SOME PHARMACOLOGICAL EFFECTS OF THE LEAF EXTRACTS OF

Vernonia lasiopus AND Maesa lanceolata: PLANTS TRADITIONALLY USED TO
TREAT COMMON AILMENTS IN HUMANS IN EAST AFRICA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN
PHARMACOLOGY OF MAKERERE UNIVERSITY.

NOVEMBER 2009
DECLARATION

I hereby declare that the work presented in this book is original. It has never been presented to any institution of learning either in full or in part, for any academic award, publication, or otherwise.

Signature ………………………………………. Date…………………………

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DEDICATION

This book is dedicated to my mother Annet Nabatanzi and my pastor Vincent Kashaija.
ACKNOWLEDGEMENT

During the preparation of this dissertation, many people assisted me in various ways and I am extremely grateful for all the assistance.

I extend gratitude to my supervisors, Prof. Willy W. Anokbonggo and Assoc. Professor Paul Waako for their encouragement, financial help and guidance throughout the study.

My gratitude is also extended to the Ministry of Health (Uganda) for paying my tuition fees. I acknowledge the help accorded to me by Mr. Lubega A. and Mr. Oddia G. during the laboratory work. Special thanks to Mr. Lubega for his encouragement and timely advice. Finally, I thank Mrs. Esther Katura and Rebecca Nakaziba and all the staff of Department of Pharmacology and Therapeutics for supporting me in various ways without which I would not have completed this work.
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<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDH</td>
<td>The British Drug Houses Ltd.</td>
</tr>
<tr>
<td>Conc.</td>
<td>Concentration</td>
</tr>
<tr>
<td>Log</td>
<td>Logarithm</td>
</tr>
<tr>
<td>Mg/ml</td>
<td>Milligram per milliliter</td>
</tr>
<tr>
<td>Mm</td>
<td>Millimeters</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>µg/ml</td>
<td>Microgram per milliliter</td>
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ABSTRACT

Background

Maesa lanceolata and Vernonia lasiopus are important traditional medicinal plants in E.Africa. *M. lanceolata* is used to treat malaria, dysentery, dermatosis, hypertension, ascariasis, difficult deliveries, etc. *V. lasiopus* is used to treat fever, abdominal pain, diarrhea, ascariasis and other ailments. Pharmacological effects of these plant extracts on the rabbit uterus and heart and guinea pig ileum were investigated in this study.

Methods

Leaves of *M. lanceolata* and *V. lasiopus* were collected from Mbarara District of Uganda. Extraction of dried plant material was done using petroleum ether, chloroform and ethanol. Effect of both plant leaf extracts of different concentrations was assessed on isolated guinea pig ileum, rabbit heart and rabbit uterus.

Results

The ether, chloroform and ethanolic extracts of *M. lanceolata* caused increase in the strength of contraction of isolated rabbit heart. The ether extract of *M. lanceolata* caused contraction of the isolated guinea pig ileum whereas the chloroform and ethanolic extracts did not. The extracts of *V. lasiopus* did not cause contraction of the isolated guinea pig ileum. Both *M. lanceolata* and *V. lasiopus* did not inhibit contraction of ileum induced by acetylcholine. The ethanolic extract of *M. lanceolata* elicited contractions of the uterus whereas the other fractions of *M. lanceolata* and *V. lasiopus* did not cause contraction of the uterus.

Conclusions

These results show that the extracts of *M. lanceolata* have a positive inotropic effect on isolated rabbit heart, which could be of clinical benefit in cardiac failure and the use of this plant in treating hypertension may be harmful. The other results suggest extracts of *M. lanceolata* may induce abortion and could cause abdominal pain and diarrhea.
CHAPTER ONE

INTRODUCTION

1.1 Background

Traditional medicine has maintained its popularity in all regions of the world and its use is rapidly spreading to industrialized countries, (WHO, 2003). In Africa, up to 80% of the population uses traditional medicine to meet their primary health care needs (WHO, 2003). Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating animal and mineral based medicines, spiritual therapies, manual techniques and exercises and plants singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. Traditional medicine is popular mainly because of its affordability and easy accessibility. Public disappointment in the fact that drugs used in modern medicine can cause serious harm, has also naturally led to a revival of interest in alternatives including traditional medicine, that promise efficacy with complete safety, (Laurence et al., 1997). It also provides an alternative to modern drugs where the development of resistance to essential antimicrobial agents has increased the cost of health care. Despite the widespread use of traditional medicine, it has some disadvantages. Many times the efficacy and safety of traditional medicine products is unknown and this exposes people to danger. For example, in the USA the herb Ma Huang (Ephedra) led to dozens of deaths, heart attacks and strokes (WHO, 2003).

The ethnobotanical and traditional healers’ survey of Kabale District, (Mubiru et al., 1993) reported some of the medicinal plants commonly used in traditional
medicine for treatment of malaria in humans including *Maesa lanceolata*, locally known as omuhanga and *Vernonia lasiopus*, locally known as omujuma. *V. lasiopus* is also used by the Kikuyu tribe of Kenya as a remedy for malaria in humans (Muregi et al., 2007). The methanolic extract of *V. lasiopus* has been found to have significant antimalarial activity in vivo tests, (Muregi et al., 2007). In another study by Irungu et al., 2007, *V. lasiopus* showed high antiplasmodial activity ranging between 1.4-35µg/ml. Both studies recommend that *V. lasiopus* warrants further evaluation in the search for novel antimalarial agents against drug resistant malaria. The extract of *M. lanceolata* on the other hand has been shown to have significant *in vitro* antiplasmodial activity. The chloroform extract had activity of IC50 of 1.6µg/ml, (Katura, 2007). Both plant extracts are therefore good candidates for further investigations in the process of developing novel antimalarial drugs.

1.2 Problem Statement

Despite the wide spread use of these plants, little work has been done to document the effects of these plants on body systems. In this study, the pharmacological effects of the leaf extracts of *M. lanceolata* and *V. lasiopus* on laboratory animals’ organ tissues have been investigated. This is part of preclinical research on extracts before they are considered for clinical trials. During this phase, the extracts under study are tested first on isolated tissues to identify their pharmacological effects. *In vivo* tests are later carried out to confirm the effects identified earlier in the *in vitro* tests. The potential usefulness
and unwanted effects of the extracts depend on their profile of activity on various tissues in both \textit{in vitro} and \textit{in vivo} tests

1.3 Conceptual Frame work
1.4 Objectives of the study

1.4.1 General Objective

To determine the pharmacological effects of extracts from *M. lanceolata* and *V. lasiopus*.

1.4.2 Specific Objectives

This study will specifically determine:

1. The *in vitro* effects of extracts of *M. lanceolata* and *V. lasiopus* on isolated guinea pig ileum.
2. The *in vitro* effects of extracts of *M. lanceolata* and *V. lasiopus* on isolated rabbit heart.
3. The effect of extracts of *M. lanceolata* and *V. lasiopus* on isolated rabbit uterus.

1.5 Significance of the study

This study provided an opportunity of establishing the effects of these plant extracts on various organ tissues of experimental animals. This is important in confirming the scientific rationale for use of these plants in traditional medicine. *In vitro* and *in vivo* studies in several animal species usually give a surprisingly good indication of the potential action of a drug in man, (AnokBonggo, 1974).
CHAPTER TWO
LITERATURE REVIEW

2.1 Plants as sources of medicines

A medicinal plant is any plant, which in one or more of its organs contains substances that can be used for therapeutic purposes or are precursors for the synthesis of useful drugs, (De Padua et al., 1999). Since time immemorial, people have used medicinal plants to treat diseases. They arrived at these treatments by trial and error and accumulated tradition and experience. Archaeological evidence for the use of herbal remedies goes back 60,000 years, (De Padua et al., 1999).

Medicinal plants are commonly available in abundance, especially in the tropics and people in Africa are using these to meet their health needs, (Njoroge et al., 2004). They offer the local population and others immediate access and affordable products for use in the treatment of illness through self-medication. A number of essential drugs used in modern medical practice are plant derived. Some of the well known examples include digoxin from the leaf extract of the foxglove plant digitalis purpurea, which is used in treating congestive cardiac failure, and reserpine, which is an alkaloid from plants of the genus Rauwolfia, (Laurence et al., 1997), used to treat mild hypertension. WHO recognizes that the credibility of use of medicinal plants in treatment of illnesses depends on developing an evidence base for safety and efficacy, (WHO, 2002). This means consolidating existing national and international studies and supporting new research to fill evidence gaps.
In Uganda, herbal medicine is widely used by many people in treatment of various illnesses including malaria as reported in several ethnobotanical surveys carried out in the country. The ethnobotanical survey by Mubiru et al., 1992 reported the use of *M.lanceolata* and *V. lasiopus* as some of the common plants used for treatment of common ailments.

### 2.1.1 *Vernonia lasiopus*

This is a medicinal plant that is used in traditional medicine in Africa. It belongs to the Asteraceae family. It is native in Africa in the following areas: Northeast Tropical Africa – Ethiopia and Sudan; East Tropical Africa – Kenya, Uganda, Tanzania, Burundi and Rwanda. It is a weedy plant. In Uganda, it is locally known as omujuma in Rukiga. It is used in traditional medicine in Kabale District to treat various illnesses, (Mubiru et al., 1993). It is reported to treat fever, abdominal pain, diarrhea, ascariasis, toothache, syphilis, anemia and jaundice. For most of these illnesses, its leaves are pounded and boiled alone or mixed with other herbs and given to the patient to drink after straining. In Kenya, this medicinal plant is used in the traditional treatment of malaria, (Muregi et al., 2007). The extract of *Vernonia lasiopus* has shown significant antimalarial activity in *in vitro* tests as reported by Muregi et al., 2007 and should therefore be further studied with the aim of developing a new antimalarial drug. Some of the studies done on the phytochemistry of this plant show that it contains elemanolides, (Koul et al., 2003). These elemanolides showed *in vitro* cytotoxicity against human cell lines in culture.
2.1.2 *Maesa lanceolata*

This is one of the medicinal plants used in western Uganda in treatment of various illnesses, (Mubiru, 1994). It belongs to the maesaceae family. It is known as omuhanga in Rukiga. Its leaves are used in the treatment of malaria, dysentery, ascariosis, hypertension, dermatosis, leprosy and management of difficult childbirth to augment labour. In Zulu land, the plant has multiple medicinal uses. The leaves are used to treat wounds, the roots to treat jaundice and as emetic, whereas the fruits and seeds treat intestinal worms, stomach pains and sore throats, (Glen et al., 2005). *M. lanceolata* is a very variable species as it can be a strangling shrub, 2 to 3m tall, or a small tree with branches almost at the ground level. *M. lanceolata* is usually a water side or mist belt resident because it needs plentiful and constant supply of moisture. It is found from the coast to about 1500m above sea level. In these habitats, it is found throughout the Eastern part of Africa, northwards to Arabia and across to India.

Phytochemical studies so far done on this plant have isolated triterpenoid saponins, (Sindambiwe et al., 1998) and benzoquinones, (Ilias et al., 2003) as some of the active compounds in the plant. The biological activities for saponins include (i) virucidal activity against enveloped viruses, (ii) haemolytic (iii) mollusciadal (iv) moderate fungistatic (v) antimutagenic properties and (vi) vasoconstriction. The benzoquinones have antitumour and antioxidant properties.
2.2 Pharmacological effects of plant extracts

Medicinal plants contain chemicals that alter the functioning of biological systems, thereby accounting for their usefulness in treating diseases or toxicity to humans and other animals. Studies done on many medicinal plants have established the effects of their extracts on various tissue sites in experimental animals and thus confirming the scientific basis for their use in traditional medicine.

In his pharmacological investigations on two Ugandan medicinal plants, Phytolacca dodecandra and Solanum terminale, Anokbonggo (1974) demonstrated, using in vitro tests, that extracts from these two plants caused contractions of uteri of various species and thus could justify the common use of these plants in labour in traditional medicine.

In the United States of America, lyophilized, partially purified extracts of the stem juice of the banana plant (Musa species) were found to augment and then block directly and indirectly evoked contractions of the mouse diaphragm and these pharmacological effects justify the use of banana stem juice as an arrow poison by African tribesmen (Sing et al., 1993).

In Iran, Heydar Parsaee et al., (2006) investigated the antispasmodic and antinociceptive effects of Teucrium polium aqueous extract. The extract inhibited acetylcholine induced contraction of the guinea pig ileum. The antinociceptive effect was also confirmed in mice. These findings showed that this medicinal plant may have clinical benefits for gastrointestinal disorders.
In Nigeria, an aqueous extract of the seed of *Acacia nilotica* was investigated for its pharmacological profile on the isolated guinea pig ileum, (Amos *et al.*, 1999). The extract displayed sustained dose-related contractile activity. The contractions were reduced by atropine and completely abolished by nifedipine. The intravenous administration of the extract to anesthetized cats produced a dose related significant elevation of blood pressure. All these studies are important in establishing the scientific rationale for the use of these extracts in treating diseases, thereby promoting evidence-based use of herbal medicines. A pharmacological profile is constructed for each plant extract basing on the effects of the extract on different isolated tissues together with *in vivo* studies. The clinical usefulness of the extract depends on its profile of activity on the various tissues. Non-beneficial effects of an extract can also be predicted using the results of these studies and decisions made whether to proceed with clinical trials in the process of developing a new drug that would benefit humans.

### 2.3 Investigation of pharmacological effects of plant extracts

Some common investigations involve qualitative and quantitative experiments. The objective is to determine whether the extract has any pharmacological activity and then compare the activity with that of standard drugs. The final stage is the determination of the mode of action of the compound. For these studies, both *in vitro* and *in vivo* preparations are used. In general, one commences with *in vitro* preparations because they enable a particular system to be studied without the complications of reflexes and multiple actions throughout the body. Activity observed *in vitro* should always be confirmed by *in vivo* studies. This is because in addition to the possible complications
mentioned above, the factors of absorption, distribution and metabolism are also relevant in the whole animal. There are a large number of *in vitro* preparations and the investigator is faced with a wide choice. In the present investigation, the traditional use of the plants suggested activity on the heart, uterus and the ileum. For each type of smooth muscle, it is necessary to study tissues from more than one species (Gosh, 1984). Toxicity studies are then performed on the plant extract. This is because the active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects (Marcus *et al.*, 2005).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials
The materials used in this study included plants, live animals, equipment, glassware and Petri dishes, chemicals and solvents.

3.1.1 Plants
The medicinal plants *M. lanceolata* and *V. lasiopus* were selected for this study on the basis of their traditional use and extent of use in western Uganda in the treatment of malaria and other illnesses. The plants were collected from western Uganda and extraction done using petroleum ether, chloroform and ethanol.

3.1.2 Animals
White New Zealand Rabbits and guinea pigs were used. Tissues from these animals were used in the experiments. Guinea pigs were laboratory bred from Natural Chemotherapeutic Research Laboratory, Wandegeya. The rabbits were acquired from the community.

The tissues included (i) the guinea pig ileum to study the effect of the extracts on its contractions (ii) the rabbit uterus to compare the effect of the extracts with oxytocin on the functioning of the uterus (iii) the rabbit heart to determine the effect of the extracts on the force of contraction in comparison with adrenaline.
3.1.3 Chemicals

Various chemicals were used in the experiments. These are presented below in Table 1 with their uses.

**Table 1: List of chemicals used in the study**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacturer</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride</td>
<td>BDH chemicals Ltd (Poole England)</td>
<td>Constituent of physiological solution</td>
</tr>
<tr>
<td>Ethanol</td>
<td>BDH chemicals Ltd (Poole England)</td>
<td>Used as extraction solvent</td>
</tr>
<tr>
<td>Glucose</td>
<td>UNILAB, Kenya Ltd</td>
<td>Constituent of physiological solution</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>Hopkin and Williams Ltd (England)</td>
<td>Constituent of physiological solution</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>BDH chemicals Ltd (Poole England)</td>
<td>Extraction solvent</td>
</tr>
<tr>
<td>Chloroform</td>
<td>BDH chemicals Ltd (Poole England)</td>
<td>Extraction solvent</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>BDH chemicals Ltd (Poole England)</td>
<td>Constituent of physiological solution</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>UNILAB, Kenya Ltd</td>
<td>Constituent of physiological solution</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Merck, Darmstadt (Germany)</td>
<td>Constituent of physiological solution</td>
</tr>
</tbody>
</table>
Table 2: Composition of Locke’s solution for Rabbit heart and uterus (20L)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount in grammes per solution (20L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>180g</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>8.4g</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>4.8g</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>3.0g</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>20g</td>
</tr>
<tr>
<td>Glucose</td>
<td>20g</td>
</tr>
</tbody>
</table>

Table 3: Composition of Tyroide’s solution for guinea pig ileum

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount in grammes per 10L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>80.0g</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>2.0g</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>2.0g</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>10.0g</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>1.0g</td>
</tr>
<tr>
<td>Sodium dihydrogen sulphate</td>
<td>0.5g</td>
</tr>
</tbody>
</table>

Table 4: Standard reference drugs used in the study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Manufacturer</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine bromide</td>
<td>Sigma chemical company (USA)</td>
<td>Muscarinic agonist</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>Sigma chemical company (USA)</td>
<td>Adrenoceptor agonist</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Sigma chemical company (USA)</td>
<td>Uterine muscle stimulant</td>
</tr>
</tbody>
</table>
Stock solutions of these drugs were prepared in distilled water and serial dilutions made to the required concentrations.

3.1.4 Equipment

Various equipments and glassware were used in extraction and separation of extracts. Conical flasks were used for cold extraction. Round-bottomed flasks and Liebig’s condensers were used for distillation. The solvents were dried under reduced pressure using a rotatory evaporator (BUCHI scientific equipments). An assembly of equipment for recording of contractions of isolated tissues was used. This consisted of (a) a water bath made of glass fitted with an electrical heater with thermostatic control; (b) a cylindrical organ bath made of glass of 20ml capacity for suspending the tissue in physiologic salt solution; (c) a coil made of glass connected to the lower end of the organ bath by means of a short length of rubber tubing to keep the salt solution warm before entering the organ bath (d) an oxygen tube cum tissue holder made of glass; (e) a writing lever; and (f) adjustable clamps, holders, grips etc, for holding the oxygen tube, writing lever and thermometer in position. Contractions were recorded by the writing lever on a smoked paper fixed round an electrically operated recording drum.

3.2 Methods

3.2.1 Collection and identification of plant specimens

Plant specimens without defects or parasitic infection were collected during day light from Mbarara district with the help of a taxonomist. Voucher specimens are deposited at the National Herbarium, Makerere University, Uganda. Leaves of both *Maesa lanceolata* and *Vernonia lasiopus* were collected.
3.2.2 Processing and extraction of plant material

Leaves of both Plants were dried at room temperature to constant dry weight. They were ground to powder using a metallic mortar and a pestle. The powdered materials were extracted by cold maceration through sequential solvent extraction using petroleum ether (fraction 1), chloroform (fraction 2), and ethanol (fraction 3). The solvents were removed using a rotatory evaporator (BUCHI scientific equipments).

3.2.3 Preparation of extract stock solution

2 grams of the ether and chloroform extract was dissolved in 4ml of 100% dimethyl sulphoxide (DMSO) and distilled water added to make 10ml of 200mg per ml of stock solution. For the ethanolic fraction, 2g of extract was added to 10ml of distilled water to make a concentration of 200 mg per ml.

3.3. Pharmacological studies

Experimental methods as documented by Ghosh [1984] were used. At least 4 doses per experiment were used with each dose tested in triplicate on the isolated tissue. An average of five animals was sacrificed to perform the recommended number of experiments.

3.3.1 Effect of extracts on perfused heart

The method of Langendorf (1895), as cited by Burn (1952) was used. In this method, the aorta of the isolated heart is secured to a canula through which warm (37°C) oxygenated Locke’s solution is supplied at constant pressure. The aortic valves are closed by this pressure and the solution perfuses through the coronary circulation.
A rabbit was sacrificed by a blow at the neck; the throat was cut and allowed to bleed. The chest was opened and ribs cut away to expose the heart completely. The heart was removed rapidly and placed in a Petri dish containing Locke’s solution. A cut was made in the aortic arch and then tied securely to the canula. A hook was attached to the apex of the ventricular wall and thread from this hook was led via two pulleys to a lever with frontal writing pen, which recorded the heart contractions on a smoked drum of the Kymograph. The extracts and adrenaline were administered through the side arm of the canula. The perfusion fluid was dosed with adrenaline starting with 0.5µg in 2-fold increasing doses (0.5, 1, 2, 4µg) and the recordings of the amplitude of the contractions taken by the writing pen on tracing paper. After the effect of adrenaline was observed, the experimental procedures above were repeated for both extracts using various doses as shown in the next chapter. The heights on the tracings were measured and recorded.

3.3.2 Effect of extracts on the rabbit uterus

A rabbit was sacrificed and the abdomen opened. Two horns of the uterus were dissected and transferred to Locke’s solution at 37°C. One of the horns was cut into small pieces of about 3cm and mounted in a 20ml organ bath containing Locke’s solution and aerated with oxygen. The solution was dosed with increasing doses of oxytocin and the procedure repeated with each of the extracts. The responses of the muscle were recorded on smoked paper using a frontal writing lever. From the tracings, the effects of the extracts on the uterus were compared to that of oxytocin. The heights in millimeters were measured and compared.
3.3.3 Effect of extracts on guinea pig ileum

Healthy adult guinea pigs were used. The animals were sacrificed by head blow, neck vessels cut and the animal bled. The abdomen was opened and the mesentery was cut away from the intestine. The intestine was cut across and the lumen of the isolated piece thoroughly cleaned by running warm Tyroide’s solution. The clean strip of the intestine was then placed in fresh warm salt solution for a short period for acclimatization before being put up. Pieces of the ileum 2.5-3cm were cut and put in a 20ml organ bath. One end was tied to the hook of the aerator, and the other to the end of a frontal writing lever. Temperature of Tyroide’s solution was kept at 37°C. Submaximal contractions were obtained with acetylcholine starting with a dose giving a bath concentration of 0.01µg/ml. Contact time was 30 seconds and then repeated every 3 minutes in 10-fold increasing doses. The above experiment was repeated for both extracts and recorded. Each experiment was repeated three times.

To ensure good quality results during these experiments, some measures were taken. These included:

- the use of freshly prepared physiological solutions for the organ baths,
- maintenance of the tissues at the correct temperatures, and
- handling of the tissues with care during removal from the donor and mounting in the organ bath, while avoiding undue tension on the tissues.

3.4 Statistical Analysis

The results were expressed as the mean ± SEM (Standard error of mean) for each group of experiments and these are presented in form of Tables and LogDose Response curves where possible. The mean % response of the extracts and the
respective controls were compared using Prism Graph Pad. The results of *M. lanceolata* extract were compared to the strength of contraction of the isolated rabbit heart, rabbit uterus and guinea pig ileum in the absence of the extract by performing the paired student t test, using SPSS-Version 13. Statistical significance was considered at P<0.05.

3.5 **Ethical and intellectual property issues**

International guidelines set by WHO governing the use of animals in experiments were conformed to. The animals were handled with utmost care before the experiments. At the time of the experiments the animals were killed by a humane method which involved painless killing. Findings of this study will be disseminated to the scientific Community and to the users of these medicinal plants in treatment of various ailments.
CHAPTER FOUR

RESULTS

The effects of the extracts of *M. lanceolata* and *V. lasiopus* on the isolated tissues were studied and compared to the effects of the standard drugs adrenaline, oxytocin and acetyl choline. The ethanolic, chloroform and ether extracts of *M. lanceolata* caused contraction of the isolated rabbit heart. The extracts of *V. lasiopus* did not affect the contractile force of the isolated rabbit heart. The ethanolic extract of *M. lanceolata* caused contraction of the isolated rabbit uterus whereas the ether and chloroform extracts of *M. lanceolata* and all the extracts of *V. lasiopus* did not cause contraction of the isolated rabbit heart. The ether extract of *M. lanceolata* caused contraction of the isolated guinea pig ileum whereas the ethanolic and chloroform extracts of *M. lanceolata* and all the extracts of *V. lasiopus* did not cause contraction of the ileum. All extracts of *M. lanceolata* and *V. lasiopus* did not inhibit the contraction of guinea pig ileum induced by acetyl choline.

4.1 Effect of adrenaline on the isolated rabbit heart

The effects of Adrenaline on isolated rabbit heart are presented in Table 5. Responses were measured in millimeters and maximum response at the dose of 4.0µg was used to calculate the percentage response at each dose.
Table 5: Effect of Adrenaline on isolated rabbit heart. Results are based on 3 experiments.

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Response (millimeters)</th>
<th>Percentage response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>9.6 ± 0.5</td>
<td>70.7 ± 1.2</td>
</tr>
<tr>
<td>1.0</td>
<td>11.3 ± 0.5</td>
<td>82.9 ± 3.7</td>
</tr>
<tr>
<td>2.0</td>
<td>12.7 ± 0.5</td>
<td>92.6 ± 0.3</td>
</tr>
<tr>
<td>4.0</td>
<td>13.6 ± 0.5</td>
<td>100 ± 0.0</td>
</tr>
</tbody>
</table>

The values of strength of contraction of the rabbit heart before dosing with adrenaline were recorded as 9mm, 10mm, 10mm and 10mm. Results in the table, show that as the dose of adrenaline increases, the strength of contraction also increases. Adrenaline has a direct myocardial stimulant effect that increases the strength of ventricular contraction (positive inotropic effect). It also increases heart rate (positive chronotropic effect). The strength of contraction was determined by measuring the height in millimeters of the contraction on the tracing.
4.2 Effect of extract of *M. lanceolata* on isolated rabbit heart

4.2.1 Effect of the ethanolic extract

The results of effect of the ethanolic extract of *M. lanceolata* are presented in Table 6. These results show that as the dose of extract increased, the response of the heart contraction as measured in millimeters also increased. This is also well illustrated in Figure 1.

**Table 6: Effect of the ethanolic extract of *M. lanceolata* on isolated rabbit heart.** Results are based on 3 experiments.

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Response (mm)</th>
<th>Percentage response</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11 ± 1.0</td>
<td>44 ± 3.2</td>
</tr>
<tr>
<td>5</td>
<td>13 ± 0.9</td>
<td>52 ± 3.3</td>
</tr>
<tr>
<td>10</td>
<td>15.3 ± 1.4</td>
<td>61.4 ± 6.7</td>
</tr>
<tr>
<td>20</td>
<td>16.7 ± 1.1</td>
<td>66.7 ± 4.9</td>
</tr>
<tr>
<td>40</td>
<td>17.3 ± 0.6</td>
<td>69.3 ± 3.4</td>
</tr>
<tr>
<td>50</td>
<td>21.6 ± 0.5</td>
<td>86.8 ± 5.3</td>
</tr>
<tr>
<td>100</td>
<td>23 ± 0.5</td>
<td>93.4 ± 2.0</td>
</tr>
<tr>
<td>200</td>
<td>25 ± 0.9</td>
<td>100 ± 0.0</td>
</tr>
</tbody>
</table>
The ED$_{50}$ estimated from the dose response curve is $4.51\mu$g/ml for the extract. The gradient for the log dose response curve of adrenaline is 0.57 and 0.68 for the *M. lanceolata* curve. The strength of contraction of the isolated rabbit heart in the presence of the ethanolic extract was significantly greater than the strength of contraction in the absence of the *M. lanceolata* extract, ($P = 0.002$). The tracing showing the effect of the ethanolic extract of *M. lanceolata* on isolated rabbit heart is displayed in Appendix 1.
4.2.2 Effect of the chloroform extract of *M. lanceolata* on isolated rabbit heart

Table 7: Effect of chloroform extract of *M. lanceolata* on isolated rabbit heart

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Response (mm)</th>
<th>Percentage response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>14 ± 0.9</td>
<td>76.5 ± 7.2</td>
</tr>
<tr>
<td>5</td>
<td>16 ± 0.94</td>
<td>87.3 ± 5.9</td>
</tr>
<tr>
<td>10</td>
<td>17 ± 0.9</td>
<td>92.7 ± 5.9</td>
</tr>
<tr>
<td>20</td>
<td>18.3 ± 0.5</td>
<td>100 ± 0.0</td>
</tr>
</tbody>
</table>

Figure 2: Dose response curves of chloroform extract of *M. lanceolata* and adrenaline on isolated rabbit heart

The results as presented in Table 7 and Figure 2 show that as the dose of chloroform extract of *M. lanceolata* was increased, the strength of contraction of the heart also increased as measured in millimeters. The strength of contraction of the isolated rabbit heart in the presence of the chloroform extract of *M. lanceolata* was significantly greater.
than the strength of contraction in the absence of the *M. lanceolata* extract, ( P = 0.003). The gradient of the *M. lanceolata* log dose response curve is 0.25.

### 4.2.3 Effect of the ether extract of *M. lanceolata* on isolated rabbit heart

**Table 8:** Showing results of effect of ether extract of *M. lanceolata* and adrenaline on isolated rabbit heart.

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Response (mm)</th>
<th>Percentage response</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>13.3 ± 0.6</td>
<td>72.3 ± 4.2</td>
</tr>
<tr>
<td>5</td>
<td>15 ± 0.9</td>
<td>81.9 ± 5.5</td>
</tr>
<tr>
<td>10</td>
<td>16 ± 0.94</td>
<td>87.1 ± 5.9</td>
</tr>
<tr>
<td>20</td>
<td>17 ± 0.9</td>
<td>92.7 ± 6.0</td>
</tr>
<tr>
<td>40</td>
<td>18.3 ± 0.54</td>
<td>100 ± 0.0</td>
</tr>
</tbody>
</table>

*Figure 3: Dose response curves of ether extract of *M. lanceolata* and adrenaline on isolated rabbit heart*
The above results show that as the dose of ether extract increases, strength of contraction measured in millimeters also increased. The strength of contraction of the isolated rabbit heart in the presence of the ether extract of \textit{M. lanceolata} was significantly greater than the strength of contraction of the isolated rabbit heart in the absence of the ether extract, \( P = 0.007 \). The gradient of log dose response curve of \textit{M. lanceolata} ether extract was estimated to be 0.57. Appendix 2 shows the results of this experiment.

4.3 Effect of extracts of \textit{V. lasiopus} on the isolated rabbit heart

4.3.1 Effect of the ether extract of \textit{V. lasiopus} on the isolated rabbit heart

The strength of contraction of the heart was 6mm at all doses of extract, and this was the strength of contraction of the heart before commencing dosing with the extract. These results show that the extract caused no change in strength of contraction of the heart.

4.3.2 Effect of the chloroform extract of \textit{V. lasiopus} on the isolated rabbit heart

The chloroform extract caused no change in the strength of contraction of isolated rabbit heart. The strength of contraction before and after dosing with different concentrations of the extract remained the same.

4.3.3 Effect of the ethanolic extract of \textit{V. lasiopus} on the isolated rabbit heart.

There was no change in the strength of contraction of isolated rabbit heart with all the doses of the extracts.

4.4 The effect of oxytocin on the isolated rabbit uterus.

The results of the study of effect of oxytocin are presented in Table 9 below.
Table 9: Effect of oxytocin on the isolated rabbit uterus

<table>
<thead>
<tr>
<th>Dose (i.u)</th>
<th>Response (mm)</th>
<th>% Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>42.3 ± 3.6</td>
<td>92.7 ± 3.8</td>
</tr>
<tr>
<td>0.04</td>
<td>38 ± 1.9</td>
<td>88 ± 8.7</td>
</tr>
<tr>
<td>0.08</td>
<td>37.3 ± 0.5</td>
<td>86.3 ± 6.8</td>
</tr>
<tr>
<td>0.16</td>
<td>41.3 ± 0.5</td>
<td>95.3 ± 3.8</td>
</tr>
</tbody>
</table>

The results above show that oxytocin causes contraction of the rabbit uterus.

4.5 Effect of extracts of *M. lanceolata* on the isolated rabbit uterus.

4.5.1 Effect of ether extract of *M. lanceolata* on isolated rabbit uterus

The ether extract had no effect on the isolated rabbit uterus. Various doses were administered to the bath fluid and no contraction of the uterus at any dose was registered. The bath fluid concentrations of extract administered were 0.1, 1.0, 10, 50, 100, and 1000 µg/ml.

4.5.2: Effect of the chloroform extract of *M. lanceolata* on isolated rabbit uterus.

The chloroform extract of *M. lanceolata* did not elicit contractions of the isolated rabbit uterus. The bath fluid concentrations of extract used were 0.1, 1.0, 10, 50, 100, and 1000 µg/ml.
4.5.3: Effect of the ethanolic extract of *M. lanceolata* on isolated rabbit uterus.

The ethanolic extract of *ML* elicited contractions of the isolated rabbit uterus at various doses.

**Table 10: Effect of the ethanolic extract of *M. lanceolata* on isolated rabbit uterus.**

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Response (mm)</th>
<th>% Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>31.7 ± 1.4</td>
<td>80.7 ± 3.0</td>
</tr>
<tr>
<td>100</td>
<td>37.3 ± 1.4</td>
<td>95 ± 4.7</td>
</tr>
<tr>
<td>1000</td>
<td>39.3 ± 0.5</td>
<td>100 ± 0.0</td>
</tr>
</tbody>
</table>

These results show that the ethanolic extract of *Maesa lanceolata* elicits contraction of the isolated rabbit uterus. The strength of contraction of the isolated rabbit uterus in the presence of the ethanolic extract of *M. lanceolata* was significantly greater than the strength of contraction of the isolated rabbit uterus in the absence of *M. lanceolata* extract, *P* = 0.077.

4.6    Effect of extracts of *V. lasiopus* on the isolated rabbit uterus.

4.6.1    Effect of the ether extract on isolated rabbit uterus

The ether extract of *V. lasiopus* did not elicit contractions of the isolated rabbit uterus. Doses of extract used ranged between 0.1 - 1000µg/ml.

4.6.2    Effect of the chloroform extract of *V. lasiopus* on isolated rabbit uterus

The chloroform extract of *V. lasiopus* did not elicit contractions of the isolated rabbit uterus.
4.6.3 Effect of the ethanolic extract of *V. lasiopus* on isolated rabbit uterus

The ethanolic extract of *V. lasiopus* did not elicit contractions of isolated rabbit uterus.

4.7 Effect of Acetylcholine on guinea pig ileum

The results of this study are presented in Table 11 below.

**Table 11: Showing results of effect of acetylcholine on guinea pig ileum**

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Response (mm)</th>
<th>% Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3.3 ± 0.5</td>
<td>21.33 ± 4.6</td>
</tr>
<tr>
<td>50</td>
<td>10.7 ± 0.5</td>
<td>67 ± 5.0</td>
</tr>
<tr>
<td>100</td>
<td>12.7 ± 0.5</td>
<td>79.33 ± 6.3</td>
</tr>
<tr>
<td>1000</td>
<td>16 ± 0.9</td>
<td>100 ± 0.0</td>
</tr>
</tbody>
</table>
Figure 4: Log Dose response curves of acetylcholine and ether extract of *M. lanceolata* on isolated guinea pig ileum.

The results above show that acetylcholine causes contraction and the strength of contraction increases with the dose. The strength of contraction of the isolated guinea pig ileum in the presence of the ether extract of *M. lanceolata* was significantly greater than the strength of contraction of the guinea pig ileum in the absence of the ether extract of *M. lanceolata*, *(P = 0.002)*.

### 4.8 Effect of *M. lanceolata* on isolated guinea pig ileum

#### 4.8.1 Effect of the ether extract of *M. lanceolata* on guinea pig ileum

The ether extract elicited contractions on isolated guinea pig ileum. Minimum dose of extract used was 50µm/ml and the maximum was 750µg/ml bath fluid. This extract did not inhibit contractions of the ileum induced by acetylcholine. From Figure 4 above, the
gradient of the log dose response curve of *M. lanceolata* ether extract is 0.29 while that of Acetycholine curve is 1.19.

### 4.8.2 Effect of the chloroform extract of *M. lanceolata* on isolated guinea pig ileum.

The chloroform extract did not cause contraction of the isolated guinea pig ileum. This extract did not inhibit contractions of the ileum induced by acetycholine.

### 4.8.3 Effect of the ethanolic extract of *M. lanceolata* on isolated guinea pig ileum

The ethanolic extract of *M. lanceolata* did not cause contraction of the isolated guinea pig ileum. Maximum dose administered to the bath fluid was 1mg/ml. This extract did not inhibit guinea pig ileum contractions induced by acetycholine.

### 4.9 Effect of extracts of *V. lasiopus* on isolated guinea pig ileum.

#### 4.9.1 Effect of ether extract on isolated guinea pig ileum.

The results indicate that the ether extract of *V. lasiopus* did not elicit contractions of the guinea pig ileum. This extract did not inhibit contractions of the ileum induced by acetycholine.

#### 4.9.2 Effect of the chloroform extract of *V. lasiopus* on isolated guinea pig ileum

The results show that the chloroform extract of *V. lasiopus* did not elicit contractions of the guinea pig ileum. The extract did not inhibit contractions of the guinea pig ileum induced by acetycholine.

#### 4.9.3 Effect of the ethanolic extract of *V. lasiopus* on the isolated guinea pig ileum.

The results show that the ethanolic extract of *V. lasiopus* did not cause contraction of isolated guinea pig ileum. Contractions of the guinea pig ileum induced by acetycholine was not affected by the extract.
CHAPTER FIVE

DISCUSSION

This study was designed to investigate the pharmacological effects of the leaf extracts of *V. lasiopus* and *M. lanceolata*, two medicinal plants that are used by people in East Africa in the treatment of malaria and other illnesses. The results of this study indicate that the three extracts of *M. lanceolata* cause increase in the strength of contraction of isolated rabbit heart. Statistical analysis of results showed that the force of contraction in the presence of *M. lanceolata* extracts was significantly greater than force of contraction in the absence with $P < 0.05$. The increase in strength of contraction induced by these extracts was dose dependent. The log dose response curves of adrenaline and the extracts of *M. lanceolata* were plotted and their slopes determined. The slope of the adrenaline curve was significantly different from the slopes of the curves of the three extracts implying that the extracts probably do not act on β receptors of the heart. From the log dose response curves, it can also be seen that adrenaline is more potent than the extracts of *M. lanceolata*. The potency of these extracts may be improved by identifying and isolating the active compounds From Figure 2 and 3, the ED50 cannot be estimated directly from the curves. However ED 50 can be established by deriving the best fit line using a computer statistical program.

Results also indicate that the ethanolic extract of *M. lanceolata* causes contraction of the rabbit uterus. The ether and chloroform extracts of *M. lanceolata* did not elicit contractions of the isolated rabbit uterus. The ether extract of *M. lanceolata* caused contraction of the isolated guinea pig ileum. The chloroform and ethanolic extracts of *M.
Lanceolata on the other hand did not cause contraction of the isolated guinea pig ileum. Study findings also show that the extracts of V. lasiopus had no effect on the strength of contraction of the isolated rabbit heart. Extracts of V. lasiopus also did not elicit contraction of the isolated rabbit uterus and isolated guinea pig ileum. The extracts of M. lanceolata and V. lasiopus did not inhibit contraction of the isolated guinea pig ileum induced by acetylcholine.

Maesa saponins isolated from M. lanceolata are responsible for some of the pharmacological effects of this medicinal plant (Francis G. et al., 2002). Saponins are well known as antifungal and antiviral agents. Saponins are also reported to have effects on the cardiovascular system. They cause vaso constriction and increased blood pressure (Anokbbongo, 1974). Maesa saponins may be responsible for the effect of extracts of M. lanceolata on the strength of contraction of the isolated heart. However, phytochemical and other studies will have to be done in future to confirm this. Catecholamines like adrenaline activate β₁ receptors of the heart leading to Ca²⁺ channels opening and thus increasing Ca²⁺ entry which increases force of cardiac contraction (Rang and Dale 1999). Other drugs so far in clinical use that increase cardiac contractility act by directly or indirectly leading to an increase in Ca²⁺ concentration intracellularly (Rang and Dale 1999). These include the cardiac glycosides e.g. digoxin. The mechanism of action of active compounds of the extracts of M. lanceolata will have to be determined in future studies.
The results of this study show that the ethanolic extract of *M. lanceolata* caused contraction of the isolated rabbit uterus. The amplitude of the contractions caused by the extract increased with dose of the extract. The use of this medicinal plant is western Uganda to augment labour may therefore have a scientific basis if the study findings are reproducible in man. Oxytocin contracts the uterus in all species (Baruga hare, 1999) and this was confirmed in this study. The uterus possesses both α and β adrenoceptors and oxytocin receptors (Rang and Dale, 1999). Stimulation of α- adrenoceptors by some drugs leads to uterine contraction. For example the drug ergotamine acts on these receptors to cause uterine contraction. It is probable that a compound in the extract of *M. lanceolata* could be acting in the same manner. Oxytocin causes uterine contraction by acting on its receptors. The physiological and pharmacological responses of the uterus vary at different stages of the menstrual cycle and pregnancy in humans, (Rang and Dale, 1999). This is due to hormonal changes that regulate the excitability of the uterine muscle. Estrogens have a definite tendency to increase the degree of uterine contractility partly because estrogens increase the number of gap junctions between the adjacent uterine smooth muscle cells (Guyton *et al.*, 2000). Progesterone on the other hand inhibits uterine contractility during pregnancy, thereby helping to prevent expulsion of the fetus. It has been postulated that the estrogen to progesterone ratio increases sufficiently toward the end of pregnancy and is responsible for the increased contractility of the uterus (Guyton *et al.*, 2000). In this study the extracts were tested on isolated pieces of the non pregnant uterus and therefore it was impossible to investigate the effect of the extracts on the hormones that regulate uterine contractility. This is a limitation of this study. However, with *in vivo* testing, the extracts could be given to the animals for a
given number of days and then histo chemical changes determined. This would help to further understand the mechanism of action of the active compound in \textit{M. lanceolata} that causes contraction of the uterus.

The ether extract of \textit{M. lanceolata} was found to cause contraction of isolated guinea pig ileum. The use of \textit{M. lanceolata} in the treatment of diarrhea may be due to other pharmacological properties of the plant other than relaxation of the gut. Results also show that acetylcholine caused contraction of the isolated guinea pig ileum. Acetylcholine stimulates muscarinic receptors of the ileum causing contraction. From the log dose response curves of acetylcholine and the ether extract of \textit{M. lanceolata} as shown in figure four, acetylcholine was more potent than the extract. Experimental procedures to determine the site of action of the active compound in the ether extract of \textit{M. lanceolata} were not carried out and therefore, no comment can be made on that issue. The extracts of both plants did not inhibit contraction of isolated guinea pig ileum induced by acetylcholine nor did they cause relaxation. This implies that they are not anti-spasmodic and their use in treating diarrhoea and dysentery may be due to other pharmacological effects of these extracts.

Results obtained on isolated tissues are not always reproducible when tested on whole animals (Gosh., 1984). This is because the factors of absorption, metabolism, excretion or interference due to nerve reflexes operate in the whole animal. This is a limitation of this study. Another limitation of this study is that due to genetic variation in response to drugs by different species, it is difficult to directly translate the results of this study to other animal species or to man. Two species of animals were used to provide isolated
tissues for this study and this is also a limitation. With more species used, more reliable predictions of the possibility of the effects observed in a study being the likely effects in man can be made.
CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The extract of *M. lanceolata* causes increase in the strength of contraction of the rabbit heart which may well be the effect in man. This could lead to increased blood pressure on the basis of the formular $BP = CO \times TPR$ where $BP =$ Blood pressure, $CO =$ Cardiac output, $TPR =$ Total peripheral resistance, and cardiac output depends partly on strength of contraction of the heart. Therefore the use of this plant in treating hypertension by people in Western Uganda may have no scientific rationale. *M. lanceolata* may be useful in treating some types of cardiac failure because of its positive inotropic effect on the heart. The ethanolic extract of *M. lanceolata* causes contraction of the isolated rabbit uterus. This effect may well be the same in man and therefore this plant may be of clinical benefit as an oxytocic drug. The use of this medicinal plant in managing difficult deliveries by people in Western Uganda may therefore have a scientific basis. The use of *M. lanceolata* may be associated with abdominal pain and diarrhea since the ether extract was shown to cause contraction of the isolated guinea pig ileum. Both *M. lanceolata* and *Vernonia lasiopus* did not inhibit the contraction of the isolated guinea pig ileum and therefore their use in treatment of gastrointestinal disorders like diarrhea and dysentery may be due to anti-microbial effects.

6.2 Recommendations

In vivo studies using different species of animals including primates should be performed for both plant extracts to confirm the pharmacological effects observed in this study. The
The effect of *M. lanceolata* on blood pressure should be studied using the anaesthetized cat model to determine the effect of this extract on blood pressure, (Gosh M.N, 1984). The use of *M. lanceolata* should be avoided in patients with cardiac disease and hypertension. Its use should also be avoided in pregnant women. However, *M. lanceolata* should be investigated for the treatment of constipation. Phytochemical and other studies to isolate the compounds responsible for the effects of *M. lanceolata* should also be done. Further studies to determine possible mechanisms of action of *M. lanceolata* and to establish safety profiles of the extract should be done. Conservation of these medicinal plants should also be undertaken since they could provide useful drugs for treating various diseases.
7.0 REFERENCES


APPENDICES

Appendix 1: Effect of the ethanolic extract of M. lanceolata on the isolated rabbit heart.

The tracing shows that as the dose of extract increased, there was an increase in the response as measured in mm.
Appendix 2: Effect of the ether extract of *M. lanceolata* on an isolated rabbit heart

The tracing above shows the effect of the ether extract of *M. Lanceolata* on isolated rabbit heart. The response of the heart increased with increase in dose of the extract.