blood glucose would be such that to achieve a desired dimunition of blood glucose concentration in individual who is producing inadequate amounts of insulin in his body, the concentration of the individual chemical compounds in the extract need not be very high. This, then, would inform on the safety of this extract even when prepared and administered in unknown and unregulated concentrations by the traditional healers.

The concentrations of copper, zinc, iron and chromium ions in CADE-A and CADE-B were found to be in between 0.034 -4.2 ppm. It can be seen that the levels of these essential elements in the extracts are significantly lower than daily reccomended allowance. The level of these elements accounts for the non-toxicity of the extracts when administered to patients. The safety and efficacy of the extracts is then assured even though the extracts are always administered in unknown and unregulated concentrations. These concentrations of trace essential elements in the extracts give mineral supplementation to the patients. The most essential are iron, zinc and chromium.

5.3 Limitations of the study

The following are constraints encountered during the study;

1. Financial constraints limited the study to only screening the extracts for hypoglycemic activity on rabbits as well as the isolated organs (ileum, heart and uterus). This is because the finances allocated to carry the study were less than comprehensive research work required to complete the data collection and analysis of the same in addition to the time required for compilation of the final report. Pure compounds were obtained in small quantities owing to a very low yield of the plant extracts and limited the study to only bioactivity screening of

the extracts on isolated rabbit and rat organs, characterization and structural elucidation of the compounds.

2. Owing to obsolete and non availability of modern analytical equipment in the Department of Chemistry of the University of Nairobi, samples had to be sent all the way to U.S.A for analysis and acquisition of spectra. The spectra obtained from the ^{13}C and $^{1}HNMR$, LC-MS, MS-FAB and IR analysis of the isolated samples of the compounds were satisfactory. Most were useful in the elucidation of the structure of the chemical compounds in the bark of *Podocarpus* sp.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The link between hypertension and diabetes has been known for decades and many hypertensives are known to become diabetic and vice versa. The results discussed in the proceeding section show that, *Podocarpus* sp contains chemical compounds which not only lead to accelerated uptake of glucose by the cells from the blood but also accelerated uptake of glucose from the gut and into the bloodstream. In this second activity, *Podocarpus* sp compounds most probably act as transmembrane at the same time as transcellular ionophores. *Podocarpus* sp extract could therefore be acting in three ways to lower blood glucose. It could be causing lowered ingestion, accelerated absorption from the intestines into the bloodstream and in addition causes/accelerates uptake of glucose from the blood into the cells. Yet *Podocarpus* sp could also be acting on this syndrome through inhibiting glycogenolysis.

The information provided by the traditional healers that both CADE-A and CADE-B are used for the treatment of high blood pressure was proved accurate by physiological experimentation. The extracts have chemical principles that not only inhibited glycogenolysis but also had both inhibitory and excitatory effect on the contractility of the smooth masculature. Therefore it also means that it was probable that these chemical principles of *Podocarpus* sp extract and CADE-B were bound onto the catecholamine binding sites on the plasma membrane of both the liver and the muscle fibre. To test this hypothesis, experiments whose aim was to establish the contractile effects of the combination of the water extract and propranolol on the heart muscle were carried out. Generally, propranolol is a non-selective-adrenergic receptor blocker, which causes marked inhibitory effects on the myocardial contractions. Propranolol augments the inhibitory activities of the extract on myocardial contractions. The difference in inhibition of contraction is distinct when the extract is used in combination with the drug as compared to the extract alone. This suggests that the *Podocarpus* sp and CADE-B inhibitory principles and propranolol compete for the same binding sites.

This also means that *Podocarpus* sp and CADE-B contains chemical compounds that bind onto the α and β-adrenergic receptocytes in the body (Mugo *et al.* 2005). The identification tests especially haemolytic effects and the bitter taste suggest the presence of saponins and sapogenins. This is confirmed by the structures of compound **A** and **B**. The presence of triterpene glycoside compounds in the extract could explain the antihypertensive activity of the extract as suggested by the activity of patrinoside. This triterpene saponin decreases the arterial pressure by decreasing the stimulating capacity of mid-brain reticular formation thereby producing a selective effect on the tension of the peripheral vessels (Swineyard, 1966).

The spectral absorption maxima at λ_{max} 472nm and λ_{max} 478nm and confirms the presence of multiple double bond in both compound **B** and compound **A** respectively, which are extended by conjugation. This was confirmed by the modified Woodward-Feiser-Khun estimation method. This is consistent with polyene cyclic ring system containing more than one conjugated double bond extending on the entire ring. β-Carotene which has 9 extended conjugated double bonds absorbs at λ_{max} 453 nm on the visible region with a ε_{cal} 19.1 $x10^4$.

The levels of trace essential elements in both CADE-A and CADE-B are low. These levels are lower than the levels required for daily intake as supplements for nutritional replenishment. Hence the trace essential element may provide mild essential mineral supplementation.

These levels agree very well with those obtained by Kinyua (1995) on the traditional drug extracts used in the treatment of epilepsy and anti-convulsion. It has already been indicated that some of these essential elements, for instance zinc are required for the healing of wounds and for proper functioning of the immune system. Iron helps in the prevention of diseases like anaemia and chromium is important in glucose metabolism in the body, just to mention but a few of the known uses of these elements.

6.2 Recommendations

Medicinal plants, as sources of biologically and especially pharmacologically active substances have yet to be utilized fully. What is needed is to further develop a scientific basis for the understanding of the uses of our floral resources. We further need to analyze these sources for pharmacologically active ingredients and improve the potency of these medicines through biotechnology. In many countries there is a shortage of modern drugs for primary healthcare. This situation has prompted people to turn to the use of traditional medicine whether or not this is government policy. In dire situations, this has meant an increase in quackery and loss in terms of human health and well being. In other countries, authorities have now recognized the importance of making room for use of herbs and other plant materials in the management of diseases.

The traditional herbalists who provided the traditional herbs used for the treatment of diabetes and hypertension seem to have had a wide knowledge of other plants used to treat many other diseases. In fact one of the traditional healers claimed to know herbal plants that can cure the dreaded AIDS. I therefore propose that an exhaustive enquiry should be carried into the knowledge held by the traditional healers in order to provide the ethnobotanical information that can be used as a basis for carrying out further biological assays and screens. By befriending these healers and tapping their knowledge- which can then be entered into a database system, a reference centre could then be established so as to facilitate further scientific investigations on a plant. whose scientific details are already on the database and which happens to be of some scientific curiosity to researchers at some future date.

Among natives of various countries, knowledge of medicine has been passed down by word of mouth from one generation to the next by priests, witchdoctors or medicine men. This is no less true in Kenya where written records in this field are almost non-existent even though the country has had a written language for over two thousand years. The method is crude and commends itself to distortion in an area where accuracy is needed. Some of the knowledge is lost through this filtration process. It could even be modified and thereby become erroneous and even dangerous to use.

The medicineman do not have any scientific data on the kind of biologically active compounds that may have been previously extracted from the plants they provided to us. For this reason it would seem highly desirable that a system for the extraction. identification of chemical compounds, besides isolation and preservation of possible pharmacologically active compounds, should be established. By introducing a system of large-scale extraction and separation of compounds, large quantities of compounds would be obtained and this would facilitate the carrying out of further research in ways of bioassays and screens.

The results obtained for the management of diabetes and hypertensions were very encouraging. I therefore recommend that further bioassays of other possible medicinal plant cures for various ailments afflicting the society should be carried out. Since in man the leading killer diseases are, malaria, hypertension, diabetes - and AIDS is becoming increasingly important, there is a need to randomly screen medicinal plant extracts for any possible curative activity for these diseases. With this kind of screening, a guide on how to come up with new bioactive compounds could then be established.

Besides all these recommendations, I also hold the opinion that I should continue with this kind of work immediately after the examination of this work is completed and is behind me in order to provide a more comprehensive output.

REFERENCES

1. Abegaz, B.M., Ngadjui, B.T., Bezabih, M., Mdee, L.K. (1999*). Novel natural products from marketed plants ofeastern and southern Africa.* Pure Appl. Chem.. Vol. 71, No. 6, pp. 919-926, 1999.Printed in Great Britain.
2. Abegaz.B.M, Bezabih, M.T. (1998). Bull. Chem. Soc. Ethiop. 12, 1-5.
3. Abiy,Y.(1997). *Chemical investigation of two Milletia and two Erythrina species (Leguminoseae) for bioactive compounds.* PhD Thesis University of Nairobi.
4. Akerele, O. (1992). *Importance of medicinal plants.* In: Natural Resources and Human Health; Plants of Medicinal and Nutritional value. Kobe 1991, Elservier, Amsterdam.
5. Achenbach, H. (1986). *Chemical investigation of West African medicinal plants.* In Advances in medicinal Biochemistry. John Libbey Great Britain.
6. Ahlquist, R.P. (1948). *A study of the adrenotropic receptors.* Amer Physiology. 153.
7. Andrzejak, R., Antonowicz J., Kochel B (1989). *Heavy metals as indirect causative factors of capillaro-therapy.* In: Frensemins J Anal Chemistry 354: 5-6
8. Bannerman, R.H.O, Burton J, Ch'en W-C.(1983). *Traditional Medicine and Health Care Coverage: A Reader for Health Administrators and Practitioners.* Geneva:World Health Organization.
9. Black, J.W., Dukkan, W.A.M., Shank R.G.,(1965). *Comparison of some properties of pronethalol and propranolol.* 25:577.
10. Block, J.H., Roche, E.B., Some, T.O., Wilson, C.O. ((1974). *Inorganic medicinal and pharmaceutical chemistry.* Lea and Febriger. Philadelphia. 48-76: 213-267.
11. Bock, S. (1990). *Staying Young the Melatonin Way.* London Press.
12. Bittner, M., Silva M., Becerra, J., Papastergiou, J., and Jakupovic, J. (1997). *Biol. Soc. Chil.* 42, 501.
13. British National Formulary (1971). *British Medical Association.* Pp35. Pharmaceutical Society of Great Britain.
14. Bruhn, R.H, Holmstedt, Chen W.C.(eds). (1983). *Ethnopharmacology objectives and principles and perspectives.* In Beal, J, L, Richard E, (eds) Natural products and medicinal agents.Hippocrates Verlag, pp405-430.

15. Buchanan, B., Gruissem,W., Jones, R. (2000). *Biochemistry and Molecular Biology of Plants. American Society of Plants Physiologists*. Maryland, United States of America.

16. Cambie, R.,Cox, D. Sidwell, D., (1984). *Phytochemistry*. 23, 333.

17. Chandler, R.F. (1985). *Canadian Pharmaceutical Journal* 118: 420-424.

18. Chopra, R.N., Chopra, I.L., Handa, K,L., Kapur, L.D. (1958) In: *Indigenous drugs of India*, 2' edn. UN Dhar and Sons Ltd. Calcutta Pp 300-329.

19. Clarkson, P.M, Haymes, E.M. (1994). *Trace mineral requirements in athletes*. Int J. Sports Nutr 1594 4(2): 104-119.

20. Corley DG, Tempesta, M.S., Twu M.M. (1985). *Tetrahedron Lett*. 26: 1615 -1618.

21. Dagne E (1996). *Bull. Chem. Soc. Ethiop*. 10, 89-103 (1996).

22. Davidson, I. W. F., Blackwell, W.L.(1968). *Proc. Soc*. Expr. Bio Med; 127: 66-72.

23. Edwards, C.R., Benediktsson, R. Lindsay, R.S., Secki, J.R. (1996). *11- beta Hydroxysteroid dehydrogenases: key enzymes in determining tissue-specific glucocorticoid effects*. Steroids Apr; 61 (4): 263-269.

24. Farnesworth, N.R. (1990). *The role of ethnopharmacology in drug development.. In:Bioactive Compounds from Plants* (Ciba Found Symp 154). John Wiley and Sons, Chichester.

25. Farnesworth, N.R. (1992).*Biotechnology and medicinal plant production*. In Natural Plants of medicinal and nutritional value. Elsevier , London 185:42-51

26. Farnesworth, N.R., Akerele, O, Bingel, A.S, Soejarto, D.P., Guo, Z.G. (1985). *Medicinal plants in therapy*. Bull WHO. 63:965 - 981.

27. Fabricant, D.S, Farnesworth, N.R.(2001). *The Value of Plants Used in Traditional Medicine for Drug Discovery*. Environmental Health Perspectives Supplements Volume 109, Number S1, March 2001.

28. Farnesworth, N.R. (1994). *Ethnopharmacology and drug development*. Ciba Found Symp 185:42-51.

29. Farnesworth, N.R. (2001). *Napralert Database*. Chicago:University of Illiniois-Chicago.

30. Feiser, L.M. (1959). Steroids.New York Reinhold : pp15-24

31. Fosmire, G.J. (1990) *Zinc toxicity.* Am. J. Clin. Nutr 1990; 51(2):225-227

32. Gueritte - Voegelein F., Guenard, D., Lavelle, F., Le Goff, M.T., Mangatal L., Portier P. (1991). *J Med. Chem.* 34: 992.

33. Hallmark M, Reynolds T, DeSouza, C. (1996). *Effects of chromium and resistive training on muscle strength and body composition.* Med Sci Sports Exerc 28 (1):139-144.

34. Harbone, J.B.(1970). *Phytochemical methods.* Chapman and Hall, London: 23-67.

35. Hironori, I., Hirosuke, E., Takara, R., Hanako, M., Hanashiro, K. (2007). *The tumor specific cytotoxicity of dihydronitidine from Toddalia asiatica Lam:*Cancer Chemother Pharmacol (2006) 58: 451–459.

36. Howell, J.M., Gunn W.M., Allen, K.A., Linlater and Wienner G. (ed) (1983). *Toxicity problems associated with trace elements in animals.* In; Trace Elements in Animal Production and Veterinary Practice.

37. Huhman D.V., Berhow M.A., Sumner L.W. (2005). *"Quantification of saponins in aerial and subterranean tissues of Medicago truncatula."* J Agric Food Chem. 53(6):1914-20. PMTD: 15769113.

38. Ibrahim, H. (1997). Pharmacognostic and Biological (Analgesic Activity) Studies of *Carissa edulis* Vahl. Ph. D. Thesis. Ahmadu Bello.University, Zaria, Nigeria. 157 – 160, 235 – 245, 306 – 307.

39. Iwu, M.M. (1992). *Traditional Igbo Medicine.* Pp 104. Institute of African Studies.University of Nigeria, Nsukka.

40. Jing-Ming L., Mu-Yun, N., Ju-Fen, F., Yon-Yon, T., Zhao-Hua W., Yu-Lin W.,Wei-Shan C. (1979) Acta. Chim. Sinica. Hal., 109:301.

41. Kato, H., Kanaoka, M. Yano, S. and Kobayashi, M. (1995). *3-Monoglucuronyl-glycyrrhetinic acid is a major metabolite that causes licorice-induced pseudoaldosteronism.* J. Clin. Endocrinol. Metab. Jun; 80 (6) :1929-1933.

42. Kapusta, I., Janda B., Stochmal, A., Oleszek W. (2005). *"Determination of saponins in aerial parts of barrel medic (Medicago truncatula) by liquid chromatography electrospray ionization/mass spectrometry."* J Agric Food Chem. 2005 Oct 5; 53 (20):7654-60. PMID: 16190612.

43. Kenya Times (1997). *Kenya Times Daily Newspaper.* 20th March 1997. Pp 12 Kenya Times Press.

44. Keith, S., Brown, J., W. Sánchez,W. (1974). *Biochem. Syst.* Ecol. 2, 11.

45. Khan, A.K., Akhtar, S., Mahtab, H., (1980). Br. Med. J: 1280: 1044-1048.

46. Khuda, M.Q., Khaleque, A., Mlah, M.A.W. (1965). *Indian Journal of Biochem - Biophysics*. 26: 400-400.

47. Kinyua, A.M. (1997). *Traditional Medicine and anti-convulsant Therapy*. PhD Thesis University of Nairobi.

48. Kokwaro, J.O. (1976). *Medicinal Plants of East Africa*. Kenya. Literature Bureau. Nairobi.

49. Kothari, C.R. (1984). *Qualitative Techniques*. 3rd Edition. Vikas Publishing House Pvt Ltd. Pp 100-165.

50. Kubo, I., Muroi, H., Himejima, M. (1992). J Nat. Prod. 55, 1436.

51. Kumar, P.G., Sudheesh S., Vijayalakshmi N.R. (1992). *Planta Med* 59: 330 - 33

52. Kurihara, Y., (1969). Life Sci 8: 537 - 543.

53. Leatherdale, B.A., Pansar, R.K, Singh. G., Atkins, T.W., Bignell A.H.C. (1981) *Br. Med. 1* 282:, 1823- 1824.

54. Lewis, W.H., Elvin- Lewis, M.P.F. (1988). *Medical Botany*. John Wiley and sons, New York Publishers.

55. Lewis, Mehl-Medrona., (2007). *Coyote medicine.Intensive Mind, Spirit, Body Healing Adventures*.University of Saskatchewan, Deparment of Medicine Canada

56. Liaquart, A., Khan, A.K.A., Mamun, M.I.R., Mozihuzzaman, M., Nar., N., Alam, M.N., Rokeya B. (1992). *Planta. Med.* 59: 408 - 412.

57. Maeda, M., Iwashita, T., Kurihara, T. (1989). *Tetrahedron.* 30: 1547 - 1550.

58. Maitai,. S., (1988). *Essential dietary elements*.In: Kenya Medical Journal, Nairobi.

59. Marini Bettolo, G.B. (1992). *New Structures from Higher Plants*. In Natural Resources and Human Health, Plants of Medicinal and Nutritional value, Kobe, 1991. Elservier Science Publishers, Amsterdam 102.

60. Manes, R.J., Farnesworth,N.R.J. (1996). *Prot.J.Bot.Med*, (3):85-135; World Health Organization: Diabetes mellitus. Report of a WHO Study Group.

61. Manguro, L.W. (1994). *Phytochemical analysis of Myrisine Africana L and Maesa Forsk and structure activity relationship studies of 2-s dehydroalkyl-benzoquinones*. PhD thesis U.o.N Pp 26.

62. Michelle, S., Alain, P., Françoise, B. (2001). *Minerals, trace elements and related biological variables in athletes and during physical activity.* Volume 312, Issues 1-2, 2001, Pages 1-11

63. Morrill,T.C., Bassler, G.C., Silverstein, R.M. (1981). Spectrometric Identification of organic compounds. 4th Ed Rochester.Carlifonia.

64. Morris, B.W., Blumsohn, A, MacNeil, S. (1992). *The trace element chromium: a role in glucose homeostasis.* Am J Clin Nutr 1992; 55(5):989-991.

65. Mugo, N.J, Kamau, N.J., Koskei, J.C. (2005). *Hypoglycaemic effect of an extract of Podorcarpus* sp. In. International Journal of BioChemiPhysics, Vol. 14, No.1, March 2005. Nairobi.

66. Mugo, N.J., Kamau, N.J., Koskei, J.C. (2005). *The antihypertensive effect of an extract of Podocarpus* sp. In. International Journal of BioChemiPhysics, Vol. 14, No.2 March 2005. Nairobi.

67. Murkherjee, K.,Ghose, N.C., Datta T. (1972.*) Indian. J. Exp. Biol.* 10:347-351

68. Nanzheng, C., Anderson. R.A., Bryden. N.A., Polansky M.M., Mertz Z.W., (1991). *Chromium interaction with nucleic acids in rat liver.* In: Trace elements in Man Animals (TEMA - 7) B.Momcilovic (ed) IMI Zagreb, Croatia, Yugoslavia, 3-8.

69. Natarajan, B., Paulsen, B.S., Pushpangadan, P. (1999). *An ethnopharmacological study from the Coimbatore district, Tamil Nadu, India*: traditional knowledge compared with modern biological science. Pharm Biol 37:378-390.

70. Natarajan, B., Paulsen, B.S. (2000). *An ethnopharmacological study from Thane District, Maharashtra, India: traditional knowledge compared with modern biological science.* Pharm Biol 38:139-151

71. Omino, E.A., Kokwaro J.O. (1993). *Ethnobotany of Apocynaceae specie in Kenya.* J. Ethnopharmacol. 4: 167 – 180.

72. Poblete, H., Rodriguez, S., Zárate, M. (1991). *Extraction and effective characterization and utilization of primary medicinal plant material.* University of Austal Chilel de Chile, Serial document N° 34.

73. Pompei, R.. (1980). *Antiviral Activity of Glycyrrhizic Acid.* Experientia 36 304- 309.

74. Prazeres, M. (1982). *Studies of some Podocarpus saligna constituents.* Thesis for the degree of Doctor of Philosophy. Department of Chemistry, London.

75. Rajab, M.S., Ogunde, M.O. Guyo., P. (1993). *Kenya Chemical Society Inaugural Conference* Proc. Pp 23 College Educations and External Studies Kikuyu Campus. Press, Nairobi.

76. Rastogi, R.P., Dhawan., B.N. (1982). *Research on medicinal plants at the Central Drug Research Institute*, Lucknow (India). Indian. J. Med Res 76 (suppl):27-45

77. Rastogi, R.P., Dhawan, B.N. (1982). *Research on medicinal plants at the Central Drug Research Institute, Lucknow (India).* Indian J. Med Res 76 (suppl):27-45.

78. Renold, Cahill (Eds). (1965). *American Physilogical Society- Handbook of Physiology*, Section 5, Williams & Wilkins, Baltimore.

79. Rivier, L., Bruhn, J.G. (1979). *Editorial. J. Ethnopharmacol* 1:1

80. Rodriguez, R., Matthei., O., Quezad, M. (1983). *Forest plants of Chile.* Universidad de Concepción, Chile.

81. Rodriguez, K., Ruiz, E. (1999). *Forest of Chile.*Faculty of Biological Sciences. University of Conception. Chile.

82. Scandalios, J.G. (1991). *Natural Resources and Human Health.* In: Plants of Medicinal and Nutritional Value. Kolbe Elsevier, Amsterdam.

83. Schroeder, P.A (1968). *Am. J. Cl*: Nut. 21:230 - 244.

84. Sharma, Y.R, Acharya, R.C. (1988). *Analytical methods in chemistry.* Kalyani 12-60

85. Sokolov, S.Y. (1986). *Advance in Medicinal phytochemistry* .21: 173 - 178. John Libbey, Great Britain.

86. Soskin, L. (1940). *Defects of carbohydrate metabolism.* Pp 35 John Wiley, London.

87. Suzuki, H., Achnine L., Xu R., Matsuda S.P.T., Dixon, R.A. (2002). "*A genomics approach to the early stages of triterpene saponin biosynthesis in Medicago truncatula.*" Plant J. 32: 1033-1048. PMID: 12492844.

88. Thomas, R.K. (1992). *Biochemistry, principles and practice.* Pp 146 John Libbey, London.

89. Turner, D.M. (1996). *Natural product source material use in the pharmaceutical industry*: the Glaxo experience. J Ethnopharmacol 51:39-43; Discussion 44 (1996).

90. Undie, A.S, Akubue, P.I. (1986). J Ethnopharmacology. 15: 133 - 144.

91. Usher, G. (1974). A dictionary of plants used by man, Constable, London

92. Verpoorte, R. (2000). *Pharmacognosy in the new millennium: Lead finding and biotechnology.* J Pharm Pharmacol 52:253-262 (2000).

93. Wallach, S. (1991). *Tissue Chromium Content in Diabetes Mellitus* and Coronary artery disease. In: Trace elements in man and animals (TEMA -7) B Momcilovic (ed). IMI Zagreb, Croatia, Yugoslavia 3 - 10.

94. Wallach, S. (1990). *Chromium and aging.* In: Proceedings of J International Symposium on Metal ions in Biology and medicine. P. Collery (ed). John Libbey and Co. Ltd. London.

95. Watt, B., Brandwijk, B. (1962). Medicinal and poisonous plants of Southern and Eastern Africa. E. & S. Livingstone Ltd. Edinburgh and London.

96. W.H.O. (1996). *Trace elements in human nutrition and health.* WHO Geneva; 22:228.

97. W.H.O. (2003). *Traditional medicine.* In: WHO Fact Sheet No. 134. Geneva.

98. Woodward, R.B. (1941). *J.Am. Chem Soc,* 63:1123, 64:72,76

99. Van Staden, L.F., S. E. Drewes, S.E. (1993). Phytochemistry 35, 685-686

Zheng, J.J., Mason, J.B., Rosenberg, I.(1993). *Measurement of zinc bioavailablity from beef and a ready-to-eat high-fiber breakfast cereal in humans application of a whole-gut lavage technique.* Am J Clin Nutr 1993; 58 (6): 902-907.

93. Zumdahl., S. (1992). *Chemical principles.* New York.

APPENDICES

Appendix 1

LC-MS separation profile (%) of the compounds in chloroform extract of *Podocarpus* sp (CADE-A)

Appendix 2

LC-MS separation profile of the compounds in methanol extract of *Podocarpus* sp bark

Appendix 3

^{1}HNMR type of proton, functional group and chemical shift (δ ppm) of compound

Appendix 4

MS-FAB of fragments of Compound **B**

Appendix 5

MS-FAB of fragments of Compound A

Appendix 6

^{13}CNMR noise decoupled chemical shift (δ ppm) of compound B

Appendix 7

Infra red spectrum of compound **B**

Appendix 8

Blood glucose (mg/100ml) in rabbits dosed with 2.5g/kg b.w. of glucose (GTT)

Appendix 9

Blood glucose (mg/100ml) in rabbits dosed with 20mg/kg b.w. of Chloroform extracts of *Podocarpus* sp (CADE-A)

Appendix 10

Blood glucose (mg/100ml) in rabbits dosed with 20mg/kg b.w. of methanol extracts of *Podocarpus* sp bark

Appendix 11

Blood glucose (mg/100ml) in rabbits dosed with 8ml/kg b.w. water extracts (CADE-A)

Appendix 12

Amplitude of contraction (mm) of the ileum when dosed with CADE-A, acetylcholine and atropine

Appendix 13

Amplitude of contraction (mm) of the ileum when dosed with CADE-B, acetylcholine and atropine

Appendix 14

Amplitude of contraction (mm) of the heart when dosed with CADE-A

Appendix 15

Amplitude of contraction (mm) of the heart when dosed with CADE-B and adrenaline

Appendix 16

Amplitude of contraction (mm) of the heart when dosed with CADE-A, propranolol and isoprenaline

Appendix 17

Amplitude of contraction (mm) of ileum when dosed with CADE-A and oxytocin

Appendix 18

Amplitude of contraction (mm) of ileum when dosed with CADE- B and oxytocin

0.80
1.55
5.16
6.91
8.01
8.58
9.01
4e5
3e5
2e5
1e5
Intensity, cps
1
2
3
4
5
6
7
8
9
10
Time, min
1.89e3 cps
+Q1: 7.21 min (149 scans) from A98MS0853-0079, subtracted (scans 45 to 67)
437.1
171.0
340.1
585.1
879.2
523.1
775.2
932.3
1057.3
1118.3
1500
1000
500
Intensity, cps
200
300
400
500
600
700
800
900
1000
1100
m/z, amu

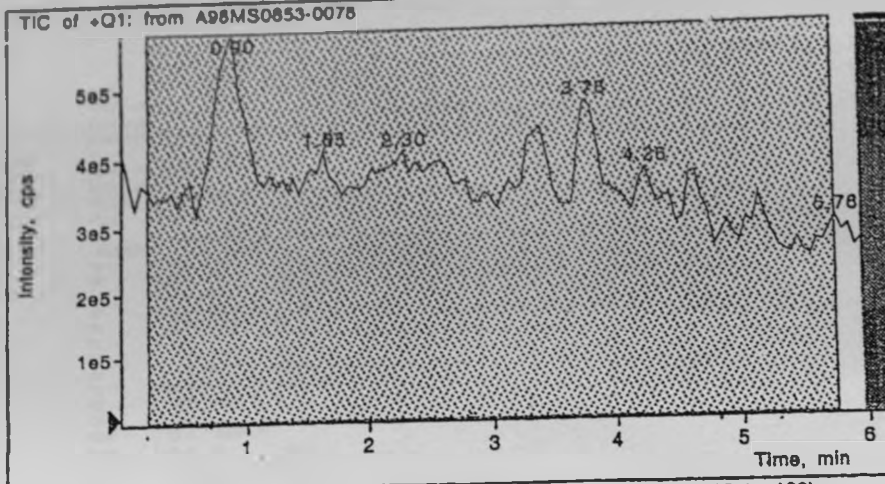
Appendix 2

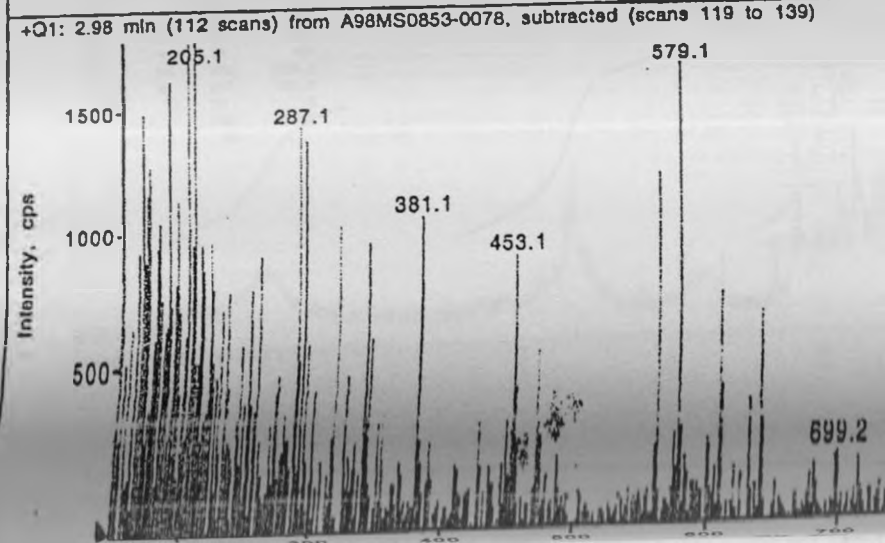
MultiView 1.4

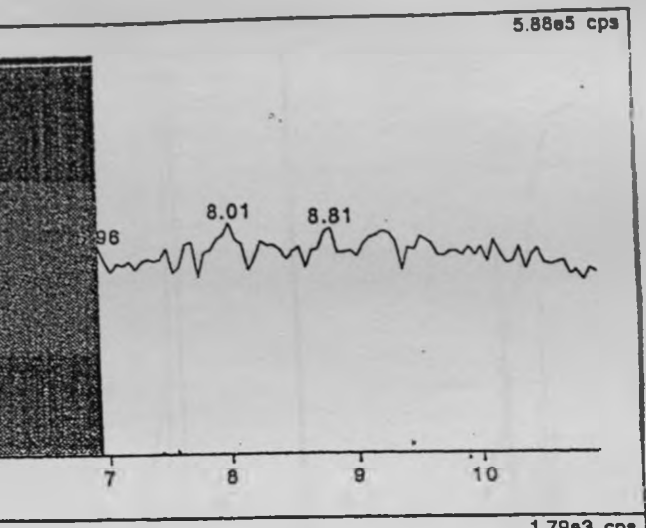
Info for pane 2: A98MS0853-0078 (333.3)

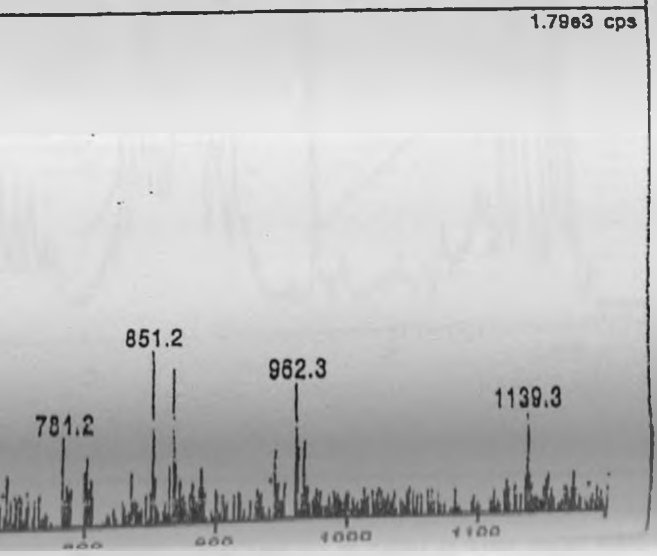
Period 1, Expt. 1; Mass range: 150.0 to 1200.3 by 1.0 amu; Dwell: 2.9 ms; Pause: 5.0 ms

Acq. Time: Thu, Dec 10, 1998 at 8:52:06 AM; Exp. Comment: Batch Exp: 12-09-98 LC

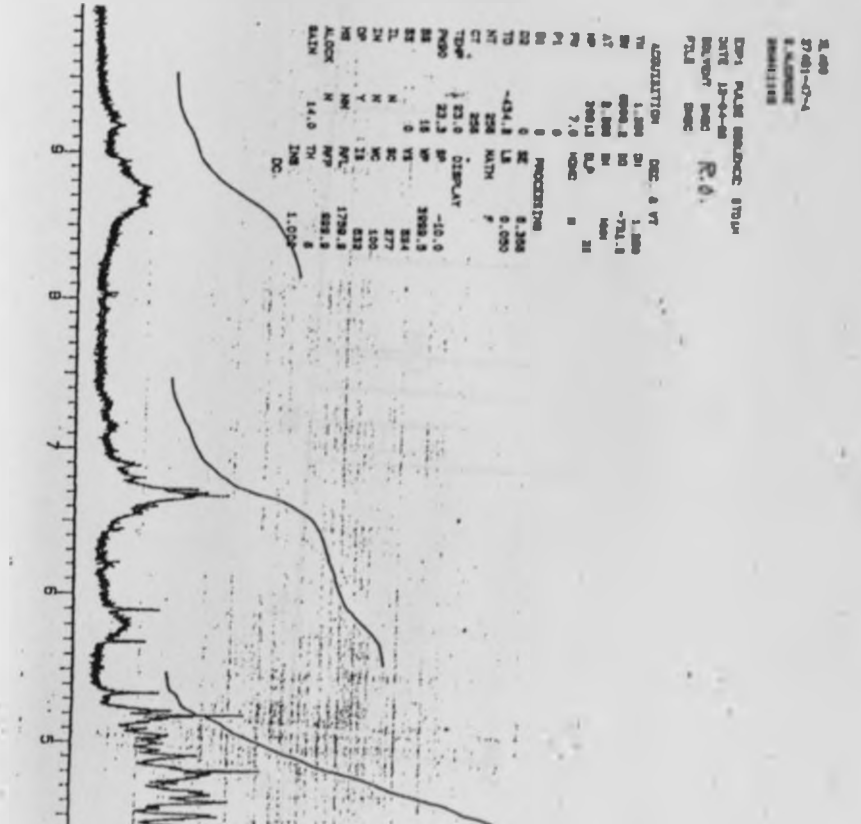




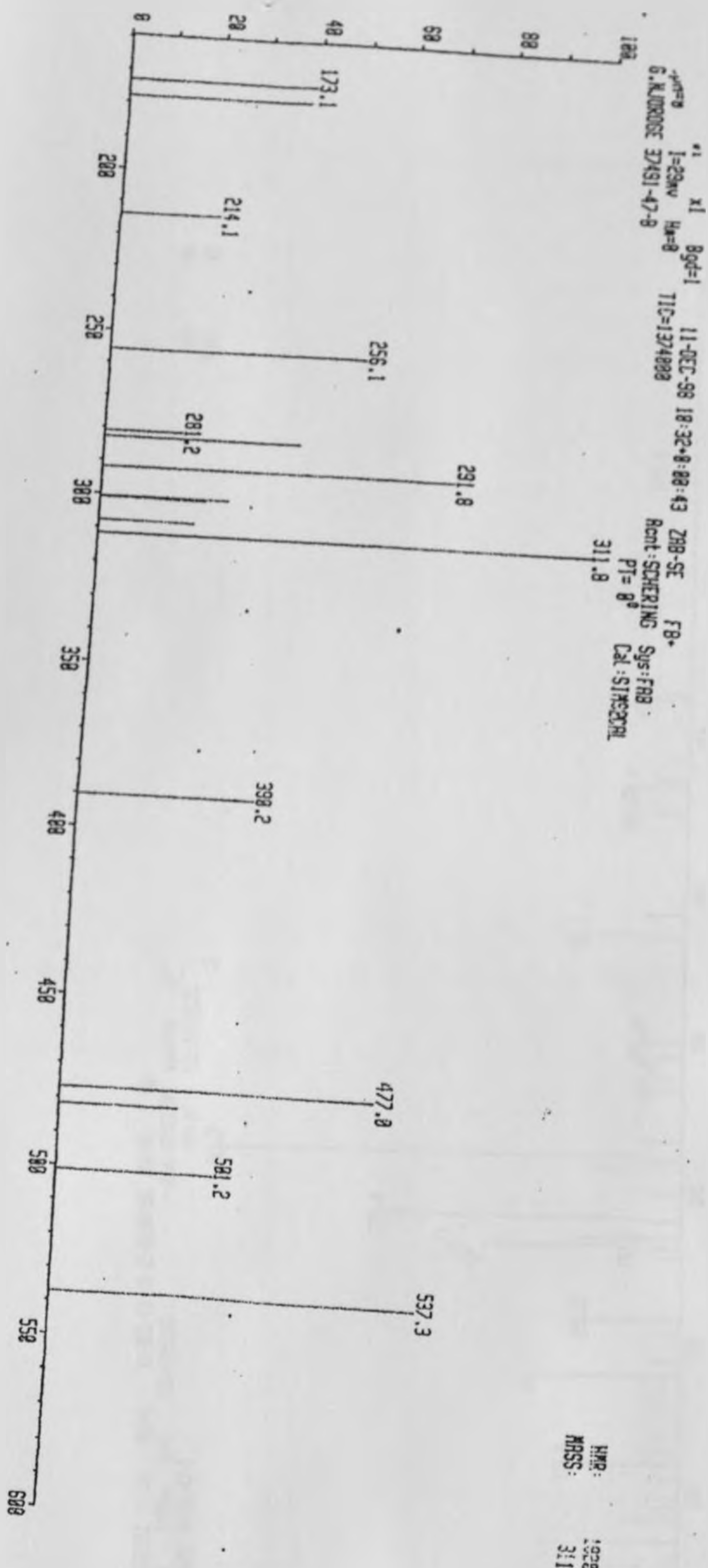

5.88e5 cps
8.01
8.81
96
7
8
9
10

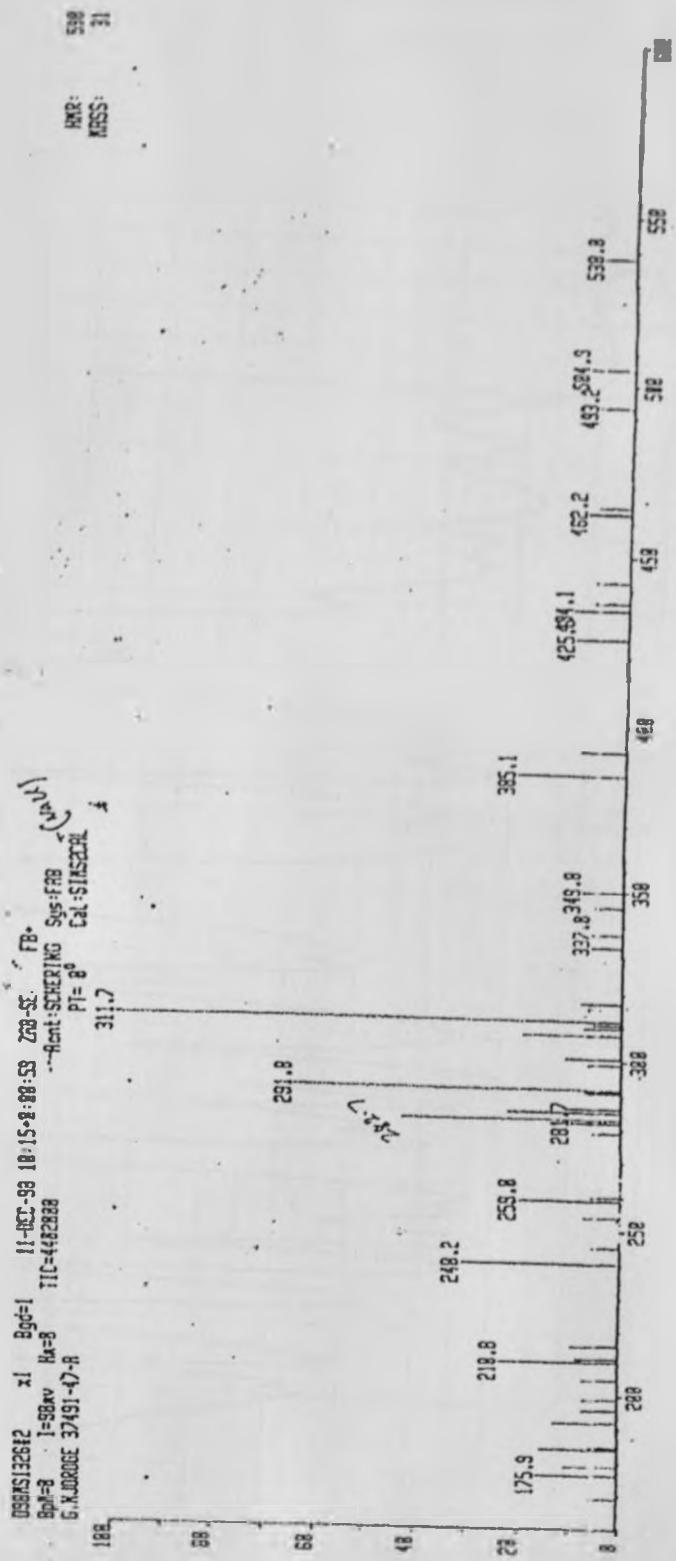

1.79e3 cps
851.2
962.3
1139.3
781.2
900
1000
1100

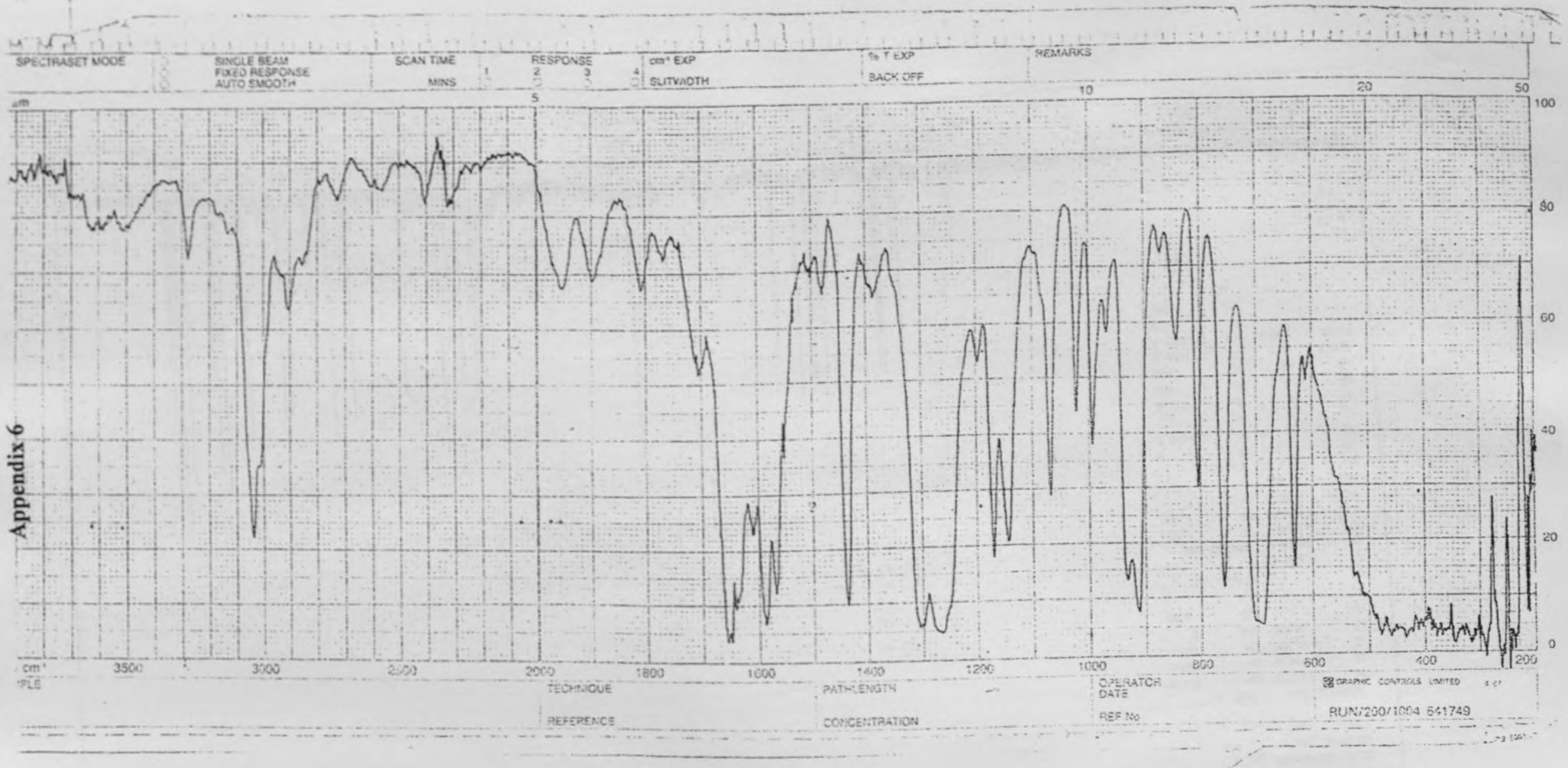
PPM



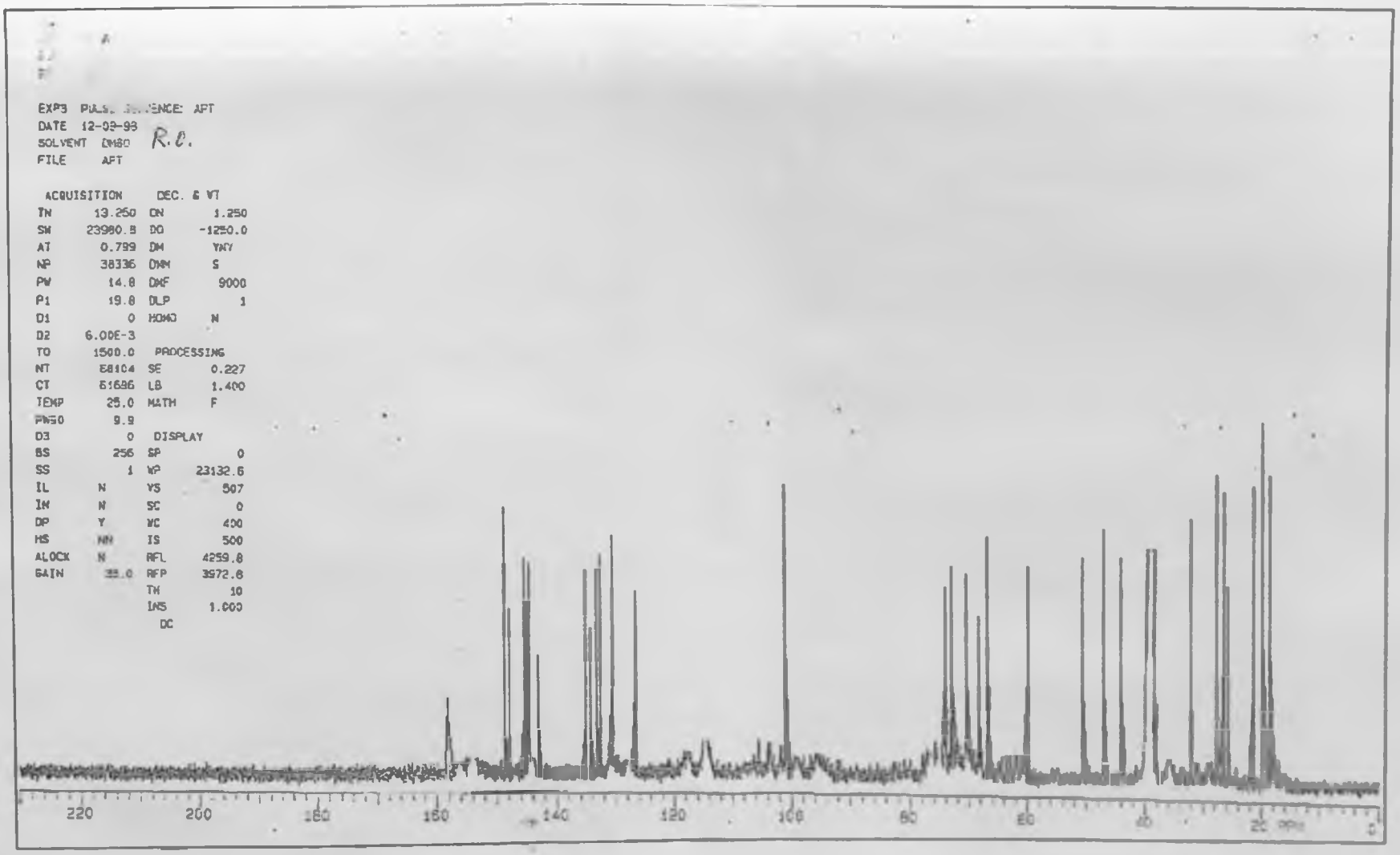
Appendix 4




DSBMS13261#2 x1 Bgd=1 11-OCT-98 10:15:8:00:53 ZAB-SE FB-
BpM=8 I=58Arv Na=8 TIC=4482888 --Ment:SCHERING Sys:FAB
G.K.DODGE 37491-47-A PT= 8.0 Cal:SIMSCAL
MAX: 500
MASS: 31
311.7
291.8
385.1
425.4
484.1
482.2
493.2
484.3
538.8
337.8
349.8
281.7
253.8
248.2
219.8
175.9
100
80
60
40
20
0
250
300
350
400
450
500
550


SPECTRASET MODE
SINGLE BEAM
FIXED RESPONSE
AUTO SMOOTH
SCAN TIME
MINS
RESPONSE
1 2 3 4
cm⁻¹ EXP
SLITWIDTH
% T EXP
BACK OFF
REMARKS
5
10
20
50
100
80
60
40
20
0
cm⁻¹
3500
3000
2800
2000
1800
1600
1400
1200
1000
800
600
400
200
TECHNIQUE
PATHLENGTH
OPERATOR
DATE
GRAPHIC CONTROLS LIMITED
REFERENCE
CONCENTRATION
REF No
RUN/200/1004 641749

Appendix 6


EXP3 PULSE SEQUENCE: APT
DATE 12-09-93
SOLVENT DMSO
FILE APT
R.D.
ACQUISITION
TN 13.250
SW 23980.8
AT 0.799
NP 38336
PW 14.8
P1 19.8
D1 0
D2 6.00E-3
TO 1500.0
NT 68104
CT 61686
TEMP 25.0
PWSO 9.9
D3 0
BS 256
SS 1
IL N
IN N
DP Y
HS NN
ALOCK N
GAIN 35.0
DEC. & VT
DN 1.250
DO -1250.0
DM YNY
DMM S
DMF 9000
DLP 1
HOMO N
PROCESSING
SE 0.227
LB 1.400
MATH F
DISPLAY
SP 0
WP 23132.6
VS 507
SC 0
WC 400
IS 500
RFL 4259.8
RFP 3972.8
TH 10
INS 1.000
DC
220
200
180
160
140
120
100
80
60
40
20 ppm
0

Appendix 8

d/f	Time (Minutes)						
	0	30	60	90	120	150	180
1	46.5	48.8	60.6	74.6	128.8	126.8	120.5
2	49.6	49.9	56.1	67.5	129.3	125.9	121.6
3	50.3	51.3	55.3	68.6	130.2	130.2	120.8
4	52.6	54.1	58.1	69.9	125.6	125.6	123.6
5	53.1	53.5	66.1	67.4	130.2	120.3	126.7
6	54.5	52.6	62.3	66.2	130.3	123.1	127.3
7	50.6	51.9	64.2	70.2	130.6	124.2	118.6
8	47.5	55.8	62.6	71.8	130.6	125.8	119.6
9	48.6	52.3	56.8	73.2	128.1	122.6	119.3
10	48.6	53.1	58.9	69.3	127.8	122.4	116.3
11	49.1	53.1	58.2	73.7	127.6	125.8	122.3
12	50	54.1	60.6	72.8	127.6	125.6	123.1
13	47.4	55.2	64.3	72.1	131.2	121.9	124.8
14	46.3	57.4	63.1	76.8	132.9	125.6	125.8
15	46.7	56.3	62.3	73.9	133.1	128.9	126
Mean	49.42667	53.29	60.63	71.2	129.59	124.98	122.42
SD	3.60	2.33	3.27	3.05	2.05	4.06	3.28

Appendix 9

d/f (n)	Time (Minutes)					
	0	30	60	90	120	150
1	50.6	118.6	138.1	110.7	99	98
2	51.7	119.3	139.2	110.6	99.2	97.1
3	51.8	118.9	143.8	110	100.1	96.2
4	49.5	117.6	138.2	108.6	103.1	96.1
5	48.3	120.1	142.9	111.3	102.6	102.1
6	50	121.3	142.6	108.6	105.2	101.2
7	51.3	120.1	140.9	109.6	104.8	100
8	52.1	122.9	140.6	108.1	102.9	99
9	51.9	122.8	140	112.6	101.2	99
10	50.6	121.9	139	112.9	100.2	99.9
11	51.6	120.5	141.8	107.6	103.1	99.6
12	51.7	119.4	141.9	110.8	102.3	98.3
13	52.1	116.6	141.2	108.6	103.4	98.2
14	50.9	117.3	140.3	112.8	102.6	98
15	49.6	115.6	140.1	112.6	103.2	97
Mean	50.91333	119.53	140.71	110.36	102.19	98.64
SD	1.13	2.17	1.69	1.82	1.88	1.73

Appendix 10

d/f (n)	Time (minutes)					
	0	30	60	90	120	150
1	48.5	80.7	92	123	113.2	101.6
2	49.5	82.6	93.8	122.9	114.6	102.1
3	52.6	82.1	92.7	123.6	116.3	101.3
4	53.1	81.5	92.6	123.2	116.2	99.7
5	50.9	81.3	91.7	128.1	115.1	99.5
6	50.8	81.3	91.8	127.3	112	101.6
7	55.5	79.6	90.9	123.1	112.6	100.2
8	54.4	79.5	89.7	124.6	112.4	99.3
9	53.3	77.1	89.5	12.2	111.9	105
10	49.9	79.6	88.4	120.6	110.7	105.9
11	49.7	79.4	89.6	125	110.2	104.6
12	48.6	78.6	90.6	125.9	116.6	103.8
13	51.3	83.4	94.8	127.3	115.6	103.2
14	50.4	82.5	93.4	126.2	114.2	102.9
15	50.6	82.5	92.6	125.6	112.6	100.8
Mean	51.27	80.78	91.61	117.24	113.61	102.10
SD	2.08	1.76	1.80	2.14	2.06	2.08

Appendix 11

d/f (n)	Time (minutes)					
	0	30	60	90	120	150
1	50.6	100.9	115.6	96.8	93.4	87.5
2	49.6	101.1	115	94.6	92.6	88.6
3	49.2	101.2	116	99.3	91.2	85.1
4	51.3	99.3	116.3	98.3	92.1	86.2
5	52	99	117.2	97.2	90.3	87.6
6	51.2	99.8	118.2	98.6	94.5	86.3
7	50.8	98.6	110.6	95	93.9	85.1
8	50.6	103.2	112.6	105.6	93.8	85.9
9	52.6	98.6	112.6	1102.1	92	87.6
10	51.8	98.6	113.2	102.3	91.9	89.1
11	51.6	97.6	114.3	100.1	91.8	85.2
12	51.2	100	115	94.9	90.6	86.8
13	50.3	101.6	114.2	97.3	90.1	87.6
14	49.3	101.2	112.3	98.6	94.2	88.4
15	50.2	101.3	112.5	99.1	95	86.5
Mean	50.82	100.1333	114.3733	165.32	92.49333	86.9
SD	1.01	1.52	2.09	4.18	1.56	1.29

Appendix 12

Time (Min)	Normal				Mean	SD	1ml				Mean	SD	2.0ml				Mean	SD
1	20	22	21	22	21	1	22	23	24	23	23	0.8	22	21	22	23	24.8	0.8
2	21	20	20	21	21	0.6	23	26	25	24	25	1.3	23	22	23	22	28	0.6
4	22	21	21	20	21	0.8	28	29	28	27	28	0.8	24	25	24	25	33.3	0.6
6	20	20	20	21	20	0.5	38	39	40	38	39	1	26	27	28	27	37	0.8
8	21	21	20	20	21	0.6	39	39	40	39	39	0.5	58	59	60	57	58.5	1.3
10	22	22	21	21	22	0.6	41	42	40	41	40	0.8	60	61	60	59	60	0.8

0.1g Acetylcholine				Mean	SD	1.0g Atropine				Mean	SD
22	21	22	23	22	0.8	38	39	40	39	38	0.8
23	22	23	22	27.5	0.6	72	71	70	73	71.5	1.1
24	25	24	25	24.5	0.6	55	79	80	77	72.8	10
26	27	28	27	24.5	0.6	101	102	103	104	103	1.1
26	25	26	27	26	0.8	97	96	97	97	96.8	0.4
25	26	26	25	25.5	0.6	72	74	75	71	74	1.6

Appendix 13

Time (Min)	Normal				Mean	SD	1ml				Mean	SD	2.0ML				Mean	SD
	1	2	3	4			1	2	3	4			1	2	3	4		
1	18	18	19	20	19	1	20	22	21	21	21	0.8	19	20	18	21	19.5	1.29
2	20	20	19	18	19	1	24	23	25	25	25	1	25	25	26	26	25.5	0.58
4	22	21	20	20	21	1	27	28	29	29	28	1	28	29	31	30	29.5	1.29
6	21	22	20	19	21	1	38	37	38	36	37	1	40	39	41	42	40.5	1.29
8	23	24	21	22	23	1.3	34	35	33	36	35	1.3	36	37	35	38	36.5	1.29
10	20	21	22	23	22	1.3	33	32	31	30	32	1.3	35	37	35	38	36.3	1.5

Appendix 14

Time (Min)	Normal								0.1 ML								0.5ML								1 ML							
	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD
1	10	11	11	10	12	11	11	####	10	11	12	13	14	10	11	1.63	11	10	11	10	10	11	10.5	0.54	11	10	10	11	11	11	11	0.5
2	12	11	12	13	11	10	11	1.05	9	10	11	10	9	11	10	0.89	11	12	11	11	10	12	11	0.75	12	12	13	14	13	11	13	1.0
4	11	12	11	10	12	13	11	1.05	7	6	7	8	9	7	6.2	1.03	9	9	8	9	9	9	8.8	0.41	14	13	14	13	13	14	14	0.5
6	10	11	12	10	12	10	10	0.98	6	7	6	8	6	6	7.5	0.84	8	8	7	7	8	8	7.7	0.52	15	15	16	15	15	15	15	0.4
8	11	12	10	11	10	12	10	0.89	4	5	5	4	5	5	5.5	0.52	7	7	8	7	8	7	7.3	0.52	14	14	14	13	15	15	14	0.7
10	11	10	11	12	10	11	10	0.75	5	6	6	5	6	5	6.3	0.55	7	7	8	9	9	8	8	0.89	14	15	15	14	14	14	14	0.5

Time (Min)	0.1g Acetylcholine				Mean	SD	1.0g Atropine						
1	40	41	42	39	41	1.3	1	19	20	21	22	21	1.3
2	72	73	74	75	74	1.3	2	20	21	22	20	21	1
4	82	83	84	82	83	1	4	19	18	17	17	18	1
6	118	119	116	117	118	1.3	6	19	20	21	20	20	0.8
8	101	100	99	97	99	1.7	8	20	21	20	22	21	1
10	60	61	62	63	62	1.3	10	21	22	21	22	22	0.6

Appendix 15

Time (Min)	Normal								1ml								2.0ML								3 ML							
	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD
1	15	16	15	15	15	16	15		15	15	15	16	16	15	15		9	9	10	10	9	9	9		6	7	7	6	6	6	6	
2	15	15	15	16	16	15	15		10	11	10	11	11	10	11		6	6	7	7	6	7	7		4	4	5	4	4	6	5	
4	15	16	16	15	16	16	16		12	12	13	12	13	12	12		5	6	6	5	6	7	6		3	3	4	3	5	4	4	
6	15	15	16	16	15	16	16		12	12	13	13	12	12	12		6	6	7	6	7	7	7		3	3	4	4	4	4	4	
8	16	16	16	15	15	15	16		11	11	11	12	12	11	11		6	7	7	6	6	6	6		3	3	3	4	4	4	4	

Time (Min)	0 1µg Adrenaline							
	1	2	3	4	5	6	Mean	SD
1	3	4	4	3	4	4	4	
2	6	6	7	6	7	7	7	
4	9	9	8	10	10	9	9	
6	10	10	11	11	10	10	10	
8	12	12	12	11	11	11	4	

Appendix 16

Time (Min)	Normal								1ml								0.1ml + 0.2µg propranolol								0.1ml + 0.1µg isoprenaline							
	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD	1	2	3	4	5	6	Mean	SD
1	10	11	12	13	11	13	11	1.21	10	11	12	13	11	12	10	1.05	10	10	10	11	11	11	11.5	0.55	10	10	11	11	10	11	11	0.55
2	10	12	11	13	10	12	11	1.21	12	13	13	14	11	11	12	1.21	12	12	13	12	13	13	12.5	0.55	12	12	11	10	12	13	12	1 03
4	11	12	13	14	10	10	11	1.64	12	12	12	13	13	12	12	0.52	13	13	13	14	14	14	13.5	0.55	25	26	28	25	25	25	25	1.21
6	11	11	12	13	10	10	11	1.17	14	14	15	14	15	14	14	0.52	13	13	14	13	13	14	13.3	0.52	22	23	23	23	24	25	23	1 03
8	11	13	12	11	11	11	12	0.84	15	15	14	14	15	14	15	0.55	15	15	15	15	14	16	15	0.63	15	15	16	15	15	16	15	0 52
10	10	10	10	11	11	12	11	0.82	10	11	10	10	11	12	11	0.82	16	16	15	16	16	15	15.7	0.52	13	12	12	12	13	12	12	0 52

Appendix 17

Time (Min)	Oxytocin								0.5ml								1.0ml								2.0ml							
	1	2	3	4	5		Mean	SD	1	2	3	4	5		Mean	SD	1	2	3	4	5		Mean	SD	1	2	3	4	5		Mean	SD
1	2	3	4	2	3	4	3	0.9	2	3	2	5	2		3	1.3	2	3	2	4	3		2.8	0 8	2	3	4	3	2		3	0 84
2	10	12	15	10	11	12	12	1.9	4	5	6	5	6		5	0.8	3	4	3	4	3		3.4	0 6	2	2	3	3	3		3	0 55
4	60	62	61	63	61	62	62	1.1	7	7	6	5	8		7	1.1	5	4	5	4	3		4 2	0 8	3	3	4	4	3		3	0 55
6	15	16	15	16	17	16	16	0.8	3	4	3	5	4		4	0.8	2	3	3	4	3		3	0 7	2	2	2	3	2		2	0 45

Appendix 18

Time (Min)	Oxytocin								1.0ml								2.0ml							
	1	2	3	4	5		Mean	SD	1	2	3	4	5		Mean	SD	1	2	3	4	5		Mean	SD
1	2	2	1	2	3		2	0.7	2	2	3	2	2		2	0.5	2	2	1	2	1		1.6	0 6
2	12	14	13	13	11		13	1.1	2	1	2	2	3		2	0.7	3	4	4	5	6		4 4	1.1
4	63	64	62	65	61		63	1 6	2	1	2	2	2		2	0 5	7	8	7	9	8		7 8	0 8