ANTIFERTILITY PROPERTIES OF SELECTED KENYAN MEDICINAL PLANTS

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ZOOLOGY (ANIMAL PHYSIOLOGY) OF SCHOOL OF SCIENCE OF UNIVERSITY OF ELDORET, KENYA.

2013

DECLARATION

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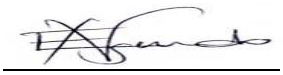
Sign  Date _____

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DEDICATION

To the many gifts bestowed on us by the almighty God, including knowledge and intellect. This work is also dedicated to my parents Mr. and Mrs. Dominic Kamita who gave me the opportunity to pursue my studies. They are the ones who made me believe that mind can achieve what it conceives or believes.

ABSTRACT

It has been reported that many deaths occur in developing countries due to pregnancy related complications as well as during illegal abortions. The abortions are mainly due to unplanned pregnancies that can be avoided through use of birth control methods. People living in the rural areas of Kenya have poor access to conventional healthcare facilities and hence heavily rely on natural methods or medicinal plants extracts as birth control agents due to their affordability and accessibility. In addition, the affordable conventional contraceptives are associated with undesirable side effect. Medicinal plants are reported in folklore to play a role as contraceptives, but these claims and/or the mechanisms of action have not been demonstrated scientifically. Very few studies have been carried out to confirm the safety and efficacy of medicinal plants used as anti-fertility agents. The aim of this study was therefore to screen selected medicinal plant extracts for anti-fertility activity, effect on the oestrus cycle as well as on the weight of the ovaries and uterus in mouse model. The acute toxicity of the active extracts in mice was determined, as well as, the determination of the chemical profiles of the bioactive extracts. Extraction of *Moringa oleifera* (aerial, seed, root bark, twigs and stem bark), *Terminalia brownii* (stem bark), *Ximenia americana* (leaves, stem bark and root bark), *Bridelia micrantha* (aerial parts), *Lippia kituensis* (root), *Rhoicissus revoilii* (aerial parts and roots), and *Ocimum masaiense* (aerial parts and roots) parts was carried out using water, methanol, ethyl acetate, dichloromethane and petroleum ether. The extracts were then administered to mice at a dose of 800 mg/kg for anti-fertility experiment and in determination of their effect on ovary and uterus weight as well as oestrus cycle. Acute toxicity was tested by administering a dose of 0 to 5000 mg/kg orally. From the study, the extracts of the leaves of *Bridelia micrantha*, and *Ximenia americana* and the seeds of *Moringa oleifera* were shown to have reversible anti-fertility effect at a dose of 800 mg/kg when administered orally to female mice. The stem bark of *Terminalia brownii* had an irreversible anti-fertility effect at a dose of 800 mg/kg when administered orally. The study on the effect of the active extracts on the oestrus cycle exhibited an arrest of the normal oestrus cycle at either the diestrous or the proestrous phase. The presence of compounds such as steroids, terpenoids, alkaloids, saponins and flavonoids found in the bioactive extracts may have contributed to the anti-fertility activity. The bioactive extracts had no significant effects on the weight of both the ovaries and the uterus. The bioactive extracts did not show severe signs of toxicity at the highest concentration tested (5000 mg/kg) except *Ximenia americana* leaves extracts which had a mortality rate of 20% at 5000 mg/kg. The study provided several medicinal plant extracts that have potential to be developed into an alternative drug for birth control. The bioactive extracts may be taken for further analysis to determine their lowest effective doses as anti-fertility agents as well as their mechanisms of action.

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LIST OF ABBREVIATIONS

BMAM	*Bridelia micrantha* (Aerial, methanol extract),
BMAW	*Bridelia micrantha* (Aerial, water extract),
COC	Combined Oral Contraceptives
FWHC	Feminist Women's Health Centre
CRNBC	College of Registered Nurses of British Columbia
EE	Ethinyl Estradiol
FEM	Positive control (Femiplan)
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
IUCD	Intrauterine Contraceptive Devices
KEMRI	Kenya Medical Research Institute
LH	Luteinizing hormone
WHO	World Health Organization
CDC	Centre for Disease Control

DEFINITION OF TERMS

Adenohypophysis	Anterior pituitary
Diestrous	The period after metestrus during which the corpus letup functions and degenerates
Oestrogen	A group of compounds that play an important role in the oestrous cycle of humans and other animals. They are the primary female sex hormones responsible for reproduction and secondary sex characteristics.
Oestrous cycle	The cycle in which, oocytes mature and are ovulated periodically in most female mammals.
Oestrus	A stage of the oestrous cycle around the time of ovulation during which a female uses behaviour to indicate that she is fertile or ready for mating.
Gonadotrophin	A hormone that stimulates the gonads (ovaries or testes) to produce gametes and hormones and also supports and maintains gonadal tissue
Granulosa cells	Somatic cells surrounding the primary oocyte of an ovarian follicle
Luteinizing hormone	A hormone produced from the anterior pituitary gland responsible for ovulation in females. In males it is known as the interstitial cell stimulating hormone responsible for production of testosterone
Metestrus	The period following oestrus during which the corpus luteum develops.

Proestrous	The period of follicular development, that precedes oestrus.
Progesterone	A sex steroid hormone secreted by the corpus luteum of most vertebrates and the placenta of eutherian mammals
Prolactin	A hormone secreted by cells in the anterior pituitary that stimulates the production of milk in female. The hormone also performs a variety of functions such as water and mineral balance, caring for the young.

ACKNOWLEDGEMENT

I express my heartfelt thanks to Almighty God who always shows a way in situations where there seem to be no way. I would like to express my deep and sincere gratitude to my supervisors Dr. E. W. Njenga, Dr. Esther Matu and Dr. James Wanga for their wide knowledge and logical way of thinking that have been of great value to me. Their understanding, encouragement and personal guidance provided a good basis for the present thesis. I thank Ms. Elizabeth M. Kigondu for facilitating this research through National Council for Science and Technology (NCST) funding.

I wish to express my warm and sincere thanks to Dr. Peter Kirira, Mr. Daniel Kiboi, Beatrice Irungu, Gabriel Amalemba, and other staff member and the entire Centre for Traditional Medicine and Drug Research where the entire project was carried out. Thank you for joyfully assisting me whenever I needed help.

I also sincerely thank my wife Emma, our dear daughter Valeria, parents Mr. and Mrs. Dominic Kamita, as well as my brother, Paul and sisters, Jennifer and Regina, for their endless encouragement, prayers and financial support even during hard times. I feel appreciated and part of a great family.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Over half a million women die due to complications that result from pregnancy, childbirth as well as during illegal abortions (Paul and Judith, 2010). Most of these deaths are reported in developing countries. In 2009, about 10% to 20% of pregnancies were reported as unplanned pregnancies. Therefore, up to 100, 000 maternal deaths are avoidable if women who are not ready to conceive could use effective contraceptives or antifertility agents (World Health Organization, 2005). People living in the rural areas have poor access to conventional health centres hence they heavily rely on natural family methods or medicinal plants as birth control agents (World Health Organization, 2005). High rates of discontinuation of existing family planning methods and complaints about their discomforting side effects are indications that contraceptive methods need to be made more acceptable (World Health Organization, 1997). Family planning in the rural parts of Kenya is usually based on use of either medicinal plants or rhythm method (African Population and Health Research Center, 2001). Rhythm method also known as Knaus-Ogino method relies on estimating a woman's likelihood of fertility, based on a record of the length of previous menstrual cycles. Pregnancy is achieved by timing unprotected intercourse for days identified as fertile, or avoided by restricting unprotected intercourse to days identified as infertile. Success of rhythm method however is subjective and requires careful record keeping and diligence (Kimball, 2012). The use of medicinal plants as contraceptives has been reported (Ravichandran *et al*., 2007) but their safety and efficacy have not been carried out exhaustively.

Family planning is a key intervention for improving the health of women, men and children. It is also an important component of reproductive health and a quality family planning method is recognized as a basic human right (Ministry of Health, 2005). It has been reported that 62.9% of the women worldwide use at least a method of birth control. Out of these 56.1%, use any form of modern contraceptives while 6.7% used any form of traditional method (United Nations, 2010), yet 28% of fertile women use at least a method of birth control out of which, 21.9% use modern method while 6.2% use traditional methods. In Africa 22.2% of people, still have unmet needs for family planning (African Population and Health Research Center, 2001). The number of women, married or in union, using birth control in Kenya is still low. For instance, in 2010, only 38.9% of the African women, most of who are in the age between 15 and 49 years, used modern contraceptive methods while 7.8% used a traditional method (United Nations, 2010).

The consistency of use of contraceptives in Kenya is very dynamic (African Population and Health Research Center, 2001). Women initiate or stop use of contraceptive in response to changes in their own circumstances and in respect to their social, health, and environment. They also choose different methods at different points in their lives. The contraceptive prevalence and method mix at any given point in time is thus as a result of a whole series of decisions made by individual women to start contraceptive use, stop use, restart use, and to choose one method over another one, a practice that results in high failure rates (African Population and Health Research Center, 2001).

Through family planning, individuals especially women, and families have enjoyed good results of contraceptives by preventing a large number of unplanned pregnancies

(Guttmacher Institute, 2002). Most of these pregnancies are unwanted and of high risk. Family planning has also contributed to women's quality of life by relieving them of the burden of consecutive pregnancies (Huezo, 1998).

Most of the current contraceptives have a number of unwanted side effects. Common side effects of Combined Oral Contraceptives (COCs) include, but not limited to spotting, breakthrough bleeding, appetite changes, breast tenderness, headaches (mild, without aura), nausea, mood changes, weight changes, and libido changes (CRNBC, 2011). Major concerns involving the use of COCs are the risk of cardiovascular disease, including stroke, myocardial infarction and cancer especially the breast, cervix, and liver cancers (Huezo, 1998). The new methods such as use of coils, which are reversible are either expensive or require expertise to use. Other methods such as voluntary surgical contraception are not reversible. Major concerns relating to Intrauterine Contraceptive Devices (IUCDs) include their mechanism of action and the risk of pelvic inflammatory disease, infertility, ectopic pregnancy and expulsion (Huezo, 1998). In addition, contraceptives are not easily accessed due to lack of enough health facilities and high cost.

Financial constraints have caused most of the health facilities not to have the capacity to provide satisfactory services. Lack of drugs, medical personnel and laboratory equipment among other necessities are inadequate in many public conventional health facilities. This has resulted in most of the poor urban and rural population relying heavily on traditional medicine for solving primary health care problems. Traditional medicine offers a health alternative that is affordable, easily accessible and culturally acceptable as compared to the conventional medicine (WHO, 2004; Payyappallimana, 2008).

Due to the side effects associated with the use of conventional contraceptives and their limited accessibility, many people fail to make use of them. This has led to many unplanned pregnancies, which carry a higher risk of morbidity and mortality often due to unsafe abortions (Farrell *et al*., 2000). The unplanned pregnancy also puts the health of the mother at risk if she gives birth so often, increase economic challenges and thus lead to high poverty levels (CDC, 2012). The correct and continuous use of contraceptives during all periods of risk can greatly reduce the likelihood of unintended pregnancy. Many women though, have difficulty adhering to such a regimen over a long period (Frost *et al*., 2007).

1.2 Statement of the Problem

The number of unwanted pregnancies worldwide is growing rapidly due to increased rates of none-use as well as discontinuation of current contraceptives. There is also an increased number of health problems associated with the use of the current contraceptives including cardiovascular diseases, breast and liver cancer. Development of safer, affordable and convenient methods of contraceptive remains a challenge to many nations. Many people especially in rural areas are still using traditional medicine to meet most of their health care needs.

1.3 Justification of the Study

The use of contraceptives in Kenya is still low despite the government's initiatives to educate the public and to make them available and affordable. Uncertainty about contraception and pregnancy, side effects, difficulties using methods such as coils and lack of satisfaction with or availability of service providers are among the reasons cited for the limited success with contraception (Frost *et al*., 2007). Although the use of modern contraceptive method especially the long-term ones is higher in urban than

in rural areas, the pattern is reverse for traditional methods (Magadi and Curtis, 2004). Studies have shown that plants are potential sources of anti-fertility agents (Johri *et al*., 2009). For instance, extracts from *Moringa oleifera* Lam. Ó demonstrated anti-fertility effects in albino female rats (Johri *et al*., 2009). Determination of the effectiveness of the plants that are traditionally used as contraceptives and standardization of their use is of paramount importance to ensuring their safe use and providing affordable and acceptable alternative to the current contraceptives.

1.4 Study Objectives

1.4.1 General Objective

i. To determine anti-fertility activity of total extracts from *Moringa oleifera, Terminalia brownii, Ximenia americana, Bridelia micrantha, Lippia kituensis, Rhoicissus revoilii,* and *Ocimum masaiense,* and then determine the safety, effectiveness on the weight of ovaries and uterus as well as the effect of the bioactive extracts on the oestrous cycle.

1.4.2 Specific Objectives

i. To screen for anti-fertility activity of total extracts obtained from selected Kenyan medicinal plants in a female Swiss mice model.
ii. To determine the effect of bioactive extracts on the reproductive cycle of female mice.
iii. To determine the effect of the bioactive extract on the weight of genital organ and body weight of mice.
iv. To test bioactive extracts for acute toxicity of bioactive plant extracts in female mice.

v. To establish the phytochemical profiles of bioactive plant extracts.

1.5 Study Hypothesis

1.5.1 Null Hypotheses

i. The extracts of *Moringa oleifera, Terminalia brownii,* Ximenia *americana, Bridelia micrantha, Lippia kituensis, Rhoicissus revoilii,* and *Ocimum masaiense* have no anti-fertility activity in a female Swiss mice model.

ii. The bioactive extracts of *Moringa oleifera, Terminalia brownii*, Ximenia *americana, Bridelia micrantha, Lippia kituensis, Rhoicissus revoilii,* and *Ocimum masaiense* have no effect on the weight of the reproductive organs of female Swiss mice.

iii. The bioactive extracts of *Moringa oleifera, Terminalia brownii*, Ximenia *americana, Bridelia micrantha, Lippia kituensis, Rhoicissus revoilii,* and *Ocimum masaiense* have no effect on the reproductive cycle of female Swiss mice.

iv. The bioactive extracts of *Moringa oleifera, Terminalia brownii*, Ximenia *americana, Bridelia micrantha, Lippia kituensis, Rhoicissus revoilii*, and *Ocimum masaiense* do not show acute toxicities when administered orally to female Swiss mice.

CHAPTER TWO

LITERATURE REVIEW

2.1 Current Contraceptive Methods

There are varieties of methods used in family planning. The Kenyan Ministry of Health (2005) has divided them into hormonal contraceptive methods, intrauterine contraceptive devices (IUCD), voluntary surgical contraception, barrier methods, lactational amenorrhea method and natural family planning.

Hormonal contraceptive methods act on the endocrine system and are composed of steroid hormones. The methods include combined oral contraceptives (COCs), progestin-only pill (POPs), emergency hormonal contraception, injectable contraceptives and contraceptive implants. Combined hormonal contraception contains ethinyl estradiol (EE) and a progestin in various doses and combinations. A low-dose COC preparation is preferred to provide effective contraception, acceptable cycle control and the least side effects for individuals (CRNBC, 2011). They are commonly referred to as “The Pill” and have to be taken daily to prevent pregnancy (Ministry of Health, 2005).

There are two categories of pills. First, the low-dose pill which comes in three types, which are monophasic, biphasic and triphasic. The monophasic pills contain equal amounts of oestrogen and progestin such as Microgynon, Lo-Femenal, Nordette and Marvelon. The biphasic contain two different dose-combinations of oestrogen and progestin for example Biphasil, Ovanon, Normovlar. The triphasic has the active pills containing three different dose-combinations of oestrogen and progestin such as

Logynon and Trinordial. In Kenya, the pill is the most used contraceptive especially the low-dose pill (Ministry of Health, 2005).

The combined hormonal contraceptives are highly effective with their effect occurring immediately. They are easy to use and can be provided by trained non-clinical service provider. The method reduces menstrual flow, decrease dysmenorrhoea as well as improve and prevent anaemia. However, the method may cause venous thromboembolism such as deep vein thrombosis and pulmonary embolism (Blanco-Molina and Monreal, 2010), nausea, spotting or bleeding in between menstrual periods, mild headaches, breast tenderness, and slight weight gain (Allen, 2012). The method also causes stroke, myocardial infarction, and their effectiveness may be lowered in the presence of gastroenteritis (Ministry of Health, 2005; La Corte *et al*., 2008).

The intrauterine contraceptive device (IUCD) is a small flexible device, which is inserted into the uterine cavity. The most widely used are the copper-bearing IUCDs, which are made of plastic with copper sleeves on the arms and copper wire wound around the stem. IUCDs do not suppress milk production in breastfeeding women. The Copper IUCD prevents pregnancy by preventing sperm from fertilizing through changing the chemical environment in the uterine cavity. The IUCD makes it difficult for the egg and sperm to fuse (Ministry of Health, 2005). According to the United Nations worldwide contraceptive use, 2009 report (Sauveur, 2012; United Nations, 2010) this is the most widely used modern reversible contraceptive method.

Advantages of IUCD include immediate effectiveness when placed, low cost (Ministry of Health, 2005). It is possible to enjoy sexual intercourse without any interruption and the method is long lasting. The IUCD method does not change the

hormone level of the body and is easy to use. The disadvantages are that the IUCD does not give protection against sexually transmitted disease and requires trained personnel to insert it. The user may experience a longer, heavier and more painful periods after insertion. IUCD may lead to infection in three weeks after insertion and may lead to higher risk of pelvic inflammatory disease (PID) that can cause infertility (Jain, 2006).

Voluntary surgical contraception (VSC) includes female and male sterilization procedures that are intended to provide permanent contraception. Surgical contraception for women also known as tubal ligation (TL) involves mechanically blocking the fallopian tubes to prevent the sperm and egg from uniting. For men a process called vasectomy involves a similar operation that blocks the *vasa deferentia* to prevent sperm entering into the ejaculated seminal fluid (STARH Program, 2001). The operation is performed under local anaesthesia. The advantages of sterilization include its effectiveness, immediate contraceptive protection, and do not require follow-up or repeat visits. This method also decreases risk of ovarian cancer (Ministry of Health, 2005). However, its disadvantage is that it is irreversible and may give rise to regret (Rutagwera, 1990).

The Lactational Amenorrhea Method (LAM) is a temporary method of family planning based on the lack of ovulation resulting from exclusive breastfeeding. This is practiced during the first 6 months postpartum only when fertility is low and the infant is fed solely on breast milk (Ministry of Health, 2005). Three criteria enable women to determine their risk of pregnancy during the natural state of infertility associated with breastfeeding (Labbok *et al*., 1994). They include a breastfeeding woman without menses since delivery also known as lactational amenorrhea (which is

a lack of menses resulting from breastfeeding), a woman must fully or nearly fully breastfeed and the infant must be less than six months old (Family Health International, 2003).

In non-pregnant and non-lactating women, hormones from the pituitary gland that are regulated by the hypothalamus (Blackburn, 2007), initiate a series of other hormonal changes that cause the development and maturation of an ovarian follicle containing an ovum or egg cell. The follicle secretes oestrogen and eventually ruptures, releasing the egg cell (Scott and Fong, 2004). The ruptured follicle forms a temporary gland known as the corpus luteum and begins to secrete progesterone in addition to the oestrogen. The oestrogen and progesterone cause the lining of the uterus to thicken in preparation for the implantation of the egg cell should it be fertilized (Scott and Fong, 2004). If the egg cell is not fertilized or if it does not implant, the uterine lining is shed during menstruation (Family Health International, 2003).

This cycle of events is sometimes modified, especially during pregnancy or breastfeeding. During breastfeeding, the stimulation of the nipple by the infant when suckling sends nerve impulses (Starr and McMillan, 2010) to the hypothalamus of the mother, which responds by changing the production of the pituitary hormones (Family Health International, 2003). The infant's suckling is the stimulus that initiates the state of lactational amenorrhea for breastfeeding women.

Advantages of Lactational Amenorrhea Method are that, LAM is universally available to all breastfeeding women and has at least 98 percent efficacy. Its protection begins immediately after giving birth and there are proven health benefits of breastfeeding for the mother and infant. LAM allows breastfeeding mothers to postpone use of other contraceptives until the infant is more mature (Petersona *et al*., 2000).

The disadvantages of using the Lactational Amenorrhea Method are that exclusive breastfeeding may be difficult for some women to maintain due to social circumstances, and offer no protection against sexually transmitted diseases, including HIV infection (Kennedy and Kotelchuck, 1998). The duration of the method is limited to a brief postpartum period and the LAM is a temporary method that can only be used by breastfeeding women (Family Health International, 2003).

Barrier methods are physical barriers that stop the sperm from coming into contact with the egg and thus preventing fertilization (Shoupe, 2011). The most common ones are condoms (both male and female), spermicides, diaphragm and cervical cap. Condoms, diaphragm and cervical cap are all mechanical barriers while the spermicides create a chemical barrier that interferes with movement of the sperm and its ability to fertilize the egg (Ministry of Health, 2005). Unlike the other modes of birth control, barrier methods are only used during sexual intercourse (WebMD, 2011).

Various risk factors are associated with the barrier methods. While using condoms, the condom may tear leading to unplanned pregnancy or risk of contracting sexually transmitted disease. In the case of diaphragm or cervical cap with spermicide, this may increase the risk of urinary tract infections. Leaving a diaphragm or cervical cap in for longer than 24 hours may also increase chances of getting toxic shock syndrome (Carlson *et al*., 2004). Spermicide on the other hand, may lead to development of sores in the vagina or on the penis especially in people who are allergic to the spermicidal (WebMD, 2011). Advantages of all barrier methods are that they are used only during sexual intercourse. The method does not affect the future fertility of the user and are known to have no other health effect such as high blood pressure or

diabetes. The method is less expensive than hormonal methods of birth control, and some are available without a prescription. Condoms and diaphragms may reduce the risk of cervical cancer and HIV (Tree, 2011). However, high failure rate, and discomfort in some women are some of the disadvantages of all barrier methods (Tree, 2011).

Natural Family Planning (NFP) is a general term for methods a woman can use to determine her fertile days of the month (NHS Choices, 2011). NFP prevents pregnancy by not having unprotected intercourse during a woman's fertile days. Different methods on NFP include use of the calendar tracking method, basal body temperature method and the cervical mucus method (Greenberg *et al*., 2010). These methods can be used together or separately. Once the body's fertile period has been determined, one can either abstain from sexual intercourse or use one of the barrier methods during that time.

The calendar tracking method uses past menstrual cycles to help estimate the fertility window. The time in between the first and last fertile days is termed the fertility window (Orshan, 2008). The basal body temperature method uses body temperature to estimate ovulation time. On the other hand, the cervical mucus method uses changes in the consistency of the cervical mucus during menstrual cycle. There are 3 to 4 dry days following a 5-day menstrual flow. For 9 days, the mucus increases daily, after which the mucus is abundant, slippery, clear and stretchy. Ovulation happens on the peak day of the stretchy mucus period and one is likely to get pregnant (American Pregnancy Association, 2011). The rules of the cervical mucus method are that no unprotected coitus should be done during and between menstrual bleeding or fertility period (Goodwin *et al*., 2010).

The natural planning method is said to have good report of success. Couples who use these natural methods correctly over one year have only a 1% to 9% chance of becoming pregnant (American Pregnancy Association, 2011). These systems are currently categorized as mucus-only, sympto-thermal, and temperature-only systems (Kippley, 2001).

Advantages of NPF include absence of side effects (De Leon, 2001), it is inexpensive and no devices or medications, or prescriptions or office visits are required. The method has a high acceptance by those couples who have religious concerns related to contraception. The disadvantages of NFP include lack of protection against sexually transmitted infections and require commitment, motivation and cooperation from both partners (Peel Public Health, 2009). The method also requires consistent and accurate record keeping and is more challenging for women with irregular cycles (Salhan, 2011).

The morning after pill, a pill taken after unprotected sex, contains Levonorgestral, a progestogen that works to prevent any unplanned pregnancy from occurring (Carlson *et al*., 2004). The pill works in three ways. First, the pill prevents ovulation from occurring by preventing the egg from being released from the ovary and thus preventing fertilization and pregnancy (Preferredrugstore, 2012). Secondly, if the ovary has already released an egg, the Levonorgestral works by preventing the sperm and egg from fertilizing. Finally, if fertilization has already occurred, then the pill works by preventing the fertilized egg from attaching itself on to the uterine lining (Carlson *et al*., 2004).

Advantages of using morning after pills include lack of interference with taking birth control pills of regular use. In addition, there is no effect on fertility in the long term

and is a safe and highly effective if taken immediately after intercourse (Mucciolo, 2000). Its disadvantages are that, the pill does not protect against sexually transmitted diseases and its effectiveness decreases with the passage of the hours after intercourse. The pill also has side effects such as nausea, dizziness, breast tenderness, bleeding, among others (Dating 360, 2012).

2.2 Anti-Fertility Effect of Medicinal Plants

A few plants have been studied for their anti-fertility effects. A strong anti-implantation and abortifacient activity have been reported in the hydroalcoholic extract of stem bark of *Ailanthus excelsa* (Roxb.) (Ravichandran *et al*., 2007). The effect of the extract of *Martynia annua* L. root on reproduction was studied on male rats where dose related reduction in the testicular sperm count, epididymal sperm count and motility, number of fertile males, ratio between delivered and inseminated females and number of pups were reported (Mali *et al*., 2002).

Ethanol extract from the seeds of *Jatropha curcas* L. have been reported to have antifertility activity when tested in adult female rats. The ethanol extracts halted the normal oestrus cycle at diestrous phase as well as significantly reducing the weight of ovaries (Ahirwar *et al*., 2010). Goonasekera *et al*. (1995) reported pregnancy-terminating effect of *Jatropha curcas* seeds in rats. In India, *Mimosa pudica* Linn (Fabaceae) is used traditionally as a birth control agent among rural people (Tiwari *et al*., 1982). The root extracts prolonged the length of the oestrous cycle when administered orally at a dose of 300 mg/kg and reduced the number of litters in albino mice. Principal hormones analysis showed that the root extracts altered estradiol secretion and gonadotropin release (Ganguly *et al*., 2007). These root extracts have

also been reported to significantly reduce the number of normal ova in rats (Valsala and Karpagaganapathy, 2002).

A study conducted by Johri *et al*. (2009) reported a low anti-implantation and anti-fertility activity of between 19% and 38% when extracts from *Moringa oleifera*, *Momordica tuberosa* (Roxb.) and *Jasminum arborescens* (Roxb.) were administered to rats. On the other hand, a 100% anti-implantation and anti-fertility activity was reported in *Cedrus deodara* (Roxb. ex D. Don) and *Peganum harmala* (L.) extracts. *Rumex steudelii* (Hochst) is traditionally used as anti-fertility plants in Ethiopia. The methanol extract from the roots of the *R. steudelii* was found to have anti-fertility activity when administered to female rats (Gebrie *et al*., 2005b). Previous studies on the same extract showed anti-implantation effect in rats. The extract also significantly reduced the litter size, prolonged the oestrus cycle especially the diestrous phase of Wistar albino rats (Desta, 1994). Phytochemical screening of the extract revealed the presence of compounds such as saponins, phytosterols and polyphenols to be present in the extract (Gebrie *et al*., 2005a).

Cissampelos pareira (Linn.) is a folk medicinal plant that is widely spread in India and is locally known as Tupukilota. The plant is traditionally used by the rural people as an agent for birth control. Leaf extracts from the plant were reported to alter the oestrous cycle pattern in female mice, prolonged the length of oestrous cycle by significant increasing in the length of diestrous stage as well as reducing the number of litters in albino mice significantly (Ganguly *et al*., 2007). Another plant used traditionally as a birth control agent is the *Ailanthus excelsa.* The petroleum ether extract from the green fruit and rhizome of *Ailanthus excelsa* have been reported to possess anti-fertility activity (Kumar *et al*., 2012).

Various compounds from plant extracts have also been reported to have anti-fertility activity. Compounds such as abridine, ergosterol peroxide, p-Coumaric acid and aristolochic acid have been reported to have 100% anti-implantation activity (Pakrashi and Pakrashi, 1978; Zia-ul-Haque *et al*., 1983; Pakrashi and Basak, 1976). In addition, Xu and Gao (1986) reported that methyl aristolate and plumbagin have a 100% and 75% abortifacient activity, respectively.

2.3 Medicinal Plants

2.3.1 *Moringa oleifera* Lam. Ó (Moringaceae)

Moringa oleifera is a small tree of 2.5-10 m in height with whitish, grey or pale buff and corky barks. The leaves are 2-3-pinnate with 4-6 pinnae. The flowers are white or cream to yellow in colour, scented and numerous in spreading panicles 8-10 cm long. The petals are oblong-spathulate with velvety pubescent at the base and the largest erect petal is 1.4-1.8 cm long and 4-8 mm wide. The others are 1-1.2 cm long and 3-8 mm in width. The fruits are 3-angled, 10-50 mm and 1.5-2.6 cm wide. The fruits are green in colour at first but later brown on maturity (Verdcourt, 1986).

Figure 2.1: 1A shows the *Moringa oleifera* tree habit and 1B shows the seed pods of *M. Oleifera* (Source Author, 2013)

The plant is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It has since been introduced in many parts of the world including East Africa. It is commonly known as the horseradish tree or drumstick tree in English, Sarangvo in Gujarati, Soanjna in Hindi, Sajna in Bengali, Sigru in Malayalam, Munaga in Telegu, mulangay, Shobhanjana in Sanskrit and Shevga in Marathi (Goyal *et al*., 2007) and Mjungu moto or Mboga chungu in Swahili (Sauveur, 2012). It is perennial softwood measuring about 10 m in height with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses (Fahey, 2005).

All parts of the tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism, and venomous bites (Goyal *et al*., 2007). The root

bark is useful in heart complaints, eye diseases, inflammation, dyspepsia and enlargement of spleen. The root and bark have also been reported to be abortifacient (Satyavati and Gupta, 1987). The leaves have been reported to have anthelmintic and aphrodisiac activities as well as the ability to cure hallucinations, dry tumours, hiccup and asthma. Leaf extracts exhibited significant abortifacient activity in rats (Nath *et al*., 1992). Aqueous extract of root and bark at a dose of 200mg/kg and 400mg/kg, respectively have been studied in rat and were shown to possess post-coital anti-fertility effect and also induced foetal resorption at late pregnancy (Prakash *et al*., 1987). The alcoholic extracts of dried barks have also been reported to have anti-fertility effect in albino female rats (Johri *et al*., 2009).

2.3.2 *Terminalia brownii* Fries (Combretaceae)

Terminalia brownii is a leafy deciduous tree with an attractive somewhat layered appearance, usually 4-15 (25) m high with a rounded, flat topped, spreading crown, and branches reaching close to the ground. The bark has dull red-brown colour and is fibrous. Young smooth bark that is whitish or old bark grey in colour, longitudinally fissured, young shoots densely hairy (Orwa *et al*., 2009a). The leaves are spirally arranged, the lamina elliptic-obtuse with a length of 6-16 cm and 3-8 cm wide. The fruits are reddish purple in colour, broadly elliptic, 3.5-5.5 cm long and 2.2-4 cm wide (Wickens, 1973).

Figure 2.2: *Terminalia brownii* plant (2A: leaves, 2B: Habitat). (Source: Author, 2012)

Some of the local names include kuuku, muvuku (Kamba), mururuku (Meru), lbukoi (Samburu), orbukoi (Maasai), and mbarao or mwalambe, in Kiswahili (Kareru *et al*., 2007; Heine and Heine, 1988). It occurs in many parts of Africa including the Democratic Republic of Congo, Ethiopia, Kenya and Tanzania (Polhill, 1973; Fyhrquist *et al*., 2002). The tree has different uses in the different areas where it is found. The leaves are used by traditional healers in Tanzania to treat diarrhoea and stomach ache, gastric ulcers, colic, and heartburn (Mbuya *et al*., 1994). It is also used by traditional healers in Kenya to treat malaria (Heine and Heine, 1988). Among the Embu and Mbeere people of Kenya, the leaves of the plant are used for treating eye infections ringworm, as well as, in family planning (Kareru *et al*., 2007). The decoction of the stem bark, trunk and branches is taken orally to treat dysmenorrhoea, nervosity, hysteria, epilepsy, beriberi, dyspepsia, stomach-ache, gastric ulcers, and colitis (Lindsay and Hepper, 1978). Stem barks are chewed to treat cough and as an emetic, infusion of barks and leaves are mixed with meat to treat hepatitis (Timberlake, 1987). Traditional healers in Ethiopia use the stem and barks to treat

jaundice, hepatitis, liver cirrhosis, and yellow fever. The bark decoction is taken for stomach and body ache (Kokwaro, 2009).

2.3.3 *Ximenia americana* L. (Olacaceae)

This is a semi-scandent bush-forming shrub or small tree 2-7 m high. Trunk diameter seldom greater than 10 cm; bark dark brown to pale grey, smooth to scaly (Booth and Wickens, 1988). The plant is usually armed with spikes. The flowers are white or greenish in colour, fragrant and the pedicels are 3-7 mm long. The number of stamens is 8 while the filaments are 2-4 mm long and the anthers are 2-4 mm long with a width of up to 0.8mm.

Figure 2.3: *Ximenia americana* leaf and fruit parts (www.zimbabweflora.co.zw)

In Kenya, the plant is found in Uasin-Gishu, Kisumu and Kilifi Counties (Lucas, 1968). The lax, usually divergent branching forms a rounded or conical crown. Branchlets are purple-red with a waxy bloom and the tree is usually armed with straight slender spines. Leaves and twigs are used to treat fevers, colds, and as a

mouthwash for toothache, a laxative and an eye lotion. The leaves are used for angina, headaches, and as a poison antidote (Beentje, 1994).

Roots are used in the treatment of skin problems, headaches, guinea worm, sexually transmitted diseases, sleeping sickness, leprosy, haemorrhoids, oedema, and act as an antidote to poison. The fruit is used in treatment of habitual constipation. The fruit eaten in large quantities acts as a vermifuge. A decoction of the roots or fruits is used to treat dysentery in calves (Orwa *et al*., 2009a; Kokwaro, 2009).

The bark is used as a decoction, dried or powdered as a cicatrisant and applied to skin ulcers; it is put on the head for febrile headache, placed in bath water for sick children, and used for kidney and heart complaints (Orwa *et al*., 2009b; Kokwaro, 2009).

2.3.4 *Bridelia micrantha* (Hochst.) Baill. (Phyllanthaceae)

Bridelia micrantha is also known as the Coastal Golden-leaf. The tree is native to Africa and belongs to the Phyllanthaceae family. It is a medium to large tree going up to 20 m (Pooley, 1993) with a dense widely spreading crown (Keay, 1958). The plant has large leaves that are alternate and simple and are found growing in coastal forests, swamp forest, and along forest margins (Keay, 1958). The truck and the branches of the plant have scattered woody thorns. The colour of the bark is silver-grey, greenish grey, brown or black.

Figure 2.4: *Bridelia micrantha* (www.plantzafrica.com)

The plants are monoecious (separate male and female flowers on the same plant) with the pedicels of the male flower being 2 mm long and 1mm wide. The male flowers also have no acute pubescent and the petals are obtriangular and 0.5mm long. The pedicels of the female flower are 1.5 mm long with a pubescent. The sepals are broadly triangular with 1.5 mm length and 1 mm width (Smith, 1987).

Extracts of *Bridelia micrantha* are used in folk medicine as an antidote, an anti-abortifacient, a laxative or purgative as well as in the treatment conditions such as headache, eye infections, abdominal pain, constipation, gastritis, common cold and scabies (Duke, 2012).

2.3.5 *Lippia kituensis*. Vatke (Verbenaceae)

A shrubby herb or 1.2-4.5 m tall with dentate and petiolate leaves. The barks are rough, corky and deeply longitudinally fissured (Verdcourt, 1992). The leaves are aromatic, ovate or elliptic acute, obtuse apex and with crenate margin. The flowers are white in colour with yellow throat and the corolla tubes are 2.4 mm long. The plant bears fruits that are red in colour. Some of the local names include Mutheithi (Kamba), Muthiriti (Kikuyu), Mwokiot (Kipsigis) and Sinoni (Somali). The fruits are edible and the leaves are sometimes used to make tea (Beentje, 1994). In Kenya, the plant is found in Kiambu and in Narok Counties (Verdcourt, 1992).

2.3.6 *Ocimum masaiense* Ayob. ex A.J. Paton (Lamiaceae)

A shrub with a height of up to 1.5 m and the leaves dentate and petiolate. The stems are erect, quadrangular with many branches. The calyx is horizontal with posterior lips that are purple in colour.

Figure 2.5: *Ocimum masaiense* herb (Source: Author, 2011)

The flowers are 2-3 mm long and uniformly pubescent (Paton *et al*., 2009). The flowers are white in colour with straight pedicel and recurved apex. The calyx are ovoid to campanulate, declined in fruit, glandular outside, glabrous or occasionally villous at throat inside, and margin winged. The corolla tube is slightly shorter than calyx, and the margin entire, flat or slightly concave. The style is longer than stamens and the nutlets ovoid, smooth or glandular foveolate, viscid when moist, with a white basal areola (Brands, 2012).

Chloroform/ethanol extracts of *Ocimum masaiense* roots have shown antinociceptive action in the formalin test (Mwangi *et al*., 2011).

2.1.7 *Rhoicissus revoilii* Planch (Vitaceae)

The plant is a woody climber with tendrils about 1-8 m in height. The plant may also appear as a shrub or a small tree of up to 10 meters in height. Their leaves are 3-foliolate with the terminal leaflets narrowly lanceolate to ovate. The lateral leaflets are

lanceolate with a length of 2-14.5 cm. The leaflets are elliptic and the laterals are very asymmetric, glabrous or hairy. The flowers are reddish brown in colour and the petals are about 3 mm long. Their fruits are black, round in shape with a diameter of 10-15 mm. The calyx is copular, 0.7-1 mm long and yellow in colour or densely ferruginous pubescent. The petals are yellow or green flushed red-purple in colour, ovate, glabrous and 1.5-2.5 mm long (Verdcourt, 1993).

Figure 2.6: *Rhoicissus revoilii* herb (Source: Author, 2012)

They are found in the margins of the forest, riverside vegetation, wooded grassland and moister types of bush land. The local names include Mgongolo (Swahili), Tarotuet in Kipsigis, Rabongo (Luo) and Ngelengei (Maasai). The Maasai community uses the plant as an antiseptic while the fibre is used in weaving (Beentje, 1994). The roots are used in the treatment of wounds while the sap from the stem is applied to cuts, burns and sores. If a cow is producing little milk, the tuberous roots are added to milk and given to a calf. The root decoction is taken as a remedy for venereal diseases and bloody constipation (Kokwaro, 2009).

2.4 Human Female Reproductive System

2.4.1 Anatomy of the Female Reproductive System

The female reproductive organs consist of the ovaries, uterine tubes, uterus, vagina, external genital organs and mammary glands. The internal reproductive organs of the female are within the pelvis between the urinary bladder and the rectum (Seeley *et al*., 2004). The uterus and the vagina are in the midline, with the ovaries to each side of the uterus. A group of ligaments holds the internal re-productive organs in place. The most conspicuous is the broad ligament, an extension of the peritoneum that spreads out on both sides of the uterus and to which the ovaries and uterine tubes are attached (Seeley *et al*., 2004).

2.4.2 Ovaries

The two ovaries are small organs about 2-3.5 cm long and 1-1.5 cm wide. This is where eggs or ova are produced (WebMD, 2012). They are situated in the abdominal cavity just ventral to the kidneys. A peritoneal fold called the mesovarium attaches each ovary to the posterior surface of the broad ligament. Two other ligaments are associated with the ovary: the suspensory ligament, which extends from the mesovarium to the body wall, and the ovarian ligament, which attaches the ovary to the superior margin of the uterus. The ovarian arteries, veins, and nerves traverse the suspensory ligament and enter the ovary through the mesovarium (Seeley *et al*., 2004).

2.4.3 Uterine Tubes

In female two uterine tubes, also known as fallopian tubes or oviducts are present. There is a uterine tube on each side of the uterus associated with an ovary. Each tube is located along the superior margin of the broad ligament (Seeley *et al*., 2004). The

part of the broad ligament most directly associated with the uterine tube is called the mesosalpinx (mesothelium of the trumpet-shaped uterine tube). The uterine tube opens directly into the peritoneal cavity to receive the oocyte from the ovary (WebMD, 2012).

2.4.4 Uterus

The uterus is the size and shape of a medium-sized pear and is about 7.5 cm long and 5 cm wide (Seeley *et al*., 2004). It is slightly flattened anteroposteriorly and is oriented in the pelvic cavity with the larger, rounded part, the fundus (bottom of a rounded flask), directed superiorly and the narrower part, the cervix, directed inferiorly. The main part of the uterus, the body, is between the fundus and the cervix (Innerbody, 2011). A slight constriction called the isthmus marks the junction of the cervix and the body. Internally, the uterine cavity continues as the cervical canal, which opens through the ostium into the vagina (Seeley *et al*., 2004).

2.4.5 Vagina

The vagina is a tube about 10 cm long that extends from the uterus to the outside of the body. The vagina is the female organ of copulation, functioning to receive the penis during intercourse, and it allows menstrual flow and childbirth (WebMD, 2012). Longitudinal ridges called columns extend the length of the anterior and posterior vaginal walls, and several transverse ridges called rugae extend between the anterior and posterior columns (Seeley *et al*., 2004). The superior, domed part of the vagina, the fornix is attached to the sides of the cervix so that a part of the cervix extends into the vagina.

2.4.6 External Genitalia

The external female genitalia, also referred to as the vulva or pudendum, consist of the vestibule and its surrounding structures. The vestibule is the space into which the vagina opens posteriorly and the urethra opens anteriorly (Seeley *et al*., 2004). A pair of thin, longitudinal skin folds called the labia minora form borders on each side of the vestibule. A small erectile structure called the clitoris is located in the anterior margin of the vestibule.

2.4.7 The Ovarian Cycle

This refers to the series of changes in the ovary during which the follicle matures, the ovum is shed and the corpus luteum develops. The hypothalamus and anterior pituitary release hormones that control these events. Numerous undeveloped ovarian follicles are present at birth but they start to mature after sexual maturity. Follicle stimulating hormone (FSH) from the anterior pituitary is primarily responsible for initiating the development of primary follicles, and as many as 25 begin to mature during each menstrual cycle. Although several follicles begin to mature during each cycle, normally only one is ovulated (Seeley *et al*., 2004). The mature follicle consists of outer cells that provide nourishment. Inside this is a fluid-filled space that contains the ovum. A mature follicle can be quite large, ranging from a few millimetres in small mammals to a few centimetres in large animals. It bulges out from the surface of the ovary before eventually rupturing to release the ovum into the abdominal cavity. Once the ovum has been shed, a blood clot forms in the empty follicle (Ollendorff, 2008). This develops into a tissue called the corpus luteum that produces the hormone progesterone. If conception takes place, the corpus luteum persists, but if there is no conception, it degenerates and a new ovarian cycle occurs (Jrank, 2012).

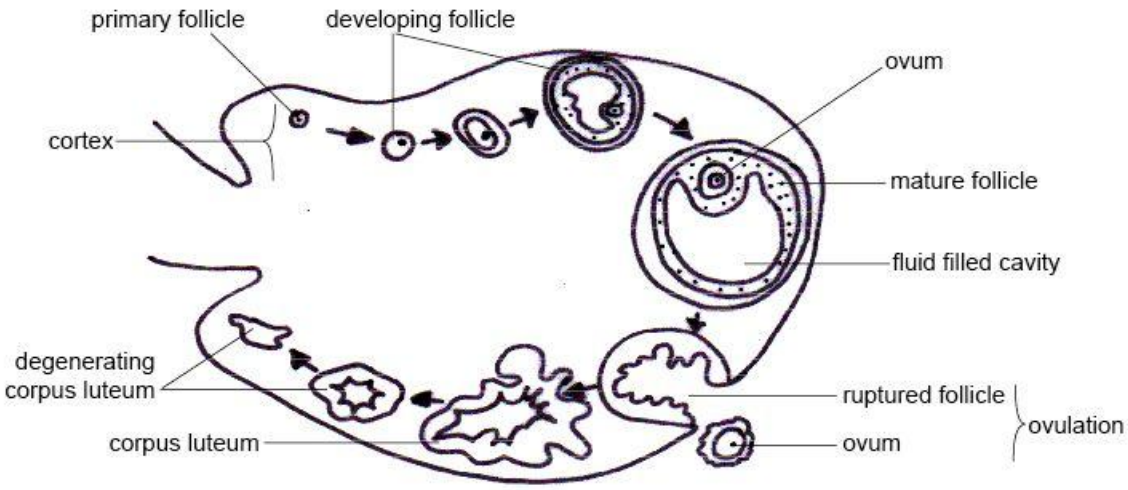

Figure 2.7: Anatomy and physiology of human's ovarian cycle

(Adopted from

http://en.wikibooks.org/wiki/File:Anatomy_and_physiology_of_animals_Ovarian_cycle_showing_from_top_left_clockwise.jpg)

2.4.8 Menstrual Cycle

The Feminist Women's Health Centre (FWHC) defines the menstrual cycle as the cyclic changes that occur in sexually mature, non-pregnant females and culminate in menses. The term menses is derived from a Latin word, meaning month. It is a period of mild haemorrhage, which occurs approximately once each month, during which the uterine epithelium is sloughed and expelled from the uterus (FWHC, 2012). The menstrual cycle is typically about 28 days long, although it can be as short as 18 days in some women and as long as 40 days in others. The menstrual cycle is divided into four phases. These phases are the menstruation, the follicular phase, ovulation and the luteal phase (Better, 2012). Menstruation is the discharge of the blood and elements of the uterine mucous membrane. The term menstrual cycle not only refers specifically to changes that occur in the uterus but also to several other cyclic changes associated

with it. These changes include the cyclic changes in hormone secretion, in the ovary, and in the uterus (Seeley *et al*., 2004).

Day 1 of the menstrual cycle is the first day of menses, which lasts 4–5 days. Ovulation occurs on about day 14 of a 28-day menstrual cycle. The time between ovulation, on day 14, and the next menses is typically 14 days. The time between the ending of menses and ovulation is called the follicular phase, because of the rapid development of ovarian follicles, or the proliferative phase, because of the rapid proliferation of the uterine mucosa (Seeley *et al*., 2004).

2.4.9 Regulation of Hormone Secretion during the Menstrual Cycle

Before ovulation, there is an increase in follicle stimulating hormone (FSH) secretion, which stimulates follicles to develop, and oestrogen secretion. Oestrogen causes the endometrium to proliferate and the hypothalamus to increase luteinizing hormone (LH) secretion, which results in the LH surge prior to ovulation (Ananth, 2010). The LH surge causes a follicle to mature and ovulate. The corpus luteum develops and secretes progesterone and some oestrogen. Approximately 7 days after ovulation, or about day 21 of the menstrual cycle, the endometrium is prepared to receive the developing embryonic mass, if fertilization has occurred.

Oestrogen causes the endometrial cells and, to a lesser degree, the myometrial cells to proliferate. It also makes the uterine tissue more sensitive to progesterone by stimulating the synthesis of progesterone receptor molecules within the uterine cells (Seeley *et al*., 2004). After ovulation, progesterone from the corpus luteum binds to the progesterone receptors, resulting in cellular hypertrophy in the endometrium and myometrium and causing the endometrial cells to become secretory cells. Oestrogen

increases the tendency of the smooth muscle cells of the uterus to contract in response to stimuli, but progesterone inhibits smooth muscle contractions (Ananth, 2010). When progesterone levels increase while oestrogen levels are low, contractions of the uterine smooth muscle are reduced. The progesterone causes hypertrophy of the endometrium and has a negative feedback effect on LH and FSH secretion. If pregnancy does not occur by day 24 or 25, progesterone and oestrogen levels decline to low levels as the corpus luteum degenerates. Consequently, the uterine lining also begins to degenerate.

The spiral arteries constrict in a rhythmic pattern for longer and longer periods as progesterone levels fall. As a result, all but the basal parts of the spiral glands become ischemic and then necrotic. As the cells become necrotic, they slough into the uterine lumen (Ananth, 2010). The necrotic endometrium, mucous secretions, and a small amount of blood released from the spiral arteries make up the menstrual fluid. Decreases in progesterone levels and increases in inflammatory substances that stimulate myometrial smooth muscle cells cause uterine contractions that expel the menstrual fluid from the uterus through the cervix and into the vagina (Seeley *et al*., 2004).

2.5 Reproductive Organs of a Female Mouse

The anatomy of a female mouse has features that are almost similar to those described above for a human female reproductive system. The Figure 2.8 illustrates the layout of the various parts in a reproductive system of mouse.

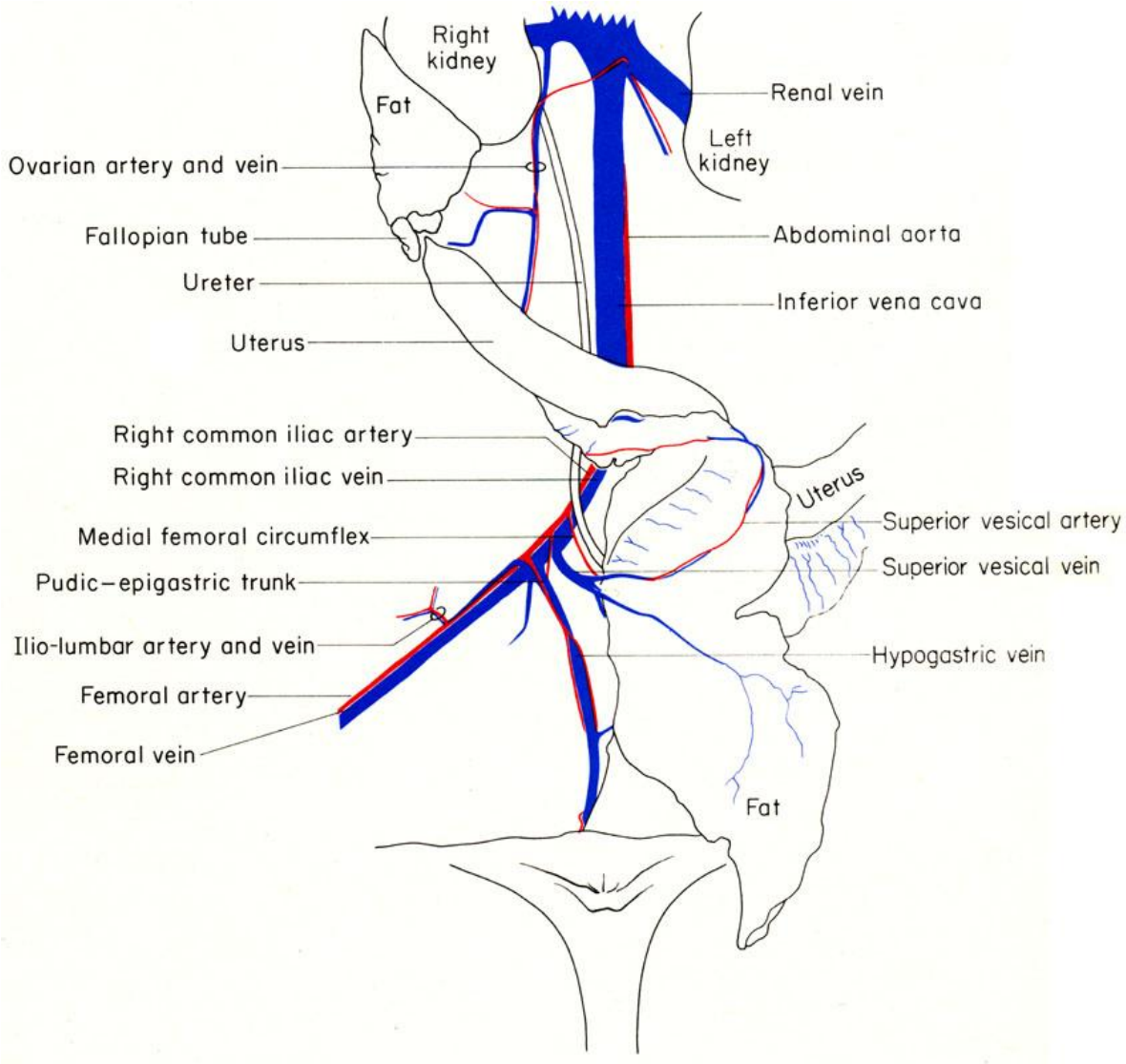

Figure 2.8: Reproductive organs of a female mouse

(Adopted from http://www.informatics.jax.org/cookbook/figures/figure122.shtml)

Figure 2.9 shows the urogenital system in mouse that combines both the urinary as well as the reproductive systems. The main organs include the uterus which serves to receive the sperm in mares and transports sperm from site of deposition to uterine tubes for fertilization. It also provides suitable environment for both the implantation of the embryo as well as the nourishment of the embryo and fetus during pregnancy.

The uterus also provides mechanical protection of the fetus and expels the mature fetus at the end of pregnancy (Charlotte, 2007).

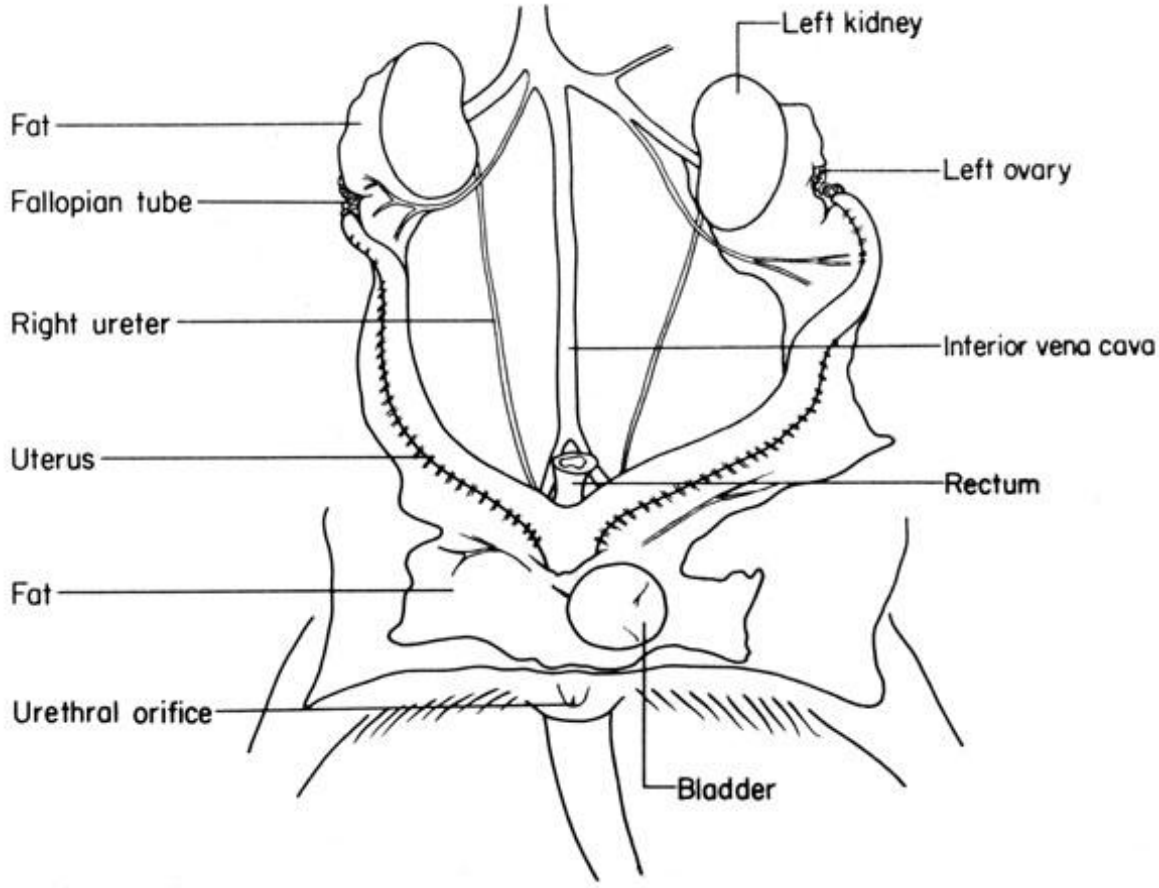

Figure 2.9: Urogenital system of a female mouse

(Adopted from http://www.informatics.jax.org/cookbook/figures/figure68.shtml)

2.6 Reproductive cycle in Female Mice

Female mammals' reproductive process is characterized by cyclic alterations in the female tract and in sexual receptivity. This leads to a recurrent period of receptivity called oestrus. Mice kept separate from males in the laboratory are able to repeat the oestrous cycle throughout the year at intervals of about four to five days. This can only be broken when the mice is subjected to pregnancy, pseudo-pregnancy (after a sterile mating), disease, or any other thing that may affect the cycle. The cycle

involves the whole of the reproductive tract, and it is possible to determine the sexual status of the female rat by examination of smears prepared from the vaginal fluid. The cycle can be divided into four main stages, diestrous, metestrus, oestrus and proestrus (Hill, 2011).

The key to cyclic reproductive activity lies in the hypothalamus. The gland communicates with the anterior pituitary through the portal system. Follicle Stimulating Hormone (FSH) is a protein, which is released by the anterior pituitary and acts to promote follicle growth. Luteinizing hormone (LH) is the other anterior pituitary protein that aids in the final development of the mature follicle and facilitates production of estrogens by the theca interna cells of the FSH-primed follicle (Bronson *et al*., 2010).

Further release of LH by the hypophysis results in rupture of the follicle and ovulation. Increasing titers of oestrogen during the later phases of follicular growth are thought to act, by way of the hypothalamus, both to suppress further release of FSH and to favour release of more LH. Progesterone, another gonadal steroid, is also produced in the ovary in small quantities during the follicular growth phases. Progesterone, in small doses, promotes ovulation by enhancing LH release. Thus the gonadal hormones produced during follicular growth act on the hypothalamus to suppress further release of FSH while promoting release of LH and hence ovulation. Functional development of the corpus luteum in the mouse is induced by mating and, when this does not occur, gonadal hormone titers decrease allowing succession of a new cycle. The prime factor allowing for the periodicity of oestrous phenomena is thought to be cyclic activity in the hypothalamus, which is reflected in LH release (Hill, 2011).

During the diestrous stage, the ovary has small follicles that are present with large corpora lutea from the previous ovulation. They secrete for only a very short time unless pregnancy or pseudo-pregnancy intervene. The uterus is small and anaemic, has low motility and the lumen is small and slit-like (Hill, 2011). The vagina has thin epithelium and the mitotic figures are infrequent. There is also abundance of leucocytes in stroma, which migrate through the epithelium into vaginal lumen. The vaginal smear is stringy mucous entangling many leucocytes and a few nucleated epithelial cells. In appearance, the vagina has small opening while the tissues have a bluish-purple colour and highly moist (Champlin *et al*., 1973).

During the proestrus stage, the ovaries have rapid growth of follicle, the uterus is vascular and its water content has increased. The uterus epithelial cells become higher and this continues into oestrus. The leucocytes disappear from mucosa and the endometrial glands are more hypertrophic (Seeley *et al*., 2004). In the vagina, the epithelium thickens and there are numerous mitoses in inner layers. The old layers of epithelium line the lumen and the leucocytes no longer migrate through the epithelium. Superficial epithelial cells slough off into lumen. The smears are largely small, round, nucleated epithelial cells, singly or in sheets. There is none or few leucocytes (Champlin *et al*., 1973).

The oestrus stage, the ovaries ovulate the eggs. Ovulation is spontaneous and occurs about 10 hours after the beginning of oestrus (Walmer *et al*., 1992). The receptivity lasts for about 13 hours and usually 10-20 eggs are ovulated each time. During this stage, the uterus gains maximum vascularisation. Here, the epithelial cells reach maximum development and there is no presence of leucocytes.

In the vagina, the outer layer of epithelial cells becomes cornified and sloughed into the lumen. In early oestrus, these cells retain their nuclei, but in later stage no nuclei visible and the cells are irregular, flat, cornified plates. The skin around the vaginal orifice also becomes swollen. The smears contain hundreds of large cornified cells with degenerate nuclei (Caligioni, 2010).

During the metestrus, there are many corpora lutea, which secrete only for a very short time, and small follicles in the ovary. In the uterus, the epithelium continues vacuolar degeneration and replacement (Walmer *et al*., 1992). There is presence of leucocytes in stroma and there is a decrease in size and vascularity of the uterus. In the vagina, the deeper layers of the oestrous epithelium line the lumen while the older, superficial layers have become cornified and sloughed off. There is also a reduction of mitotic activity in epithelium. There are leucocytes in the stroma, which migrate through the epithelium into the lumen. The smears have many leucocytes and a few cornified cells. During oestrus, metestrus and diestrous, the plasma circulation of LH and FSH are low (Caligioni, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Study Site

The study involved Machakos, Meru, Kajiado and Elgeyo Marakwet Counties in Kenya where the plants for the study were collected.

3.1.2 Collection and processing of the plant material

Collection of plants for the study was based on ethno-botanical information (herbalists who visited Centre for Traditional Medicine and Drug Research (CTMDR), at the Kenya Medical Research Institute (KEMRI) Headquarters, Nairobi, Kenya) and literature (Kokwaro, 2009). Other plants collected for previous studies were also included in the study. These were *Lippia kituensis, Ocimum masaiense, Rhoicissus revoilii* and *Terminalia brownii* parts. A plant taxonomist was engaged during the field study to identify all the plant materials collected. A voucher specimen of each collected species was then deposited at the East African Herbarium, National Museum of Kenya. The summary of the information on the plants collected is given in Table 3.1.

The collected plant parts were taken to CTMDR for further processing. All the plant parts were cleaned using distilled water, dried at room temperature for two weeks, and weighed. The plant materials were then ground into powder using an electrical mill and the powder stored in labelled airtight bags for storage waiting extraction.

Table 3.1: Plant names, locality, parts used and their voucher number

PLANT	PART(S) (VOUCHER NUMBER)	LOCATION
Bridelia micrantha (Hochst.) Baill.	Aerial Parts (712)	Ngong
Lippia kituensis. Vatke	Aerial Parts	Ngong
Moringa oleifera Lam. Ó	Aerial (710), seed (710), Root bark (710), and stem bark (710).	Kibwezi
Ocimum masaiense Ayob. ex A.J. Paton	Aerial parts and roots	Kerio valley
Rhoicissus revoilii Planch	Root and Aerial parts	Kerio valley
Terminalia brownii Fries	Stem bark	Meru
Ximenia americana L.	Leaves (711), Stem bark (711) and root bark (711)	Kerio valley
Lippia kituensis. Vatke	Root	Kerio valley

3.2 Methods

3.2.1 Organic extraction

The sample (50-100 g) was soaked separately in the methanol, petroleum ether, dichloromethane and ethyl acetate (approx. 200 mL) and left to stand for 48 hours. Samples were then filtered using Whatman® (No.1) filter paper. The filtrate was kept in a conical flask while the residues were re-soaked for another 48 hours. The samples were then filtered again and the filtrate added to the previous filtrate. The filtrates were then concentrated under reduced pressure using a rotatory evaporator at controlled temperature of between 40-60^{o}C and then transferred into weighed vials (Mallikharjuna *et al*., 2007). The samples were then left to dry for two to three weeks depending on the solvent of extraction with methanol extracts taking the longest time. After drying, the weight of the sample was obtained in grams and the percentage yield from the plant parts calculated using the formula $\frac{W2-W1}{W0} \times 100$. Where W_2 is the

weight of the extract and the container, W_1 the weight of the container alone and W_0 is the weight of the powdered sample (Anokwuru *et al*., 2011).

3.2.2 Aqueous extraction

Fifty grams of the samples was weighed and transferred into a conical flask. The materials were then covered with distilled water (100 mL) and placed in the water bath at 60^{o}C for 2 hours. After two hours, the samples were filtered and divided into two 50 ml portions and transferred into round bottomed flasks. After freezing the extracts in dry ice bath, the extracts were freeze-dried using a freeze drying machine. The percentage yield was calculated and recorded.

3.3 *In vivo* bioassays

3.3.1 Animal handling

A total of 80 healthy Swiss mice of both sexes, weighing 21-25 g from KEMRI animal house were used in the study. The animals were housed spaciously in standard polypropylene cages clearly labelled with experimental details. The mice were maintained at room temperature and 60-70% relative humidity range appropriate for their species. This enabled them to acclimatize with minimal stress and physiological alteration. The mice were fed on commercial rodent food and water *ad libitum* (Adeneye *et al*., 2006). A cannula was used for oral extract administration. The size of the needle that was used to draw blood was 25 gauges. After the experiment, the mice were sacrificed by euthanizing in diethyl ether. The euthanized mice were then placed in biohazard disposable bags for incineration.

3.3.2 Extract constitution

On the day of extract administration, each of the organic extract was freshly prepared by dissolving in a solution consisting of 70% Tween 80, 30% ethanol and diluted 10 folds with double distilled water. The aqueous extracts were prepared using distilled water (Houghton and Raman, 1998). Both the organic and water extracts were prepared to make a dosage of 800mg/kg (Mishra, et al., 2009). A conventional drug (Femiplan®) was used as the positive control. The effective concentration of the femiplan was determined by administering three doses of 180 mg/kg, 90 mg/kg and 45 mg/kg to determine the lowest effective dose of the drug in mice. Both 180 mg/kg and 90 mg/kg were effective in controlling the fertility of the mice. However, 45 mg/kg concentration was not effective in controlling the fertility of the mice. For the study 90 mg/kg was taken to be the lowest effective dose and was used as the positive control.

3.3.3 Fertility test

A total of 80 female mice were divided into 5 mice per cage. In each cage one male and a total of 16 male mice were introduced to the 8 weeks virgin female mice and were allowed to mate. After two weeks, the male mice were withdrawn. Pregnancy sign (bulging stomach) was noted in some of the female mice. The expectant females were allowed to go to term and deliver and the number of pups delivered noted.

3.3.4 Screening for the anti-fertility effect in mice

This study was done according to the method described by Ganguly *et al* (2007). Twenty six groups (each group containing 3 mature female mice) were selected for the study. Twenty three groups received the extracts while 3 groups received distilled

water, Tween 80 and Femiplan as controls. The extract administration was first carried out for 8 days before the introduction of males. All the experimental mice were then allowed to mate with mature fertile male mice, and the extract administration continued for 21 days. The number of litters was determined after the completion of one gestation period of 21 days in all experimental groups. The study was independently repeated and the average of the two studies determined.

3.3.5 Reversibility Test

The reversibility of the anti-fertility effect of the extract was also studied in the treated groups according to the method described by Salhad *et al* (1997). Briefly, the extracts were administered at 800 mg/kg continuously for 21 days, and then withdrawn. After 21 days of extract withdrawal, animals were allowed to mate with male mice. The number of litters was determined after the completion of one gestation period. The study was independently repeated and the average of the two studies determined.

3.3.6 Effect of the extract on the oestrous cycle

Five mature female mice were employed for the study. Vaginal smear from each animal were examined under a microscope every morning for 21 days. This accounted for about 4 - 5 cycles. Each mouse was held in a supine position and the vaginal secretion was collected after cleansing with 0.2 ml of normal saline (NaCl 0.9%) contained in a smooth plastic pipette (Marcondes *et al*., 2002). The smears were then placed in a tube and taken to the laboratory for assessment. A small drop of the cell suspension was then placed on a clean glass slide covered with a cover slip and examined under a light microscope at x10 and x40 magnification. Vaginal smears were assessed once each day between 9.00 and 10.00 a.m. The smears were evaluated to determine the phases of oestrous cycle using the proportion of characteristic cell

types such as the leucocytes, cornified and epithelial cells (Abu and Uchendu, 2011; Malaivijitnond *et al*., 2006). The duration of the oestrous cycle together with that of the various phases was determined as described by Makonnen *et al*. (1997) for 21 days. All mice then received test extracts whose activity had been determined during screening at the active concentration every day orally for another 21 days and the same parameters were determined.

3.3.7 Effect of the extract on the weight of genital organ and body weight

The experiment was done according to Makonnen *et al*. (1997) with some few modifications. Five groups of 5 mature female mice in each group were employed. The experimental mice received the test extract at 800 mg/kg for 10 days through the oral route. The control groups received distilled water and 10% Tween 80 for the same number of days by the same route. On the 11th day, all the animals in all the groups were weighed and sacrificed using diethyl ether anaesthesia. The ovaries and uteri were dissected out, separated from the surrounding tissues, and then blotted on aluminium foils (Gebrie *et al*., 2005b). The organs were weighted and a ratio calculated by dividing the weight of the ovary, as well as, that of the uterine in milligrams by body weight in grams. The rise in the uterine ratio was an indication of the estrogenic effect of the extract as described by Vogel (1997).

3.3.8 Acute toxicity assay

The acute toxicity effect of the bioactive extracts was carried out according to the method described by Mukinda and Syce (2007). Briefly, 5 animals per group were housed in a well-ventilated room with 12 h cycle of day and night light conditions and at room temperature. The bioactive extracts were aseptically reconstituted in water while the organic extracts were dissolved in 10% Tween 80 and orally administered in

single doses orally of 0, 1000, 2000, 3000, 4000 and 5000 mg/kg. The general behaviour of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h especially the first 4 hours (Hilaly *et al*., 2004), and then daily thereafter, for a total of 14 days. Changes in the normal activity of mice and their weights were monitored and the time at which signs of toxicity or death appeared recorded.

3.4 Phytochemical screening of the bioactive extracts

Phytochemical screening of the bioactive extracts was carried out using of simple qualitative and quantitative methods. The methods tested for the presence of secondary metabolites such as alkaloids, phenols, steroids, terpenoids, anthraquinones, flavonoids, cardiac glycosides, and saponins in the test sample. The methods as described by Harborne (1998) with slight modifications were used using Thin Layer chromatography (TLC) techniques. Approximately 50 mg of the extracts were reconstituted using the solvents that were used during extraction. The TLC plate was then placed in a tank with the solvent system as indicated in Table 3.2 to enable separation of compounds in the extracts (Mallikharjuna *et al*., 2007). The solvent systems used to develop the TLC plates for the various extracts are shown in

Table 3.**2** below.

Table 3.2: The solvent systems that were used in the development of TLC plates

Type of Extract	**Solvent system**	**Ratio**
Dichloromethane (DCM) extracts	Petroleum Ether: DCM: Methanol	6:3:1
Petroleum Ether Extracts	Petroleum Ether: DCM	7:3
Ethyl Acetate Extracts	Petroleum Ether: DCM: Methanol	5:3:2
Methanol Extracts	Butanol: Acetic acid: Water	4:1:1

3.4.1 Test for alkaloids

To determine the presence of alkaloids in the extracts, 2.5 g of the extracts was heated on a boiling water bath with 5 ml of 2N HCl. After cooling, the extract mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with few drops of Mayer's reagent. The other portion was treated with few drops of Wagner's reagent. The samples were observed for the presence of precipitation or turbidity (Mojab *et al.*, 2010).

For TLC, the test was carried out using Dragendroffs reagent as explained by Harborne (1998) by combining reagent I and reagent II. Reagent I was prepared by dissolving 0.85 g of bismuth sub-nitrate in a solution of 10 ml of acetic acid and 40 ml of distilled water. On the other hand, Reagent II was prepared by dissolving 8 g of potassium iodide in 20 ml of distilled water.

Equal portions, 1 ml each, of reagent I and reagent II were mixed with 2 ml of fresh acetic acid and 10ml of distilled water to make the testing solution. The test for the presence of alkaloids and other nitrogenous compounds was done by spraying the mixture on the TLC plate. The appearance of an orange colour gave an indication of the presence of alkaloid compounds in the samples.

3.4.2 Test for Phenols

In a test tube, 1 ml of the extract and 2 ml of distilled water were added followed by addition of few drops of 10% ferric chloride ($FeCl_3$). Appearance of blue or green colour was an indication of the presence of phenols (Ganatra *et al.*, 2012). Using TLC, the test solution was prepared by making a solution of ferric chloride in distilled water and another solution of 0.1 g of potassium ferricyanide in distilled water. The two solutions were mixed to form a blue solution. The test was then carried out by spraying the solution onto the TLC plate using a spraying gun. The appearance of blue spots gave an indication of the presence of phenolic compounds.

3.4.3 Test for Sterols

In this test, Salkowaski test was used. The test involves taking 10 mg of extract and adding 2 ml of chloroform and 2ml of concentrated sulphuric acid from the side of the test tube. The test tube was shaken for few minutes and development of red colour in chloroform layer indicated the presence of sterols (Devmurari, 2010).

Using TLC, the testing solution was prepared by placing 5 ml of acetic anhydride solution in a conical flask followed by 5 ml of sulfuric acid and finally 50 ml of absolute ethanol. The TLC plate was then sprayed with the solution and heated at $90^{o}C$ for 15 minutes. The appearance of brown or orange colors was an indication of presence of steroid compounds.

3.4.4 Test for Terpenoids

The test employed the Knollar's test in determining the presence of terpenoids. Five grams of the extract was treated with 2ml of 0.1% anhydrous stannic chloride in pure

thionyl chloride. A change of colour from purple to red indicated the presence of terpenoids (Devmurari, 2010).

Using TLC, a solution for the testing of terpenoids was prepared by dissolving 1 g of vanillin in 100 ml of concentrated sulfuric acid. The TLC plate was sprayed and heated at 100^{o}C for 5 minutes. Purple spots and streaks indicated the presence of terpenoids in the sample.

3.4.5 Test for Anthraquinones

In a test tube, 0.5 g of the extract was added and boiled with 10 ml of sulphuric acid (H_2SO_4) the extract mixture was filtered while hot and the filtrate shaken with 5 ml of chloroform. The chloroform layer was transferred into another test tube using a pipette and 1 ml of dilute ammonia added. The resulting solution was observed for colour changes to pink showed the presence of an anthraquinone (Ayoola, *et al*., 2008).

Using TLC, this test was done by using methanolic potassium hydroxide solution. This solution was made by dissolving 1 g of potassium hydroxide in 10 ml of methanol. The TLC plate was sprayed and heated at 100^{o}C for 5 minutes. Purple spots and streaks indicated the presence of anthraquinones in the sample.

3.4.6 Test for Cardiac Glycosides

To test for the presence of glycoside, 5 ml of the extract was added in a test tube and treated with 2 ml of glacial acetic acid that contained ferric chloride ($FeCl_3$) solution. The mixture was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). Presence of a brown ring of the interface indicated a de-oxy sugar characteristic of cardenolites (Ganatra *et al.*, 2012).

Using TLC, the testing reagent was *p*-anisaldehyde reagent. The reagent was prepared by mixing 1 ml of *p*-anisaldehyde and 1 ml of sulfuric acid in 18 ml of ethanol. The TLC plate was sprayed and heated at 110^{o}C for 5 minutes. Presence of green-yellow spots was an indication of the presence of sugars and glycosides.

3.4.7 Test for Flavonoids

About 1 g of the extracts was treated with a few drops of concentrated HCl and 0.5 g of magnesium turnings. Development of pink or magenta-red colour within 3 minutes was an indication of the presence of flavonoids (Mojab *et al.*, 2010).

Using TLC, the test was carried out by exposing the TLC plate with the sample to ammonia solution in a tank. The presence of brown colour was an indication of the presence of flavonoids.

3.4.8 Test for Saponins

The extracts were dissolved in water and shaken vigorously to froth and then allowed to stand for 15-20 minutes. The saponin content was classified as negative when no froth was formed, weakly positive when the formed froth was less than 1 cm, positive when the froth was 1.3 cm long and strongly positive when froth was more than 2 cm high (Mojab *et al.*, 2010).

3.5 Statistical analysis

Results for the litter size and organ/body weight ratio were expressed as mean ± standard error of mean (S.E.M.) or as mean ± standard deviation (S.D). A significant difference between control and experimental groups was assessed by the use of Student's t-test while the analysis of variance (ANOVA) was used to assess any

significant difference among the groups. The level of significance was set at ρ- values less than 0.05.

CHAPTER FOUR

RESULTS

4.1 Percentage Yield of the Plant Extracts

The amount of plant samples and solvents used to do extraction were as recorded in Table 4.1. The calculated percentage yields of extracts are also indicated.

Table 4.1: Percentage yield of the medicinal plant extracts used in the study

Solvent	**Sample**	**Weights in Grams**		**Percentage Yield (%)**
		Amount Soaked	**Amount Extracted**	
Methanol	*Bridelia micrantha* (Aerial)	100	16.4	16.4
	Lippia kituensis (Aerial)	100	2.7	2.7
	Moringa oleifera (Aerial)	100	2.3	2.3
	Moringa oleifera (Seed)	100	1.6	1.6
	Moringa oleifera (Twigs)	50	2.4	4.8
	Moringa oleifera (Root bark)	100	3.4	3.4
	Ocimum masaiense (Aerial)	100	1.9	1.9
	Ocimum masaiense (Root)	20	1.1	5.7
	Rhoicissus revoilii (Aerial)	100	4.0	4.0
	Rhoicissus revoilii (Root)	50	2.8	5.6
	Terminalia brownii (Stem bark)	100	14.3	14.3
	Ximenia americana (Leaves)	50	11.4	22.9
	Ximenia americana (Root bark)	50	7.0	14.0
	Ximenia americana (Stem bark)	100	7.0	7.0
	Lippia kituensis (Root)	50	1.6	3.2
Dichloro-methane	*Bridelia micrantha* (Aerial)	100	4.6	4.6
	Bridelia micrantha (Leaves)	100	3.0	3.0
	Lippia kituensis (Aerial)	100	0.1	0.1
	Moringa oleifera (Aerial)	100	3.1	3.1
	Moringa oleifera (Root bark)	100	1.1	1.1
	Moringa oleifera (Seed)	100	19.9	19.9
	Moringa oleifera (Twigs)	50	0.9	1.8
	Ocimum masaiense (Aerial)	100	0.7	0.7
	Rhoicissus revoilii (Aerial)	100	7.5	7.5
	Ximenia americana (Stem bark)	100	0.8	0.8
Petroleum	*Moringa oleifera* (Aerial)	100	1.4	1.4
	Moringa oleifera (Seed)	100	13.7	13.7

Ether	*Moringa oleifera* (Root bark)	100	0.7	0.7
	Moringa oleifera (Twigs)	50	0.2	0.4
	Bridelia micrantha (Aerial)	100	3.0	3.0
	Lippia kituensis (Aerial)	100	0.3	0.3
	Terminalia brownii (Stem bark)	100	0.3	0.3
	Ocimum masaiense (Aerial)	100	0.5	0.5
	Rhoicissus revoilii (Aerial)	100	1.1	1.1
	Ximenia americana (Stem bark)	100	0.8	0.8
Ethyl acetate	*Bridelia micrantha* (Aerial)	100	3.9	3.9
	Lippia kituensis (Aerial)	100	0.4	0.4
	Moringa oleifera (Aerial)	100	1.0	1.0
	Moringa oleifera (Root bark)	100	0.4	0.4
	Moringa oleifera (Seed)	100	15.0	15.0
	Moringa oleifera (Twigs)	50	0.5	1.1
	Ocimum masaiense (Aerial)	100	0.9	0.9
	Ximenia americana (Stem bark)	100	0.6	0.6
	Terminalia brownii (Stem bark)	100	4.4	4.4
	Rhoicissus revoilii (Aerial)	100	1.0	1.0
Water	*Bridelia micrantha* (Aerial)	50	5.3	10.5
	Lippia kituensis (aerial)	50	3.1	6.3
	Moringa oleifera (seed)	50	5.5	11.1
	Moringa oleifera (Twigs)	50	3.3	6.5
	Moringa oleifera (Root bark)	50	6.7	13.4
	Ocimum masaiense (Aerial)	50	3.0	6.0
	Rhoicissus revoilii (Root)	50	4.1	8.3
	Terminalia brownii (stem bark)	50	8.3	16.6
	Ximenia americana (leaves)	50	9	18
	Ximenia americana (Root bark)	50	9	18

The highest yield was of ethyl acetate extracts of the seeds of *Moringa oleifera* (19.9%) followed by methanol extracts of the aerial parts of *Bridelia micrantha*. The lowest percentage yield was recorded in the dichloromethane extract of the aerial parts of *Lippia kituensis* (0.079%). The seeds of *Moringa oleifera* gave an oily extract and had high yields with all the solvents other than methanol, which did not yield an oil extract. The extraction of *Moringa oleifera* seeds, dichloromethane, petroleum ether, ethyl acetate and water gave 19.867%, 13.683%, 15.014% and 11.08% yield respectively.

4.2 *In Vivo* Bioassays

4.2.1 Fertility Test

Determination of the potent female mice gave a high rate of fertility in the female mice. Out of the 80 mice that were selected, 48 of them were confirmed fertile. The results of fertility test (Table 4.2) suggested that some of the mice were not receptive for the 21 days that the male mice were in the cage, and thus did not give birth during the first gestation period. The litter size per mouse recorded an average of 6 litters. The 48 mice that gave birth were the ones that were considered as fertile and were used in the screening for anti-fertility activity of the candidate plants.

Table 4.2: Fertility of female mice after one gestation period (Male: Female ratio, 1:5)

Group	No of mice per groups	No of fertile mice	Total Litter	Litter size ±S.D
1	5	3	16	5.33±0.58
2	5	4	24	6.00±0.82
3	5	3	18	6.00±1.0
4	5	3	18	6.00±1.0
5	5	3	11	3.67±0.58
6	5	4	31	7.75±1.26
7	5	4	23	5.75±0.96
8	5	4	19	4.75±0.96
9	5	3	24	8.00±1.0
10	5	2	16	8.00±0.71
11	5	3	30	10.00±1.0
12	5	2	10	5.00±1.414
13	5	2	9	4.50±0.71
14	5	2	16	8.00±0.71
15	5	2	14	7.00±1.41
16	5	4	31	7.75±0.5
Total	80	48	310	6.46

4.2.2 Screening for the anti-fertility effect

The organic root extracts of *Lippia kituensis*, seed extracts of *Moringa oleifera*, and stem bark extract of *Terminalia brownii* had anti-fertility effect (Table 4.3). On the other hand, aqueous extracts from the leaves of *Ximenia americana*, and aerial parts of *Bridelia micrantha* showed anti-fertility activity. Femiplan, used as a positive control also proved to reduce the fertility of mice at a concentration of 90 mg/kg when dissolved in distilled water. The water extracts of *Terminalia brownii* were toxic to

the mice at 800, 600 and 400 mg/kg. Mice died within the first week of extract administration in all cases. Fertile mice gave birth at an average of 7 litters per mouse. However, there was no significant difference observed in the litter size in any group with a ρ value of 0.2119. All delivered pups were normal and healthy. The results for the anti-fertility screening are as shown in Table 4.3. The data is an average of two independent experiments.

Table 4.3: Fertility of female mice after 21 days of treatment with 800 mg/kg of test extract (Male: Female ratio, 1:3)

Extract	Solvent	Extract Dose mg/kg	No. of fertile/ treated	Total no. of pups	Litter size ±S.D
Lippia kituensis **(Root)**	Methanol	800	0/3	0	0
	Water	800	3/3	16	5.30±0.58
Moringa oleifera **(seed)**	Pet ether	800	2/3	16	8.00±1.0
	Ethyl acetate	800	0/3	0	0
	DCM	800	3/3	27	9.00±0.58
	Water	800	3/3	20	6.67±0.33
Moringa oleifera **(Twigs)**	Methanol	800	3/3	22	7.33±0.33
Moringa oleifera **(Root bark)**	Methanol	800	3/3	18	6.00±0.58
	Water	800	2/3	12	6.00±0.50
Moringa oleifera **(aerial)**	Methanol	800	3/3	18	6.00±0.58
Terminalia brownii **(stem bark)**	Ethyl acetate	800	0/3	0	0
Terminalia brownii **(stem bark)**	Water	800	0/0	0	0
		600	0/0	0	0
		400	0/0	0	0
Ximenia americana **(leaves)**	Methanol	800	3/3	21	7±1.00
	Water	800	0/3	0	0
Rhoicissus revoilii **(Root)**	Methanol	800	2/3	14	7.00±0.50
	Water	800	2/3	16	8.00±1.00
Lippia kituensis **(aerial)**	Methanol	800	2/3	21	10.50±0.50
Bridelia micrantha **(Aerial)**	Methanol	800	2/3	10	5.00±1.00
	Water	800	0/3	0	0
Ocimum masaiense **(Aerial)**	Methanol	800	2/3	18	9.00±1.00
	Water	800	2/3	12	6.00±1.00
Negative control	Water	N/A	3/3	24	8.00±0.58
	Tween 80	10%	2/3	12	6.00±1.00
Positive control (Femiplan^{TM})		90	0/3	0	0

4.2.3 Reversibility Test

Extracts that exhibited anti-fertility effect were subjected to a reversibility test to check for the reversibility of their anti-fertility effect. From the experiment, ethyl acetate, and water extracts from the seeds of *Moringa oleifera,* methanol extracts from the leaves of *Ximenia Americana,* methanol extracts from the roots of *Lippia kituensis* and water extracts from the leaves of *Bridelia micrantha* had reversible anti-fertility effect (Table 4.4). However, ethyl acetate extract of the stem bark of *Terminalia brownii* had a permanent anti-fertility effect and the mice that were treated with the extract did not give birth after withdrawal. The group that was administered with femiplan also showed reversibility after the withdrawal of the extract. The data is an average of two independent experiments.

Table 4.4: Fertility of female mice after 21 days of treatment and then withdrawal of the treatment

Extract	Solvent	Extract Dose mg/kg	No. of fertile/ treated	Total no. of pups	Litter size ± S.D
***Moringa oleifera* (seed)**	Ethyl Acetate	800	2/3	10	5±1.41
***Terminalia brownii* (stem bark)**	Ethyl Acetate	800	0/3	0	0
***Lippia kituensis* (Root)**	Methanol	800	2/3	12	6±1.41
***Bridelia micrantha* (Leaves)**	Water	800	2/2	10	5±1.41
***Ximenia americana* (leaves)**	Water	400	2/2	11	5.5±0.71
Femiplan	Water	180	3/3	18	6±1.00
	Water	90	3/3	22	7.3±0.58

4.2.4 Effect of the Extract on the Oestrous Cycle

In this study, mice in the control group exhibited regular oestrous cycle of 4 to 5 days (Table 4.5). The length of the oestrous cycle in all the treatment groups was significantly changed as compared with the control group that received distilled water. All the extracts arrested the normal oestrus cycle at either the diestrous or the proestrous phase. The diestrous and proestrus phases of the cycle of the treated mice were prolonged in the treated groups. However, the oestrous and metestrus phases of the treated mice were highly reduced in the treatment group. Most of the days during the administration of extraction of *Terminalia brownii* and *Moringa oleifera*, the mice exhibited diestrous phase followed by proestrus phase and only one day for some of the mice exhibited the oestrus and metestrus phases.

Comparing the effect of the different extracts on mice, there was no significant difference among the mice subjected to different extracts ($\rho = 0.854$). The test was done at a confidence level of 95%.

Table 4.5: Effect of the bioactive plant extracts (800 mg/kg) on oestrus cycle of female mice (n = 3)

Phases (days)	**Control**	***Bridelia micrantha* (Leaves)**	***Ximenia americana* (leaves)**	***Terminalia brownii* (stem bark)**	***Moringa oleifera* (seed)**
Oestrous cycle	4.33	0	0	0	0
Proestrus	1.15	5	8.33	7.00	4.33
Oestrus	1.46	0	0.00	0.33	0.33
Metestrus	1.23	0	0.00	0.33	0.33
Diestrous	1.08	16	12.67	13.33	16.00

4.2.5 Effect of the Extract on the Weight of Genital Organ and Body Weight

Oral administration of the extracts of the leaves of *Bridelia micrantha*, stem bark of *Terminalia brownie*, leaves of *Ximenia americana* and the seeds of *Moringa oleifera* did not have suppressive effect on the ovary as well as the uterus. The results were expressed in mg/1000 mg of body weight (Table 4.7).

The administration of water extracts of *Bridelia micrantha* (leaves), at a concentration of 800 mg/kg for 10 days did not affect the uterine wet weight ($\rho = 0.0913$), as well as, the fresh weight of the ovaries ($\rho = 0.122$). In addition, administration of ethyl acetate extracts of *Terminalia brownii* (stem bark), at a concentration of 800 mg/kg for 10 days did not affect the uterine wet weight ($\rho = 0.292$), as well as, the wet weight of the ovaries ($\rho = 0.217$). Likewise, administration of water extracts of *Ximenia americana* (leaves) at a concentration of 800 mg/kg for 10 days did not affect the uterine wet

weight (ρ= 0.808), as well as, the wet weight of the ovaries (ρ= 0.198). Administration of ethyl acetate extracts of *Moringa oleifera* (seed) at a concentration of 800 mg/kg for 10 days did not affect the uterine wet weight (ρ= 0.077), as well as, the wet weight of the ovaries (ρ= 0.145). The body, ovaries and uterus weights were as recorded in Table 4.6.

Table 4.6: The body, ovaries and uterus weights after 10 days of extract administration

Extract	Mouse No.	Weight		
		Mouse (g)	Ovaries (mg)	Uterus (mg)
***Bridelia micrantha* (Leaves)**	I	23	7.5	29
	II	22	8.5	33
	III	26	11.1	43
	IV	21	10.3	40
	V	24	6.2	24
***Terminalia brownii* (stem bark)**	I	21	8.5	34
	II	27	4.3	18
	III	24	8	31
	IV	23	12	48
	V	28	12.9	58
***Moringa oleifera* (seed)**	I	23	9	30
	II	25	11	42
	III	24	10	38
	IV	25	11	43
	V	27	9	35
Control	I	22	10.2	40
	II	24	14	58
	III	23	12.2	48
	IV	23	10	40
	V	25	9	36
***Ximenia americana* (leaves)**	I	20	10	40
	II	25	13	30
	III	21	15	59

Two mice in *Ximenia americana* treatment died in the course of the experiment. There was also no significant difference among the different treatments at ρ=0.05 significant level on the size of the ovary with a ρ value of 0.0611. In the same way,

there was no significant difference among the different treatments at ρ=0.05 significant level on the size of the uterus with a ρ value of 0.364.

Table 4.7: The effect of the plant extracts in comparison with the control on wet weight of mice uterus and the ovaries

Treatment	Ovary/Body Weight± S.E.M	Uterus/Body Weight ± S.E.M
CONRTOL	0.474±0.043	1.900±0.185
BML	0.378±0.045	1.464±0.175
TBSB	0.376±0.070	1.547±0.297
MOS	0.404±0.022	1.517±0.102
XAL	0.578±0.084	2.003±0.569

The data is presented as Mean ± SEM. Where BML is *Bridelia micrantha* (Leaves), TBSB is *Terminalia brownii* (stem bark), MOS is *Moringa oleifera* (seed) and XAL is *Ximenia Americana* (leaves). Results are expressed as uterine or ovarian ratio ± S.E.M, with n=5.

The average ratio of ovary/body weight and uterus/body weight were represented in a bar graph as shown in Figure 4.1.

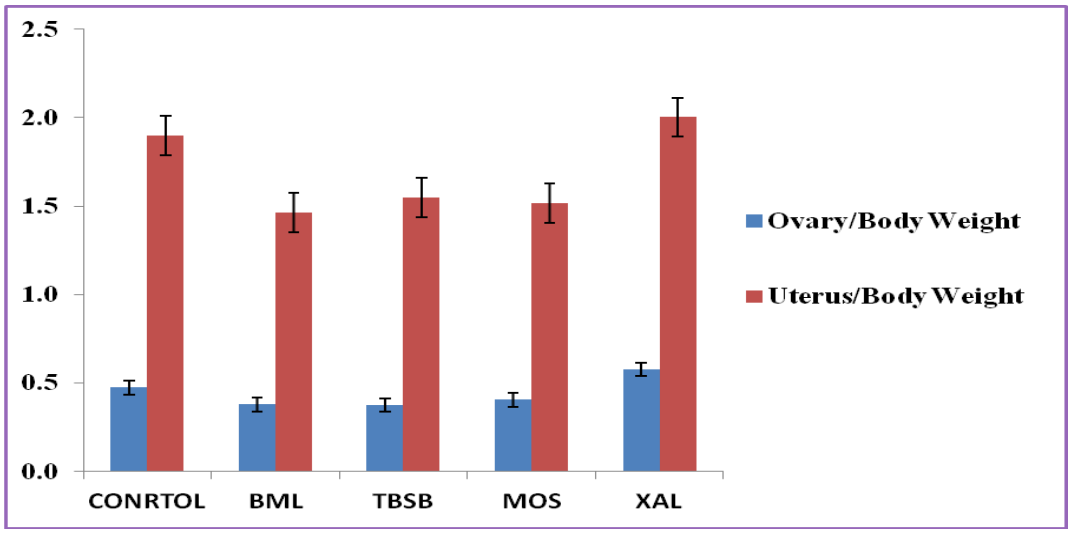

Figure 4.1: A graph of ratio of the weight of the organs/ body weight against extracts

4.2.6 Acute Toxicity Assay

In this study, there were no deaths or any signs of toxicity observed after the oral administration of the bioactive extracts of *Bridelia micrantha* leaves (water), *Terminalia brownii* stem bark (ethyl acetate) at any dose level up to the highest dose tested (5000 mg/kg). Administration of the ethyl acetate extracts of *Moringa oleifera* seed showed no death or signs of toxicity up to 4000mg/kg. However, at the highest tested dose (5000 mg/kg) there were mild signs of toxicity that included hypo-activity, low appetite and piloerection for both the ethyl acetate extracts of *Moringa oleifera* and leaf extracts of *Ximenia americana.* The mortality rate, as well as, the signs of acute toxicity of the orally administered water extracts of the leaves of *Ximenia americana* increased progressively with the increasing dose. The water extracts of the leaves of *Ximenia americana* had a mortality rate of 0% up to a dose of 4000 mg/kg and of 20% at 5000 mg/kg. The mortality at 5000 mg/kg was noted after 48 hours after extract administration.

The mild effects were noted in ethyl acetate extracts of *Moringa oleifera* for 1 hour and after the second hour after extract administration most of the mice had regained their normal activity as well as appetite.

4.3 Phytochemical Screening of the Extract

The results reported here are for phytochemical screening that was conducted using Thin Layer Chromatography (TLC) and different spraying reagents. The results (Table 4.8) revealed the presence of terpenoids and sterols in the seeds of *Moringa oleifera* as well as in the leaves of *Bridelia micrantha*. The stem bark of *Terminalia brownii* contained saponins, flavonoids, terpenoids and sterols. *Ximenia americana* leaves contained terpenoids, glycosides, sterols, and phenols. However, anthraquinones, cardiac glycosides and alkaloids were not present in any of the tested extracts.

Table 4.8: Phytochemical constituents of the plant extracts

	Extracts			
Compounds	MOS Ethyl acetate	BML Water	TBSB Ethyl acetate	XAL Water
Terpenoids	+	+	+	+
Anthraquinones	-	-	-	-
Flavonoids	-	-	+	-
Cardiac Glycosides	-	-	-	-
Saponins	-	-	+	-
Sterols	+	+	+	+
Alkaloids	-	-	-	-
Phenols	-	-	-	+

Where MOS is *Moringa oleifera* seed, BML- *Bridelia micrantha* leaves, TSBS- *Terminalia brownii* and XAL- *Ximenia Americana*, +, positive and - negative

CHAPTER FIVE

DISCUSSION

The methanol extracts generally gave the highest yield for most of the plant parts as compared to the other organic solvents, while ethyl acetate gave the least. For example, *Bridelia micrantha* produced 16.4% using methanol and 3.9% using ethyl acetate. Water extraction gave higher yields than the organic solvents, underscoring its efficiency as a universal solvent.

The result of the percentage yield provides a suggestion that methanol is a better organic solvent for the extraction of aerial parts of *Bridelia micrantha*, stem barks of both *Terminalia brownii* and *Ximenia Americana*, the leaves and the root bark of *Ximenia americana.* The results of higher yield using methanol have also been reported (Anokwuru *et al*., 2011; Singh *et al*., 2002) and this is usually due to the high polarity of methanol as compared to other solvents. The use of methanol alone as an extraction solvent resulted in greater yield when compared to a mixture of different solvents (Jayaprakasha *et al*., 2001).

Mice just like rats exhibit a characteristic short oestrus cycle of 4 to 5 days in phases (Mandl, 1951) making both of them ideal for reproductive studies (Marcondes *et al*., 2002). Production of ovarian hormones is involved in governing the stages of the oestrus cycle. The inter-conversion of these stages is under control of oestrogen and progesterone which are the ovarian hormones secreted by the cells of membrana granulose of the matured follicles and carpus luteum. The secretion of these hormones is under the control of the secretion of pituitary gonadotropins and hypothalamic-releasing factors (Ahirwar *et al*., 2010). Vaginal cornification occurs due to the

oestrogen in the adult mice and is involved in the induction of cornified cells in sterilized female mice, as well as, rats. Inhibition of cornification of vaginal epithelium is an important measure in the detection of the anti-estrogenic effect in a compound (Lerner, 1969).

Administering the bioactive extracts of the *Bridelia micrantha, Ximenia americana* arrested the cycle of the mice at the diestrous phase and at the proestrous phase while showing an average of 5 and 8.33 times in the proestrus phase while in 16 and 12.67 days the mice showed the diestrous phase in *Bridelia micrantha, Ximenia americana* respectively. The mice did not show either the oestrous or the metestrus phase during the 21 days of extract administration. The diestrous and proestrus phases of the cycle in the treated mice were longer in the treated mice than it was in the control group. The oestrous as well as the metestrus phases were reduced in all the groups that were treated with the extracts. This is an indication that the administered extracts had compounds that have anti-estrogenic activity. Administration of anti-estrogenic compound orally to cyclic mice resulted in arresting of the oestrus cycle in diestrous stage as well as lowering the number of the cornified cells in the vaginal smear (Prakash, 1978).

Most of those mice exhibited either oestrus or metestrus phases during the first days of extract administration probably before the extract could take effect. The extracts thus exhibited strong anti-estrogenic property. This suggests that the extracts posed negative influences on the oestrous cycle by reducing the number of days that ovulation was to take place. This could be due to the presence of high level of phytoestrogen compounds such as saponins and essential oils (Oluyemi *et al*., 2007).

This inhibitory effect of steroidal saponin on the oestrus cycle has been reported by Tamura *et al*. (1997).

The results of this study confirm the reports of the ability of some plant extracts to prolong the oestrus cycle as well as the diestrous phase of the cycle (Shibeshi *et al*., 2006). However, Shukla *et al*. (1987) reported complete abolition of the proestrus phase, shortened diestrous and prolonged oestrus phases of the cycle after butanolic extract of *Pueraria tuberosa* was administered in rats. Shivalingappa *et al*. (2001) also reported similar observations on the prolongation of the oestrous cycle when ethanolic extract of *Rivea hypocrateriformis* was administered in rats. Similarly, extract of *Anethum graveolens* prolonged the oestrus cycle in rat (Monsefi *et al*., 2006). Prolonging the cycle reduces fertilization in the affected experimental animals (Uchendu *et al*., 2000).

In the determination of the effect of the bioactive extract on the weight of genital organ and body weight of mice, there was no extract that showed uterine and ovary increase in weight. The rise in the uterine ratio gives an indication of the estrogenic effect of the extract as is described by Vogel (1997). However, none of the extracts showed a rise in the uterine or even ovarian ratio and thus no estrogenic effect and the extracts may be concluded to have anti-estrogenic effect. This suggests that the extracts prevented the ovaries as well as the uterus from undergoing the normal preparations during oestrous cycle. This could be due to the presence of high level of phytoestrogen compounds such as saponins and essential oils (Oluyemi *et al*., 2007). The deaths that were recorded in *Ximenia americana* group may be attributed to idiopathic causes and were not related to the drug administered.

Although the medicinal plants tested had credible biological activities in mice, there is generally very little information about their toxicity. In this study, there were no severe signs of toxicity observed after the oral administration of the most of the bioactive extracts. Mortality was only observed at the highest dose administered of extracts of the leaves of *Ximenia americana.* In a previous toxicity study, methanol extracts of the leaves of *Ximenia americana* showed that the extract was not toxic and caused no death up to a concentration of 5000 mg/kg orally (Siddaiah *et al*., 2011). Toxicity of the water extract of the leaves of *Ximenia americana* may have been due to difference in compounds extracted by water from those extracted by methanol.

The no-observed-adverse-effect level (NOAEL) (Alexeeff *et al*., 2002) for *Bridelia micrantha, Terminalia brownii* and *Moringa oleifera* was 5000 mg/kg while that of *Ximenia americana* was 4000 mg/kg. In a study done to test the effect of *Moringa oleifera* seed extract on red blood cells among other indices showed no change in these indices. The absence of significant changes on these indices suggested that the extract does not possess toxic substances that can be lethal to the rats (Ajibade *et al*., 2012). This observation is in agreement with the report of Jahn (1988) where no toxic effects were observed when extracts of *Moringa oleifera* were administered to Wistar rats.

Unlike the methanolic extract of the bark of the *Terminalia brownii* that has been reported, to have mortality of 66% at a dose of 1000mg/kg in rat orally (Thoria *et al*., 2012) the ethyl acetate extract of the same was safe at the highest concentration of 5000mg/kg. This could have been because of different compounds that are extracted by the different solvents. Compounds extracted by ethyl acetate may be safer than those extracted using methanol.

The phytochemical studies indicated presence of compounds such as sterols, glycosides, terpenoids, saponins, phenols and alkaloids in the extracts. Some of these extracts have been shown to have anti-fertility activity (Hiremath and Rao, 1990) and may thus have contributed to the anti-fertility effect of the extracts. Saponins content of *Albizia lebbeck* bark have been reported to have significantly reduced the sperm concentration of testes and epididymides in rats. The saponins reduced by 100% the fertility of male rats (Gupta *et al*., 2005). This compound is also present in *Terminalia brownii* and could be responsible for the 100% reduction of fertility in the experimental mice. Presence of abundant amounts of saponins and flavonoids in stems of *Terminalia brownii* has also been reported by Omer and ElNima (1999).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The results of this study conclude that the extracts of the leaves of *Bridelia micrantha*, stem bark of *Terminalia brownii*, leaves of *Ximenia americana* and the seeds of *Moringa oleifera* have anti-fertility effect at a dose of 800 mg/kg when administered orally to mice. This effect is reversible in the case of *Bridelia micrantha*, *Ximenia americana* and *Moringa oleifera.* However, the anti-fertility effect of *Terminalia brownii* was not reversible as the mice did not get pregnant even after test extracts was withdrawn.

The study on the effect of the bioactive extracts on the oestrus cycle confirmed that the extracts were able to control fertility by having the anti-estrogenous activity. While the mice in the control group exhibited regular oestrous cycle of 4 to 5 days, the mice in the experimental groups exhibited an arrest of the normal oestrus cycle at either the diestrous or the proestrous phase. On the other hand, the diestrous and proestrus phases of the cycle of the treated mice were prolonged in the treated groups. The extracts therefore have strong anti-estrogenous activity. The presence of compounds such as sterols, terpenoids, alkaloids, saponins and flavonoids found in the active extracts may attributed to the anti-fertility activity of the tested extracts.

The results of the effect of the active extracts on the weight of both the ovaries as well as the uterus showed no significant change. The weight of the uterus as well as that of the ovaries did not differ in any significant way. The extracts are therefore not harmful to the reproductive organs of the mice.

Finally, the active extracts did not show severe signs of toxicity at the highest concentration tested (5000 mg/kg) other than the *Ximenia americana* leaves which had a mortality rate of 20% at 5000 mg/kg. However, the extract was not lethal at 4000 mg/kg and was there considered safe at this concentration.

6.2 Recommendations

This study led to various conclusions from which the following recommendations were made.

1. The concentration of the bioactive extract should be reduced in order to establish the lowest effective dose of the extracts.
2. Water extracts of *Terminalia brownii* still toxic to the mice at a concentration of 400 mg/kg. It is thus recommended that the highest nontoxic concentration be determined and their anti-fertility activity determined.
3. The permanent effect of ethyl acetate extracts of *Terminalia brownii* be monitored for any chance of reversibility of the effect in the future.

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APPENDICES

Table A.1: The oestrus cycle phases before extract administration

Oestrus cycle before extract administration																					
		Day																			
Extract	Mouse	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A	I	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D
	II	E	M	D	P	E	M	D	P	E	M	M	D	P	E	M	D	P	E	M	D	P
	III	E	E	D	P	E	M	D	P	E	E	M	D	P	E	M	D	P	E	E	D	P
B	I	E	M	M	D	P	E	M	M	D	D	P	E	E	M	D	P	E	M	M	D	P
	II	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M
	III	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D
C	I	E	M	D	P	E	M	M	D	P	E	E	M	D	P	E	M	D	P	E	E	M
	II	D	P	D	E	E	M	D	P	E	E	M	D	P	E	E	M	D	P	E	E	M
	III	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P
F	I	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E
	II	D	P	E	M	D	D	P	E	M	D	P	E	M	D	D	P	E	M	D	D	P
	III	E	M	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M
VE	I	M	D	P	P	E	M	D	P	E	M	D	P	E	D	P	E	M	D	P	E	M
	II	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E
	III	M	D	P	E	E	M	D	P	E	E	M	D	P	E	E	M	D	P	E	E	M

Where A = *Bridelia micrantha* (Leaves), B = *Terminalia brownii* (stem bark), C = *Moringa oleifera* (seed), F = *Ximenia americana* (leaves) and VE = Negative control (Tween 80), P = Proestrous, E = Oestrous, D = Diestrous, and M= Metestrus.

All the groups showed the four phases of oestrous cycle taking 4-5 days for a single cycle.

Table A.2: The oestrus cycle phases during extract administration

Oestrus cycle during Extract administration																						
		Days																				
Extract	Mouse	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A	I	P	D	D	D	D	P	D	D	P	D	D	D	D	P	P	P	D	D	D	D	D
	II	E	D	D	D	D	P	D	P	D	D	D	D	D	P	D	D	P	P	P	P	P
	III	E	D	D	D	D	D	D	D	D	D	D	D	D	P	D	D	D	P	D	D	D
B	I	E	D	D	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
	II	M	D	P	P	D	P	D	D	P	P	P	P	P	P	P	P	P	P	P	P	P
	III	P	D	D	D	D	P	D	D	D	D	D	D	D	D	D	D	D	P	D	D	P
C	I	M	D	D	D	D	P	D	D	P	D	P	D	D	P	D	D	D	D	D	D	D
	II	D	D	D	D	D	D	P	P	D	D	P	P	D	D	D	D	D	D	D	D	D
	III	E	D	D	P	P	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	P
D	I	M	D	D	D	D	P	D	D	D	D	P	P	D	D	D	D	D	D	D	D	D
	II	E	D	D	D	D	P	P	D	P	D	P	P	P	P	P	P	P	P	P	P	P
	III	D	D	D	D	D	D	D	P	P	D	D	D	P	D	D	D	P	P	D	D	P
VE	I	D	P	E	M	D	P	E	M	D	P	E	M	P	E	M	D	P	E	M	P	E
	II	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M	D	P	E	M
	III	D	P	E	E	M	D	P	E	E	M	D	P	E	E	M	D	P	E	E	M	D

Where A = *Bridelia micrantha* (Leaves), B = *Terminalia brownii* (stem bark), C = *Moringa oleifera* (seed), F = *Ximenia americana* (leaves) and VE = Negative control (Tween 80), P = Proestrous, E = Oestrous, D = Diestrous, and M= Metestrus.

Table A.3: Fertility test of 16 groups of 5 mice in each group

Descriptive Statistics

	N	Mean		Std.
	Statistic	Statistic	Std. Error	Statistic
Group1	3	5.33	.333	.577
Group2	4	6.00	.408	.816
Group3	3	6.00	.577	1.000
Group4	3	6.00	.577	1.000
Group5	3	3.67	.333	.577
Group6	4	7.75	.629	1.258
Group7	4	5.75	.479	.957
Group8	4	4.75	.479	.957
Group9	3	8.00	.577	1.000
Group10	2	8.50	.500	.707
Group11	3	10.00	.577	1.000
Group12	2	5.00	1.000	1.414
Group13	2	4.50	.500	.707
Group14	2	7.50	.500	.707
Group15	2	7.00	1.000	1.414
Group16	4	7.75	.250	.500
Valid N (listwise)	2			

Table A.4: The first anti-fertility-screening test on 3 mice in each group

Descriptive Statistics

	N	Mean		Std.
	Statistic	Statistic	Std. Error	Statistic
LKRM	3	.00	.000	.000
LKRW	3	5.00	.577	1.000
LKRP	2	8.00	1.000	1.414
MOSE	3	.00	.000	.000
MOSD	3	9.00	.577	1.000
MOSW	3	6.67	.333	.577
MOTM	3	7.33	.333	.577
MORBM	3	6.00	.577	1.000
MORBW	2	6.50	.500	.707
MOAM	3	6.00	.577	1.000
TBSBE	3	.00	.000	.000
XALM	3	.00	.000	.000
XALW	3	.00	.000	.000
RRRW	2	7.50	.500	.707
RRRM	2	8.00	1.000	1.414
LKAM	2	10.50	.500	.707
BMAM	2	5.00	1.000	1.414
BMAW	3	.00	.000	.000
OMAM	2	9.00	1.000	1.414
OMAW	2	6.00	1.000	1.414
WATR	3	8.00	.577	1.000
TWEEN	2	6.00	1.000	1.414
FEM	3	.00	.000	.000
Valid N (listwise)	1			

Table A.5: The first reversibility test on 3 mice in each group

Descriptive Statistics

	N	Mean		Std.
	Statistic	Statistic	Std. Error	Statistic
MOSE	2	5.00	1.000	1.414
TBSBE	3	.00	.000	.000
XALM	2	8.00	1.000	1.414
LKRM	2	6.00	1.000	1.414
BMLW	2	5.00	1.000	1.414
XALW	2	5.50	.500	.707
FEMIPLAN	3	6.00	.577	1.000
Valid N (listwise)	2			

Where LKRM= *Lippia kituensis* (Root, methanol), LKRW = *Lippia kituensis* (Root, water extract), LKRP = *Lippia kituensis* (Root, petroleum ether extract), MOSE= *Moringa oleifera* (seed, ethyl acetate extract), MOSD = *Moringa oleifera* (seed, DCM extract), MOSW = *Moringa oleifera* (seed, Water extract), MOTM= *Moringa oleifera* (Twigs, methanol extract), MORBM = *Moringa oleifera* (Root bark, methanol extract), MORBW = *Moringa oleifera* (Root bark, water extract). MOAM = *Moringa oleifera* (aerial, methanol extract), TBSBE = *Terminalia brownii* (stem bark, ethyl acetate extract), XALM=*Ximenia americana* (leaves, methanol extract), XALW=*Ximenia americana* (leaves, water extract), RRRW= *Rhoicissus revoilii* (Root, methanol extract), RRRM = *Rhoicissus revoilii* (Root, water extract), LKAM= Lippia kituensis (aerial, methanol extract). BMAM= *Bridelia micrantha* (Aerial, methanol extract), BMAW= *Bridelia micrantha* (Aerial, water extract), OMAM= *Ocimum masaiense* (Aerial, methanol extract), OMAW= *Ocimum masaiense* (Aerial, water extract), WATR=Negative control (distilled water), TWEEN= Negative control (Tween), and FEM/ FEMIPLAN =Positive control (Femiplan).

Table A.6: The repeat of the anti-fertility-screening test on 3 mice in each group

Descriptive Statistics

	N	Mean		Std.
	Statistic	Statistic	Std. Error	Statistic
MOSE	3	.00	.000	.000
TBSBE	3	.00	.000	.000
XALM	3	7.00	.577	1.000
BALW	3	.00	.000	.000
XALW	3	.00	.000	.000
FEMH	3	.00	.000	.000
FEMM	3	.00	.000	.000
FEML	3	8.33	.667	1.155
DDW	3	6.33	.882	1.528
TWEEN	3	7.33	.882	1.528
Valid N (listwise)	3			

Table A.7: The reversibility test for the repeat screening for anti-fertility activity on 3 mice in each group

Descriptive Statistics

	N	Mean		Std.
	Statistic	Statistic	Std. Error	Statistic
MOSE	3	5.33	.333	.577
TBSBE	3	.00	.000	.000
BALW	2	5.50	.500	.707
XALW	3	6.33	.882	1.528
FEMH	3	6.67	.333	.577
FEMM	3	7.33	.333	.577
Valid N (listwise)	2			

Where MOSE= *Moringa oleifera* **(**seed, ethyl acetate extract), TBSBE = *Terminalia brownii* (stem bark, ethyl acetate extract). XALM=*Ximenia americana* (leaves, methanol extract)**,** XALW=*Ximenia americana* (leaves, water extract**),** BMLW= *Bridelia micrantha* (leaves, water extract)**,** FEMH=Positive control **(**Femiplan High dose of 180mg/kg), FEMM = Positive control **(**Femiplan Medium dose of 90mg/kg), FEML= Positive control **(**Femiplan low dose of 45mg/kg). DDW= Negative control (Distilled water) and TWEEN = Negative control (Tween 80).

Table A.8: ANOVA results for the significant difference in any of the groups from one another on the effect of the bioactive extracts on the weight of genital organs. The green value indicates the ρ value.

Anova: Single Factor for Uterus/Body Weight						
Groups	Count	Sum	Average	Variance		
BML	5	7.32	1.463	0.122		
TBSB	5	7.74	1.547	0.353		
MOS	5	7.58	1.516	0.0415		
XAL	3	6.01	2.003	0.647		
Contol	5	9.50	1.900	0.136		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.00	4	0.250	1.152	**0.364**	2.93
Within Groups	3.91	18	0.217			
Total	4.91	22				
Anova: Single Factor for ovaries/Body Weight						
Groups	Count	Sum	Average	Variance		
BML	5	1.889	0.377	0.008		
TBSB	5	1.88	0.375	0.0195		
MOS	5	2.02	0.404	0.00197		
XAL	3	1.73	0.5781	0.014		
Contol	5	2.37	0.474	0.0074		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.107	4	0.0268	2.737	**0.061**	2.927
Within Groups	0.1766	18	0.0098			
Total	0.283	22				

Table A.9: The T-test results for the significant difference between the experimental group and the control group in the ratio of weight of the ovary to weight of the mice. The green value is the ρ value

t-Test: MOS (Ovary) and Control			**t-Test: TBSB (Ovary) and Control**		
	Control	MOS		Control	TBSB
Mean	0.474	0.404	Mean	0.474	0.375
Variance	0.007	0.00198	Variance	0.00744	0.0195
Observations	5	5	Observations	5	5
Pooled Variance	0.005		Pooled Variance	0.0135	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	8		df	8	
t Stat	1.617		t Stat	1.341	
P(T<=t) one-tail	0.072		P(T<=t) one-tail	0.108	
t Critical one-tail	1.86		t Critical one-tail	1.859	
P(T<=t) two-tail	**0.145**		P(T<=t) two-tail	**0.216**	
t Critical two-tail	2.306		t Critical two-tail	2.306	
t-Test: BML (Ovary) and Control			**t-Test: XAL (Ovary) and Control**		
	Control	BML		Control	XAL
Mean	0.474	0.3778	Mean	0.474	0.578
Variance	0.007	0.0081	Variance	0.00744	0.0141
Observations	5	5	Observations	5	3
Pooled Variance	0.008		Pooled Variance	0.00963	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	8		df	6	
t Stat	1.732		t Stat	-1.446	
P(T<=t) one-tail	0.061		P(T<=t) one-tail	0.099	
t Critical one-tail	1.86		t Critical one-tail	1.943	
P(T<=t) two-tail	**0.122**		P(T<=t) two-tail	**0.198**	
t Critical two-tail	2.306		t Critical two-tail	2.447	

Table A.10: The T-test results for the significant difference between the experimental group and the control group in the ratio of weight of the uterus to weight of the mice. The green value is the ρ value

t-Test: MOS and Control			t-Test: BML and Control		
	Control	MOS		Control	BML
Mean	1.900	1.517	Mean	1.900	1.46
Variance	0.137	0.042	Variance	0.137	0.122
Observations	5.000	5.000	Observations	5.000	5
Pooled Variance	0.089		Pooled Variance	0.129	
Hypothesized Mean Difference	0.000		Hypothesized Mean Difference	0.000	
df	8.000		df	8.000	
t Stat	2.032		t Stat	1.919	
P(T<=t) one-tail	0.038		P(T<=t) one-tail	0.046	
t Critical one-tail	1.860		t Critical one-tail	1.860	
P(T<=t) two-tail	**0.077**		P(T<=t) two-tail	**0.091**	
t Critical two-tail	2.306		t Critical two-tail	2.306	
t-Test: TBSB and Control			**t-Test: XAL and Control**		
	Control	TBSB		Control	XAL
Mean	1.900	1.547	Mean	1.900	2.003 17
Variance	0.137	0.353	Variance	0.137	0.647 65
Observations	5.000	5.000	Observations	5.000	3
Pooled Variance	0.245		Pooled Variance	0.307	
Hypothesized Mean Difference	0.000		Hypothesized Mean Difference	0.000	
df	8.000		df	6.000	
t Stat	1.128		t Stat	-0.255	
P(T<=t) one-tail	0.146		P(T<=t) one-tail	0.404	
t Critical one-tail	1.860		t Critical one-tail	1.943	
P(T<=t) two-tail	**0.292**		P(T<=t) two-tail	**0.808**	
t Critical two-tail	2.306		t Critical two-tail	2.447	