EVALUATION OF ANTI-FERTILITY POTENTIAL OF SELECTED MEDICINAL PLANTS OF TANA RIVER COUNTY, KENYA.

A thesis submitted in fulfillment for the award of Doctor of Philosophy degree of the University of Nairobi (Reproductive Biology)

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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ABSTRACT

Approximately 200 million women in the developing world have unmet contraceptive need. Globally 20 million women procure unsafe abortions yearly due to the unmet contraceptive need. In Kenya; 465000 unsafe abortions were carried out in 2012 largely due to unplanned pregnancies. Conventional contraceptives though potent antifertility agents are not devoid of side effects. The search for novel affordable, reversible, safe and potent antifertility agents with minimal side effects is imperative. The overall objective of the study was to evaluate the anti-fertility properties of two selected medicinal plants (*Croton menyharthii* and *Uvariodendron kirkii*) in female Winstar rats. The study documented medicinal plants used for reproductive dysfunctions and also determined the pharmacological efficacy, phytochemical compounds, effect of extracts on reproductive parameters, reproductive hormonal profile and ovarian and uterine histomorphology of the selected plants. An ethnobotanical survey was carried out using questionnaires and focused group discussions. A total of 80 herbalists were interviewed. Pharmacological efficacy and phytochemical compounds of two most frequently mentioned plants namely *Croton menyharthi* and *Uvariodendron kirkii* were determined. The antifertility efficacy of the two plants on mating success, fertility index, gestation length, litter size and body weight was evaluated using 3 treatment regimes on normocyclic female winstar rats aged between 50-60 days. Cyclicity was monitored by daily vaginal smears and only rats with regular estrus cycles were used for the study. Male rats were introduced into female cages at the ratio of 1 male per 2 females at the appropriate time. A total of 96 rats were used. These were divided into 3 groups (1, 2 and 3) with 32 rats each. The 32 rats in each group were further divided into 4 subgroups (A, B, C, D) with 8 rats each. Group1, sub group A and B, received 500 and 800 mg/Kg of *Croton*

menyharthii respectively. Subgroup C and D of group 1 received 500 and 800 mg/kg *Uvariodendron kirkii* respectively. These doses were administered for 14 days through intra-abdominal gavage after which they were mated. The first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope. Group 2 animals were first mated after which sub group A and B received 500 and 800 mg/Kg of *Croton menyharthii* aqueous extract respectively and Subgroup C and D received 500 and 800 mg/Kg *Uvariodendron kirkii* respectively for 14 days. Group 3 sub group A, B, C and D were treated in a similar manner as group 1 except extract administration was continued after mating until end of gestation. Control groups consisted of eighteen negative control rats that received 0.5ml physiological saline through intra-abdominal gavage daily in 3 treatment protocols as in the experimental animals above. Six positive control rats received a subcutaneous injection of estrogen/ progesterone combination (15µg estradiol / 0.15 mg progestrin) once. Both negative and positive control animals were then mated. Gestation length, litter sizes as well as body weights of all animals were recorded. The effect of *Croton menyharthii* and *Uvariodendron kirkii* extracts on estrus cycle, implantation index, reproductive hormonal profiles, uterine and ovarian histomorphology was also carried out.The reproductive dysfunctions identified were pregnancy related complications, dysmenorrhea, menorrhagia, amenorrhea, oligomenorrhea, fibroids, infertility and fertility regulators in women. Forty eight plant species distributed in 40 genera and 29 families were documented as being important for the management of pregnancy related complications, menstrual disorders, infertility, fibroids and as fertility regulators. Thirteen (27.08%) plants were used to treat infertility and eleven plants (22.92%) were used as female fertility regulators. *Uvariodendron kirkii* and *Croton menyharthii* significantly disrupted the estrus cycle in rats.

Both plant extracts caused a prolonged duration of metestrus ($P<0.01$) and diestrus ($P< 0.001$) phases compared to the control. There was a significant decline in estrus ($P< 0.05$) and proestrus ($P<0.05$) phases. The plant extracts caused a dose related significant reduction in fertility index and implantation index. *Croton menyharthii* at 500mg/Kg and *Uvariodendron kirkii* at 800mg/Kg caused a significant ($P<0.05$) prolongation of the gestation length compared to the control (22 ± 1). U*variodendron kirkii* and *Croton menyharthii* aqueous extracts caused a significant reduction in litter size in group 1 and 3 at both dose levels compared to negative control. Both plant extracts however caused a non-significant reduction in litter size in group 2 at both dose levels. *Uvariodendron kirkii* and *Croton menyharthii* caused a significant reduction in FSH levels compared to the negative control. 17β estradiol serum levels significantly reduced in treated groups compared to positive and negative controls in a dose dependent manner by *Croton menyharthii* and *Uvariodendron kirkii* extracts. *Croton menyharthii* caused an increase in LH levels however *Uvariodendron kirkii* caused a non-significant reduction in LH levels at 500 and 800 mg / Kg compared to the negative control. Progesterone levels significantly increased ($P<0.01$) in treated groups compared to negative controls in a dose dependent manner by both plant extracts. Ovarian histomorphometry was disrupted with a significant finding being the loss of ovum in all ovaries in a dose dependent manner. *Croton menyharthii* caused 18% ($P< 0.05$) and 48% ($P<0.01$) loss of ovum at 500 mg/Kg and 800mg/Kg respectively. *Uvariodendron kirkii* caused the most significant ($P<0.001$) percentage loss of ovum; 39% at 500mg/Kg and 67% at 800mg/Kg. The study has shown that *Croton menyharthi* and *Uvariodendron kirkii* have potential as anti-fertility agents. The reversible anti fertility properties of both plants should however be evaluated in laboratory animals.

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LIST OF ABBREVIATION

AR-Androgen Receptor

CGC-condensed granulosa cell layer

CL-Corpus luteum

CTC-compromised theca cells

Cx37- Connexin 37

Cx43-Connexin 43

ED-Erectile Dsyfunction

ER-Estrogen Receptor

ES- Estrogen

FSH- Follicle Stimulating Hormone

FSHR- Follicle Stimulating Hormone Receptor

GnRH- Gonadotrophin Releasing Hormone

HSD-Hypoactive Sex Drive

IFAD-International Fund for Agriculture Development

IK-Indigenous Knowledge

LH-Luteinizing Hormone

LHR-Luteinizing Hormone Receptor

MSD- Male Sexual Dysfunction

P4- Progesterone

PGCs- Primordial Germ Cells

PG-Prostaglandin

PMS- Premenstrual syndrome

PPH-Post Partum Hemorrhage

PRL-Prolactin

RAB-Retained After Birth

SO-shrunken ooplasm

TMPs - Traditional Medicinal Practitioners

WHO- World Health Organization

CHAPTER 1

1.0 INTRODUCTION

Tremendous improvement in the reproductive health of women has been achieved in the developing world and an increase in the use of conventional fertility regulating methods noted. Nonetheless, many women in rural parts are not able to access information, supplies and services that could facilitate preventing unplanned pregnancies and planning the number and timing of desired pregnancies. Globally approximately 137 million women have unmet need for contraception (Gill et al., 2007), and this unmet need is particularly high in sub Saharan Africa where contraception use remains low, due generally to lack of access to contraceptive options in particular (Adebisi and Bello, 2011).

Unintended pregnancy combines unwanted and mistimed pregnancies. Many married women in developing countries do not have access to contraceptive method of choice in order to space or limit family size and the options are even more limited for unmarried women and adolescents who rarely have access to reproductive health information, counseling and are often excluded from contraceptive services. Some of the barriers to contraceptive use in developing countries are; fear of side effects and health concerns, inadequate access to reproductive health information and services, religious beliefs, fear of social disapproval, opposition from spouses and family, cost is an issue for the poor. The strong desire to regulate fertility combined with lack of access to effective contraceptive results in largest number of unintended pregnancies which in turn leads to unsafe abortions. Globally 205 million pregnancies are unintended and approximately 20 million unsafe abortions are carried out yearly resulting in a 13% maternal mortality rate. Ninety seven percent of these unsafe abortions occur in developing countries (Sedgh and Henshaw, 2010) where 67,000 women die

yearly as a result of unsafe abortions (Ahman and Shah, 2007). In a Kenyan health survey of 2008 to 2009, 43% of births in the preceding five years, were reported by women as unwanted or mistimed (Kenya Demographic Health Survey 2008/09) and a total of 465,000 unsafe abortions were reportedly procured, where 266 out of 100 000 women die yearly due to unsafe abortions, mostly resulting from unintended pregnancy (Izugbara et al., 2013). A gap exists between actual and desired family size resulting in unintended pregnancies. Sixty six percent of unintended pregnancies occur among women who are not using any method of contraception. Modern contraceptive methods are safe and effective when used according to directions. Many steroidal and non-steroidal compounds have been used as contraceptive and anti-ovulatory agents to control fertility. The drugs, though potent anti-fertility agents are not devoid of side effects. Skilled health care workers are often unavailable in resource poor settings, so options that allow for non-medical staff delivery might increase access to contraceptives. Recently, there has been renewed interest, spearheaded by World Health Organization (WHO), in the use of medicinal plants for primary healthcare needs. This interest has led to increased research on traditional medicines. To alleviate the unmet contraceptive need in Tana River, this study was carried out to validate the anti-fertility potential of *Croton menyhathii* and *Uvariodendron kirkii* traditionally consumed by women in Tana River. Contraceptive properties of a medicinal plant can be as a result of hypothalamo - pituitary gonadal axis hormonal disruption.

1.2 JUSTIFICATION

Reproductive issues and ailments constitute 18% of the global burden of disease for women of reproductive age and are the number one cause of maternal mortality in developing countries (WHO, 2003). Female reproductive ailments range from pregnancy and related

complications, fertility issues and menstrual complications. TMPs by their nature do not keep records and most of the knowledge they have is passed on verbally from generation to generation (Giday et al., 2010). There is thus need not only to capture this indigenous knowledge but also to study the plants in order to provide credible evidence to support therapeutic efficacy claims by herbalists (Sofowora, 1993). In Tana River County (Figure 1) TMPs are routinely consulted because of their wide indigenous medicinal knowledge base (Kaingu et al., 2011), a tradition that has persisted in many rural communities due to inequitable health provision. In Kenya, 75% of health facilities and personnel are concentrated in urban areas (WHO, 2005). The national doctor patient ratio is 1: 20,000; but in Tana River County with 57 health facilities and a population of 240075 (Kenya population and Housing census report, 2009); the doctor patient ratio is 1: 95,500 emphasizing a serious shortage of both health facilities and staff in the County (Tana River District Strategic Plan, 2005-2010). On the other hand, the ratio of TMPs to patients is 1: 987 (Kenya Population and Housing census report, 2009), suggesting that the TMPs are more readily accessible. The locals are 90% of the time attended to by clinical officers who refer emergency cases to Malindi District hospital 90 km away. In General, the health sector including reproductive health faces a number of challenges. According to the Tana River District Strategic Plan (2005 - 2010); issues of major concern are; unsafe motherhood, high maternal mortality rates and inadequate family planning services. Globally 205 million pregnancies are unintended. Approximately 20 million unsafe abortions are carried out yearly. Ninety seven percent of the unsafe abortions are carried out in developing countries. Some of the current contraceptive technologies cause undesirable side effects while others are culturally unacceptable. It is

therefore important to undertake research and explore the anti-fertility properties of medicinal plants in order to offer alternative forms of contraceptive technologies.

1.3 OVERALL OBJECTIVE

The overall objective of the study was to evaluate the anti-fertility properties of two selected medicinal plants (*Croton menyharthii* and *Uvariodendron kirkii*) in female Winstar rats.

1.3.1 Specific Objectives

1. To identify and document medicinal plants used by Traditional Medicinal Practitioners (TMPs) for reproductive health management in Tana River County; with particular emphasis on female fertility regulation.
2. Evaluate the anti-fertility efficacy of the two selected plant extracts on female rat reproductive parameters.
3. Evaluate the effect of the two plant extracts on the histomorphology of ovaries and uterus.
4. Determine the effect of the two plant extracts on reproductive hormonal profiles.
5. Determine the acute toxicity as well as phytochemical compounds of the two plants.

1.4 NULL HYPOTHESIS

Croton menyharthii and *Uvariodendron kirkii* plants do not have fertility regulating effect.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1.0 Overview of the mammalian female reproductive process

2.1.1 Oogenesis and Ovulation

Oogonia originate from primordial germ cells (PGCs). PGCs migrate to the genital ridge, proliferate by mitosis and give rise to oogonia (Sánchez and Smitz, 2012). Migration, proliferation and colonization of PGCs to the developing gonads depend on the interaction between PGCs and their surrounding somatic cells (Tingen et al., 2009). Following colonization of the gonad, PGCs undergo mitotic proliferation leading to the formation of 'germ cell nests' (Tingen et al., 2009; Pepling and Spradling, 2001). With time and before follicle formation, mitotic divisions stop and germ cells initiate meiosis to become primary oocytes (Tingen et al., 2009; Pepling and Spradling, 2001). Meiosis is arrested at prophase 1 until LH induces final oocyte maturation. Prophase events that are hormone dependent, are vital for germ cell survival hence endocrine-disrupting compounds may interfere with oogenesis by causing disturbances in spindle formation, interfering with microtubule polymerization and inducing multipolar spindles as seen in mouse oocytes (Tingen et al., 2009; Sánchez and Smitz, 2012). Therefore plant secondary compounds might disrupt meiosis; compromise oogenesis and result in infertility. Germ cell nests breakdown to form somatic pre-granulosa cells that surround oocytes thus initiating the formation of primordial follicles around the time of meiotic arrest. Somatic follicle formation occurs immediately after birth in the mouse and rats (Pepling and Spradling, 2001; Pepling, 2006).

Hormones play a key role in maintenance of germ cell line. Jefferson et al., (2002) pointed out that estrogens maintain germ cell nests via the estrogen receptor (ER)- β. With time

primordial follicles are activated and continuously recruited in cohorts to initiate folliculogenesis (Sánchez and Smitz, 2012). The activation is a very dynamic and tightly controlled process, but despite the enormous progress that has been made, many molecular mechanisms are still not fully understood. Early pre-antral follicles are independent of follicle stimulating hormone for their initial growth, as evidenced by the fact that development to the primary and secondary stage can take place in the absence of hormones (Adriaens et al., 2004; Sánchez and Smitz, 2012). Instead, primary to secondary follicle transition is driven by local intra-ovarian paracrine factors produced by oocytes, their companion granulosa cells and theca cells (Kol and Adashi, 1995, Sánchez and Smitz, 2012). Several studies using specific knock-out mice have also confirmed the importance of intra-ovarian factors in progression of primary to secondary follicles (Nilsson and Skinner, 2002; Dong et al., 1996; Elvin et al., 1999, Otsuka et al., 2000; Yan et al., 2001; Wang et al., 2001; Walters et al., 2008). It therefore follows that a disruption of intra ovarian factors would impede this progression. Communication among granulosa cells and between granulosa cells and the oocyte is crucial at all stages of folliculogenesis. This is achieved through gap junction proteins also known as connexins 43 and 37 (Cx43 and Cx37). Cx43 expressed on granulosa cells forms gap junctions between granulosa cells (Juneja et al., 1996; Gittens et al., 2003; Gittens et al., 2005; Sánchez and Smitz, 2012), whereas Cx37, expressed on oocytes at all stages of follicle development, is crucial for an oocyte–granulosa cell communication. Mice ovaries lacking Cx37, exhibit arrested folliculogenesis at the early antral stage and oocyte meiotic competence is compromised (Carabatsos, 2000; Simon et al., 1997; Dan et al., 2007). Therefore any compound that disrupts the intra ovarian factors involved in oogenesis and

folliculogenesis might lead to incompetent oocyte development that may result in ovulation, and/or fertilization failure.

Follicular development throughout the very early stages has been considered to be gonadotropin- independent and essentially driven by locally secreted factors. When follicles reach the preantral stages, development throughout this period and progression to the early antral stage still rely primarily on intraovarian factors; however, unlike in earlier stages, follicles express functional FSH and LH receptors and are able to respond to gonadotropins. Antral follicle development starts with antrum formation and the differentiation of granulosa cells into the cumulus and mural cell compartments, which confers to the oocyte the competence to resume meiosis. The progression throughout the antral stages and ovulation is dependent on pituitary-secreted gonadotropin (FSH and LH) support. FSH is the driver for antral development. FSH induces luteinizing hormone receptor expression in mural cells. The expression is required for follicles to respond to LH, the latter being crucial for triggering the ovulatory process (Sánchez and Smitz, 2012). Action of both gonadotropins in the ovary is mediated by binding and activation of their receptors (LH receptor, LHR and FSH receptor, FSHR). In LHR knockout mice, follicle development does not progress beyond the antral stage; the mice become infertile due to low estrogen production and anovulation (Zhang et al., 2001).

Mice deficient in FSH (FSH- β knockouts) are infertile due to a block of the follicle development prior to the antral stage. Several studies have demonstrated that in the absence of FSH, cultured follicles are arrested in their development; do not support antrum formation and exhibit apoptosis, all of which can be prevented by FSH supplementation (Nayudu and Osborn, 1992; Cortvrindt et al., 1996).

Thus, although gonadotropins are essential during antral stages in vitro, a-dose-fine-tuning is critical to obtain an appropriate antral development (Sánchez and Smitz, 2012). A disruption of LH and FSH concentration levels is detrimental to folliculogenesis and oogenesis (Sánchez and Smitz, 2012). Therefore interference with the hormonal profiles can result in infertility.

Under the influence of gonadotropins, follicles synthesize steroid hormones such as androgens and estrogens, which contribute to follicular development, by inducing granulosa cell proliferation and differentiation via the androgen receptor (AR) and estrogen receptor (ER), respectively (Sánchez and Smitz, 2012; Findlay and Drummond, 1999; Drummond et al., 1999; Findlay et al., 2000). Through the two-cell, two-gonadotropin model, theca cells produce androgens under the influence of LH, whereas granulosa cells produce estrogens using androgens as a substrate, under the influence of FSH (Sánchez and Smitz, 2012; Hillier et al., 1994). One of the major functions of pre-ovulatory granulosa cells is the synthesis of estradiol. In the follicle, estradiol is produced via the enzyme aromatase, and it enhances the response of granulosa cells to the gonadotropins. Intra ovarian factors (activins and inhibins) produced by granulosa cells play essential paracrine roles by regulating the LH-induced androgen synthesis by theca cells (Sánchez and Smitz, 2012), and therefore ensure estradiol supply.

Therefore exogenous factors or compounds that disrupt the physiological function of the granulosa and theca cell will disrupt steroid hormone production leading to compromised oogenesis and folliculogenesis resulting in infertility. Finally, preovulatory follicles containing fully-grown oocytes are ready to undergo ovulation, which is induced by the pre-ovulatory LH surge. Ovulation is characterized by the rupture of the follicle wall and the release of the cumulus–oocyte complex; at this time the oocyte has resumed meiosis and has

progressed to the metaphase II stage of meiosis. After ovulation, granulosa and theca cells become luteal cells and are responsible for the production of estradiol and progesterone, the latter predominantly expressed in the corpus luteum (Kwintkiewicz and Giudice, 2009; Sánchez and Smitz, 2012). Oocyte meiotic maturation involves a cascade of processes that is initiated with the pre-ovulatory LH surge, leading to the progression of the oocyte to the metaphase II stage and ending with the extrusion of the first polar body. After the LH surge, an event that is essential for the oocyte to resume meiosis is the expansion of cumulus cells, which is caused by the production of hyaluronic acid produced by the cumulus cells in response to gonadotropins. Cumulus expansion is dependent on the stimulation of LH-induced epidermal growth factor (EGF)-like peptides. Therefore a disruption of gonadotropin concentrations will disrupt physiological functions within the ovary leading to infertility. Oocyte–granulosa cell interaction mediated by either paracrine signals and by gap junctional communication from early stages of development determines the rate of follicle growth and differentiation. Furthermore, after differentiation, oocyte–cumulus cell interaction is essential to promote oocyte nuclear and cytoplasmic maturation, which determine the capacity of the oocyte to support early embryo development (De La Fuente and Eppig, 2001; Sánchez and Smitz, 2012).

Therefore exogenous factors or compounds that disrupt the critical oocyte–granulosa cell interaction will interfere with oocyte nuclear and cytoplasmic maturation thereby compromising fertility. Gap junctions play a crucial role in the bidirectional communication between oocytes and cumulus cells, allowing the passage of molecules of different types, such as amino acids and metabolites such as pyruvate from the cumulus to the oocyte (Su et al., 2009). Indeed expression of gap junction proteins Cx43 and Cx37 in granulosa cells and

oocytes, respectively, has been shown to be of vital importance in guaranteeing oocyte and follicle development (Gittens et al., 2005). A disruption of the critical gap junction communication between oocyte and granulosa cell will compromise developmental competence of the oocyte and interfere with ovulation, thereby compromising fertility.

2.1.2 Fertilization

Successful fertilization requires complex spermatozoa-ova interactions. Fertilization involves many sequential steps, beginning with the binding of spermatozoa to the zona pellucida, followed by the acrosome reaction and penetration of spermatozoa through the zona pellucida, and ending with the fusion of the sperm and egg pronuclei (Sánchez and Smitz, 2012). The zona pellucida is vital for viable oocyte development, for fertilization (e.g., in gamete recognition at the zona pellucida), in the prevention of polyspermia and for the protection of early embryos before implantation (Conner et al., 2005; Pang et al., 2011, Wassarman, 2008; Matzuk et al., 2002). The blastocyst for example, becomes competent for implantation only after shedding the zona pellucida (Sánchez and Smitz, 2012). It therefore follows that shedding of the zona pelucida or an interference with the structural intergrity of the zona pelucida will interfere with nutrients and other molecular transfer through the gap junctions that transverse the zona pelucida.

2.1.3 Implantation and establishment of pregnancy.

Successful implantation depends on the quality of the blastocyst, a receptive endometrium and the synchronization between the developmental stages of the embryo itself. Implantation is a complex process involving coordinated effects of autocrine, paracrine and endocrine factors and is modulated by various factors some of which are still being elucidated (Mohan

et al., 2011). It therefore follows that implantation failure can be due to compromised blastocyst quality and or compromised endometrial receptivity.

Animal models have provided valuable insights into the molecular mechanisms that occur during embryo implantation (Wang et al., 2004). However despite extensive research in this field, the majority of pregnancy losses occur before or during implantation. Every ninth couple in Europe and the USA is affected by implantation disorders and pregnancy wastage (Krussel et al., 2003). The success of implantation depends on achieving the appropriate embryo development to the blastocyst stage (Kubota et al., 2016) and at the same time the development of an endometrium that is receptive to the embryo. Endometrium is known to become receptive only for short periods in rodents and humans. Beyond this period of receptivity, the embryo is unable to successfully establish contact with a refractive endometrium. Following fertilization, locally acting hormones and growth factors mediate the initial dialogue between the free floating blastocyct and a receptive endometrium (Lopata et al., 2002). In every ovulatory cycle, when the endometrium is exposed to estrogen and progesterone in an orderly manner, the endometrium becomes 'decidualized' during the second half of the cycle to allow implantation of the embryo. Decidualizalion which requires both estrogen and progesterone is an irreversible process, and if implantation does not occur programmed cell death (apoptosis) ensues. Thus progesterone and estrogen mobilize several molecular modulators which support embryo implantation (Lim et al., 2002). These hormones exert their effects on the endometrium via nuclear estrogen (ER) and progesterone (PR) receptors (Wang and Dey, 2006). The steroid hormones and the presence of viable blastocyst in uterus, induce the endometrial stromal cells to undergo decidualization. During decidualization, the endometrial tissue also undergoes a secretory transformation of the

uterine glands and vascular remodeling (Gellersen et al., 2007; Ma et al., 2003). It is therefore possible that abnormal progesterone and estrogen synthesis compromises decidualization leading to implantation failure (Mohan et al., 2011).

Prostaglandins play important roles in reproductive processes such as ovulation and implantation (Kang et al., 2005). Cyclooxygenases are enzymes responsible for various prostaglandin syntheses (Mohan et al., 2011). Cyclooxygenase-2 (Cox-2) expression is regulated by ovarian steroid hormones and during blastocyst adhesion this expression is critical for successful implantation (St-Louis et al., 2010). Achache (2010) reports that recurrent implantation failure in humans is closely associated with low levels of Cox-2 possibly as a result of reduced progesterone synthesis. It therefore follows that an interference in the concentration levels of one or both hormones (Kubota et al., 2016) compromises implantation and leads to infertility. Further understanding of the molecular factors that modulate implantation might be useful in the management of recurrent implantation failures.

2.1.4 Hormonal regulation overview

The hypothalamus through its secretion of gonadotropin releasing hormone drives anterior pituitary gonadotropin production (Beshay and Carr, 2012). FSH and LH act on the ovaries to promote follicle development, ovulation, formation of corpus luteum and secretion of ovarian steroid hormones. Nuclear estrogen receptor and progesterone receptor mediate many of the actions of estrogen and progesterone hormones (Kubota et al., 2016). Mice lacking β estrogen receptor (ERβ) exhibit lack of progression from pre to antral follicles (Emmen et al., 2005). Steroid synthesis inhibitors decrease survival and growth of antrum follicles and reduce hormone production by secondary follicles (Rodrigues et al., 2015). Estrogen is required for normal follicle health; it inhibits granulosa cell apoptosis, diminishes follicle atresia in rats

(Piyali et al., 2012) and increases the number and size of rodent and bovine follicles (Rodrigues et al., 2015). Progesterone is also anti-apoptotic but inhibits follicle growth at high doses. Indeed studies in the rat suggest that progesterone is not necessary for antral follicle growth (Piyali et al., 2012). Rats possessing null mutations at the progesterone receptor (null female) exhibit failure in ovulation and ovulatory processes and are thereby infertile (Kubota et al., 2016).Under the influence of estrogen and progesterone, the endometrium undergoes a transition and acquires an appropriate morphological and functional state; referred to as the 'window of implantation' during which the blastocyst attaches to the endometrium (Beshay and Carr, 2012). Estradiol is critical for the growth and thickening of the endometrium; while progesterone is responsible for endometrial gland proliferation and secretion (Kubota et al., 2016) which has nutritive value to the embryo and also essential for implantation.

The establishment of pregnancy requires the presence of a functional corpus luteum (CL) that is able to produce sufficient progesterone. A viable conceptus sends specific signals to a "pregnancy-ready" uterus; these signals rescue the corpus luteum from luteolysis. Maternal recognition of pregnancy in rodents involves activation of the non-functional CL of the estrous cycle into the functional CL of pregnancy. The formation and maintenance of CL and production of progesterone require two events. First, mating induces the release of prolactin (PRL) from the anterior pituitary, which increases LH receptors on luteal cells to form the CL and suppress 20α-hydroxysteroid dehydrogenase activity; this transition prevents the conversion of progesterone to 20α-hydroxyprogesterone, which will not support pregnancy. Second, the lactogenic hormones that are produced by the uterine decidua and placenta act through prolactin receptors on the luteal cells to maintain their function and the production of progesterone throughout gestation. LH is key in luteolization of the remaining granulosa cells

following ovulation. A disruption of LH release will therefore compromise CL formation, lead to inadequate levels of progesterone which in-turn interferes with decidualization of the endometrium leading to failed implantation (Pang et al., 2011).

2.1.5 Phytoestrogens.

Environmental estrogens are derived mainly from phytoestrogens or synthetic estrogens (Burton and Wells, 2002). Phytoestrogens are naturally occurring phytochemicals present in plants and are structurally and functionally similar to isoflavones (17β-oestradiol) or synthetic estrogens such as diethylstilboestrol (lignins). Examples of isoflavone include coumestrol, genistein, diadzen and equol (Whitten et al., 1995). According to Whitten et al. (1995) phytoestrogens exert their biological activity by; mimicking the action of endogenous estrogens, acting as estrogen antagonists, altering the pattern of synthesis and metabolism of endogenous hormone and modifying hormone receptor values. However, in the absence of endogenous estrogen, isoflavones have a weakly estrogenic effect while exhibiting an anti-estrogenic property in their presence. From previous studies, phytoestrogens were shown to exhibit uterotrophic effect (increased uterine weight), reduced ovarian weight and hyperplasia of the endometrium (El Samannoudy et al., 1980; Whitten et al., 1992). For example, neonatal rats exposed to either coumestrol or genistein had increased uterine wet and dry weight until day 10 after post natal life (Medlock et al., 1995a: 1995b; Santell et al., 1997). Furthermore, phytoestrogens like coumestrol and diethylstibestrol were shown to induce significant long term abnormalities in the reproductive tract that included endometrial squamous metaplasia, absence of corpora lutea, decreased number of graffian follicle and increased number of atretic follicles in mice (Murthy and Venkaiah, 2010). Whitten et al. (1993) further showed that the animals exhibited persistent cornification of the vaginal epithelial cells which is

indicative of persistent estrous stage of the estrus cycle. Moreover, these animals failed to respond to priming by estrogen and progesterone with an LH surge at the expected time. This implies that exposure to phytoestrogens during the neonatal period could affect the hypothalamo- hypophysial- ovarian axis (Murthy and Venkaiah, 2010). In the adult mice, intra peritoneal administration of benzene extract from flowers of *Hibiscus rosa sinensis* induced irregular estrous cycle with a dose dependent prolonged estrus and metestrus stage (Murthy and Venkaiah, 2010). Resveratrol, a phytoestrogen naturally found in grape with a structure similar to diethylstilbestrol decreased body weight, disrupted estrous cyclicity and increased ovarian weight in intact female rats (Henry and Witt, 2002). In humans, phytoestrogens exert an effect on the menstrual cycle; however, these findings are inconsistent (Whitten and Naftolin, 1998). For instance, Cassidy et al. (1994) revealed that a diet containing soy protein delays the onset of menstruation and prolongs the follicular phase of the cycle, suppresses the mid cycle LH and FSH peak plasma concentrations, increases plasma concentrations of estradiol and decreases cholesterol concentration in the follicular phase. On the other hand, Duncan et al. (1999) illustrated that a diet rich in soy isoflavone reduced plasma estrone concentration but had no impact on the length of the menstrual cycle, follicular or luteal phases. The effect of phytoestrogens in the female genital tract may be dependent on the age and duration of exposure (Burton and Wells, 2002). The presence of high levels of phytoestrogens in the body, originating from herbal remedies, could thus lead to endogenous endocrine disruption that could be detrimental to reproductive performance. This is an area that can be exploited through research for fertility regulation.

2.1.6 Rat estrus cycle, Gestation length and Litter size

The estrus cycle of the rat (like any mammal) is characterized by proesrus (nucleated epithelial cells), estrus (a-nucleated epithelial cells), metestrus (leukocytes, cornified and nucleated epithelial cells in equal proportions) and diestrus (mainly leukocytes) within the vagina and lasts an average of 4-5 days (Paccola et al., 2013; Hamid and Zakaria, 2013). Ovulation occurs from the beginning of proestrus until the end of estrus. There are fluctuations in the level of reproductive hormones during the estrus cycle (Goldman et al., 2007). For instance, in the rat, the level of prolactin, LH and FSH remain low during most of the estrous cycle, and increase in the second half of proestrus, while estradiol peaks in early proestrus, dips to baseline values and rises again during mid estrous before returning to baseline levels (Paccola et al., 2013). Progesterone peaks in early diestrus and then at mid Proestrus. The varying amounts of hormones, particularly estradiol-17β and progesterone during the different stages of the estrous cycle induce cyclic changes in the cell morphology and histology of the uterine epithelium and ovaries of the rat. At diestrus, the uterus is small and inactive with a slit-like lumen lined with low cuboidal or columnar epithelium showing occasional degenerate cells. Within the ovaries, the corpora lutea attains maximum size with vacuoles at the centre and the presence of early formation of fibrous tissue (Westwood, 2008). During proestrus, the endometrium is lined by tall cuboidal or columnar epithelium with frequent mitosis and little or no epithelial cell degeneration of the gland or epithelium. The vasculature of the endometrium becomes prominent, the lumen becomes dilated, and there is the presence of edema in the stroma. In the ovaries, degeneration of the corpora lutea occurs with marked presence of central fibrous tissue formation (Westwood, 2008). In estrus, there is cellular degeneration in the gland and epithelial lining of the uterus, loss of mitotic activity

and leucocyte infiltration, while the dilation of the lumen may persist until late estrus. At this stage the ovaries, present degenerated corpora lutea and sometimes newly formed corpora lutea with central fluid filled cavity, devoid of fibrous tissue formation (Westwood, 2008). During metestrus, there is continued vacuolar degeneration in the epithelium of the uterus with marked return of mitotic activity. The uterine endometrial morphological changes are driven by ovarian steroids. Therefore a disruption or interference in estrogen and progesterone ratios compromises these changes thereby compromising fertilization and implantation. The rat has a short gestation length (21± 2 days) and is therefore an ideal model for studying effects of chemicals on reproductive parameters (Jacobsein and Stanleya, 2013). The litter size of a rat is 9±3. Hamid and Zakaria (2013) have reported on lengthening of the rat gestation length due to effects of endocrine disruptors, while the litter size was not significantly affected. The present study aims at studying the effect of fertility regulating plant extracts on gestation length and litter size.

2.1.7 Female reproductive health management using herbal remedies

Herbal medicines have been used for the treatment of human ailments for thousands of years (Yakubu et al., 2007a; Yakubu and Bukoye, 2009). In developing countries 80% of the population still relies on traditional medicine to meet their healthcare needs (WHO, 2003). It is no wonder therefore that World Health Organization is pushing for renewed research interest on medicinal plants used by traditional medicine practitioners (TMPs) the world over. The importance of herbal remedies is further emphasized by the fact that globally 30% of the pharmaceutical preparations used for conventional medicine preparation are based on plants (Shinwari et al., 2006).

Reproductive issues and ailments constitute 18% of the global burden of disease for women of reproductive age and are the number one cause of maternal mortality in developing countries (WHO, 2003). Female reproductive ailments range from pregnancy and related complications, fertility issues and menstrual complications. TMPs by their nature do not keep records and most of the knowledge they have is passed on verbally from generation to generation (Giday et al., 2010). There is thus need not only to capture this indigenous knowledge but also to study the plants in order to provide credible evidence to support therapeutic efficacy claims by herbalists (Sofowora, 1993). In Tana River County (Figure 1) TMPs are routinely consulted because of their wide indigenous medicinal knowledge base (Kaingu et al., 2011), a tradition that has persisted in many rural communities due to inequitable health provision. In Kenya, 75% of health facilities and personnel are concentrated in urban areas (WHO, 2005). The national doctor patient ratio is 1: 20,000; but in Tana River County with 57 health facilities and a population of 240 075 (Kenya Population and Hosuing census report, 2009); the doctor patient ratio is 1: 95,500 emphasizing a serious shortage of both health facilities and staff in the County (Tana River District Strategic Plan, 2005-2010). On the other hand, the ratio of TMPs to patients is 1: 987 (Kenya Population and Housing census report, 2009), suggesting that the TMPs are more readily accessible. The locals are 90% of the time attended to by clinical officers who refer emergency cases to Malindi District hospital 90 Km away. In General, the health sector including reproductive health faces a number of challenges. According to the Tana River District Strategic Plan (2005 - 2010); issues of major concern are; unsafe motherhood, high maternal mortality rates and inadequate family planning services. The study will document and identify two plants with the highest use value for further *in vivo* physiological tests in female rats.

2.1.7.1 Infertility

Infertility, which is defined as the inability to conceive after one year of unprotected intercourse in humans has a global prevalence of 9% Boivin et al. 2007. Among infertile couples, it is estimated that the cause is predominantly feminine in 38% of the cases and primarily masculine in 20% of the cases while 27% have both male and female abnormalities, and the remaining 15% have no identifiable cause (Bretveld et al., 2007). Infertility affects 50 to 80 million people globally, out of which 20 to 35 million couples in Africa experience this problem. The prevalence of infertility is particularly high in sub-saharan Africa ranging from 20% to 60% (Unuane et al., 2011). The common causes of female infertility include ovulatory disorders, endometriosis, pelvic adhesions, tubal blockage or damage, and hyperprolactinemia (Unuane et al., 2011). Based on several interactions between thyroid hormone and the female reproductive system (Poppe and Velkeniers, 2004) hypothyroidism has been associated with reproductive disorders ranging from abnormal sexual development to menstrual irregularities and infertility. Gamete production to intrauterine development of the embryo is believed to be susceptible to endocrine disruption, triggering morphological and functional abnormalities (Diamanti-Kandarakis et al., 2009; Caserta et al., 2011). Caserta et al. 2011 reports on female reproductive disorders including infertility, endometriosis, uterine and ovarian disorders, such as premature ovarian failure and polycystic ovary syndrome caused by endocrine disruptors of plant origin. Soy for instance, contains genistein a phytoestrogen (Cederroth et al., 2012) and could thus be an endocrine disruptor hence the need for further research to explore these possibilities.

2.1.7.2 Pregnancy and related issues

More than 500,000 women die annually of pregnancy related complications, eighty six percent from sub Saharan Africa (WHO, 2003). 150,000 of these maternal deaths result from bleeding complications (Satoko et al., 2006, De Bernis., 2003). In Kenya maternal mortality due to pregnancy complications remains unacceptably high at 590 per every 100,000 live birth. Globally, post-partum hemorrhage is the leading single direct cause of maternal mortality Rajan and Wing. (2010). In Tana River County, Kenya, threatened abortion, protracted labor, premature labor, retained after birth, post- partum hemorrhage, inadequate milk production are some of the pregnancy related complications handled by TMPs where retained placenta is again the commonest cause of post-partum hemorrhage. Generally, rural women who deliver at home are more vulnerable to post-partum hemorrhage (Prata et al., 2012) and therefore medicinal plants used for their hemostatic properties may represent an alternative to conventional hemostatic in such resource poor communities. Some of these plants are *Musa sapientum* L, *Jatropha multifida* L, *Rauvolfia vomitoria* Afzel, *Annona senegalensis* L, *Macrosphyra longistyla* DC and *Newbouldia leavis P. Beauv.*

2.1.7.3 Inadequate milk production

Galactogogues are substances that augment established lactation. Galactogogues may be synthetic, plant-derived or endogenous products. These medications increase prolactin secretion by antagonizing dopamine receptors (Gabay, 2002). Dopamine agonists and antagonists regulate prolactin synthesis and secretion through interaction with the hypothalamus and anterior pituitary and thereby control milk production (Behera et al., 2013). *Medicago sativa, Nigella sativa; Cnicus benedictus; Borago officinalis; Carum carvi* seeds, *Trigonella foenum graecum, Foeniculum vulgare, Rubus idaeus, Urtica dioica, Utica urens,*

Cnicus benedictus, Vitex agnus-castus, Medicago sativa, Cimicifuga resebosa, Galega officianalis and *Anethum graveolens* seeds are some of the plants with established galactogogue effects (Zuppa et al., 2010; Sharrif, 2011; Farhadi et al., 2012; Jana and Shekhawat, 2010). The Ethnobotanical study carried out in Tana River revealed the use of certain plants in milk letdown induction following parturition (Kaingu et al., 2013a).

2.1.7.4 Mastitis

Mastitis is a complex disease with multiple causative agents inclusive of host and pathogen factors. Antimicrobials have good prognostic value but due to emerging multi drug resistant micro-organisms it is important to explore phytochemicals as potential candidates for new antibiotics. Phytochemical compounds might come in handy as they inhibit bacterial growth by different mechanisms than the presently used antibiotics (Aires et al., 2016). Plant bio active compounds belong to chemical structural classes like phenolics, terpenoids, other essential oil constituents, alkaloid, lectins, polypeptides and poly acetylenes.

2.1.7.5 Reproductive tract ailments

2.1.7.5.1 Menses

Menstruation occurs as a regular cyclic event. Disruption of the regulated sequence at cellular level can lead to a range of menstrual disorders including menorrhagia, amenorrhea, hypomenorrhoea, oligomenorrhoea and dysmenorrhea Mukta and Patki. (2012). The median menstrual cycle length is 28 ± 3 days in human and the average duration of menstrual flow is 5 ± 2 days. This cyclic process is regulated by complex changes in the concentrations of gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone. Menstrual irregularities can be due to

hypothalamic disorders, significant weight loss, strenuous exercise, substantial changes in sleeping or eating habits and severe stress. *Menorrhagia* is menstrual bleeding longer than seven days or in an amount exceeding 80 ml from normal secretory endometrium Diaz et al. 2006. It affects 10-30% of the menstruating women. *Metrorrhagia* is irregular intra menstrual cycle uterine bleeding while *polymenorrhea* is menstruation that occurs too frequently. *Oligomenorrhea* is an abnormally infrequent menstrual bleeding characterized by 3-6 menstrual cycles per year. When menstrual bleeding does occur, it can be profuse and prolonged or decreased in amount. *Primary amenorrhea* should be considered for any adolescent who has not reached menarche by the age of 15 years or has not done so within three years of thelarche. There are three types of dysmenorrhea: Primary, secondary and membranous. *Primary dysmenorrhea* is characterized by the absence of an organic etiology, while *secondary dysmenorrhea* is associated with specific diseases or disorders, such as endometriosis, ovarian cysts, pelvic inflammatory disease, adenomyosis, cervical stenosis, fibroid polyps and possibly uterine displacement with fixation. *Membranous dysmenorrhea* (uterine cast) is rare and causes intense cramping pain due to the passage of the intact endometrial cast through an undilated cervix (Gerbie, 1987). In women with dysmenorrhea, the concentrations of prostaglandins (PG), both PGF2α and PGE, in menstrual blood are significantly increased compared to those in women without dysmenorrhea.

2.1.7.5.2 Fibroids

Uterine fibroids are the most common nonmalignant tumors in women of reproductive age (Liu et al., 2013). They are also referred to as leiomyomata, fibromyoma or myomas. The fibroids are growths of muscular and fibrous cells within or attached to the wall of the uterus. They are categorized as submucosal or subserosal. They could grow as single or in clusters

and the diameter could vary from one to eight inches and above. Approximately 30% of women within reproductive age group have clinical symptoms of uterine fibroids (Liu et al., 2013). The commonest symptoms are; heavy and or painful menses, prolonged menses, bleeding between periods, pelvic or low abdominal pain, fullness in lower abdomen and reproductive problems including infertility, multiple miscarriages and or early onset of labor during pregnancy. Other women do not display any symptom. In developed countries, fibroids can affect up to 77% of women within ages 15-49 years (Liu et al., 2013) and black women are at 3-5 times greater risk of developing fibroids compared to whites. Obese and women who have never had children are also at greater risk. Though causative factor for fibroids is unknown, genetic, hormonal, immunological and environmental factors are thought to play a role. Treatment depends on characteristic of the fibroid. The fibroids that are small in size and where no symptoms are exhibited are not treated but the patient is frequently monitored. Mild symptoms are managed with non-steroidal anti-inflammatory drugs; for example Ibuprofen. Surgery is treatment of choice for patients exhibiting serious symptoms. Surgical therapy is considered effective. It could either be myomectomy (removal of fibroid only) or hysterectomy. Use of herbal medicine to treat fibroids is a common clinical practice (Khan et al., 2014) by the Chinese. Chinese practitioners recognize uterine fibroids as disturbances of the endocrine system and blood circulation. The treatment is said to relieve symptoms and shrink the fibroids without adverse effects.

2.1.7.5.3 Vaginal rash

Mangifera indica L. is a medicinal plant used to manage vaginal rash and inflammation (Zahra and Mahmood, 2014). The flowers of *chamomile* contain 1–2% volatile oils including alpha-bisabolol, alpha-bisabolol oxides A and B, and matricin (usually converted to

chamazulene and other flavonoids which possess anti-inflammatory and antiphlogistic properties. A study in human volunteers demonstrated that chamomile flavonoids and essential oils penetrate below the skin surface into the deeper skin layers. This underlies their usefulness as topical antiphlogistic (anti-inflammatory) agents to manage cases like vaginal rash. Indeed in Tana River County, vaginal rash is reportedly managed by several herbs including *Pluchea ovalis* (Kaingu et al., 2013a).

2.1.7.5.4 Vaginal warts

External genital warts, also known as *condyloma accuminatum*, are extremely common, with between 500,000 to one million new cases diagnosed each year in developed countries. Study has revealed genital warts to be benign cellular proliferations of the anogenital skin and mucosa in response to a viral invasion. Recent advances in molecular biological techniques have allowed for the successful identification of the offending virus, Human Papilloma Virus (HPV), as the source of genital wart outbreaks. Herbal treatments include *Pluchea ovalis*, *Cissus rotundifolia*, *Ademia gummifera* (Kaingu et al., 2013a); *Kaalaani kalimpu* in India (Amuthan et al., 2015).

2.1.7.6 Fertility regulation

The world population is 7.3 billion and is projected to reach 9.7 billion by 2050. This will pose critical strain on resources even in resource rich countries and more so in resource poor developing countries. In developed countries, there are estimated to be 100 abortifacients of plant origin (Mills et al., 2013). Medicinal plant contraceptives use in India is widespread and the plants used include; *Hibiscus rosasinensis, Embelia ribes, Daucus carota, Butea monosperma, Sapindus trifoliatus, mentha arvensis*, *Ferula jaeschkeana*, *Gossypium*

herbaceum, Tripterygium wildfordii (Mills et al., 2006). Indeed a few of the biochemical compounds isolated from some of these plants are being used as novel contraceptives (Talwar et al., 1997). In developing countries, several studies have verified the anti-fertility properties of medicinal plants (Mutreja et al., 2008; Ravichandran et al., 2007; Montaserti et al., 2007; Shibeshi et al., 2006a). Researchers have demonstrated the effect of many plant extracts on fertility in rodents (Montaserti et al., 2007; Gebri et al., 2005; Nivsarkar et al., 2005). Gebri et al. (2005) for instance, reported that methanolic extract of *Rumex steudelii* decreased the number of implantation sites significantly but did not affect the serum estrogen-progesterone ratio in rats. Nivsarkar et al. (2005) showed that *Hibiscus rosa-sinensis* flowers had antifertility, abortifacient activity and exhibited anti-estrogenic activity in rats. Kulkarni et al. (2005) on the other hand, reported that the alcoholic extract of lemon seeds exerted reversible anti-fertility effect in female mice by virtue of its anti-zygotic action. In Africa several studies have also reported the traditional use of medicinal plants as contraceptives. Such plants include *Leonotis ocymifolia* in Ethiopia (Geremerew et al., 2005), *Abrus precatorius L., Carica papaya, Senna alata, Citrullus lanatus, Citrus limon, Curculigo pilosa, Macrosphyla longistyla, Ricinus comunis* and *Sorghum bicolor* in Nigeria (Bablola, 2009). Some of these plants are reported to have reversible contraceptive, anti-implantation and abortifacients properties. *In vivo* studies using laboratory animals have confirmed these properties but clinical studies in humans are yet to be carried out. Therefore the search for an affordable, safe, effective and accessible herbal contraceptive is still on.

2.1.8 Contraceptive history in Kenya

Rapid population growth is one of the major challenges facing developing countries, with its inevitable consequences on all aspects of development, especially employment, education,

housing, health care, sanitation and environment (Ochako et al., 2015). Family planning services in Kenya began in 1976. By 1996, family planning services were still considered weak because of lack of demand. Despite the low demand, the total fertility rate (TFR) reduced from 8 in the 1970s to 7 in 1980s. The TFR decline in Kenya has been one of the highest in Africa (Kenya Demographic Health Survey 2008/2009). By comparison however, use of family planning services in Africa is still the lowest globally. Some of the contributing factors are inadequate numbers of trained health care providers, inadequate client counseling and unreliable supplies of contraceptive options. Access to high quality family planning and reproductive health services is thus a growing concern in sub Saharan Africa (Tsui et al., 2010) which also has the highest population growth rate (3% per annum). The Governments are understandably increasingly concerned about the adverse effects of such rapid population growth on development efforts (Tsui et al., 2010). Women in Africa start having children early and in large numbers (Singh and Darroch, 2012; Darroch and Singh, 2013) and yet an estimated 22 million in the region have unmet contraceptive need (are currently not using any fertility regulating method even though they would wish to delay or avoid future pregnancies) Singh and Darroch. (2012).

Forty percent of the annual 215,000 maternal deaths occur in Africa due to reproductive health dysfunction stemming from unsafe abortions and increased sexual activity by the adolescents. This has enhanced a growing interest in and response to family planning and reproductive health programs (Tsui et al., 2010; Adams and Garcia, 2006; Ahmed et al., 2012). Despite the unmet contraceptive need those family planning services that do exist are often underutilized especially in rural parts of the countries; probably due to poor service delivery (Darroch and Singh, 2013). It might also be due to low levels of motivation for

women to avoid pregnancies in certain communities (Ochako et al., 2015, Sedgh et al., 2007a; 2007b). Several studies have reported on barriers to contraceptive uptake/continuation being disapproval from family members especially husbands/partners (Ochako et al., 2015; Adetunji, 2011); religious beliefs (Shraboni and Singh, 2015); culturally unacceptability (Ochako et al., 2015; Darroch, 2013) and/or undesirable side effects (Ochako et al., 2015; Darroch and Singh, 2013). It is therefore imperative for Government and Non-governmental organizations to collaborate and come up with viable solutions on how to improve access and supply of high demand contraceptives in rural parts of Kenya. At the same time increase funding for research exploring novel contraceptive options.

2.1.9 Review of current female contraceptive methods

The oral pills and injectable hormones are the most common reversible contraceptive options in Kenya for preventing unintended pregnancies. Frequent inter cycle bleeding which is culturally unacceptable in some communities and health fears that the method could cause irreversible in-fertility are some of the existing barriers to this method (Darroch, 2013). Inadequate supply, overburdened health system; inadequate client counseling and follow-ups are some of the factors that lead to non-uptake or discontinuation of the method (Adetunji, 2011) in rural communities despite the high demand. Darroch and Singh. (2013) report on failure of the method as partly being due to refusal by some health care providers to give hormonal methods to non-menstruating women; others turn away those who are late for injection. Some discontinuations are due to family disapproval (Adetunji, 2011). All these factors play in a field where women do not always have control over the use of contraceptives.

Long acting permanent contraceptive methods that are reversible are ideal for those who wish to delay, space or limit their pregnancies (Adetunji, 2011). These include; intrauterine devices considered effective whose long standing barrier has been the risk to the client and/or health provider especially when dealing with HIV^+ persons. The method requires a health provider to physically insert the device into reproductive tract and exposes one to risk of HIV infection. Although implants are highly effective and more convenient, and with unmatched effectiveness compared to other contraceptive methods (Ochako et al., 2015), they are not easily accessible in rural communities (Adetunji, 2011). Globally, access is also lower among poorer, less educated, rural, and younger women (Ochako et al., 2015). Implants release ultra-low amounts of progestin continuously into the bloodstream (Jacobstein and Stanleya, 2013). Currently, 3 implants are available: Implanon, Jadelle, and Sino-implant II. They are convenient, immediately effective and offer 3 to 5 years of extremely reliable contraceptive protection (Bradley et al., 2011). Long acting permanent methods are more attractive due also to lack of pelvic examinations and laboratory tests as a requirement. Further more implants can be used discreetly, do not interfere with sexual intercourse, and return to fertility upon removal is not delayed or negatively affected with only 1 unintended pregnancy occuring among every 2,000 implant users in the first year of use (Ochako et al., 2015). In contrast, failure rates in the first year of typical use of the commonly used resupply methods are considerably higher at 180 unintended pregnancies per 1,000 users of male condoms, 90 unintended pregnancies per 1,000 users of pills, and 60 unintended pregnancies per 1,000 users of the progestin-only injectable Depo-Provera. Thus, implants are 120 times more effective than the injectable, 180 times more effective than the pill, and 360 times more effective than the condom.

A woman in sub-Saharan Africa faces a 1 in 39 lifetime risk of maternal death, while a woman in South Asia has a 1 in 150 lifetime risk (WHO, 2010). In contrast, the lifetime risk of maternal death in industrialized countries is 1 in 4,700. Nearly all maternal deaths (99%), occur in low-resource countries (Finlayson and Downe, 2013) where women have less access to modern contraception including implants. Access is also more constrained for young women, among whom 44% of all unintended pregnancies in sub- Saharan Africa occur (Robinson and Ross, 2007). Satisfying unmet need for contraception could reduce maternal mortality by 29%, preventing more than 100,000 maternal deaths each year (WHO, 2010). If only 1 of 5 sub-Saharan African women now using pills or injectables (less effective hormonal contraception) were to switch to an implant, more than 1.8 million unintended pregnancies would be averted in 5 years, resulting in almost 600,000 fewer abortions and 10,000 fewer maternal deaths (Hubacher et al., 2011)

Permanent fertility regulating methods include; female sterilization and vasectomy whose popularity are gradually increasing (Bertrand et al., 2014). Barrier contraceptive methods include the male and female condom and the diaphragm. Barrier methods also protect against sexually transmitted infections including HIV. Another contraceptive option is the use of topical spermicidal compounds that kill or disable sperms.

Calendar method is preferred by women from North Eastern Kenya (Ochako et al., 2015), who have resisted modern contraceptives due to religious affiliation. The method advocates for avoidance of sex during unsafe days of the cycle (day 8 to 18^{th}) after commencement of menses. As the Government and other nongovernmental agencies undertake situation analysis to identify and address weaknesses and challenges in the current contraceptive supplies and services in order to improve program delivery, majority of women in rural parts of Kenya

continue to suffer unintended pregnancies, unsafe abortions and untimely death. It is therefore important to explore fertility regulating potential of plants as alternative contraceptive options to the millions of women in rural parts of Kenya who lack access to modern contraceptives.

2.2.0 Fertility regulating medicinal plants

Fertility regulation is not a new concept. Through-out history women have tried to regulate their fertility using various means in order to limit the number of children or space their births and passed the information to their children (Gangwar et al., 2010). The need for effective fertility regulation as already mentioned, is driven by the rising population especially in developing countries. Consequently intensive efforts are being made to control birth rate by various means (Benitez et al., 2010). In an effort to find new orally active non- steroidal compounds; extensive research is being carried out all over the world. These efforts are encouraged by World Health Organization who also strongly advocates for legitimization of traditional practices on herbal contraceptive use (Cominsky, 1986) to counter synthetic oral contraceptives that cause serious side effects; such as hormonal imbalance, hypertension, increased risk of cancer and weight gain. There is thus an urgent need to replace the synthetic agents with safe, effective, affordable and accessible alternatives. Plants are an important source of novel compounds as evidenced by the fact that 25% of current antibacterial, anti-malarial and anti-tumor prescriptions contain active principles of plant origin (Dinesh et al., 2012). For instance; Nitisinone derived from *Callistemon citrinus* is used as an anti tyrosinaemia; galantamine derived from *Galanthus nivalis* used in the management of Alzhemers disease; apomorphine from *Papaver somniferum* for the management of Parkinson disease and capsaicin from *Capsicum annuum* as a pain reliever (Veeresham, 2012). Plants therefore, hold a great promise for the discovery of new and effective antifertility agents.

Globally several studies have reported contraceptive properties of several plants such as; *Citrus bergamia, Cuscuta reflexa Roxb, Datura metei Linn, Derris brevipes, Dloscora pentaphylla Linn, Duckesia verrucosa, Ehretia cymosa Thonn, Eriosema crinitum, Ficus religosa, Ficus wassa, Huperzia saururus, Hymenaea stigonocarpa, Indigofera linnaei, Justicia simplex, Mentha arvensis, Mentha longifolia Linn, Mouriri pusa, nardostachys gradiflora, Persea Americana, Petroseltnum crispum, Ricinus communis, Phoradendron macrophyllum, Pouzolzia hypoleuca, Senecto aureus Linn, Solanum incanum, Trichosanthes tricuspidata* (Shah et al., 2009; Priya et al., 2002; Badami et al., 2003; Jain et al., 2005; Rodrigues, 2007; Trillo et al., 2010; Gangwar et al., 2010; Duke, 2009; Rainer and Ashley, 2010; Toledo et al., 2007; Gonzalez et al., 2010; Sewani-Rusike, 2010; Kamble et al., 2010). In India plant contraceptives include *Hibiscus rosasinensis, Embelia ribes, Daucus carota, Butea monosperma, Sapindus trifoliatus, mentha arvensis, Ferula jaeschkeana, Gossypium herbaceum, Tripterygium wildfordii* (Mills et al., 2013). In Africa several studies have also reported the traditional use of medicinal plants as contraceptives. Such plants are; *Leonotis ocymifolia* in Ethiopia (Geremerev et al., 2005), *Abrus precatorius L., Carica papaya, Senna alata, Citrullus lanatus, Citrus limon, Curculigo pilosa, Macrosphyla longistyla, Ricinus comunis* and *Sorghum bicolor* in Nigeria (Bablola, 2009), *Markhamia zanzibarica, Combretum illairii, Ricinus communis, Croton menyharthii, Suregada zanzibariensis, Plectranthus barbatus, Ficus natalensis, Ximenia Americana, Citrus sinensis, Harrisonia abyssinica, Grewia villosa willd* and *Cissus rotundifolia* in Kenya (Kaingu et al., 2013a). All these plants are said to have reversible contraceptive, anti-implantation and abortifacient properties that have been confirmed through animal model studies (Mills et al., 2013). Plants reported to be abortifacients for instance include; *Acalypha wilkestana, Acorus calamus Linn,*

Acosmium dasycarpum, Adhatoda vasica, , Adiantum capillus, Aerva lantana, Ambrosia cumanenesis, Ananas comosus, Annona squamosa, Artemisia siverstana (Gangwar et al., 2010, Rodrigues, 2007, Shah et al., 2009, Benitez et al., 2010, Mitra and Mukhajee, 2009, Murthy and Venkaiah, 2010). In developed countries, there are estimated to be 100 abortifacients of plant origin (Mills et al., 2013). Other studies by Rainer and Ashley. (2010); Smith-Oka.(2008) and Jain et al. (2004) have reported on the ability of *Aa paleacea, Blechnum orientale, canarium indicum, Grewia colunnaris* and *Persea American* to cause sterility in females. Several studies have also verified the anti-fertility properties of plants, such as S*triga lutea,* B*arleria prionitis, Nelumbo nucifera, Coriandrum sativum, Dodonea viscose, Melia azedarach* in developing countries (Mutreja et al., 2008; Ravichandran et al., 2007; Montaserti et al., 2007; Shibeshi et al., 2006b).

Fertility regulation can also be achieved through interference with sperm activity. *Piper nigrum* for instance had antispermatogenic effect while *Azadirachta indica* showed both anti spermatogenic and significant reduction of spermatozoa motility (Parohit et al., 2008). *Tinospora cordifolia* caused 100% suppression of spermatogenesis and significant reduction of male fertility (Gupta and Sharma, 2003) while *Barleria prionitis* caused 100% antifertility effect in males. The contraceptive properties of plants could therefore be effected through various physiological processes. For instance, the plant compounds could be anti-ovulatory, anti-fertilization, abortive, anti-implantation, emmenagogue (promote bleeding), reduce fertility index or oxytocic (cause a contraction of reproductive tract smooth muscle thereby preventing implantation (Dinesh et al., 2012; Saurabh et al., 2011). *Hibiscus rosa-sinensis* caused 100% anti-implantation effect (Neeru and Sharma, 2008). *Butea monosperma* reduced fertility and caused 100% anti-implantation activity, *Ocimun sanctum; Striga orobanchioides;*

Ricinus communis, Punica granatum and *Calotropis procera* all had anti implantation properties (Khanna and Chaudhury, 1968). Other plants with reported anti-implantation activity were *Mentha arvensis, Lawsonia inermis, Juniperus communis, Hagenia abyssinica, Crotalaria juncea, Cicer arietinum* and *Citr us limonum* (Maurya et al., 2004; Kulkarni et al., 2005). According to Vasudeva and Sharma. (2006), *Achyanthes aspera* had both anti-implantation and 100% abortifacient activities, while–*Plumbago zeylanica* had antifertility properties. *Hibiscus rosa-sinensis* flowers had antifertility, abortifacient activity and exhibited anti-estrogenic activity (Nivsarkar et al., 2005).

Other plants work by disrupting endocrine regulation of the reproductive process. Plants such as M*ormodica charantia* caused a significant reduction of estrogen and progesterone (Osonuga et al., 2014); *Nigella sativa* increased levels of LH, testosterone and enhanced fertility index in male rats (Rahmatollah et al., 2012); *Cnidosiolous aconitifolius* reduced FSH, LH and Estrogen in rats (Musa et al., 2008); *Andragraphis paniculata* reduced FSH, LH, estrogen and Progesterone in rats and mice (Sakila et al., 2009); *Martynia annua* caused a significant reduction of LH and testosterone in rats (Mali et al., 2002); *Juniperis communis* had significant antiprogestational properties (Sandhya et al.,1990) whereas *Quassia amara* reduced FSH, LH, testosterone, and epididymal sperm counts (Raji and Bolarinwa,1997) in rats. Medicinal plants contain several active compounds which are responsible for their various activities and efficacy. Many of these compounds have been and continue to be isolated and formulated into novel medicinal products. A few of the biochemical compounds isolated from plants are already being used as novel contraceptives in India (Joshi et al., 2003). Indeed, many plant compounds have been tested and reported to affect fertility in rodents (Montaserti et al., 2007). Gebri et al. (2005) for instance reported that methanolic

extract of *Rumex steudelii* decreased the number of implantation sites but did not affect the serum estrogen-progesterone ratio. Alcoholic extract of lemon seeds exerted reversible anti-fertility effect in female mice by virtue of its anti-zygotic action (Kulkarni et al.,2005). Oxymethyl anthraquinone from *Polygonum hydropiper* Linn caused 60% inhibition of ovulation (Kapoor et al., 1974). Glycosides and cardenolides from *Calotropis gigantea* had significant anti-implantation activity. *Striga lutea's* active principles are acacetin, luteolin, and flavone (Hiremath et al., 1990). *Spondias mombin* flavonoids caused significant anti-conceptive activity (Chukwuka and Uchendu, 2008).

Medicinal plants therefore present enormous possibilities for improved reproductive health provision if properly studied and documented. Thus the aim of this study was to evaluate the contraceptive efficacy of selected medicinal plant extracts by elucidating their effects on selected reproductive parameters, reproductive hormonal profiles as well as effects on uterine and ovarian histomorphology. The study will also demonstrate the biological activity of the plant extracts using the brine shrimp bioassay and establish the phytochemical compounds and acute toxicological profile of the selected plants extracts.

CHAPTER 3

3.0 ETHNOBOTANICAL SURVEY ON TRADITIONAL MANAGEMENT OF REPRODUCTIVE HEALTH IN TANA RIVER

3.1 INTRODUCTION

Reproductive issues and ailments constitute 18% of the global burden of disease for women of reproductive age and are the number one cause of maternal mortality in developing countries (WHO, 2003). Female reproductive ailments range from pregnancy and related complications, fertility issues and menstrual complications. In Tana River County, TMPs are routinely consulted because of their wide indigenous medicinal knowledge base (Swaleh, 1999), a tradition that has persisted in many rural communities due to inequitable health provision.

In Kenya, 75% of health facilities and personnel are concentrated in urban areas (WHO, 2005). The national doctor patient ratio is 1: 20,000; but in Tana River County with only 57 health facilities, the doctor: patient ratio is 1: 95,500 emphasizing a serious shortage of both health facilities and staff in the County (Tana River District Strategic Plan, 2005 - 2010). On the other hand, the ratio of TMP to patients is 1: 987 (Kenya Population and Housing Census report, 2009), suggesting that the TMPs are more readily accessible. In General, health sectors including reproductive health face a number of challenges. According to the Tana River District Strategic Plan (2005 - 2010); issues of major concern in reproductive health sector are; unsafe motherhood, high maternal/child mortality rates and inadequate family planning services.

Sexual dysfunction afflicts 10% of men of all ages, ethnicities and cultural background. Reproductive health is one of the most prevalent health care problems in Africa. However, advocates of reproductive health care have been focusing mainly on women and disregarding men. Thus, some diseases such as sexual impotence and erectile dysfunction that deserve mention have not been given due regard, to the detriment of families and societies as a whole (Kamatenesi-Mugisha and Oryem-Origa, 2005). In Tana River County a large percentage of reproductive health ailments are managed by traditional healers. Unfortunately, in traditional medicine practice, there is no documentation and information is passed on verbally from generation to generation. The aim of this section was to identify and document plants that are used for the management of male sexual dysfunctions and infertility.

The study also aimed to identify and document medicinal plants used for the management of other illnesses in males and females. The plant parts, route of administration, method of preparation, dose and whether the plant was administered as a concoction was also documented.

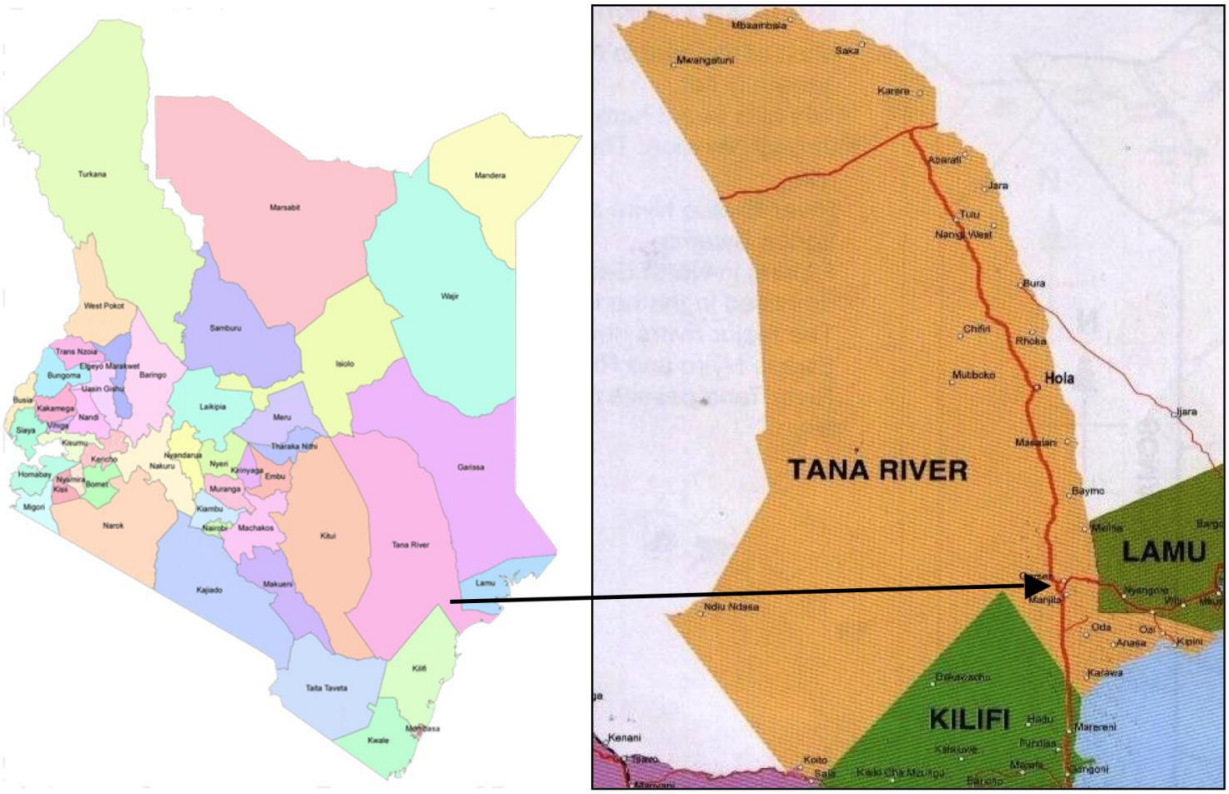


Figure 1: The Map of Tana River County.

(Source: Department of Geography University of Nairobi). Arrow points at area of study: Garsen, Itsowe and Ngao subdivisions.

3.2 MATERIAL AND METHODS

An ethno botanical survey was carried out in Tana River County to identify and document the plants that are used by traditional medicinal practitioners (TMPs) for the management of female reproductive ailments with particular emphasis on but not restricted to female fertility regulation. The survey also collected information on male sexual dysfunctions and infertility as well as the management of other reproductive tract related illnesses. The plant parts, route of administration, method of preparation, dose and whether the plant was administered as a decoction or concoction was documented.

3.2.1 Study area identification and description

A reconnaissance survey was undertaken in Tana River County in March 2012, to identify key informants for the study. Local administrators were key resource persons in providing information on TMPs. Discussions with these key informants led to Garsen, Itsowe and Ngao subdivisions being chosen as most suitable study areas due to widespread use of herbal medicine and in accessibility to health facilities. Tana River shares boundaries with Kitui to the West, Mwingi to the North West, Garissa to the North East, Ijara to the East, Meru North and Isiolo to the North, Lamu to the South East and Malindi to the South West. It also borders the Indian Ocean to the South with a coastal strip of 35Km. The total land size is 38, 782 Km^2. The County lies between latitude 0° and 3° South and longitudes 38°30’ east and 40°15’ east. According to the population and housing census report 2009; the County has a population of 240 075 persons. Ninety six percent of Tana River County lies in the coastal lowland zone six which is characterized by low, erratic rainfall and high temperature. The rainfall is low, bimodal and erratic with a mean annual range of 300-500mm. Long rains occur in the months of April and May while short rains occur in the months of October and

November. The average annual temperatures are approximately 30˚C. On the other hand, along the coastal line there are humid conditions. The coastal region receives upto 1200mm of rain annually although it varies and is highly unreliable. The levels of poverty are very high. It is estimated that 72% of the total County population live below the poverty line. Acute droughts often accompanied by destitution and ethnic conflict revolving around sharing of natural resources are partly responsible for the high poverty incidence. Both drought and ethnic conflict retard development, consequently entrenching poverty Omosa. (2005). The County is inhabited by various ethnic groups; the main ones being Pokomo, Wardei, Somali, Malakoti, Munyoyaya, Wata, Bajuni and Mijikenda. The Pokomo, Munyoyaya, Malakote and Mijikenda are involved in farming activities while the Orma, Wardei and Somali are mainly cattle keepers. Most villages are found along the River Tana where farming is favorable. The pastoralists are mainly found in the hinterland and live in *manyattas* concentrated around watering points like dams, wells and boreholes.

3.2.2 Target population, study design and data collection

The target population for the ethno botanical survey was TMPs and the County has a high number of these. The TMPs were derived from the main tribes living in the study area namely Pokomo, Ormas and Giryama. The University of Nairobi Biosafety, Animal welfare and Ethics Committee reviewed and approved the research protocol. The study design was a cross sectional survey where a systematic random sampling method was used to identify 80 practicing herbalists as participants. Semi structured questionnaires (appendix 1) were used to document medicinal plants used by TMPs for the management of female reproductive ailments in Garsen, Itsowe and Ngao subdivisions, by a team comprising local translators, botanist and researchers. TMPs were asked to give signed informed consent before,

participating in the study. The objectives of the study were clearly stated. Quantitative and qualitative data collection methods were applied. Structured questionnaires were administered to the TMPs, and focused group discussions were conducted that allowed for detailed exploration of individual's knowledge and practices about reproductive health ailments and management. The questionnaires were designed to be responsive to the objectives of the study. Interviews with informants were conducted in Pokomo, Orma and Giryama languages assisted by local translators, and responses were recorded in English. A pilot study was conducted earlier to test and re-design the research tools appropriately. The TMPs study variables included age, marital status, education levels, number of years in practice, how they acquired their knowledge and the interventions used to manage reproductive health ailments.

3.2.3 Sample size determination and statistical analysis

Fisher et al. (1998) formula was used to determine sample size; n=Z2 pq/ d2 whereby n= the desired sample size; z = the standard normal deviate at the required confidence level; p = the proportion in the target population estimate to have characteristics being measured; q = 1-p; d = the level of statistical significance set. The data was analyzed qualitatively.

3.2.4 Plant identification

The plants were identified by a taxonomist and voucher specimens deposited at the University of Nairobi Herbarium. The information gathered included vernacular name of plant, species and ailment treated. Plant part, route of administration, method of preparation, dose, duration and whether the remedy was administered as a concoction or decoction was also documented.

3.3 RESULTS

3.3.1 Demographic data for traditional medicinal practitioners

A total of 80 herbalists from the three subdivisions were interviewed. The herbalists were mostly elderly people aged 45 years and above and mostly illiterate (53%), with only 5 having completed primary school and another 2 completed secondary school. Majority of the herbalists (68%) were males while ten of the female herbalists practiced also as Traditional Birth Attendants (TBAs). All the herbalists had been in practice for 15 years or more and practically all had acquired their knowledge from relatives (Table 1).

Table 1: Demographic data for Traditional Medicinal Practitioners

Male	Female	Married	Widowed	Age groups			
				Below 45years	45-57 years	57-84 years	Over 84 years
54 (67.5%)	26 (32.5%)	25 (31.2%)	55 (68.8%)	None	35 (43.8%)	43 (53.8%)	2 (2.5%

Education levels					Religion	
Never attended school	Incomplete primary education	Completed Primary education	Incomplete secondary education	Completed secondary education	Christian	Muslim
42 (52.5%)	30 (37.5%)	4 (5%)	2 (2.5%)	2 (2.5%)	62 (77.5%)	18 (22.5%)

Number of years in practice			How the herbalists acquired medicinal plant skills	
> 15yrs	> 30yrs	> 50yrs	Through family inheritance	Acquired through divine intervention
30 (37.5%)	42 (52.5%)	8 (10%)	79 (98.75%)	1 (1.25%)

3.3.2 Traditional management of female reproductive health

Forty eight medicinal plants were used for the management of reproductive health ailments (Table 2). The plants belonged to 29 families, the commonest based on family use value being Euphorbiaceae, Capparaceae, Labiatae,Annonaceae, Leguminoceae, Tiliaceae, Salvadoraceae, Combretaceae, Olacaceae, Moraceae (Fig. 2). Fig. 3 gives the distribution of reproductive health ailments and the percentage of plants used for their management. A total of 27 plants (56.3%) were identified for the management of pregnancy and related problems (Table 1). Sixteen plants (32.65%) were presented for prevention of threatened abortion, 10 plants (20.8%) were used to alleviate post-partum hemorrhage, 10 plants (20.8%) to manage retained afterbirth, 3 plants (6.25%) to alleviate protracted labor, 1 plant (2.08%) was used to augment or induce labor, 1 plant (2.08%) to arrest premature labor, 1 plant (2.08%) to manage breach birth and 1 plant (2.08%) was used to induce retraction of the uterus after birth. Twenty two (45.8%) plants were identified for the management of menstrual disorders; 8 plants (16.7%) were presented for the management of menorrhagia, 4 plants (8.3%) were used to treat dysmenorrhea, 5 (10.42%) to treat amenorrhea and 5 (10.42%) to manage irregular menses. Thirteen (27.08%) plants were used to treat infertility or to enhance fertility. Eleven plants (22.92%) were used as contraceptives to suppress fertility after delivery, 6 plants (12.50%) were presented for management of fibroids, 1 (2.08%) to induce milk letdown and 1 plant (2.08%)) to treat mastitis. The data on herbal preparations, mode of administration and part used is presented in Table 1. The most common method of preparation involved boiling or soaking the fresh or dried plant parts in water (decoctions) or ground into powder and taken orally or as infusions. The water extracts were prepared just before consumption or just before steam bath. The most frequent route of administration as reported by the herbalist was oral at

93%, followed by topical application at 7%. Most of the remedies were prepared as concoctions of more than one plant in combination with the principal plant. The most common plant part used was the root (71%), followed by the leaf (22%), the root bark (6%), the stem (4%) and the fruit (2%).

Table 2: Medicinal plants used for the management of female reproductive dysfunctions.

Family	Plant species	Local name	Traditional use in Tana River	Method of preparation, route of administration and dose.
Alocaceae	*Aloe volkensii Engl*. CK027	Hargeis, D'aar (Orma)	Infertility	Leaves squashed in water. Decoction used to wash genital area 3 times daily until effective.
Anacardiaceae	*Lannea schweinfurthii* (Engl.) Engl. CK001	Mumongoo (pokomo)	RAB, PPH	Roots boiled in water and decoction taken orally. Half glass daily for 2 days.
Annonaceae	*Uvariodendron kirkii Verc.*CK008	Msaidizi (Giryama)	Fertility regulator	Root bark boiled in water and decoction taken orally. One glass daily for 30 days. Every 7 days fresh root bark boiled in water.
Annonaceae	*Uvaria acuminate oliv* CK023	Mundagoni Murori (pokomo)	PPH, menorrhagia, dysmenorrhea	Roots boiled in water and concoction taken orally. Half glass daily for 5 days. Usually mixed with *Markhmia zanzibarica*.
Annonaceae	*Uvaria leptocladon*	Sholole (Orma)	Threatened abortion, infertility, breach birth, RAB	Roots boiled in water and concoction taken orally. Half glass 3 times daily for 3 days. Mixed with *Croton dichagamus*.
Apocynaceae	*Hunteria zaylanica (zets) Gard ex thr var* CK041	Mutsungutsungu (Pokomo)	Induces milk letdown after delivery	Root bark boiled in water and decoction taken orally. One glass daily for 3 days after parturition.
Biaceae	*Pergularia daemia*	Mpovu (Pokomo)	RAB	Roots or leaves boiled in water

	(forsk) chiov CK046			and decoction taken orally. One glass daily for 3 days.
Bignoniaceae	*Markhamia zanzibarica* CK014	Mubwoka (Pokomo)	Threatened abortion, infertility, menorrhagia, dysmenorrhea, amenorrhea, RAB, Fertility regulator, Fibroids.	Roots or leaves boiled in water and concoction taken orally. Half glass twice daily for 5days. Mixed with *Salvadora persica* and *Uvaria acuminate* oliv.
Burserecеae	*Commiphora habessinica* (O.Berg) Engl.CK050	Mutsutsu (Pokomo)	RAB, PPH, post-partum retraction of uterus.	Roots boiled in water and decoction taken orally. Half glass for 4 days.
Capparaceae	*Thylachium thomasii Gilg* CK024	Uhiya, kukube (Orma)	Threatened abortion	Roots boiled in water and decoction taken orally. Half glass daily for 3 days.
Capparaceae	*Boscia coriaceae pax.* CK025	Kalkacha (0rma)	Threatened abortion, menorrhagia, dysmenorrhea, amenorrhea, irregular menses, RAB, PPH	Roots boiled in water and concoction taken orally. Half glass daily for 5 days. Usually mixed with *Uvaria leptocladon* and *Combretum hereroense Schinz.*
Capparaceae	*Cadaba ruspolii Gilg* CK032	Ilkavate (Orma)	Threatened abortion	Roots boiled in water and decoction taken orally. Half glass for 3 days.
Capparaceae	*Cadaba glandulosa forsk.* CK037	Alakal (Orma)	Infertility	Roots boiled in water and decoction taken orally. Half glass daily for 5 days.
Capparaceae	*Cadaba farinose* CK035	Kumis (Orma)	Infertility	Roots boiled in water and decoction taken orally. Half glass daily for 2 days.
Combretaceae	Combretum hereroense Schinz CK035	Konkon(Orma)	Threatened abortion, menorrhagia, dysmenorrhea,	Roots boiled in water and decoction taken orally. Half glass 3 times daily for 6 days.

			amenorrhea, irregular menses, RAB, PPH	Mixed *Uvaria leptacladon* roots.
Combretaceae	Combretum illairi Engl. CK049	Mshinda alume (Pokomo)	Infertility, PPH, Fertility regulator	Roots boiled in water and decoction taken orally. Half glass 2-3 times daily for 14 days.
Compositae	Pluchea ovalis (Pers.) Dc CK010	Msasa (Pokomo)	Vaginal rash	Leaves boiled in water and decoction used to wash genitalia for 1 week.
Euphobiaceae	Ricinus communis L. CK016	Mubonye, Mbono (Pokomo)	Fertility regulator	Two dried fruits swallowed daily for 30 days. The same dose repeated after 1 year.
Euphorbiacea	*Acalypha volkensii Pax* CK020	Mupunga mbuu (Pokomo)	Threatened abortion	Root bark boiled in water and decoction taken orally. Half glass daily for 3 days.
Euphorbiacea	*Croton menyharthii pax* CK021	Mualikaji, Munyuma (Pokomo)	Fertility regulator, PPH, Threatened abortion, Infertility, menorrhagia, Irregular menses	Root and or leaves boiled in water and decoction taken orally. Half glass 2-3 times daily for 5 days.
Euphorbiacea	*Suregada zanzibariensis Boull.* CK022	Mudimu tsaka (Giryama)	Fertility regulator	Roots boiled in water and decoction taken orally. Half glass 3 times daily for 4 days
Euphorbiacea	*Croton dichagamus* CK031	Qashin a' adha, Muuqaadhi (Orma)	Threatened abortion, Infertility	Roots boiled in water and concoction taken orally. Half glass 3 times daily for 6 days. Sometimes mixed with *Uvaria leptocladon* roots.
Euphorbiacea	*Euphorbea uhligiana pax* CK044	Daliid (Orma)	Threatened abortion, PPH	Roots boiled in water and decoction taken orally. Half glass daily for 2 days.
Fabaceae	Prosopis juliflora	Mathenge	Threatened abortion,	Root bark boiled in water and

	CK051		Infertility	concoction taken orally. One teaspoonful daily for 5 days. Mixed with *Zanthoxylum usamel* root bark.
Labiatae	*Plectranthus barbatus* Andr. CK015	Papaha (Pokomo)	Threatened abortion, RAB, PPH Fertility regulator, menorrhagia, amenorrhea, Irregular menses, Infertility	Roots boiled in water and concoction taken orally. Half glass daily for 30 days. Mixed with *Cissus rotundifolia* roots for the first 4 days.
Labiatae	*Ocimum kilimandscharicum Gurke* CK018	Vumba kuu (Pokomo)	Threatened abortion,	Roots boiled in water and decoction taken orally. Half glass daily for 3 days
Labiatae	*Hoslundia opposite* Vahl CK045	Mtserere	Infertility	Roots boiled in water and decoction taken orally. Half glass 2-3 times daily for 2 days.
Leguminosaceae	*Acacia zanzibarica* (S. Moore) *Taub. Var Zanzibarica* CK004	Muryela (Pokomo), muhegakululu (Giryama), Wachu (Orma)	Irregular menses, Mastitis	Roots boiled in water and decoction taken orally. Half glass daily for 3 days.
Leguminosaceae	*Cassia occidentalis L.* CK009	Muchoyoko (Pokomo)	RAB, PPH	Roots or leaves boiled in water and decoction taken orally. Half glass daily for 3 days.
Lythraceae	*Lawsonia inermis* L. CK048	Musuruja (Pokomo)	Fibroids	Roots boiled in water and decoction taken orally. Half glass daily for 30 days. After every 7 days fresh roots boiled.
Malvaceae	*Thespesia danis* Oliv. CK006	Mudanisa (Pokomo)	Fibroids	Roots or leaves boiled in water and decoction taken orally. Half glass daily for 3 days.
Menispermaceae	*Cissampelos*	Chovi, Kivila kya	Protracted labor,	Roots boiled in water and

	micronata. A. Rich CK040	mani (Pokomo), Kashikiropaka (Giryama	Threatened abortion	concoction taken orally. One glass 3 times daily for 4 days. Mixed with *Cassia abbreviate* and *Strychnos henningsii* roots.
Mimosaceae	*Acacia robusta* CK058	Munga (Pokomo)	Fibroids	Roots or leaves boiled in water and concoction taken orally. One glass 2 times daily for 5 days. Mixed with *Cissus rotundifolia* roots.
Moraceae	*Ficus natalensis* Hochst CK013	Mgandi (Pokomo)	Fertility regulator	Roots boiled in water and decoction taken orally. Half glass daily for 30 days. After every 7 days fresh roots are boiled.
Moraceae	*Ficus sycomorus* L. CK052	Mukuyu (Pokomo)	Augment labor, protracted labor	Leaves boiled in water and decoction taken orally. Half glass daily for 30 days. After every 7 days fresh leaves boiled.
Olacaceae	*Ximenia americana L.* CK033	Muntuntuda, Mtundukula (Pokomo), Huda hudo (Orma)	Fertility regulator	Roots boiled in water and concoction taken orally. Half glass daily for 5 days. Mixed with *Ochna holstii* roots.
Olacaceae	*Capparis sepiaria Var. caffra* CK039	Hamwalika (Pokomo), Mugwada paka (Giryama)	Fibroids	Roots boiled in water and concoction taken orally. Half glass daily for 3 days. Sometimes mixed with *Grewia plagiophylla* roots
Passifloraceae	*Adenia gummifera* (Harv.) Harms	Mujoka (Pokomo)	Menorrhagia, Infertility, Fibroids	Roots and or stems boiled in water and decoction taken

	CK019			orally. Half glass daily for 3 days
Pedaliaceae	*Pedalium murex L.* CK005	Mbigili (Pokomo)	protracted labor	Roots boiled in water and decoction taken orally. Half glass daily for 3 days
Rutaceae	*Citrus sinensis* (L) Osbeck CK012	Mudimu (Giryama)	Fertility regulator, infertility	Roots and or stem bark boiled in water and concoction taken orally. One glass 3 times daily for 3 days. Mixed with *Acacia robusta* and *Cissus rotundifolia* roots.
Salvadoraceae	*Salvadora persica L.*CK017	Muswaki, Mujungu moto (Pokomo) A'adhey (Orma)	Excessive bleeding	Roots boiled in water and decoction taken orally. Half glass daily for 5 days.
Salvadoraceae	*Dobera glabra (forsk.) poir* CK034	Garas (Orma)	RAB	Roots boiled in water and decoction taken orally. One glass 2 times daily for 2 days.
Sapidaceae	*Allophylus pervilleria* (A.Rich) Engl. CK047	Mnyanga kitswa (Pokomo)	Infertility	Roots boiled in water and decoction taken orally. One glass daily for 3 days.
Simorobaceae	*Harrisonia abyssinica Oliv A.* CK042	Musabini, Muyengwa, Chewa, (Pokomo)	Fertility regulator	Roots boiled in water and concoction taken orally. One glass 2-3 times daily for 3 days. Mixed with *Cassia abbreviate* and *Cissampelos micronata* roots.
Tiliaceae	*Grewia villosa Willd* CKK026)	Ogomdi (Orma	Threatened abortion, Fertility regulator	Roots boiled in water and decoction taken orally. Half glass daily for 30 days.
Tiliaceae	*Grewia tenax*	Deeka (Orma),	Infertility, PPH	Roots boiled in water and

	(forssk.) Fiori. CK028	Mubavubavu, Mukawa wa guba (Pokomo)		concoction taken orally. Half glass 3 times daily for 6 days. Mixed with *Combretum illairii* roots.
Usambarenseceae	*Zanthoxylum usamel* CK011	Msafaraji (Pokomo)	Threatened abortion	Root bark boiled in water and concoction taken orally. One teaspoonful daily for 5 days. Mixed with *Prosopis juliflora* root bark.
Vitaceae	*Zanthoxylum usamel* CK011	Mkwembe, Maneke, Neke (Pokomo), Arma (Orma)	Threatened abortion /premature labor, Fertility regulator	Leaves boiled in water and concoction taken orally. Half glass 3 times daily for 4 days. Mixed with *Plectranthus barbatus* leaves

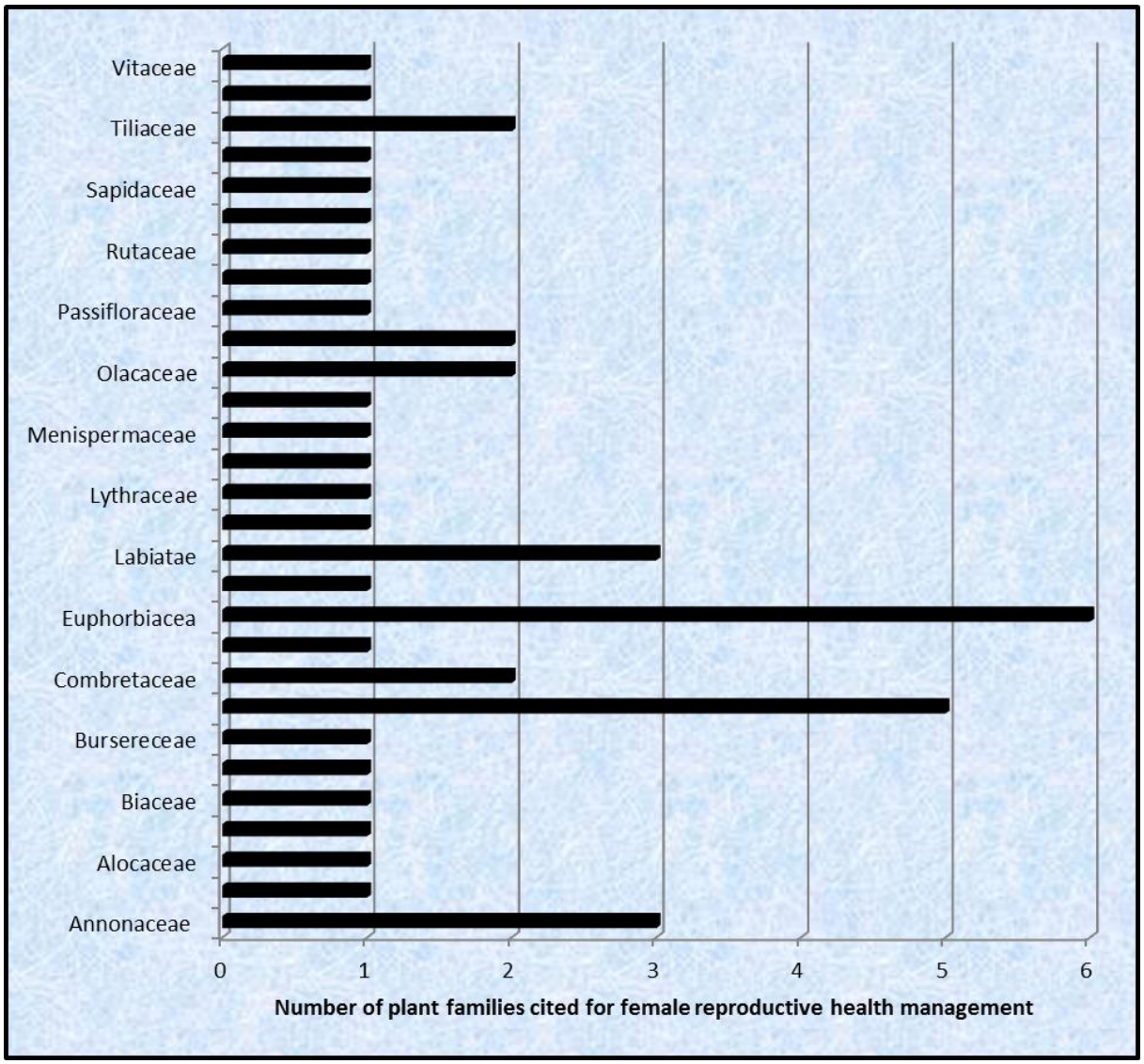


Figure 2: Number of plant families cited for female reproductive health management.

The figure shows the commonest plant families for the management of female reproductive health dysfunctions.

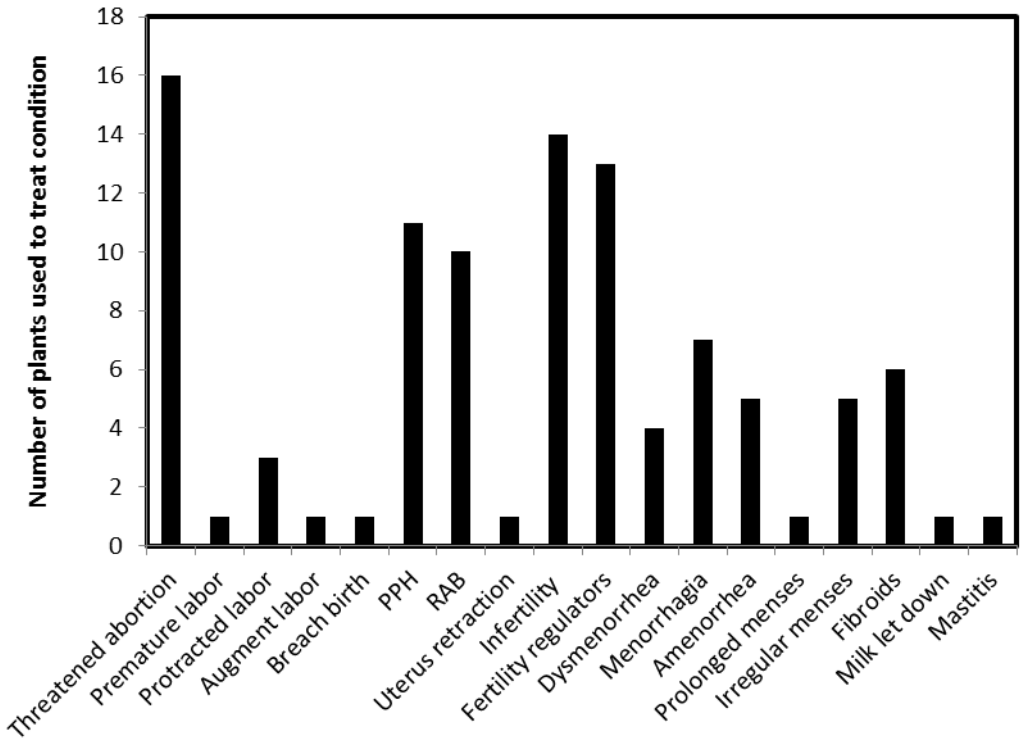


Figure 3: Female reproductive dysfunctions managed by TMPs.

The Figure shows the female reproductive dysfunctions managed by Traditional Medical Practitioners in Tana River County.

Key: PPH- postpartum hemorrhage; RAB- retained after birth.

3.3.3 Traditional management of male reproductive dysfunctions.

A total of nineteen plant species in 15 genera and 13 families, all collected from the wild, were mentioned for the treatment of male sexual dysfunction. Seven plants (46.7%) were used as aphrodisiacs to increase sexual drive, 6 plants (40.0 %) for the treatment of ED/impotence, 6 plants (40.0 %) to treat infertility and 2 plants (13.3%) were reported for the treatment of all three conditions ED/impotence, HSD and infertility (Table 3 and Fig 5). The family Capparaceae was the most represented with 3 species, followed by Annonaceae, Combretaceae, Labiatae, Tiliaceae with 2 species each, and the rest with 1 species each (Fig 5). The species frequency of mention ranged from 18.75% to 3.75%, all of the plants having been mentioned by 3 or more herbalists from the three communities (Table 3). The plant parts mostly used were roots (84%) and root bark (16%) and these were used fresh; plant part chopped and boiled. All preparations were administered orally (Table 3). Thirteen (68%) of the plant preparations were concoctions of 2 or 3 plants.

Table 3: Male reproductive dysfunctions managed by TMPs in Tana River, method of preparation and administration with cross reference to reproductive use in published literature.

Family	Species, voucher number	Local name	Traditional use	Method of preparation	Documented reproductive medicinal use	Frequency of mention
Annonaceae	*Uvaria acuminata* oliv CKK023	Mundagoni, murori (Pokomo)	Aphrodisiac	Roots are used in combination with *Markhamia zanzibarica*. Roots boiled in water and concoction taken orally. One glass daily for 5 days.	Probably being reported for first time for reproductive use. Documented reproductive use could not be found	12.5
Annonaceae	Uvaria leptocladon CKK029	Sholole (Orma)	Impotence	Roots are used in combination with *Boscia coriaceae* and *Combretum hereroense* Schinz. Roots boiled in water and concoction taken orally. Half glass daily for 5 Days.	Probably being reported for first time for reproductive use. Documented reproductive use could not be found	6.25
Bignoniaceae	*Markhamia zanzibarica* CKK014	Mubwoka (Pokomo)	Aphrodisiac	Roots used in combination with *Uvaria acuminate* roots. Roots boiled in water and concoction taken orally. One glass daily for 5 days.	Probably being reported for first time for reproductive use. Documented reproductive use could not be found	12.5
Caesalpiniaceae	*Cassia abbreviate* CKK059	Mubaraka wa guba (Pokomo)	Impotence	Roots used in combination with *Cissampelos micronata* roots. Roots boiled in water and concoction taken	Probably being reported for first time for reproductive use. Documented reproductive use could not be found	

				orally. One glass 3 times daily for 4 days.		
Capparaceae	*Boscia coriaceae* pax. CKK025	Kalkacha (Orma)	Impotence	Roots used in combination with *Uvaria leptacladon* and *Combretum hereroense* Schinz. Roots boiled in water and concoction taken orally. Half glass daily for 5 Days.	*Boscia senegalensis* aphrodisiac in Nigeria (Kokwaro, 1993)	6.25
Capparaceae	*Cadaba glandulosa* forsk. CKK037	Alakal (Orma)	Infertility	Roots boiled in water and decoction taken orally. Half glass daily for 5 days.	No documented reproductive use could be found. Probably being reported for the first time for reproductive use.	7.5
Capparaceae	*Cadaba farinose* CKK038	Kumis (Orma)	Infertility	Roots boiled in water and decoction taken orally. One glass daily for 3 days	Emmenagogue, painful menses (Korir et al., 2012)	5
Combretaceae	*Combretum Illairii* Engl. CKK049	Mshinda alume (Giryama)	Infertility, impotence	Roots used in combination with *Grewia tenax*. Root bark boiled in water and concoction taken orally. One glass daily for 7 days	Infertility (Korir et al., 2012)	18.75
Combretaceae	*Combretum hereroense* Schinz. CKK035	Konkon(Orma)	impotence	Roots used in combination *with Uvaria leptacladon*. Roots boiled in water and concoction taken orally. One glass daily until effective.	Dysmenorrhea, infertility in women (McGaw et al., 2001)	6.25

Labiatae	*Plectranthus barbatus* Andr.CKK015	Papaha (Pokomo)	Impotence	Roots boiled in water and concoction taken orally. Half glass daily for 30 days. Sometimes used with *Cissus rotundifolia* (forsk.) for the first 4 days.	Infertility (Verissimo et al., 2011) Aphrodisiac, emmenagogue (Fernando and Ione., 2000; Ramachandran et al., 2004; Almeida and Lemonica, 2000)	12.5
Labiatae	*Hoslundia opposita Vahl* CKK045	Mtserere) (Giryama	Infertility	Roots boiled in water and decoction taken orally. One glass 2-3 times daily for 14 days.	No documented reproductive use could be found. Probably being reported for the first time for reproductive use.	7.5
Menispermaceae	*Cissampelos micronata*. A. Rich CKK040	Chovi, Kivila kya mani (Pokomo) Kashikiro paka (Giryama)	Aphrodisiac	Roots sometimes used in combination with *Cassia* abbreviate. Roots boiled in water and concoction taken orally. Half glass daily for 3 days.	Menstrual problems, infertility, azoospermia, Uterine contraction, abortion, RAB (Amri and Kisangau, 2012)	15
Olacaceae	*Capparis sepiaria* Var. caffra CKK039	Hamwalika (Pokomo), Mugwada paka (Giryama)	Aphrodisiac	Root bark used in combination with *Grewia plagiophylla*. Root bark boiled in water and concoction taken orally. Half glass daily for 10 days.	*Capparis zaylanica* roots aphrodisiac (Padal et al., 2010; Ribeiro et al., 2010)	10
Palmae	*Phoenix reclinata* Jacq. CKK054	Mukindu (Pokomo)	Aphrodisiac	Roots boiled in water and decoction taken orally. One glass daily for 3 days.	Erectile dysfunction and impotence (Kamatenesi-Mugisha and Oryem-Origa., 2005	3.75
Papilionaceae	*Abrus precaterius*	Mudanda, mutuririri,	Aphrodisiac	Roots boiled in water and	Seeds alter oestrus cycle patterns and block	11.25

	L.sp africana verde CKK055	mudwadwa (Pokomo		decoction taken orally. One glass daily for 3 days.	ovulation, aphrodisiac, abortifacient, antifetility (Vijay Kumar et al., 2012)	
Sapidaceae	*Allophylus pervilleria* (A.Rich) Engl. CKK047	Munyanga kitwa (Pokomo)	Infertility	Roots boiled in water and decoction taken orally. One glass 2-3 times daily for 7 days.	No documented reproductive use could be found. Probably being reported for the first time for reproductive use.	7.5
Tiliaceae	*Grewia plagiophylla*. K. Schum CKK053	Mkoi (Pokomo)	Aphrodisiac	Root bark used in combination with *Capparis sepiaria*. Root bark boiled in water and concoction taken orally. Half glass daily for 10 days.	No documented reproductive use could be found. Probably being reported for the first time for reproductive use.	10
Tiliaceae	*Grewia tenax* (forssk.) Fiori. CKK028	Deeka (Orma), Mubavubavu, mukawa wa guba (Pokomo)	Aphrodisiac,infertility, Impotence	Root bark Used in combination with *Combretum illairii*. Roots bark boiled in water and concoction taken orally. One glass daily for 7 days.	No documented reproductive use could be found. Probably being reported for the first time for reproductive use	18.75
Vitaceae	*Cissus rotundifolia* (forsk.) CKK030	Mkwembe, Maneke, Neke (Pokomo), Arma (Orma)	Impotence	Roots Sometimes mixed with *Plectranthus barbatus* (forsk.) for the first 4 days. Roots boiled in water and concoction taken orally. Half glass daily for 30 days.	No documented reproductive use could be found. Probably being reported for the first time for reproductive use.	12.5

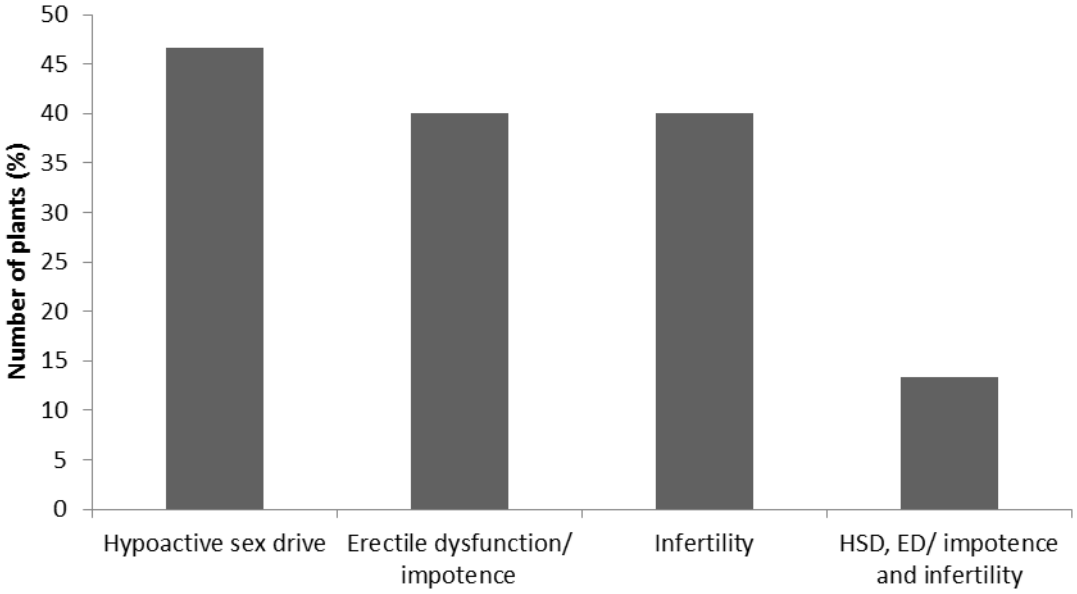


Figure 4: Percentage number of plants used to treat male sexual dysfunction.

The figure shows percentage number of plants used to treat male sexual dysfunction**.**

Key: HSD hypoactive sex drive; ED-erectile dysfunction

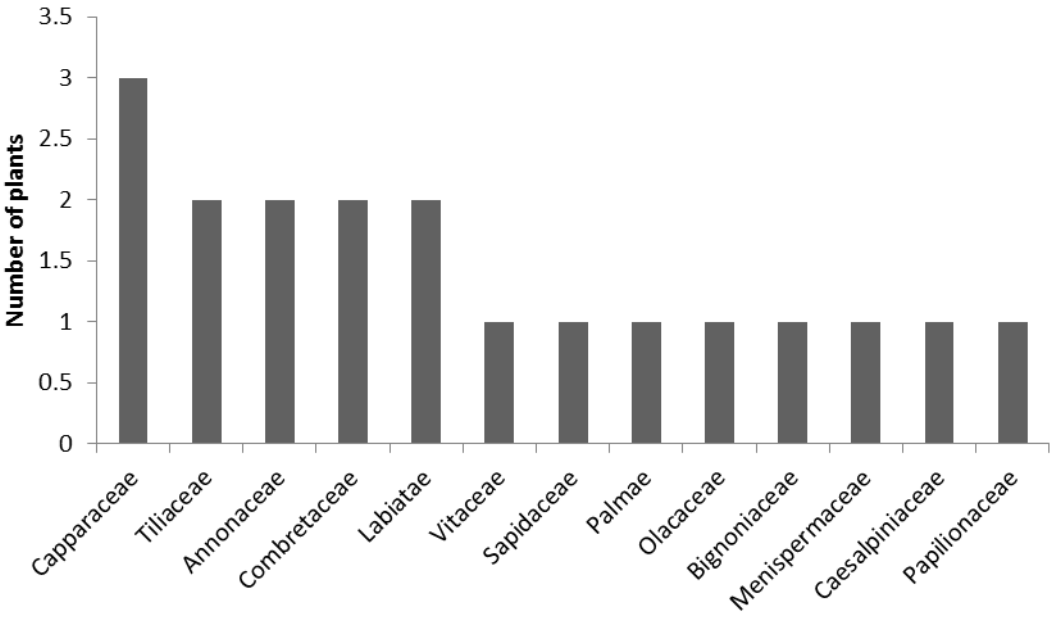


Figure 5: Plant families for management of male sexual dysfunction and infertility.

The Figure shows plant families used to manage male sexual dysfunction and infertility.

3.3.4 Traditional management of other illnesses

Thirty one plant species belonging to 25 plant families were identified for the management of illnesses (Table 4). The commonest plant family was Euphobiaceae, Salvadoracea, Leguminosaceae, Olacaceae Compositae, Bignonaceae, Mimosaceae, Vitaceae, Tiliaceae, Capparaceae, Loganiaceae, Simorobaceae, Lythraceae, Apocynaceae, Burseraceae, Labiatae, Solanaceae, Pedaliaceae, Papilionaceae, Malvaceae, Palmae, Rutaceae, Passifloraceae, Amaranthaceae and Caesalpiniaceae.

The commonest ailments managed by the TMPs were pneumonia, arthritis, kidney problems, fibroids, typhoid, breast cancer, toothache, boils, stomache ache, mastitis, asthma, high blood pressure, malaria and diabetis (Fig. 6 and Table 4).

Table 4: Medicinal plants used for general illness management and their ethnotherapeutic use value.

Family	Species, voucher number	Local name	Preparation method, route and dose	Ethno-therapeutic use	Frequency of mention (FV)	Use value (SU_v)
Amaranthaceae	*Achyranthes aspera L.* CKK060	Kinamaha (Pokomo	Roots boiled in water and decoction taken orally. Half glass daily for 2 days.	Typhoid, arthritis	1	2
Apocynaceae	*Hunteria zaylanica (zetz.) Gard ex thr var* CKK041	Mutsungutsungu, (pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days.	Induces milk letdown after delivery	1	1
Bignoniaceae	*Markhamia zanzibarica* CKK014	Mubwoka (Pokomo)	Roots or leaves boiled in water and decoction taken orally. Half glass twice a day for 5 days.	Fibroids, breast cancer	1	2
Burseraceae	*Commiphora habessinica (O. Berg) Engl.* CKK050	Mutsutsu (Pokomo)	Roots boiled in water and decoction taken orally. Half glass taken once.	Toothache	1	1
Caesalpiniaceae	*Cassia abbreviate* CKK059	Mubaraka wa guba (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days. The decoction also used to wash the boils.	Boils	1	1
Capparaceae	*Thylachium thomasii Gilg* CKK024	Uhiya, kukube (Orma)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days.	Arthritis	1	1

Compositae	*Sonchus oleraceus L.* CKK061	Mtsunga (Pokomo)	Leaves squeezed to obtain sap. One teaspoonful taken once.	Tonsils	2	1
Compositae	*Pluchea ovalis* (Pers.) DC CKK010	Msasa (Pokomo)	Leaves boiled. The decoction used to wash the affected area of body daily for 2 weeks.	Body warts	2	1
Euphobiaceae	*Suregada zanzibariensis Boull* CKK022	Mudimu tsaka (Giryama)	Boil fresh roots in water and decoction taken orally. Half glass 3 times daily for 4 days	Stomach ache	3	1
Euphobiaceae	*Flueggea virosa* (Willd.) Voigt ssp. virosa CKK062	Mupambaa (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 2 days.	Kidneys	3	1
Euphobiaceae	*Ricinus communis* L. CKK016	Mubonye, Mbono (Pokomo)	2 dried fruit split and crushed. Mixed with water. Daily for 7 days.	Migraine	3	1
Labiatae	*Hoslundia opposite* Vahl CKK045	Mtserere (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 2 days.	Kidney problems	1	1
Leguminosaceae	*Albizia gummifera* CKK063	Habecho (Orma)	Boil roots in water and decoction taken orally. One glass per day for 2 days.	Typhoid	2	1
Leguminosaceae	*Acacia zanzibarica* (S. Moore) Taub. Var Zanzibarica CKK004	Muryela (Pokomo), muhegakululu (Giryama), Wachu (Orma)	Root bark boiled in water and decoction taken orally. Half glass daily for 3 days	Arthritis, mastitis	2	2

Loganiaceae	*Strychnos henningsii* CKK057	Mumalindi	Leaf and stem bark boiled in	Kidneys, resistant typhoid, pneumonia, malaria	1	4
Lythraceae	*Lawsonia inermis* L. CKK048	Musuruja (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 30 days	Immune booster, fibroids, treats poisoning and constipation through causing diarrhea	1	4
Malvaceae	*Thespesia danis* Oliv. CKK064	Mudanisa (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days. Leaves squeezed and one teaspoonful mixed with the root	Pneumonia, high blood pressure	1	2
Mimosaceae,	*Acacia robusta* CKK058	Munga (Pokomo)	Roots or leaves boiled in water and decoction taken orally. Half glass daily for 5 days. Smear part of fluid on affected joints.	Fibroids, boils, arthritis	1	3
Olacaceae	*Ximenia americana L.* CKK033	Muntuntuda, mtundukula (Pokomo), huda hudo (Orma)	Roots boiled in water and decoction taken orally. Half glass daily for 5 days	Anti-diarrhea	2	1
Olacaceae	*Capparis sepiaria Var. caffra* CKK039	Hamwalika (Pokomo), mugwada paka (Giryama)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days	Fibroids, pneumonia	2	2
Palmae	*Phoenix reclinata Jacq.* CKK054	Mukindu (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days.	Toothache	1	1
Papilionaceae	*Abrus*	Mudanda,	Roots boiled in water	Asthma,	1	2

	precaterius L.sp africana verde CKK055	muturituri, mudwadwa (Pokomo)	and decoction taken orally. Half glass daily for 2 days.	pneumonia		
Pedaliaceae	*Pedalium murex L.* CKK005	Mbigili (Pokomo)	Roots boiled in water and decoction taken orally. Half glass daily for 3 days.	Kidneys, vaginal warts	1	2
Passifloraceae	*Adenia gummifera* (Harv.) Harms CKK019	Mujoka (Pokomo)	Roots or stem bark boiled in water and decoction taken orally. Half glass daily for 3 days.	Fibroids	1	1

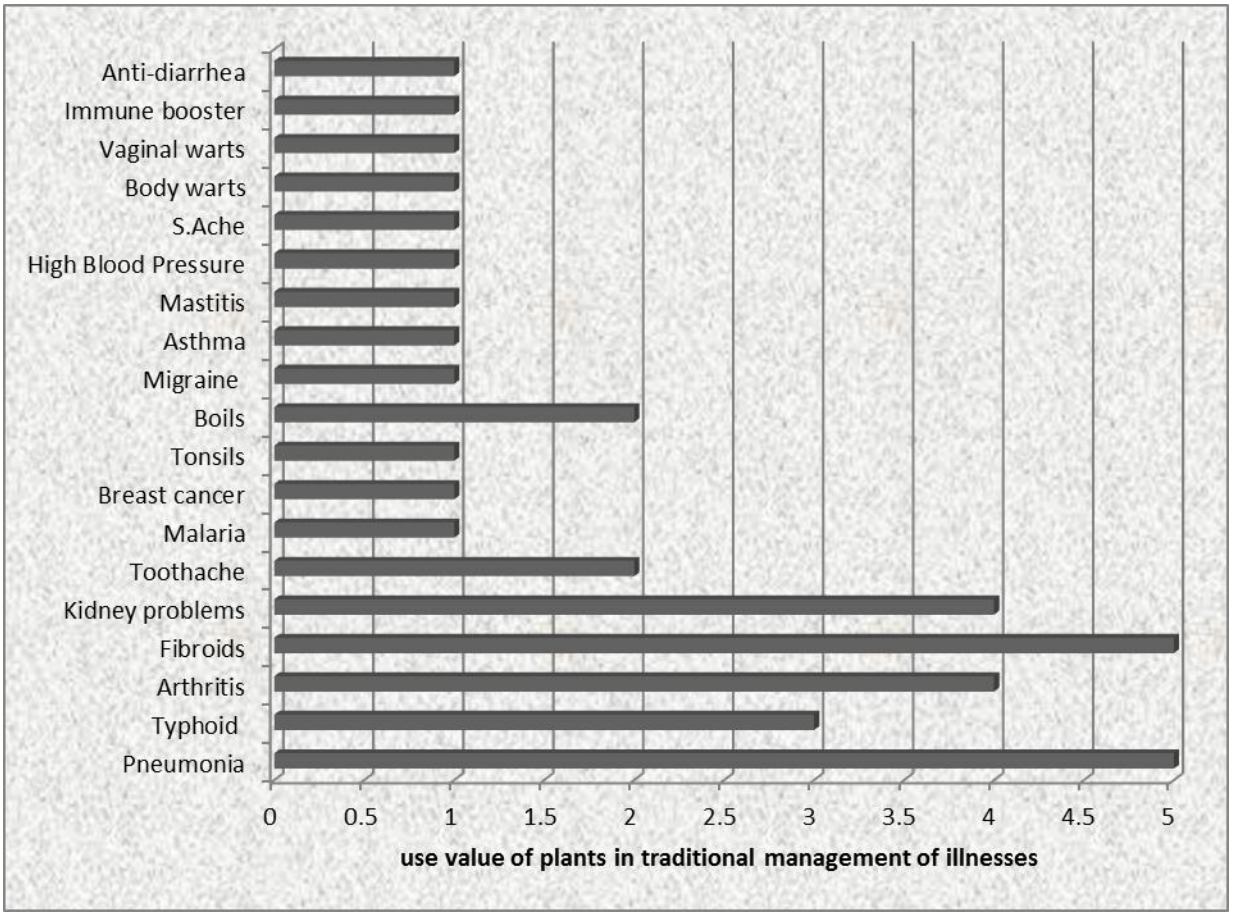


Figure 6: Plants use value in traditional management of various illnesses in Tana River County.

The figure shows use value of plants in traditional management of various illnesses in Tana River County.

3.4 DISCUSSION

3.4.1 Female reproductive health dysfunctions

The present study has revealed that traditional medicine practice is not only common in Tana River County of Kenya but is socio-culturally acceptable. Traditional healers are known and respected members of the same community in which they practice (Swaleh, 1999). Reproductive health issues that drive women in Tana River County, to visit TMPs are many but similar to those found in other rural parts of Kenya (Kaingu et al., 2011). Several studies have reported that long distances to hospital, unreliable public transport system and lack of financial support are the main constraints that drive people in the rural areas to consult TMPs (Barton and Wamai, 1994; Chuang et al., 2009; Cigand and Laborde, 2003; Kaingu et al., 2011). TMPs are cheap and will rarely deny treatment to patients due to lack of payment. This makes them the most likely to be consulted by the majority rural poor (Kaingu et al., 2013_b; Kazerooni et al., 2006; Rapkin, 2003). The study has established that in Tana River County, the custodians of traditional knowledge, including reproductive health knowledge, were all elderly men and women aged over fifty years with long years of practice. Considering that their knowledge was acquired through inheritance from practicing relatives, coupled with the migration of youth to major towns (according to practicing parents and grandparents), there is danger of this knowledge not being passed on to the younger generations for posterity (Kamatenesi-Mugisha and Oryem-Origa, 2005). The lack of documentation is coupled with the lack of systematic conservation to preserve the plants. In this study, the plant part mostly used was the root thereby issues of plant conservation becomes a priority. Female reproductive ailments managed by traditional healers in Tana River County are shown in Table 2. The commonest ailments were pregnancy and related complications,

menstrual problems, infertility and contraception (Fig. 3). The study revealed that pregnant women with signs of threatened abortion readily consulted herbalists and used herbal remedies. Threatened abortion was the most commonly mentioned pregnancy related problem in the community (Fig. 3). A similar finding was reported by (Kaingu et al., 2011; Chuang et al., 2005 and 2007). Post-partum hemorrhage (PPH) and retained afterbirth (RAB) are the leading cause of maternal mortality and morbidity in developing countries (Rajan and Wing, 2010) and a concern in developed countries (WHO, 2003, WHO, 2006). Excessive bleeding requires emergency services that would involve administration of uterotonic agents to facilitate the delivery of the placenta (afterbirth). In rural parts of the developing world, such emergency services are non-existent (WHO, 2006; Rajan and Wing, 2010). The role played by TMPs in handling PPH and RAB is therefore crucial.

A few plants were reported for the management of delayed and protracted labor and in these cases; TMPs used herbal remedies to induce labor with hardly any hospital referrals (Kaingu et al., 2013a). This contrasts similar studies (Kaingu et al., 2011), where some TMPs referred such patients to hospital. Menstrual disorders were the second most mentioned ailments in this study. Literature indicates that numerous effects including physical, hormonal and emotional disorders can disrupt the normal menstrual cycle resulting in complications such as absence or abnormal cessation of menstruation (amenorrhea), heavy menstrual bleeding, (menorrhagia), and dysmenorrhea (severe painful menses) (McEvoy et al., 2004; Meduri and Touraine, 2003; Rapkin, 2003). Herbal remedies have proven effective in relieving the pain and discomfort of menstrual disorders. In the present study, menstrual problems were managed by 22 plants (45.8%) suggesting a high prevalence of such ailments. Similar high prevalence of menstrual disorders has also been reported in other studies (Yassin, 2012). The most common menstrual

complaints in this study were menorrhagia, irregular menses, amenorrhea, and dysmenorrhea respectively. This agrees in part with previous studies conducted in Israel (Goldestein et al., 2006), Turkey (Talatu and Egbunu, 2007), England (Houston et al., 2006) and Egypt (Yassin, 2012) where dysmenorrhea and premenstrual syndrome (PMS) were the most prevalent menstrual complaints. Infertility was the third commonest problem in this study. WHO estimates that approximately 8 - 10% (50 - 80 million people worldwide) of couples experiences some form of infertility problems whose prevalence varies from region to region (Nagendra and Jayachandran, 2010). Many women consulted herbalists in order to enhance their fertility. The treatment for infertility by use of herbs is worldwide (Deka and Kalita, 2011). However, no individual herb is considered especially useful for promoting fertility. In Africa, India and China for instance, a lot of plants have been used in various combinations to treat infertility (Deka and Kalita, 2011; Ugwah-Oguejiofor et al., 2011; Nagendra and Jayachandra, 2010).

In the present study, although 13 plants were presented for the treatment of infertility, 6 of those plants were also used for the treatment of fibroids. Fibroids are linked not only to painful menses and excessive bleeding but also to infertility. The herbalists seemed able to diagnose the presence of fibroids and claimed to not only control their growth but also shrink the large growths. Herbal practitioners in this study presented eleven plants which they claimed were used to suppress fertility by preventing conception rather than as abortifacients. The importance of plants as fertility regulators (contraceptive drugs) has been investigated by many researchers for years (Yakubu et al., 2007a; Yakubu et al., 2007b) and availability of such plants with anti-fertility properties would be of great benefit in developing countries because such plants with contraceptive properties would be easily available and affordable. The minimal uptake of modern contraceptive methods among rural communities in sub-Saharan Africa is generally due

to lack of access to orthodox medicine and contraceptive options in particular (Adebisi and Bello, 2011). In Tana River County the presence of a district hospital at Ngao that offers family planning services did not prevent women from consulting herbalists and instead emphasized the dependence of rural communities on traditional remedies perhaps due to safety considerations.

3.4.2. Male sexual dysfunction

Male sexual dysfunction in the local context can mean a lot of different conditions. Erectile dysfunction for instance can sometimes imply "impotence," especially among communities like those in Tana River County, where proper distinctions between the two are not made. The word "impotence" may also be used to describe other problems that interfere with sexual intercourse and reproduction, such as lack of sexual desire and problems with ejaculation or orgasm. One study for instance, defines impotence as the inability to finish sexual intercourse due to lack of penile erection (Pamplona-Roger, 2000). These variations make defining sexual dysfunction in general and ED in particular difficult. It would also make estimating the incidence of sexual dysfunction /ED difficult. This indeed was the situation in this study where the herbalists described sexual dysfunction collectively as 'upunguvu wa nguvu za kiume' or reduced male vigor with no clear classification of the conditions or symptoms. The fact that nineteen plants were presented for the management of sexual dysfunction would suggest that this is a real problem for males in Tana River County. Most societies especially in the developing world are yet to embrace sexual dysfunction as a true medical condition. The description of impotent men in western Uganda among the Banyankore ethnic group for instance, is literally translated as the persons without legs, implying that the penis is dead (cannot bear children) (Kamatenesi-Mugisha and Oryem- Origa, 2005). Other societies describe such men with sexual impotence and/or ED and unable to reproduce as worthless. In Uganda and perhaps other parts of Africa,

such men were not supposed to be given positions of responsibility or leadership because they were regarded as abnormal. It was therefore not surprising that in this study most of the men, according to the herbalists, consulted without the knowledge of their wives or partners. Culturally, in certain societies, the impotent men married wives and entrusted their wives to very close friends or relatives to bear them children and save them from the shame (Kamatenesi-Mugisha and Oryem-Origa, 2005). Erectile dysfunction and/or impotence are therefore difficult conditions to accept or live with for most males regardless of race or ethnicity. Even among the elite and educated only the few who can afford it seek modern medical care be it privately and secretly. The rest have no option but to turn to traditional healers. In this study impotence was mentioned to mean ED and vice versa. A study by Kamatenesi- Mugisha and Oryem-Origa reported similar difficulties with definitions (Kamatenesi-Mugisha and Oryem-Origa., 2005). Erectile dysfunction and/or impotence have profound and devastating effect on psychological well being of the victim. They can lead to low self-esteem, depression, negative effect on relationships and reduced life satisfaction; reducing the victims worthiness in the society (Kamatenesi-Mugisha and Oryem-Origa., 2005). In this study, a total of seven plants were reported for the management of ED/impotence. A further seven plants were presented with aphrodisiac properties, and while this would suggest that low sex drive was understood as being a separate condition from ED/ impotence, it was treated by the herbalists as a possible contributing factor in the prevalence of ED/impotence as well as infertility. Six plants were used to treat infertility, described by the herbalists as the inability to father children. This inability was not necessarily due to ED/impotence or low libido since some of their clients claimed to have no issues with ED or HSD. However the link between sexual dysfunction and infertility is not disputed and in this study, 2 plants were presented for the management of all three conditions,

thus demonstrating this link. Erectile dysfunction, impotence and reduced fertility are old problems and traditionally, the indigenous knowledge had ways of managing them (Kamatenesi-Mugisha and Oryem-Origa, 2005). Studies carried out elsewhere indicate that some of the plants presented in this study for the management of erectile dysfunction and impotence may indeed be potent on reproductive problems like infertility and other ailments (Table 3) (Ribeiro et al., 2010). This presents an opportunity to further explore the plants not only in terms of phytochemistry but also for the management of sexual dysfunction. Phytochemical reports are important as they give an indication of the kind of biological activity expected from the plant. Kamatenesi- Mugisha and Oryem-Origa. (2005) for instance reported that the stimulant effect of caffeine was due to the presence of alkaloids.

3.4.3. Other illnesses in Tana River

Six plant species (19%) were used to treat pneumonia; probably the condition is very common amongst the 3 communities in the study area and this might account for more plant species treating the condition. Kaingu et al. (2011) reported a similar finding in Machakos District, Kenya. Arthritis was also very common in Tana River and 16% of the plant species were reported to be effective in treating the condition (Figure 6). It was also interesting; that 16% of the medicinal plants were used to manage kidney problems, 13% to manage fibroids, 13% to treat typhoid, 9.7% to treat breast cancer, 9.7% to treat toothache,6.5% to treat boils, 6.5% to treat malaria and 6.5% to treat diabetes (Figure 6). Some of the remedies might contain antimicrobial properties, emetics, excitants and digestives. These were used to manage convulsions, stomach aches, constipation, poisoning, cholera, diarrhea, mastitis, migraine, tonsillitis, stomach ulcers, asthma, high blood pressure, urinary incontinence, body warts and to induce milk letdown as shown in Table 4. The most popular plant species in decreasing order

were *Harrisonia abyssinica*, *Strychnos henningsii*, *Lawsonia inermis*, *Acacia robusta*, *Solanum incunum* that were cited by herbalists for the management of three to five conditions as calculated by their use values (Table 4). Considering that for 83% of the plant species, the most commonly used plant part was the root; issues of conservation should be looked into. In the current study, the herbalists were not worried about conserving or using alternative plant parts as they had easy access to the bushy woodland as reported in other studies Muthee et al. (2011), Nanyingi et al. (2008). The respondents administered different doses of remedy and durations for similar conditions. The present study has shown that the people in Tana River County have a very good knowledge base on herbal remedy for primary health care. Traditional healers still rely largely on naturally growing species in their locality. Furthermore, the documented medicinal plants can be used as a basis for future phytochemical and pharmacological studies.

3.5 CONCLUSION

Reproductive dysfunction is a major obstacle to social-economic development amongst the inhabitants of Tana River County. Tana River has a pool of TMPs with a wealth of indigenous knowledge that needs to be exploited. The plants used to treat dysmenorrhea for example may be important analgesic agents that need further investigation while those reported as fertility regulators may contain steroidal phyto chemical compounds. Such species therefore need further investigation to establish their effect on female reproductive parameters, hormonal profiles and ovarian histogy.

The plant remedies described in this study represents valuable indigenous knowledge (IK) and could prove useful as treatment for male sexual dysfunctions and infertility. Major issues that need to be addressed are proper research on the efficacy and safe use of the plants,

standardization of doses and quality of the products. These should be documented followed by advocacy for sustainable utilization of the plants before the knowledge is lost, particularly when the roots of such plants are used.

In this chapter, the study has successfully identified and documented medicinal plants used for management of female and male reproductive health dysfunctions and general illnesses in the community for future posterity. Two fertility regulating plants (*Croton menyharthii* and *Uvariodendron kirkii*) with the highest frequency of mention by the TMPs were selected for further *in-vivo* investigation.

Three papers have been published in International peer reviewed journals (Appendix 4, 5 and 6).

CHAPTER 4

4.0 EFFECTS OF *CROTON MENYHARTHII* AND *UVARIODENDRON KIRKII* EXTRACTS ON SELECTED REPRODUCTIVE PARAMETERS.

4.1 INTRODUCTION

Medicinal plants disrupt estrus cycle and cause significant anti-ovulatory and anti-implantation effect in rats (Dinesh et al., 2012). Contraceptive effect by plants could either be through prevention of ovulation, fertilization and implantation or through causing abortion. Studying the effect of plant extracts on reproductive parameters helps determine the contraceptive interphase. A disruption of estrus, mating success, fertilization and/or implantation might cause infertility which was a desired effect by some women in Tana River County. It is prudent to scientifically validate the fertility regulation claims by TMPs. The chapter evaluates the antifertility efficacy of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on reproductive parameters of the adult female rats. The reproductive parameters evaluated were estrus cycle, mating success, fertility index, gestation length, litter size and body weight. Mating was said to be successful once a vaginal plug was established or the presence of spermatozoa from a vaginal smear was microscopically observed. Fertility index was calculated as number of non-pregnant animals divided by total number of animals successfully mated multiplied by 100.

4.2 MATERIALS AND METHODS

4.2.1 Laboratory animals

Mature female winstar rats weighing between 170-210g were used for the study. The animals were purchased from the Department of Biochemistry and kept in the animal house, Anatomy

and Physiology Department, of the University of Nairobi, Kenya. They were caged in pairs and were maintained under standard environmental conditions of 12 hours light and 12 hours darkness at 24-25 °C. The rats were fed on commercially obtained diet pellets and tap water was provided *ad libitum*. They were monitored daily for the first 10 days using vaginal smears to ascertain cyclicity. Only those with regular 4-5 day estrous cycles were used for the experiment.

4.2.2 Plant collection, identification and preparation

Medicinal plants used for the management of reproductive dsyfunctions in Tana River County were harvested and brought to the University of Nairobi, School of Biological Sciences for botanical identification and voucher specimens were preserved for future reference. Six commonly mentioned plants used as female fertility regulators in Tana River County were harvested for further investigation. However for the purposes of this study, only two most frequently mentioned plants were selected for *in-vivo* physiological tests. The six plants were *Croton menyharthii*, *Uvariodendron kirkii*, *Ficus natalensis*, *Cissus rotundifolia*, *Ximenia Americana* and *Citrus sinensis* whose fresh roots were cut into small pieces using a knife. The roots were then kept under shade and dried at room temperature for a period of two weeks. The roots were ground into powder in a fume chamber using a Cunningham grinder (Gakuya, 2001). The plant powder was packed in 300g satchets and stored in cool and airy cupboards away from direct sunlight. Out of the six, the two selected plants for further studies were *Croton menyharthii* and *Uvariodendron kirkii*.

4.2.3 Aqueous extract preparation of *Croton menyharthii* and *Uvariodendron kirkii*

300g of *Croton menyharthii* root bark powder was weighed using (Lark digital weighing balance LP502A, 500G/0.01g). The root bark powder was macerated in distilled water at a ratio of 1 to 6

(w/v) in a volumetric flask. The suspension was rotated on a shaker for 24 hours at room temperatures and left to soak for 48 hours. Filtration was carried out using whatman filter paper (number 4). The filtrate was freeze dried for 48 hours and the extract weighed to determine yield. *Uvariodendron kirkii* aqueous extract was prepared in a similar manner. The aqueous extract yield for *Croton menyharthii* and *Uvariodendron kirkii* was 83.89 and 118.93 grams respectively.

4.3. EFFECTS OF AQUEOUS EXTRACTS OF *CROTON MENYHARTHII* AND *UVARIODENDRON KIRKII* PLANT EXTRACTS ON REPRODUCTIVE PARAMETERS.

4.3.1 Effect on estrus cycle

The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on estrus cycle was evaluated using twenty five normocyclic female rats divided into 5 groups of 5 rats each. These animals were not mated even though male rats were kept in the same room but in different cages. The rats were monitored daily for the first 10 days to ensure cyclicity through specific cytological features that distinguished the four stages of the estrus cycles (Diestrus, Proestrus, Estrus and Metestrus). Only rats with regular estrus cycles were used for the study. The findings were recorded. Thereafter, negative control group 1 with five rats received 0.5ml physiological saline through intra-abdominal gavage for 20 days. Groups 2 and 3 received 500 and 800 mg/ Kg *Croton menyharthii* aqueous extract respectively through intra-abdominal gavage daily for 20 days; group 4 and 5 received 500 and 800 mg/Kg *Uvariodendron kirkii* aqueous extract respectively through intra-abdominal gavage daily for 20 days. Vaginal wash samples were collected daily from all the rats between 9 and 10am and examined for estrus cycle cytological features. The findings were recorded.

4.3.2 Effects on mating success, fertility index, gestation length, litter size and body weight

The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extracts on reproductive parameters namely, mating success, fertility index, gestation length, litter size and body weight was evaluated using 3 treatment regimes on normocyclic female rats aged between 50-60 days. Male rats were kept in the same room but in different cages and were introduced into female cages at the ratio of 1 male per 2 females at the appropriate time. A total of 96 rats were used. These were divided into 3 groups (1, 2 and 3) with 32 rats each. The 32 rats in each group were further divided into 4 subgroups (A, B, C, D) with 8 rats each. Group 1, sub group A and B received 500 and 800 mg/Kg of *Croton menyharthii* respectively. Subgroup C and D of group 1 received 500 and 800 mg/kg *Uvariodendron kirkii* respectively. These doses were administered for 14 days through intra-abdominal gavage after which the rats were mated. The first day of gestation was taken to be the day spermatozoa were detected in the vaginal smear under the light microscope. Group 2 animals were first mated after which sub group A and B received 500 and 800 mg/Kg of *Croton menyharthii* aqueous extract respectively while Subgroup C and D received 500 and 800 mg/Kg *Uvariodendron kirkii* respectively for 14 days. Group 3 sub group A, B, C and D were treated in a similar manner as group 1 except extract administration was continued after mating until end of gestation. Control groups consisted of eighteen negative control rats that received 0.5ml physiological saline through intra-abdominal gavage daily in 3 treatment protocols as in the experimental animals above. Six positive control rats received a subcutaneous injection of estrogen/ progesterone combination (15µg estradiol / 0.15 mg progestrin) once. Both negative and positive control animals were then mated. Gestation length, litter sizes as well as body weights of all animals were recorded.

4.3.3 Effects of *Croton menyharthii* and *Uvariodendron kirkii* extracts on implantation

Effects of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on implantation was determined using three randomly selected rats from each of the subgroups of groups 1, 2 and 3, giving a total of 36 animals. The selection was done on day 7 of pregnancy and given 0.25% Evans blue dye via the tail vein. Fifteen minutes later, the rats were sacrificed. The uteri were quickly opened and assessment of implantation sites was carried out by counting the number of uterine dye sites in each uterine horn. Anti-implantation activity (%) was calculated using the formula [(A-B) /A] x 100 where A = number of implantation sites in control group, B = number of implantation sites in test group.

4.3.4 Statistical Analysis

The data was analyzed using one-way ANOVA to compare group means and recorded as X ± SEM.

4.4 RESULTS OF BOTH PLANTS EXTRACTS ON ESTRUS CYCLE

4.4.1 Effect of 500 mg/kg *Uvariodendron kirkii* extract on estrus cycle

At 500 mg/kg *Uvariodendron kirkii* aqueous extract disrupted the estrus cycle with increased frequency of observation of metestrus and diestrus stages respectively compared to the control (Figure 7, 8 and Table 5). Subsequently there was a decline in frequency of observation of estrus and proestrus stages over the 20 day treatment period.

DAYS	NEGATIVE CONTROL	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5
DAY 1						
DAY 2						
DAY 3						
DAY 4						
DAY 5						
DAY 6						
DAY 7						
DAY 8						
DAY 9						
DAY 10*						
DAY 11						
DAY 12						
DAY 13						
DAY 14						
DAY 15						
DAY 16						
DAY 17						
DAY 18						
DAY19						
DAY 20						
DAY 21						
DAY 22						
DAY 23						

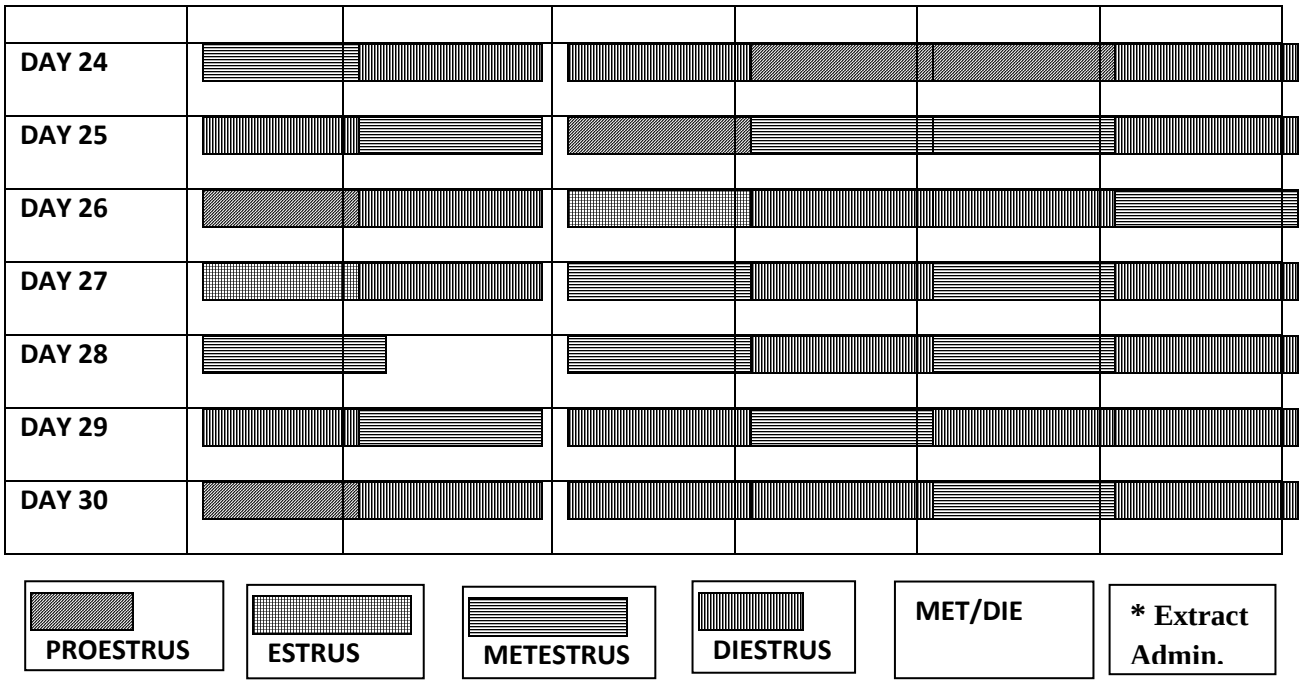


Figure 7: Effect of 500mg/kg *Uvariodendron kirkii* on estrus cycle stage appearance over a 20 day period.

Table 5: The frequency of appearance of each estrus cycle stage over a period of 20 day *Uvariodendron kirkii* extract administration (500mg/Kg).

	Control	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean for test groups
Proestrus	8	5	6	6	5	5	5.4*
Estrus	7	4	5	4	5	4	4.4*
Metestrus	8	10	9	9	9	8	9**
Diestrus	7	12	10	11	11	13	11.4***

The Table shows the observation frequency of estrus cycle stages over a 20 day treatment period. The frequency of observation of metestrus and diestrus were significantly ($P<0.001$) increased. Similarly estrus and proestrus frequency of observation were significantly ($P<0.05$) reduced compared to the control. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

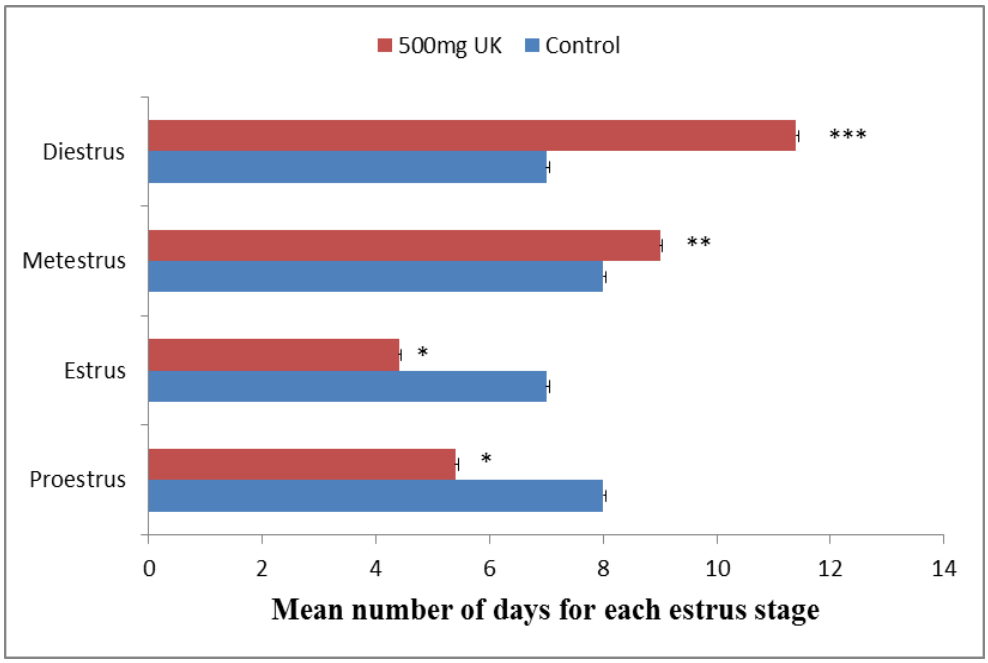
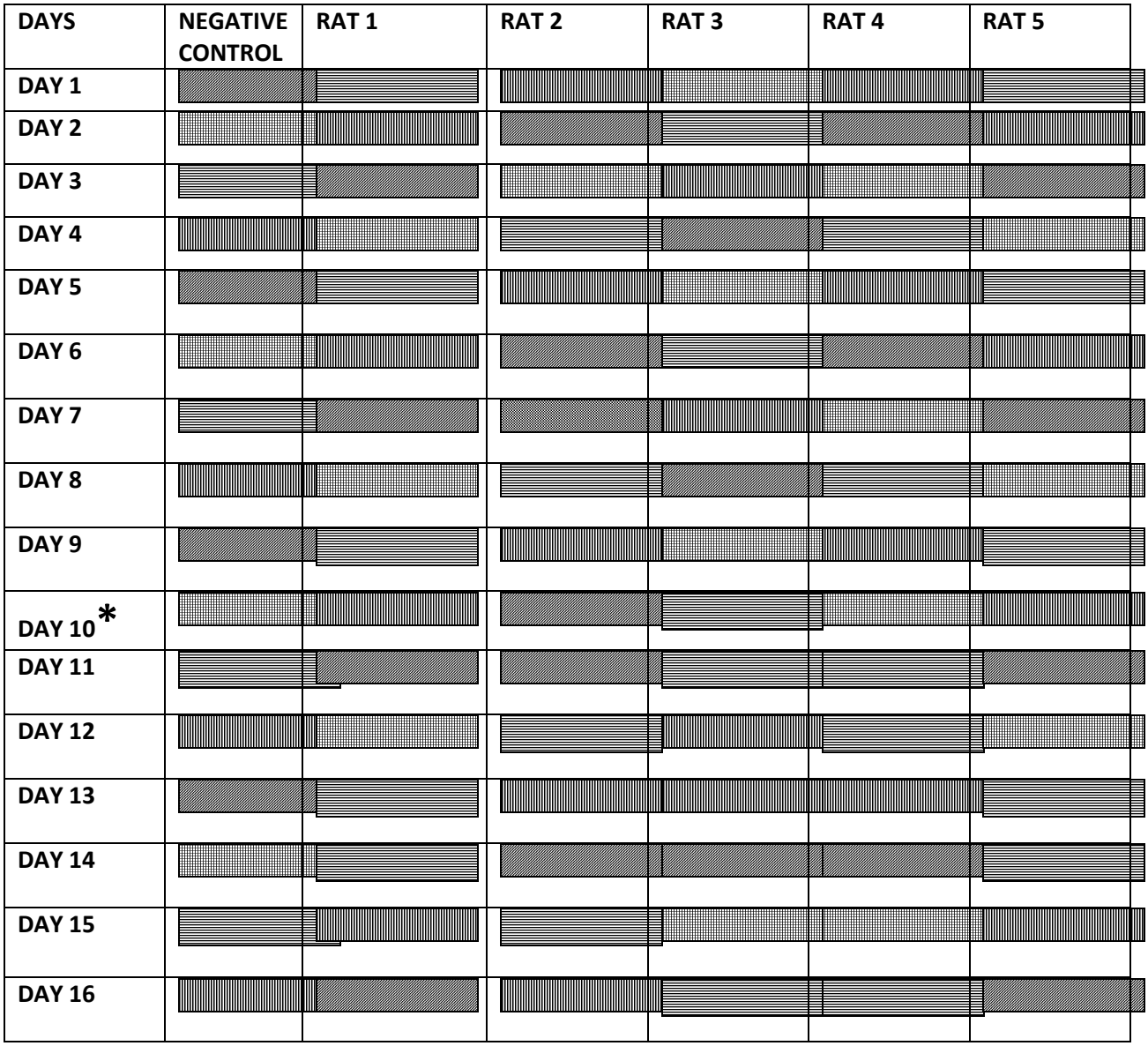


Figure 8: The frequency of appearance of each estrus cycle stage over the 20 day *Uvariodendron kirkii* extract administration (500mg/Kg).

Figure 8 shows the graphic presentation of the effect of *Uvariodendron kirkii* aqueous extract at 500 mg/kg on the frequencies of estrus cycle stages. Within 20 days of extract administration; there was a disruption of estrus cycle stages with a significant ($P< 0.01$, $P<0.001$) increase in frequency of observation of metestrus and diestrus respectively compared to the control. Subsequently there was a significant ($P<0.05$) reduction in frequency of estrus and proestrus phases. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

4.4.2 Effect of 800 mg/kg *Uvariodendron kirkii* extract on estrus cycle

Figure shows the frequency of appearance of each estrus cycle stage over the 20 day 800mg/Kg *Uvariodendron kirkii* extract administration.The estrus cycle was disrupted; the frequency of observation of estrus and proestrus declined while that of metestrus and diestrus increased (Figure 9,10 and Table 6).



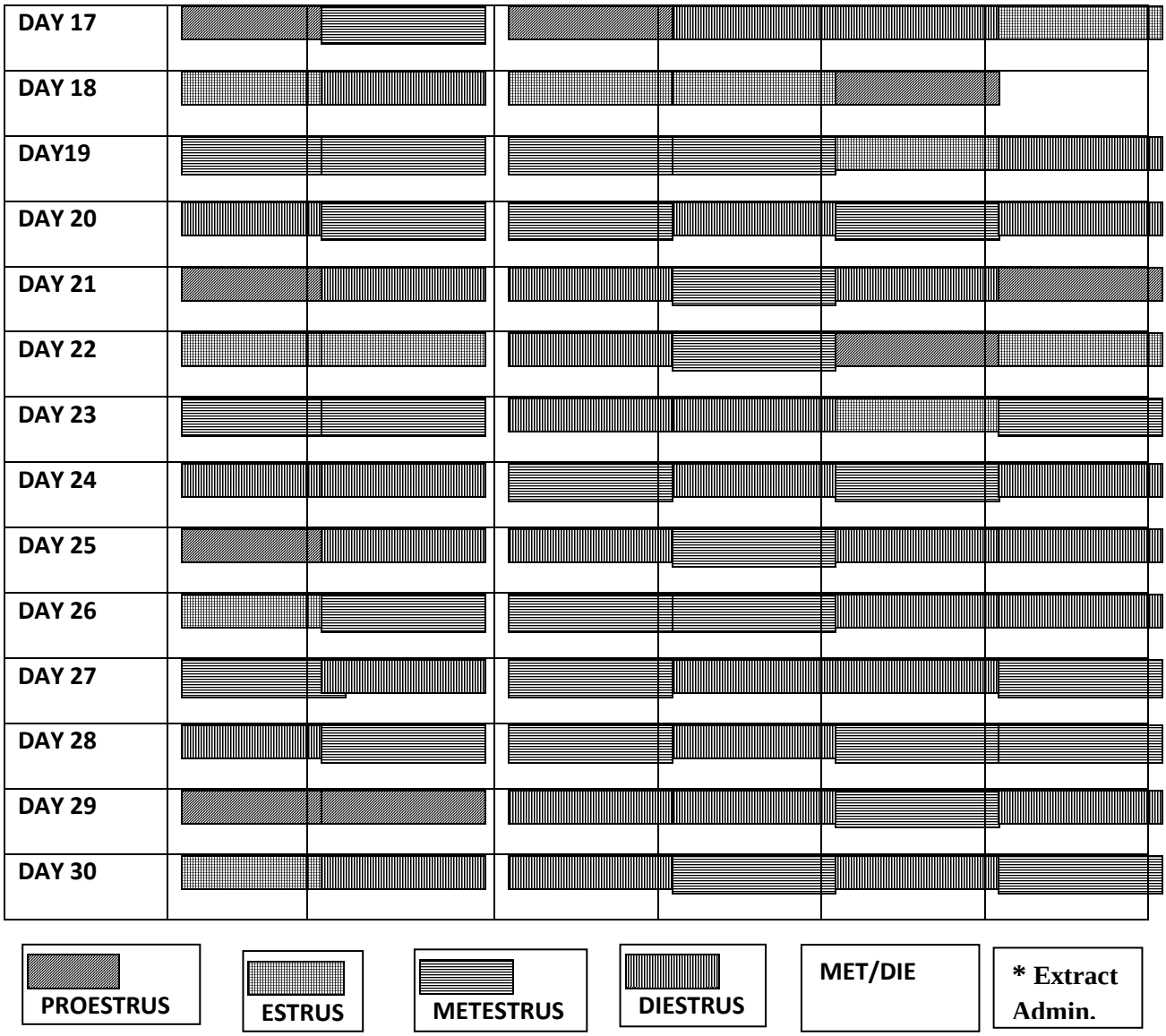


Figure 9: Effect of 800mg/kg *Uvariodendron kirkii* extract administration on estrus cycle stage appearence.

Table 6: The frequency of appearance of each estrus cycle stage over the period of 20 day *Uvariodendron kirkii* extract administration (800mg/Kg).

	Control	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean for test groups
Proestrus	8	5	6	3	5	5	4.8**
Estrus	8	4	3	5	6	5	4.6**
Metestrus	7	11	10	12	9	10	10.4***
Diestrus	7	10	11	10	10	11	10.4***

The Table shows a disruption of the estrus cycle with a frequently observed metestrus ($P< 0.001$) and Diestrus ($P<0.001$) stages of the estrus cycle compared to the control. Consequently the estrus and proestrus frequency of appearence were significantly reduced ($P< 0.05$) compared to the control. $P<0.05$* $P<0.01$ ** $P<0.001$ ***

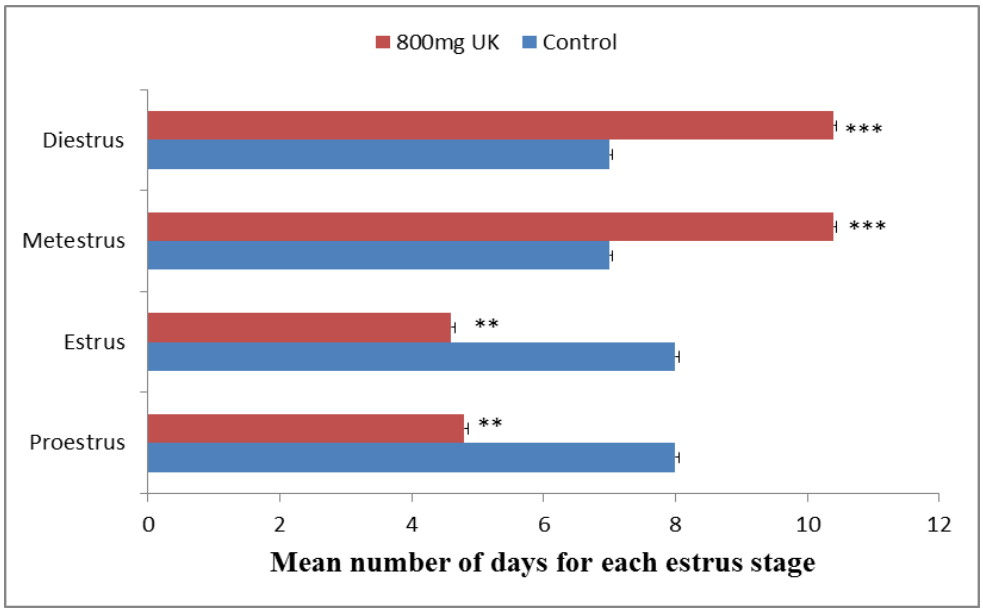


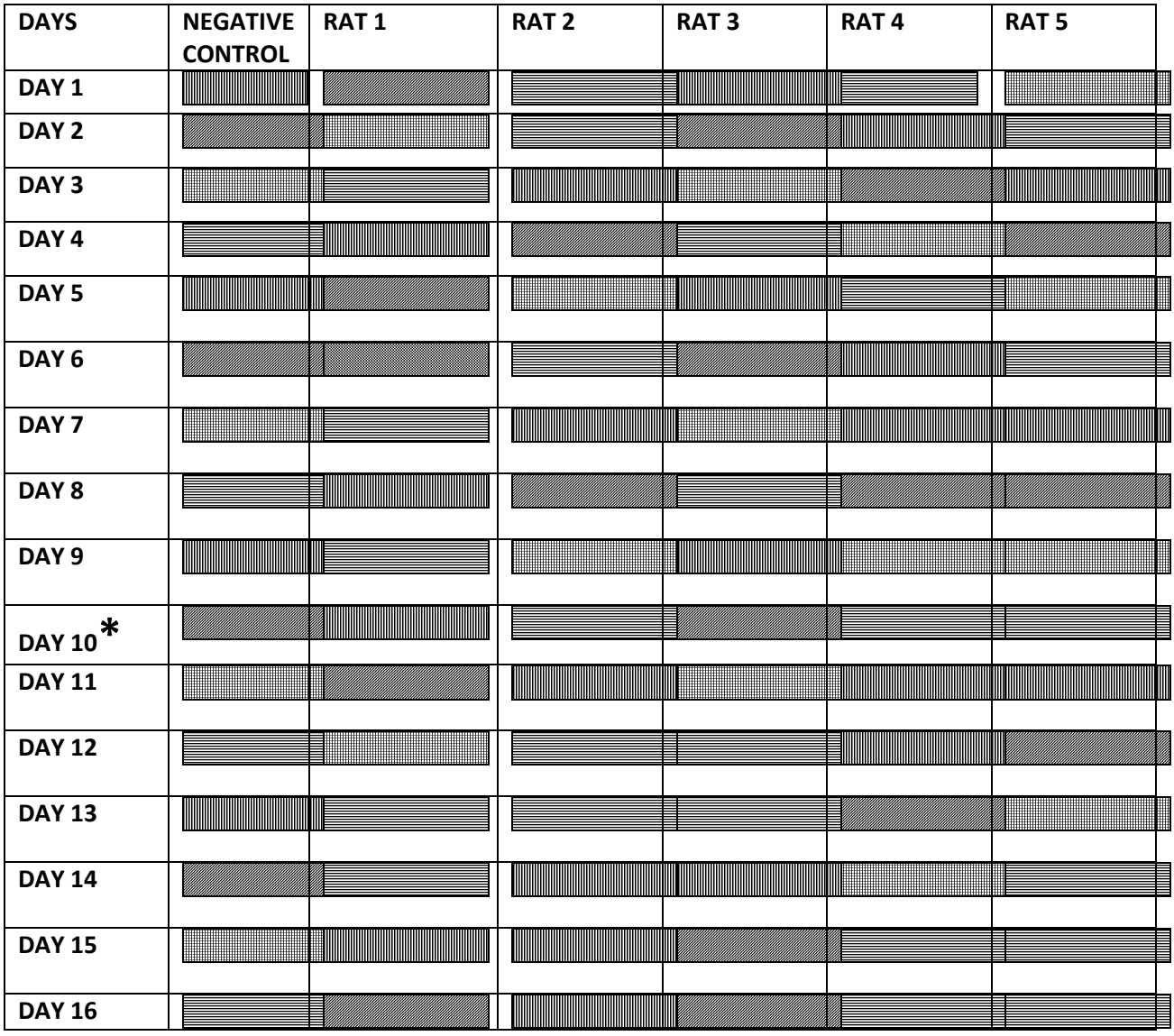
Figure 10: The frequency of appearance of each estrus cycle stage over the period of 20 day *Uvariodendron kirkii* extract administration (800mg/Kg).

The Figure shows the effect of 800mg/Kg *Uvariodendron kirkii* aqueous extract on frequency of appearance of each estrus cycle stage. After a 20 day treatment regime; the estrus cycle was disrupted with a significant ($P<0.01$) reduction in frequency of appearance of estrus and Proestrus compared to the control. Subsequently frequency of appearance of diestrus and metestrus significantly ($P<0.001$) increased compared to the control.

*$P<0.01$ ** $P<0.001$ *** ($P<0.01$).

4.4.3 Effect of 500mg/kg *Croton menyharthii* extract on estrus cycle

The Figure shows the appearance frequency of each estrus cycle stage over a 20 day period of extract administration. *Croton menyharthii* extract at 500mg/kg similarly caused a significant disruption of the estrus cycle with an increased appearance frequency of metestrus and Diestrus stages compared to the control. Estrus and proestrus appearance frequencies were similarly reduced compared to the control (Figure 11, 12 and Table 7).



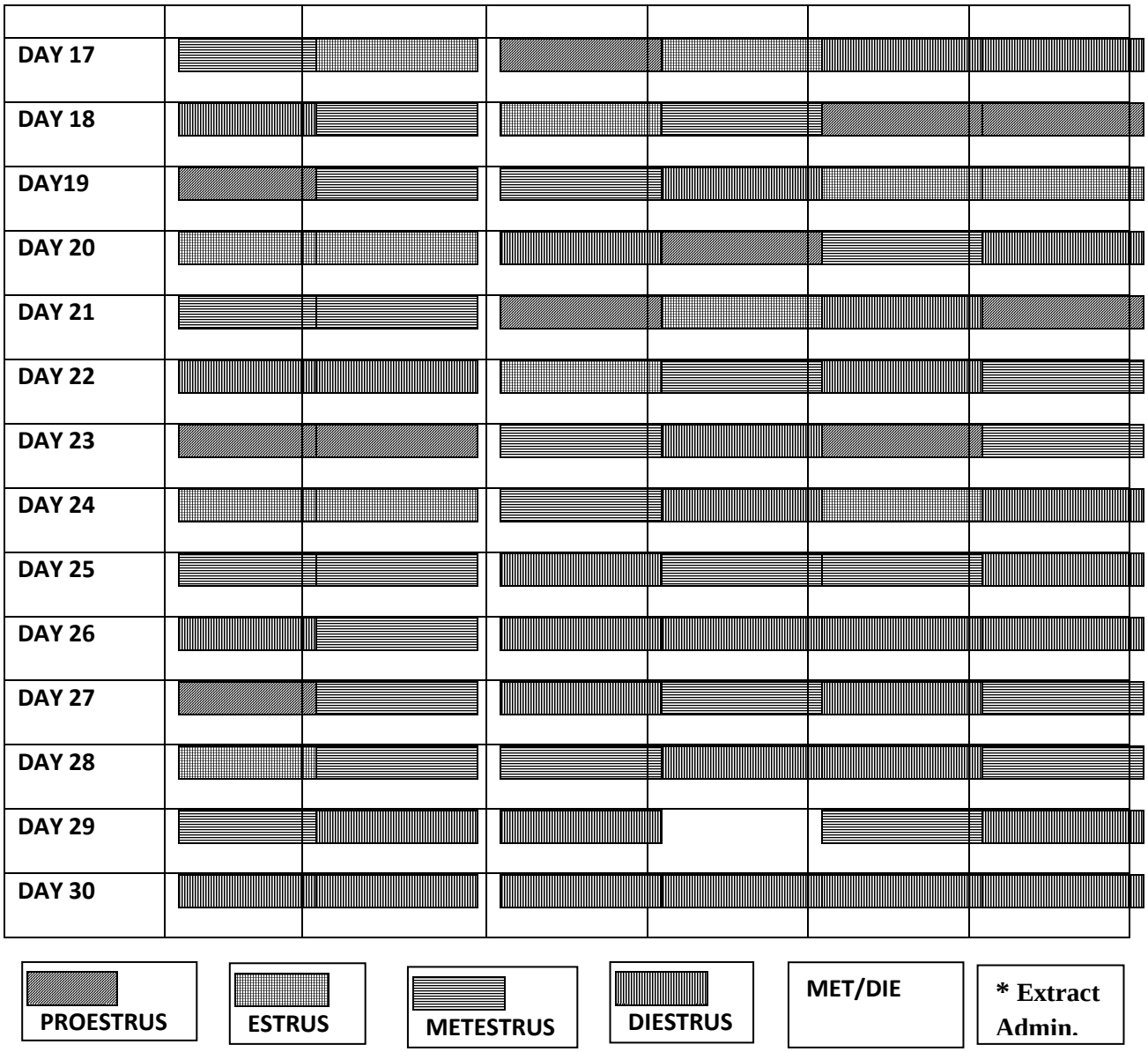


Figure 11: Effects of 500 mg/Kg *Croton menyharthi* extract on estrus cycle stage appearance frequency.

Table 7: The frequency of appearance of each estrus cycle stage over a period of 20 day *Croton menyharthii* extract administration (500mg/Kg).

	Control	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean for test groups
Proestrus	7	5	3	6	5	5	4.8*
Estrus	7	6	4	5	5	5	5*
Metestrus	8	12	10	9	8	10	9.8***
Diestrus	8	7	12	9	12	10	10***

The Table shows a disruption of the estrus cycle with a frequently observed metestrus ($P< 0.001$) and Diestrus ($P<0.001$) stages of the estrus cycle compared to the control. Consequently the estrus and proestrus frequency of appearence were significantly reduced ($P< 0.05$) compared to the control. $P<0.05$* $P<0.01$ ** $P<0.001$ ***

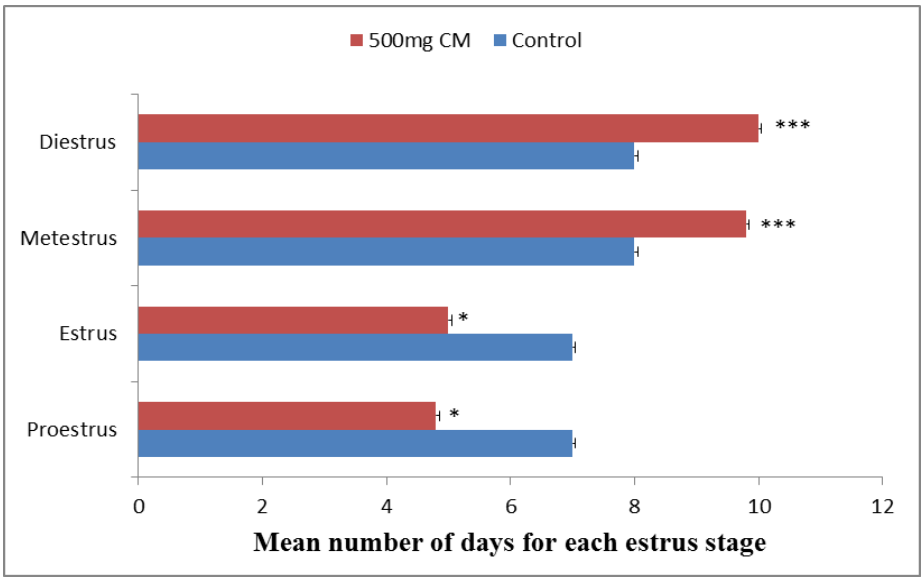


Figure 12: The frequency of appearance of each estrus cycle stage over a period of 20 day *Croton menyharthii* extract administration (500mg/Kg).

The Figure shows the effect of a 20 day extract administration regime. 500mg/kg *Croton menyharthii* extract caused a disruption of the estrus cycle with a significant appearance frequency of metestrus ($P< 0.001$) and diestrus ($P<0.001$). Consequently the frequency of appearance of estrus and proestrus stages were significantly reduced ($P< 0.05$).

$P<0.05$* $P<0.01$ ** $P<0.001$ ***

4.4.4 Effect of 800mg/kg C*roton menyharthii* aqueous extract on estrus cycle.

Figure 13 shows the effect of a 20 day extract administration regime. The estrus cycle was disrupted with an increased appearance frequency of metestrus and diestrus stages compared to the control (Figure 13, 14 and Table 8). Consequently the appearance frequency of estrus and proestrus stages were reduced.

DAYS	NEGATIVE CONTROL	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5
DAY 1						
DAY 2						
DAY 3						
DAY 4						
DAY 5						
DAY 6						
DAY 7						
DAY 8						
DAY 9						
DAY 10*						
DAY 11						
DAY 12						
DAY 13						
DAY 14						
DAY 15						
DAY 16						
DAY 17						
DAY 18						
DAY19						
DAY 20						
DAY 21						
DAY 22						
DAY 23						

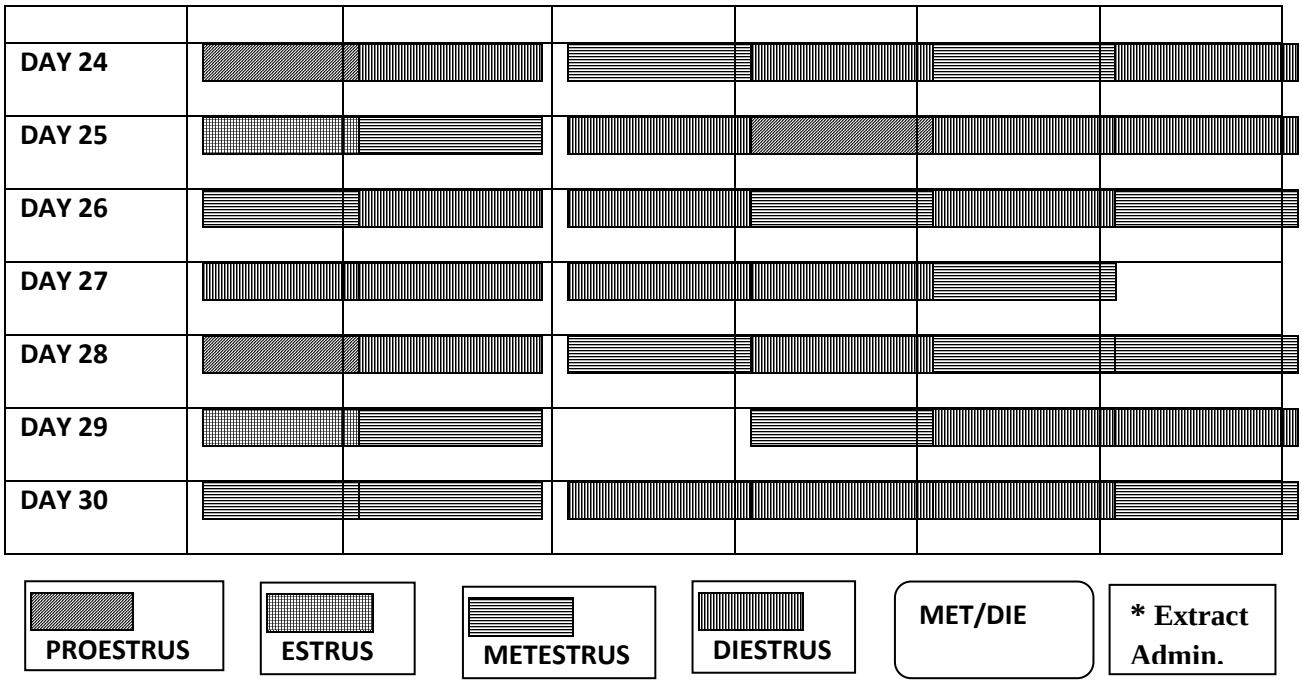


Figure 13: Effect of 800mg/kg *Croton menyharthi* extract on appearance frequency of estrus cycle stages.

Table 8: Appearance frequency of each estrus cycle stage after a 20 day *Croton menyharthii* extract administration (800mg/Kg).

	Control	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Mean
Proestrus	7	5	5	4	5	5	4.8*
Estrus	6	4	3	4	4	4	3.8**
Metestrus	8	11	11	12	10	11	11***
Diestrus	8	10	12	10	11	11	10.8***

After a 20 day treatment regime; the estrus cycle was disrupted with a significant appearance frequency of metestrus ($P< 0.001$) and diestrus ($P<0.001$) stages compared to the control (Figure 14). Estrus and Proestrus frequency of observation were similarly disrupted ($P<0.01$; $P<0.05$). $P<0.05$* $P<0.01$ ** $P<0.001$

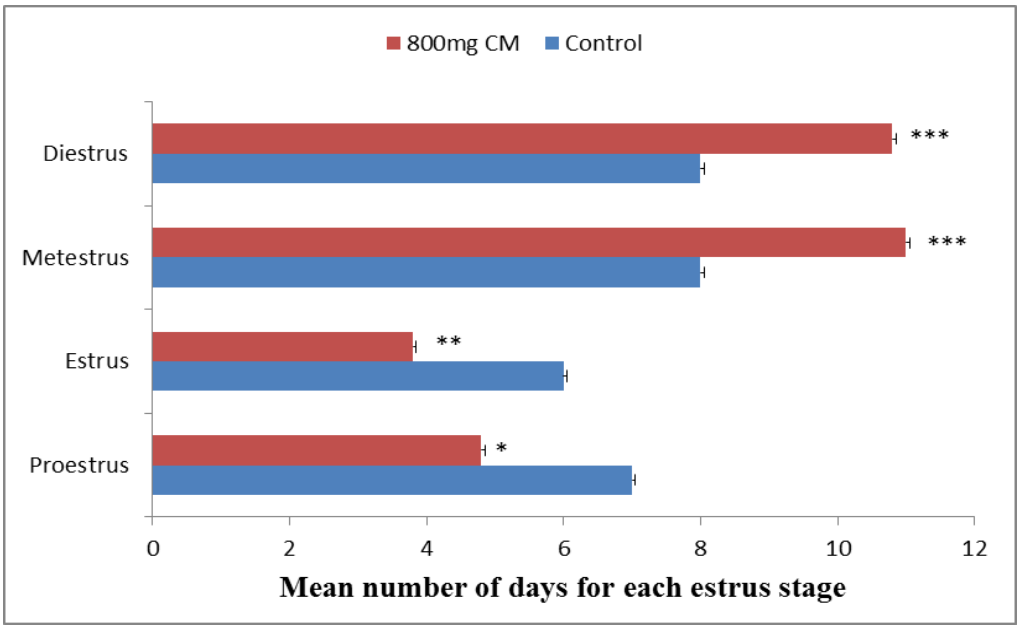


Figure 14: The appearance frequency of each estrus cycle stage over a period of 20 days *Croton menyharthii* extract administration (800mg/Kg).

The Figure 14 shows the effect of a 20 day *Croton menyharthii* extract administration on estrus cycle stages. The estrus cycle was disrupted with a significant ($P<0.001$) finding being increased appearance frequency of metestrus and diestrus cycle stages compared to the control. Consequently there was a significant ($P<0.01$; $P<0.05$) reduction in appearance frequency of estrus and proestrus stages. $P<0.05$* $P<0.01$ ** $P<0.001$ ***

4.4.5 Summary of the effect of C*roton menyharthii* and U*variodendron kirkii* extracts on estrus cycle.

Figure 15 summarizes the effect of *Uvariodendron kirkii* and *Croton menyharthii* at 500 and 800mg/kg each respectively on the estrous cycle. Both plants caused a significant disruption of the estrus cycle ($P<0.01$; $P<0.001$). There was an increase in frequency of observation of metestrus and diestrus stages compared to the control; with subsequent lowering of the frequency of observation of estrus and proestrus stages.

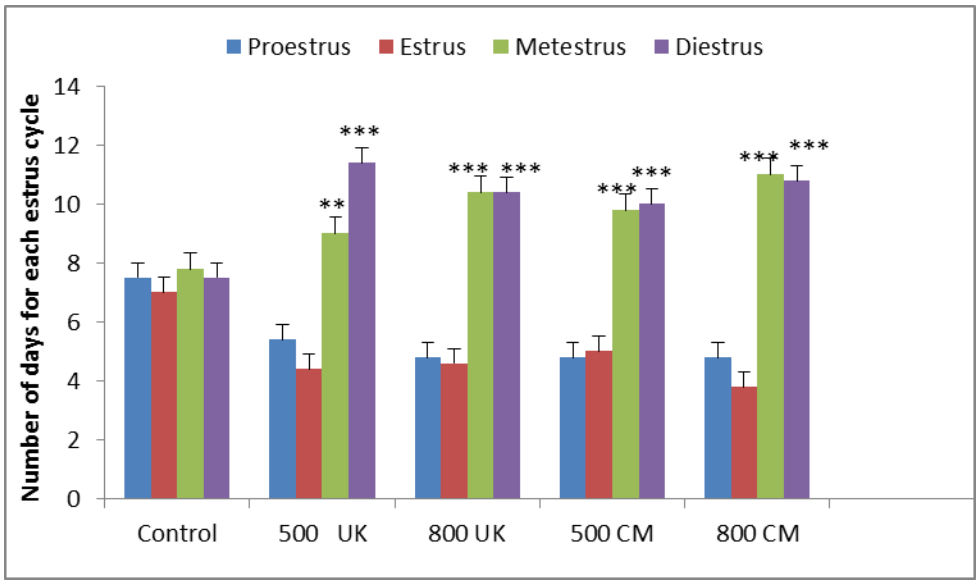


Figure 15: Summary of the effect of *Croton menyharthii* and *Uvariodendron kirkii* extract on appearance frequency of estrus cycle stages.

Figure 15 shows the disrupted estrus cycle. *Uvariodendron kirkii* at 500 and 800mg/kg caused a significant ($P<0.01$; $P<0.001$) increased appearance frequency of metestrus and diestrus cycle stages compared to the control. Consequently there was a lowering of the appearance frequency of estrus and proestrus stages. *Croton menyharthii* aqueous extract at 500 and 800 mg/kg caused a significant ($P<0.001$) disruption of the estrus cycle with increased frequency of metestrus and diestrus stages with subsequent lowering of the appearance frequency of estrus and proestrus stages. The results are Mean ± SEM. $P<0.05$* $P<0.01$ ** $P<0.001$ ***.

4.5 RESULTS OF BOTH PLANTS EXTRACTS ON MATING SUCCESS

4.5.1 The effect of *Croton menyharthii* and U*variodendron kirkii* extract on mating success.

Uvariodendron kirkii aqueous extract at 800 mg/Kg caused a 20 day delay before the female rats successfully mated compared to the negative control. *Uvariodendron kirkii* at 500 mg/Kg and *Croton menyharthii* aqueous extract at 800 mg/Kg caused a 15 and 16 day delay respectively before successful mating. *Croton menyharthii* aqueous extract at 500 mg/Kg caused a 10 day delay before successful mating compared to the negative control animals that mated within four days of the first estrous cycle.

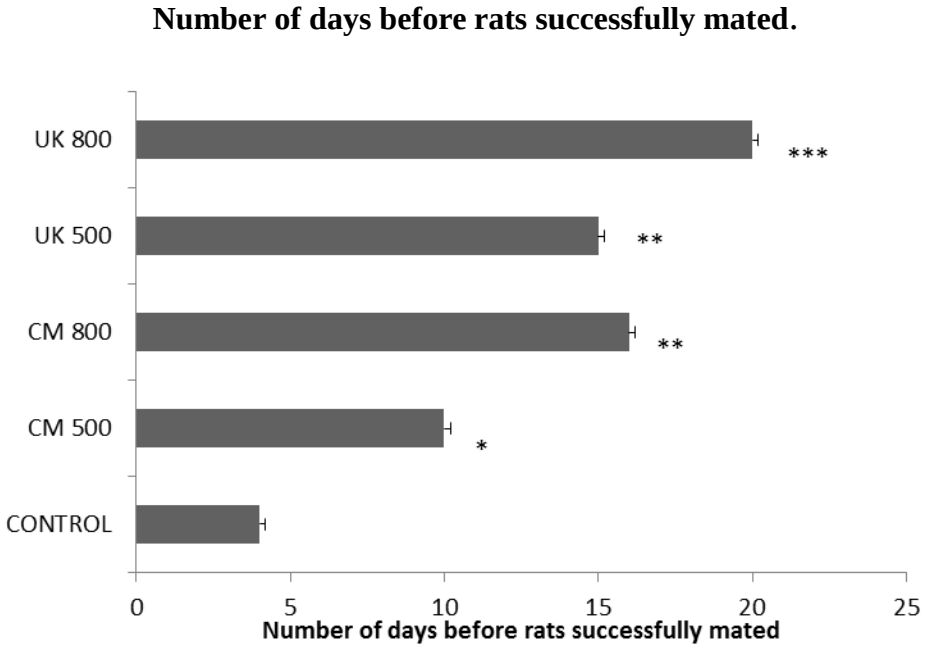


Figure 16: Effect of *Croton menyharthii* and *Uvariodendron kirkii* extracts on number of days before successfully mating.

Figure 16 shows significantly prolonged number of days before rats successfully mated. *Uvariodendron kirkii* aqueous extract at 800 mg/Kg caused a 20 day delay before rats successfully mated compared to the negative control. *Uvariodendron kirkii* at 500 mg/Kg and *Croton menyharthii* aqueous extract at 800 mg/Kg caused a 15 and 16 day delay respectively before successful mating. *Croton menyharthii* aqueous extract at 500 mg/Kg caused a 10 day delay before successful mating compared to the negative control. The results are Mean ± SEM ***P<0.001, **P<0.01, *P<0.05.

UK-*Uvariodendron kirkii* CM-*Croton menyharthii*

4.6 RESULTS OF THE EFFECTS OF *CROTON MENYHARTHII* AND *UVARIODENDRON KIRKII* EXTRACT ON REPRODUCTIVE PARAMETERS.

4.6.1 Effects of both plant extracts administered before mating (Group 1).

Croton menyharthii at 500 and 800 mg/Kg had no effect on mating success but caused a significant ($P<0.05$; $P<0.01$) reduction in fertility index at 50% and 40% respectively. *Uvariodendron kirkii* aqueous extract at 500 and 800 mg/Kg had a 20% and 0% reduction in mating success respectively with a significant ($P<0.01$; $P<0.001$) reduction in fertility index at 33% and 20 % respectively (Table 9). The reduction in fertility index was dose dependent for both plants, with a significant reduction at 500 mg/Kg ($P< 0.01$) and at 800 mg/Kg ($P< 0.05$) for *Croton menyharthii* and at 500 mg/Kg ($P<0.01$) and 800 mg/Kg ($P< 0.001$) for *Uvariodendron kirkii* respectively (Figure 15). *Croton menyharthii* at 800mg/Kg and *Uvariodendron kirkii* at 500mg/Kg caused a significantly ($P<0.05$) prolonged gestation length compared to the control. *Uvariodendron kirkii* and *Croton menyharthii* aqueous extract at 500 and 800mg/Kg caused a significant reduction ($P<0.001$) in litter size (Table 9) compared to the control. The rats gained weight during the 14 days premating extract administration. $P<0.05$* $P<0.01$ ** $P<0.001$ ***

Table 9: The effect of administering extracts of *Croton menyharthii* and *Uvariodendron kirkii* before mating on reproductive parameters.

	Mating success	Fertility index	Gestation length (days)	Litter size	Body weight
Negative control	100 %	100 %	22 ± 0.05	10 ± 0.12	231± 0.01
Positive control	Nil	Nil	Nil	Nil	262.3 ± 0.11
CM 500 mg/Kg	100 %	40 % **	22.5 ± 0.15	5 ± 0.05**	271.6 ± 0.16
CM 800 mg/Kg	100 %	50 % *	26 ± 0.22*	6 ± 0.15**	268.4 ± 0.03
UK 500 mg/Kg	80 %	33 %**	24 ± 0.14*	3 ± 0.03***	272.5± 0.15
UK 800 mg/Kg	100 %	20 % ***	22 ± 0.03	2 ± 0.05***	249.8 ± 0.11

Table 9 shows the effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract administered before mating in Group1 on reproductive parameters. There was a dose dependent significant reduction in fertility index and litter size and a significantly increased gestation length.

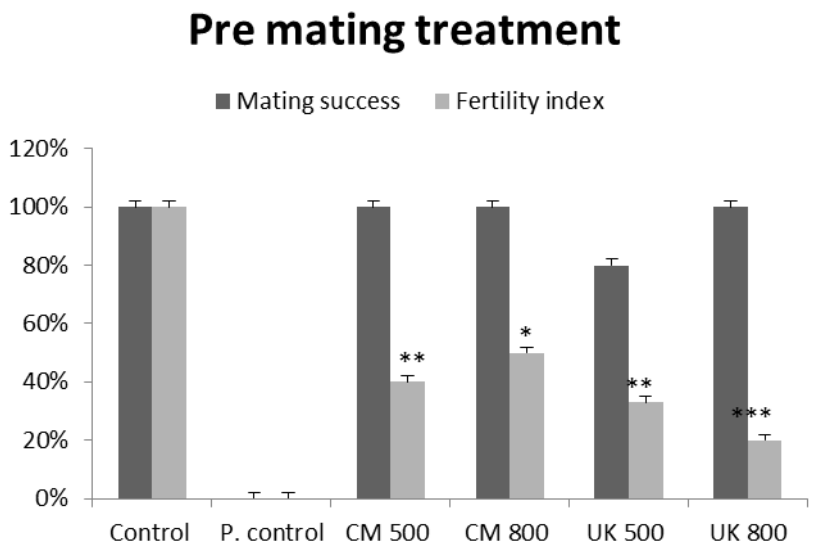


Figure 17: The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract administered before mating on fertility index and mating success.

The Figure shows a significant dose dependent reduction in fertility index by both plant extracts. *Croton menyharthii* at 500 and 800 mg/Kg caused a significant reduction in fertility index-($P<0.01$ and $P<0.05$) respectively compared to the negative control (Table 9). *Uvariodendron kirkii* at 500 caused a 20% reduction in mating success. *Uvariodendron kirkii* at 500 and 800 mg/Kg also caused a significant reduction in fertility index ($P<0.01$ and $P<0.001$) respectively compared to the negative control (Table 9). The results are Mean ± SEM .$P<0.05$* $P<0.01$ ** $P<0.001$ ***

4.6.2 Effects of both plant extracts administered after mating (Group 2).

Croton menyharthii at 500 and 800 mg/Kg had no effect on mating success with a non-significant reduction in fertility index at 97% and 90% respectively compared to the negative control. *Uvariodendron kirkii* aqueous extract at 500 and 800 mg/Kg also had no effect on mating success with a non-significant reduction in fertility index at 96% and 93 % respectively compared to the negative control. *Croton menyharthii* at 500 and 800 mg/Kg caused a significantly ($P<0.001$) prolonged gestation length compared to the negative control (22 ± 1). *Uvariodendron kirkii* at 500mg/Kg caused a significantly ($P<0.01$) prolonged gestation length compared to the negative control (22 ± 1). Both plant extracts caused a non-significant alteration of the litter size compared to the negative control (Table 10). Both plant extracts caused significant ($P<0.05$; $P<0.01$) increase in body weight compared to the negative control.

Table 10: Effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract administration on reproductive parameters after mating

	Mating success	Fertility index	Gestation length (days)	Litter size	Body weight
Control	100 %	100 %	22 ± 0.1	10 ± 0.12	231± 0.01
Positive control	Nil	Nil	Nil	Nil	239 ± 0.05
CM 500 mg/Kg	100%	97%	31 ± 0.2 ***	9 ± 0.2	256 ± 0.11 **
CM 800 mg/Kg	100%	90%	34 ± 0.15***	10 ± 0.5	248 ± 0. 41*
UK 500 mg/Kg	100%	96%	27± 0.21**	8 ± 0. 6	261 ± 0.18**
UK 800 mg/Kg	100%	93%	22 ± 0.14	8 ± 0.34	244 ± 0.13*

Croton menyharthii at 500 and 800 mg/Kg had no effect on mating success with a non-significant reduction in fertility index at 97% and 90% respectively compared to the negative control. *Uvariodendron kirkii* aqueous extract at 500 and 800 mg/Kg also had no effect on mating success with a non-significant reduction in fertility index at 96% and 93 % respectively compared to the negative control. *Croton menyharthii* at both doses caused a significantly ($P<0.001$) prolonged gestation length compared to the negative control (22 ± 1). *Uvariodendron kirkii* at 500mg/Kg caused a significantly ($P<0.01$) prolonged gestation length compared to the negative control (22 ± 1). Both plant extracts caused a non-significant alteration of the litter size compared to the negative control

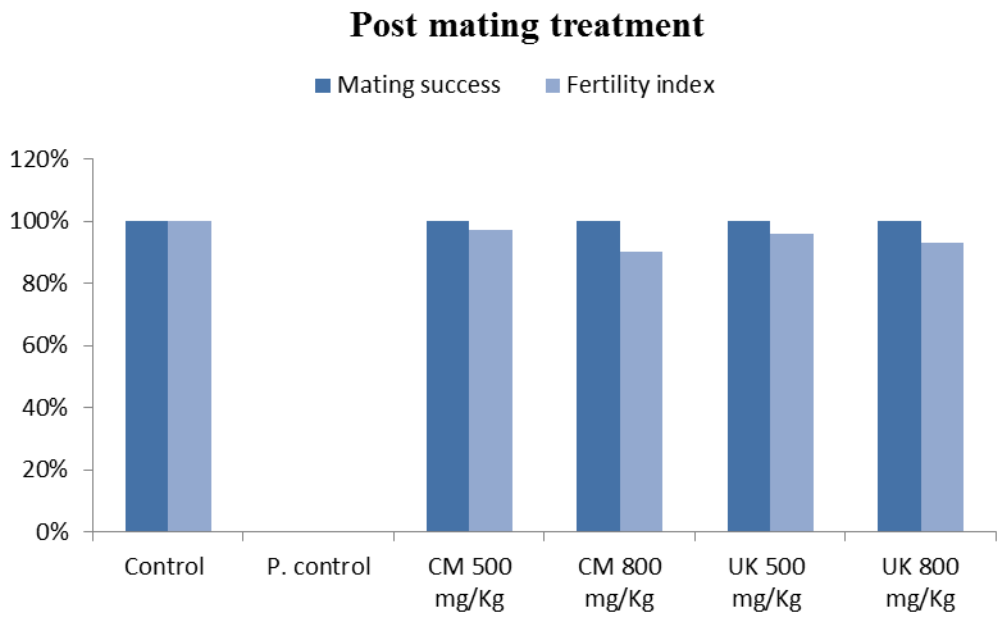


Figure 18: Effect of *Croton menyharthii* and *Uvariodendron kirkii* extract administered after mating on mating success and fertility index.

Figure 18 shows a non-significant reduction in fertility index by both plant extracts. Both plants caused 100% mating success**.** The results are Mean ± SEM.

4.6.3 Effect of both plant extracts administered before and after mating (Group 3).

Croton menyharthii at 500 and 800 mg/Kg caused 80% and 90% reduction in mating success respectively with a significant ($P<0.001$) reduction in fertility index at 0% compared to the negative control. 500 and 800 mg/Kg *Uvariodendron kirkii* aqueous extract had a 100% reduction in mating success and fertility index with none of the mated rats littering. There was significant loss in weight in all rats (Table 11).

Table 11: The effect of "before and after mating' *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract administration on reproductive parameters.

	Mating success	Fertility index	Gestation length (days)	Litter size	Body weight
Control	100 %	100 %	21-23	9 ± 0.02	231± 0.05
Positive control	Nil	Nil	Nil	Nil	246 ± 0.47
CM 500 mg/Kg	20%***	0%	Nil	Nil	215 ± 0.4
CM 800 mg/Kg	10%***	0%	Nil	Nil	221 ± 0.15
UK 500 mg/Kg	0%	0%	Nil	Nil	215 ± 0.5
UK 800 mg/Kg	0%	0%	Nil	Nil	209 ± 0.13

Croton menyharthii at 500 and 800 mg/Kg caused 80% and 90% reduction in mating success respectively with a significant ($P<0.001$) reduction in fertility index at 0% compared to the negative control. 500 and 800 mg/Kg Uvariodendron kirkii aqueous extract had a 100% reduction in mating success and fertility index with none of the mated rats littering. There was significant loss in weight in all rats (Table 11).

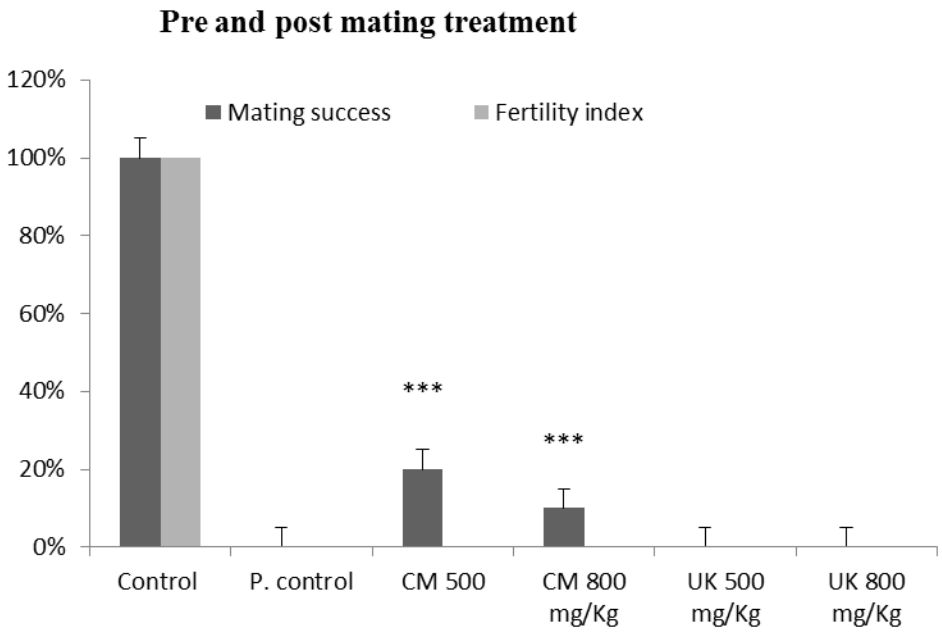


Figure 19: The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract administration before and after mating on mating success and fertility index.

The Figure shows that *Croton menyharthii* at 500 and 800 mg/Kg caused 80% and 90% reduction in mating success respectively with a significant ($P<0.001$) reduction in fertility index at 0% and 0% respectively compared to the negative control. *Uvariodendron kirkii* at 500 and 800 mg/Kg aqueous extract caused a 100% reduction in mating success and 100% effect on fertility index. None of the mated rats littered. The results are Mean ± SEM. $P<0.001$ ***

4.7 RESULTS OF ANTI-IMPLANTATION EFFECT OF BOTH PLANT EXTRACTS

4.7.1 Anti implantation effect of C*roton menyharthii* and U*variodendron kirkii* extract administered before mating (Group 1).

Croton menyharthii and *Uvariodendron kirkii* premating extract administration had a significant anti-implantation activity (Table 12) at all extract dose levels. At 500 and 800 mg/Kg *Croton menyharthii* caused a significant (P<0.01) anti-implantation effect of 58.7% and 48.5 % respectively compared to the negative control. At 500 and 800 mg/Kg *Uvariodendron kirkii* caused a significant (P<0.001) anti-implantation activity of 67.1% and 81.3% respectively compared to the negative control (Table 12).

Table 12: Anti-implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* extract administered before mating.

Treatment groups	Number of pregnant rats	Mean implantation sites	Anti-implantation activity (%)
Control	3	9.7 ± 0.11	0
Positive control	None	0.00	100%
CM 500 mg/Kg	3	4.0 ± 0.2	**58.7%****
CM 800 mg/Kg	3	6.0 ±0.13	**48.5%****
U.K 500 mg/Kg	3	3.3 ± 0.5	**67.1%*****
U.K 800 mg/Kg	3	1.9 ± 0.31	**81.3%*****

Table 12 shows anti-implantation activity of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract. *Uvariodendron kirkii* at 500 and 800 mg/Kg significantly (P<0.001) caused an anti-implantation activity at 67.1 and 81.3% respectively. *Croton menyharthii* at 500 and 800 mg/Kg caused a significant (P<0.01) anti-implantation activity at 58.7 and 48.5% respectively. Mean implantation values are expressed as Mean ± SEM (n=3/ group). *** P< 0.001 **P<0.01 *P<0.05.

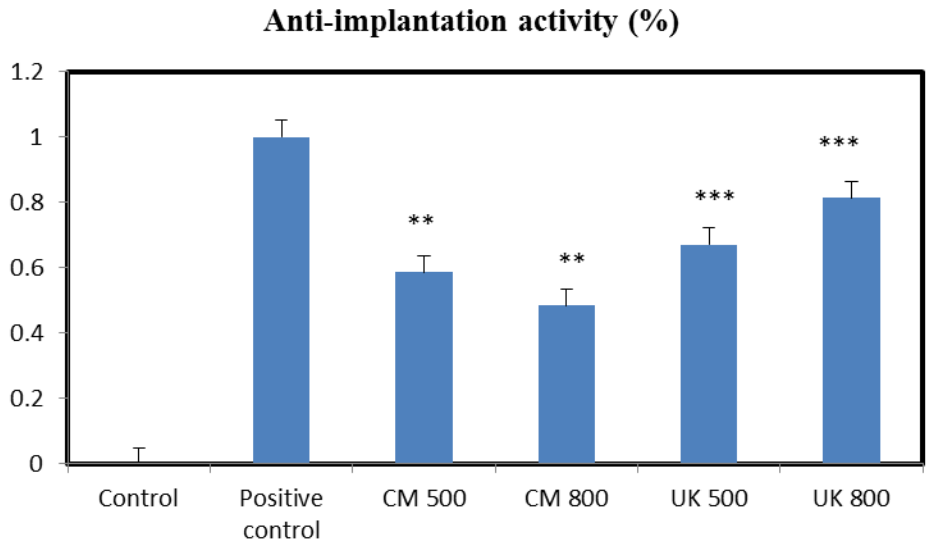


Figure 20 Anti-implantation effect of *Uvariodendron kirkii* and *Croton menyharthii* extract administred before mating.

The Figure shows anti-implantation activity of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract when administered before mating. The anti-implantation activity was significant (P<0.001) at 500 and 800 mg/Kg *Uvariodendron kirkii* at 67.1 and 81.3% respectively. *Croton menyharthii* at 500 and 800 mg/Kg on the other hand had a significant (P<0.01) anti implantation activity at 58.7 and 48.5% respectively.

Mean implantation values are expressed as Mean ± SEM (n=3/ group). *** P< 0.001 **P<0.01 *P<0.05.

4.7.2. Anti implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* extract administered after mating (Group 2).

The effect of administering extracts of *Croton menyharthii* and *Uvariodendron kirkii* at 500 and 800 mg/Kg respectively post mating on anti-implantation activity. The results were not significant at all extract dose levels (Table 13). Mean implantation values are expressed as Mean ± SEM (n=3/ group).

Table 13: Anti-implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract administred after mating.

Treatment groups	Number of pregnant rats	Mean implantation sites	Anti-implantation activity (%)
Control	3	9.7 ± 0.11	0
Positive control	None	0.00	100%
CM 500 mg/Kg	3	9.6 ± 0.2	1.0 %
CM 800 mg/Kg	3	8.7 ±0.13	10 %
U.K 500 mg/Kg	3	9.5 ± 0.5	2.0 %
U.K 800 mg/Kg	3	9.1 ± 0.31	6.2 %

Table 13 shows anti-implantation activity of post mating administration of *Croton menyharthii* and *Uvariodendron kirkii* extract at 500 and 800 mg/Kg. The results were not significant at all extract dose levels compared to the negative control.

Mean implantation values are expressed as Mean ± SEM (n=3/ group). *** P< 0.001 **P<0.01 *P<0.05.

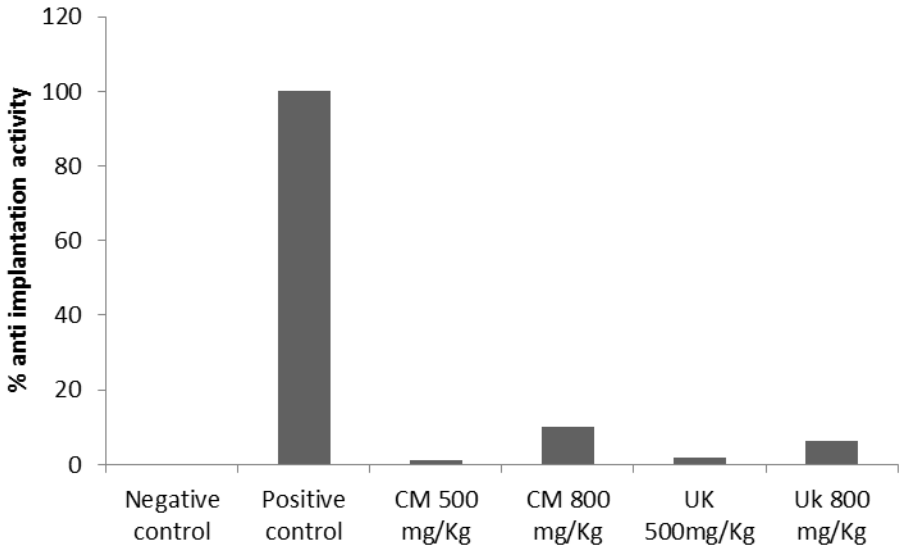


Figure 21: Post mating anti-implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract.

Figure 21 shows anti implantation activity of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract at 500 and 800 mg/Kg when administered after mating. The results were not significant at all extract dose levels compared to the negative control.

Values are Mean ± SEM (n=3/ group). *** P< 0.001 **P<0.01 *P<0.05.

4.7.3 Anti implantation effect of C*roton menyharthii* and U*variodendron kirkii* extract administered before and after mating (Group 3).

Pre and post mating *Croton menyharthii* and *Uvariodendron kirkii* extract administration caused a significant (P<0.01; P<0.001) anti implantation effect at both dose levels. *Croton menyharthii* at 500 and 800mg/Kg caused a 68 and 100% anti-implantation activity respectively compared to the negative control.

Table 14: Anti- implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* extract administred before and after mating

Treatment groups	Number of pregnant rats	Mean implantation sites	Anti-implantation activity (%)
Negative control	3	9.7 ± 0.11	0
Positive control	None	0.00 ±	100%
CM 500 mg/Kg	3	3.1 ± 0.2	**68 %****
CM 800 mg/Kg	3	0.0 ± 0.13	**100 %*****
UK 500 mg/Kg	3	0.0 ± 0.5	**100 %*****
UK 800 mg/Kg	3	0.0 ± 0.31	**100 %*****

The results were significant (P<0.05) and (P<0.001) at 500 and 800 mg/Kg of *Croton menyharthii* respectively. At 500 and 800mg/Kg *Uvariodendron kirkii* the results were significant (P<0.001) respectively compared to the control. Mean implantation values are expressed as Mean ± SEM (n=3/ group). *** P< 0.001 **P<0.01 *P<0.05.

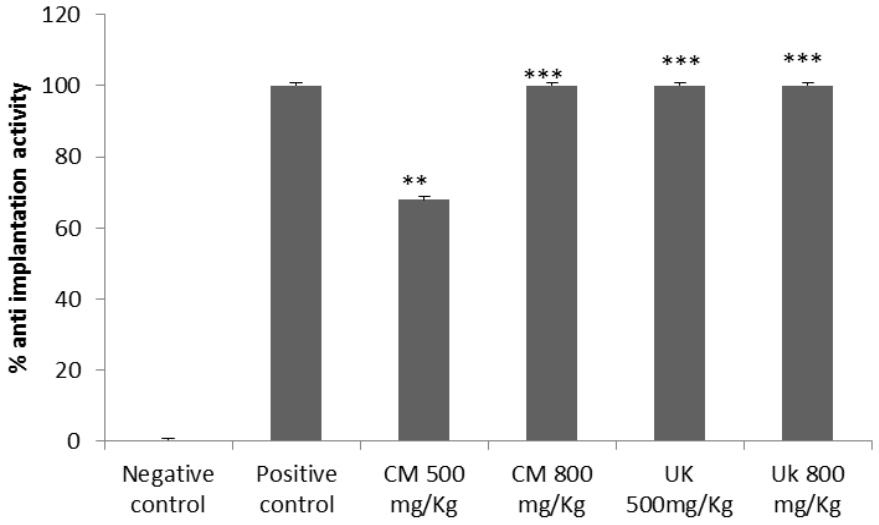


Figure 22: Anti-implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* extract administered before and after mating.

Figure 22 shows the anti-implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* extracts at 500 and 800 mg/Kg respectively compared to the negative control. *Croton menyharthii* and *Uvariodendron kirkii* at 500 and 800 mg/Kg caused a significant (P<0.01; P<0.001) reduction of implantation compared to the negative control. Mean anti implantation activity is expressed as Mean ± SEM (n=3/ group). *** P< 0.001 **P<0.01 *P<0.05.

4.8 DISCUSSION

4.8.1 Estrus Cycle

This study has shown that at 500mg/Kg and 800mg/Kg *Uvariodendron kirkii* significantly disrupted the estrus cycle. Both doses caused a prolonged duration of metestrus ($P<0.01$) and diestrus ($P< 0.001$) phases compared to the control (Table 5 and 6) Figures 8 and 9). The aqueous extract also caused a significant reduction in frequency of estrus ($P< 0.05$) and proestrus ($P<0.05$) phases compared to the control. At 500mg/kg and 800 mg/Kg *Croton menyharthii* aqueous extract caused a significant disruption of the estrus cycle with a prolonged metestrus ($P< 0.001$) and diestrus ($P<0.001$) phase of the estrus cycle. From the 14th day of extract administration, the frequency of estrus and proestrus phases were significantly reduced ($P< 001$) (Figure 11 and 13). The estrus cycle is driven by both pituitary gonadotropins and ovarian steroid hormones. A disruption / disturbance of hormonal balance especially estradiol disrupts the estrus cycle. Levels of estradiol start increasing as FSH secretion gradually increases and initiates antral follicular growth and maturation. Rising levels of estradiol plays a major role in endometrial receptivity to implantation. Varshney et al., (2016) corroborated this study by showing that *Embelia ribes, Azadirachta indica, Mormodica charantia, Curcuma longa, Jatropha gossipifolia, Cynodon dactylon, Garcinia cola, Cissampelos pareira, Plumbago zaylanica* and *Anethum gravelens* all disrupted estrus cycle with a significant change in diestrus duration. In this study the estrus cycle was disrupted perhaps as a direct effect of *Croton menyharthii* and *Uvariodendron kirkii* on folliculogenesis and oogenesis or it might be due to a disruption of gonadotropins or ovarian steroids.

4.8.2 Effects of extracts on mating success and fertility index

Mating was said to be successful once a vaginal plug was established or the presence of spermatozoa in a vaginal smear was microscopically observed. Fertility index was calculated as number of non-pregnant animals divided by total number of animals successfully mated multiplied by 100. The hormones produced locally within the follicle, such as progesterone and estradiol are involved in a variety of follicular modifications and signaling cascades. In this study premating administration of both plant extracts had significant results (Table 9). Both plant extracts caused a dose dependent significant reduction in fertility index (Figure 15). *Croton menyharthii* at 500 and 800 mg/Kg had no effect on mating but had a significant reduction in fertility index at 50% and 40% respectively. *Uvariodendron kirkii* aqueous extract at 500 and 800 mg/Kg had a 20% and 0% reduction in mating succes but caused a 33% and 20 % significant ($P<0.001$) reduction in fertility index (Figure 15, Table 9). Since 80 to 100% of the rats were successfully mated as evidenced by presence of spermatozoa plugs; when exposed to extract administration prior to being mated (Group 1) it suggests that ovulation was probably occurring. In every estrus cycle; a group of follicles are recruited to grow and mature. In this study ovulation occurred and those rats successfully mated. It is possible that fertilization and/or implantation was disrupted as shown by the significant reduction in fertility index (Figure 15). Female fertility is determined by the developmental competence of oocyte; in its ability to be fertilized and give rise to a viable embryo and for that embryo to successfully implant. In this study; *Uvariodendron kirkii* had the most significant ($P<0.001$) anti fertility activity (Table 14) compared to the control. Other studies have reported on abortive properties of several medicinal plants. *Aerva lantana, Annona reticula* (Mitra and Mukhajee, 2009); *Alangium salvifoltum* (Meena and Rao, 2010); *Ananas comosus* (Murthy and Venkaiah, 2010); *Artemisia siverstana*

willd (Uniyal et al., 2006); *Acorus calamus Linn* (Gangwar et al., 2010); *Adiantum capillus veneris* (Benitez et al., 2010). These results are further supported by (Daniyal and Akram, 2015 Dinesh et al., 2012; Okoko et al., 2010; Azamthulla et al., 2015; Hu et al., 1985) who reported on anti-implantation effect of several medicinal plants such as *Acalypha indica, Ailanthus excelsa, Aristolochia bracteolate, Azadirachta indica, Bambusa vulgaris, Butea monosperma, Citrus emedica, Dalbergia saxatilis Linn, Vicoa indica, Plumbago zaylanica, Nelumbo nucifera, Hibiscus rosa-sinensis, Heliotropium indicum, Gloriosa superba, Ferula hermonis, Polygonum hydropiper Linn, Ocimum sanctum, Striga orobanchioides, Ricinus communis, punica granatum, Calotropis procera, Mentha arvensis, Lawsonia inermis, Juniperus communis, Hagenia abyssinica* and *Cicer arietinum*. The redution in fertility index was dose dependent with a significant reduction at 500 mg/Kg ($P< 0.01$) and 800 mg/Kg ($P< 0.05$) *Croton menyharthii*; 500 mg/Kg ($P<0.01$) and at 800 mg/Kg ($P< 0.001$) *Uvariodendron kirkii* respectively (Figure 13). The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on reproductive parameters provides a pointer towards possible anti fertility properties of the extracts. Contraceptive properties of drug compounds could be anti-ovulatory, anti - fertilization, disrupt embryo implantation or cause abortion (Daniyal and Akram, 2015). A disruption of pituitary gonadotropins or ovarian steroids compromises folliculogenesis and oogenesis and leads to infertility. The presence of spermatozoa plugs was evidence that mating did occur and perhaps fertilization took place. The effects of *Croton menyharthii* and *Uvariodendron kirkii* on fertility index suggests anti implantation properties of the extracts. The post mating extract administration treatment group further supported this theory. The anti- implantation activity of both plant extracts administered post mating was 1.0%, 10%, 2.0% and 6.2% at 500 and 800 mg/Kg *Croton menyharthii* and at 500 and 800 mg/Kg *Uvariodendron kirkii* respectively

(Figure 16; Table 10). Suggesting that post mating extract administration might not have significant effect at disrupting implantation and establishment of gestation. The pre and post mating extract administration treatment regime on the other hand, had the most significant effect on mating and fertility index. Mating only occurred in 20% and 10% of the rats administered with 500 and 800 mg/Kg *Croton menyharthii* respectively (Table 11; Figure 17). None of these rats littered (fertility index 0%). The rats exposed to *Uvariodendron kirkii* never mated suggesting that a pre and post mating extract administration regime could have had a significant effect on folliculogenesis, oogenesis, ovulation or implantation. Probably very few rats were ovulating suggesting an anti- ovulatory and anti- implantation properties of both plant extracts. Daniyal and Akram, (2015) and Dinesh et al., (2012) support our results by reporting on anti-ovulatory and anti-implantation properties of medicinal plants.

4.8.3 Effect of both plant extracts on gestation length, litter size and body weight

Croton menyharthii at 500mg/Kg and *Uvariodendron kirkii* at 800mg/Kg caused a significantly ($P<0.05$) prolonged gestation length compared to the control (Table 9). *Uvariodendron kirkii* aqueous extract significantly reduced ($P<0.001$) litter size (Table 9). *Croton menyharthii* aqueous extract also significantly reduced ($P<0.01$) litter size compared to the control (Table 9).The significant reduction in litter size supports the anti-implantation theory seen in the previous section 4.5.2. The increase in body weight (Table 9; Table 10) in Group 1 and Group 2 experimental animals in the present study suggests that other physiological functions within the body were normal. Both plant extracts however caused a significant reduction in the body weight of Group 3 experimental animals (Table 11). It is possible there was compromised feeding in this group as extract administration was sustained for a longer duration compared to the other experimental animals (Group 1 and 2).

Both plant extracts however caused a significant reduction in the litter size compared to the negative control (Table 10). This is in contrast to the findings by Olayaki et al., (2009) who reported on significant reduction in maternal body weight but an increase in litter size on oral administration of *Cajanus cajan* aqueous extract. *Quassia amara* caused a significant reduction in litter size and body weight (Yinusa et al., 2010). Aqueous extract of *Anacardium occidentale* caused a significant reduction of litter size (Dare et al., 2011) but no significant change of gestation length. However aqueous extract of *Allium sativum* caused a non- significant change of litter size. *Hibiscus sabdariffa* aqueous extract caused a significant reduction in litter size (Iyare and Adegoke, 2011). In group 2 (post mating treatment group) *Croton menyharthii* at 500 and 800 mg/Kg caused a significantly ($P<0.001$) prolonged gestation length compared to the negative control (Table 10), while *Uvariodendron kirkii* at 500mg/Kg caused a significantly ($P<0.01$) prolonged gestation length compared to the negative control at 22 ± 1 days. This is in contrast to (Iyare and Adegoke, 2011) who reported that *Hibiscus sabdariffa* aqueous extract caused a non-significant change in gestation length. However**;** *Indigofera trifoliata* leaves caused no change in gestation length (Okoyea et al., 2015**).** *Carica papaya* causes non-significant change of litter size, body weight and gestation length. On the other hand, both plant extracts caused a significant ($P<0.05$; $P<0.01$) increase of body weight compared to the negative control suggesting normal physiological functions within the body. In group 3; (pre and post mating treatment) none of the mated rats littered (Table 11). There was a significant loss of body weight in all treated animals (Table 11), suggesting the possibility of other physiological functions of the body being affected by the extracts. This is in contrast to Okoyea et al., 2015 who reported on methanolic leaf extract of *Telfairia occidentalis* that caused a non- significant difference in litter size, body weight and gestation length.

4.8.4 Anti implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* extracts

The anti-implantaion activity of both plants extracts when administered before mating was significant. At 500 and 800mg/kg *Uvariodendron kirkii* caused a significant ($P<0.001$) anti implantation activity (Table 12). At 500 and 800mg/kg *Croton menyharthii* caused a significant ($P<0.01$) anti implantation activity (Table 12). This suggests that pre mating extract administration had significant effect on either implantation process or the hormones that control the process. The anti-implantation activity of both plant extracts when administered after mating was not significant and stood at 1%, 10%, 2% and 6.2% at 500 and 800 mg/kg of *Croton menyharthii* and *Uvariodendron kirkii* respectively (Table 13). This suggests that post mating extract administration had minimal effect on hypothalamus pituitary gonadal axis which is responsible for GnRH, gonadotropins release and ovarian steroids synthesis. FSH and LH are key in folliculogenesis, oogenesis and an LH surge responsible for ovulation. Ovarian steroids facilitate endometrium receptivity. Probably after mating, the concentration levels of the extract did not affect the endometrium milieu and had minimal effects on implantation. In the Post mating treatment regime; ovulation and fertilization had already occurred. Probably the ovarian steroids levels were optimal thereby leading to established gestation. When extracts were administered before and after mating; the most significant effect on mating and fertility index (Table 14) occurred. Mating only occurred in 20 and 10% of the rats administered with 500 and 800 mg/Kg *Croton menyharthii* respectively. None of these rats littered (fertility index 0%). The rats exposed to *Uvariodendron kirkii* extract did not mate at all (Table 14), suggesting that a pre and post mating extract administration had the most significant effect on fertility perhaps due to an anti-ovulatory or anti-implantation property of both plants. Extract administration through-out the follicular and luteal phase of estrus cycle had the most significant effect on fertility. This

might probably be due to the accumulative effect of the extract as is the case in practice. TMPs administer the aqueous extract for are duration of about 3 months to effect fertility regulation. Daniyal and Akram (2015); Aarthi et al., 2012 and Dinesh et al., (2012) support the results of this study by reporting on anti-ovulatory and anti- implantation properties of *Acalypha indica, Ailanthus excelsa, Aristolochia bracteolate, Azadirachta indica, Bambusa vulgaris, Butea monosperma, Nelumbo nucifera, Hibiscus rosa-sinensis, Heliotropium indicum, Gloriosa superba, Ferula hermonis, Polygonum hydropiper Linn, Ocimum sanctum, Striga orobanchioides, Ricinus communis, Hagenia abyssinica* and *Cicer arietinum*. Other studies have also reported on the anti-implantation effect of plants. Our results corroborate Srivastava et al., 2007; Neeru and Sharma, 2008; Daniyal and Akram, 2015; Pallavi et al., 2011; Neetesh et al., 2016; Pokharkar et al., 2010; Olayaki et al., 2009; Hiremath et al., 2000; Kulkarni et al 2005 and Shibeshi et al., 2006b who report on anti-implantation properties of *Hibiscus rosa-nensis*, *Calotropis gigantea*, *Ocimun sanctum*, *Abroma angusta*, *Adhatoda vasica* Nees, *Carica papaya*, *Daucus carota*, *Gossipum herbacium*, *Grewia asiatica*, *Sapindus trifoliatus*, *Striga orobanchioides*, *Citrus limonum* and *Achyranthes aspera*. Ethanolic extract of seeds of *Ricinus communis*, fruits of *Punica granatum*, roots of *Calotropis procera*, roots of *Polygonum hydropiper*, leaves of *Mentha arvensis*, leaves of *Lawsonia inermis*, seeds of *Juniperus communis* , roots of *Hagenia abyssinica*, seeds of *Crotalaria juncea*, and roots of *Cicer arietinum* all had strong anti-implantation activity (Maurya et al.,2004). The anti-implantation effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract seen in this study suggests that the main anti-fertility property of both plant extracts could be their anti-implantation property. The post mating treatment regime had the least effect on fertility. Positive control had 100% anti-implantation (Table 14) demonstrating the role of hormones in fertility

regulation and supporting the results of this study that possible mechanism of action was anti implantation effect by both plants.

4.9 CONCLUSION

The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on reproductive parameters provides pointers towards the possible contraceptive properties of the extracts. From our results aqueous extract of both plants have anti implantation effects which should be explored further. The effect might either be at the ovaries and/or uterus or probably on reproductive hormones. In this chapter, the study has successfully evaluated the effect of *Croton menyharthii* and *Uvariodendron kirkii* on reproductive parameters. A manuscript has been submitted for peer review in the *Journal of Ethnopharmacology***.**

Kaingu C.K., Oduma J.A., Mbaria J.M. and **Kiama S.G (2016).** Antifertility and anti-implantation properties of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract in female Winstar rats.

CHAPTER 5

5.0 EFFECT OF C*ROTON MENYHARTHII* AND *UVARIODENDRON KIRKII* AQUEOUS EXTRACT ON OVARIAN AND ENDOMETRIAL HISTOMORPHOLOGY.

5.1 INTRODUCTION

Female fertility is driven by the developmental competence of the oocyte in its ability to undergo meiosis, be fertilized and give rise to a viable embryo. Ovarian folliculogenesis, oogenesis and ovulation are regulated by pituitary gonadotropins; FSH and LH. The recruitment, growth and maturation of pre antral follicles are independent of pituitary gonadotropins. However the development of the antral follicle is dependent on FSH. As the antral follicle grows, the theca cells under the influence of LH secrete androgens which are converted within granulosa cells into estradiol. Organization and functioning of the ovary is dependent on very close interactions between oocyte and surrounding follicle cells (Kidder and Vandenhyden, 2010). Communication between the oocyte and surrounding follicles is bi-directional, follicle cells regulate oocyte growth and oocyte regulates follicular development. It therefore follows that a disruption of the bidirectional communication between the oocyte and surrounding somatic cells; a disruption of either the stroma cells or the oocyte intergrity will interfere with folliculogenesis and oogenesis and compromise fertility.

Estradiol plays a key role in the cyclic growth of the endometrium layer; which undergoes differentiation under the influence of progesterone hormone and undergoes a short period of receptivity to embryo implantation. Many studies on the physiological and molecular changes of

the endometrium have been undertaken but many processes that govern uterine receptivity still remain unknown. Successful implantation requires coordinated interactions between the blastocyst and uterus. A compromised endothelium will interfere with implantation and lead to infertility. This chapter evaluates the effect of graded doses of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on ovarian and uterine lining histomorphology.

5.2 MATERIAL AND METHODS

Twenty five mature normocyclic female winstar rats were used for the study. The rats were divided into 4 groups (1, 2, 3 and 4) and treated as follows. Group 1 and 2 received 500 and 800 mg/Kg *Croton menyharthii* while group 3 and 4 received 500 and 800 mg/Kg *Uvariodendron kirkii* aqueous extract respectively, daily for 28 days (6 estrus cycles) through intra-abdominal gavage. Five control animals received 0.5ml physiological saline for 28 days. Before sacrificing estrus cycle staging of all rats (including control rats) was done in order to standardize the histomorphology findings. All rats were humanely sacrificed using diethyl ether anaesthesia on the last treatment day. Whole blood was collected from all sacrificed animals through cardiac puncture, centriguged at 3000rpm and serum stored at -20ºC and later assayed for Progesterone, Estradiol 17β, Follicle Stimulating Hormone and Luteinizing hormone in chapter 6. Physiological saline was used to flush the body of all the rats and immediately thereafter left ovaries and uterine horns were harvested and processed for histology and morphometry. Ovaries were fixed, cut in sections of 8 micron thickness and stained with Hematoxylin & Eosin and observed under a light microscope.

5.2.1 Tissue processing protocol

Tissue processing of both ovaries and uterine horns were carried out as per the protocol described by Hanneia et al., (2013).

5.3 RESULTS

5.3.1 Effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on the ovaries

5.3.1.1 Effect of *Croton menyharthii* extract on follicles

Figures 23B to 27B are representative slides that show the changes observed in the ovaries. *Croton menyharthii* aqueous extract at 500 mg/Kg led to a significant reduction in primary follicles (Fig 23B). The primary follicles were showing degenerative changes as from the 7^{th} day of extract administration. There was the presence of degenerating pre antral follicles compared to the control (Fig 23A). The cytoplasm was condensed and there was a disruption in arrangement of granulosa cells, shrinkage of ooplasm and zona pellucida disruption (Fig 23B) compared to control (Fig 23A). The theca cell layer of the primary follicle was disrupted. The major degenerative changes were however observed within secondary follicles (Fig 24B) compared to the control that had a well demarcated granulosa cell layer, zona pellucida and viable oocyte (Fig 24A). There was lack of an oocyte and zona pelucida (Fig 24B). Granulosa cells were condensed giving an appearance of loss of cytoplasm. There was presence of pyknotic cells in the inner lining of the granulosa cell layer and a disrupted theca layer compared to the control (Fig 24A). The mean number of antral follicles decreased significantly ($P < 0.05$) in all treatment groups in comparison with the control group. Figure 26B shows an atretic antral follicle. The follicle has shrunken ooplasm, disrupted zona pelucida, condensed granulosa cell layer and a disrupted theca interna layer. Most of the ovarian follicles in different stages of development and also the

matured Graffian follicles exhibited degenerative changes with different degrees of severity (24B, 25B, 26B, 27B) compared to the controls (24A, 25A, 26A, 27A). In treated ovaries, the follicles were found to be undergoing degenerative changes and they had lost their normal shape and arrangement of granulosa cells. There was total absence of zona pellucida (24B, 25B). It was a conspicuous finding that all the ovarian follicles including primordial follicles had undergone degenerative changes. Pre antral and antral follicles (Figs. 24B, 26B and 27B) of the treated ovaries had undergone degenerative changes simultaneously. None of the follicles could be seen with intact ovum and its normal nucleus (Figs. 25B and 27B). The granulosa cells were well arranged in Graafian follicle of control ovaries, with a demarcated area between the ovum and the granulosa cells on one hand and between granulosa cell and theca interna, theca externa and stroma of the ovary on the other hand (Fig. 23A, 24A, 25A, 26A and 27A). However, in the treated ovaries the granulosa cells had lost their typical arrangement and were randomly scattered except a few of them poorly aligned around the site of ovum (Figs. 24B, 25B, 26B and 27B). In histological study of treated ovaries the principal observation was degeneration of ova in all the follicles simultaneously, which were in different stages of their development. The first sign noticed during the atresia in a follicle was pyknosis and fragmentation of the inner granulosa cells (Fig. 25B, 27B).

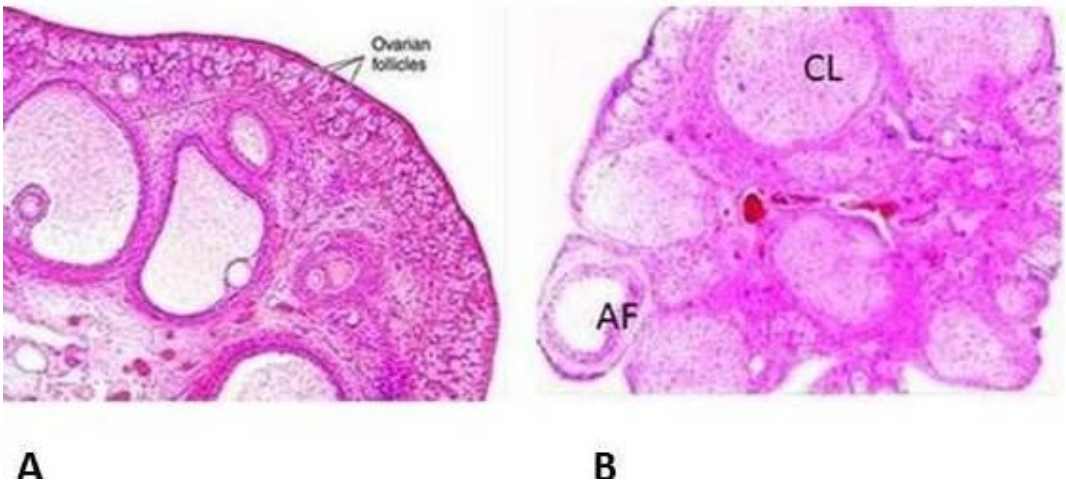


Figure 23: The effect of C*roton menyharthii* on ovaries.

23A: Control, presence of primordial, preantral, antral and Graffian follicle. 23B: Significant reduction in primary follicles and presence of a degenerating antral follicle compared to the control. Magnification ×100. Key: AF-antral follicle; CL-corpus luteum.

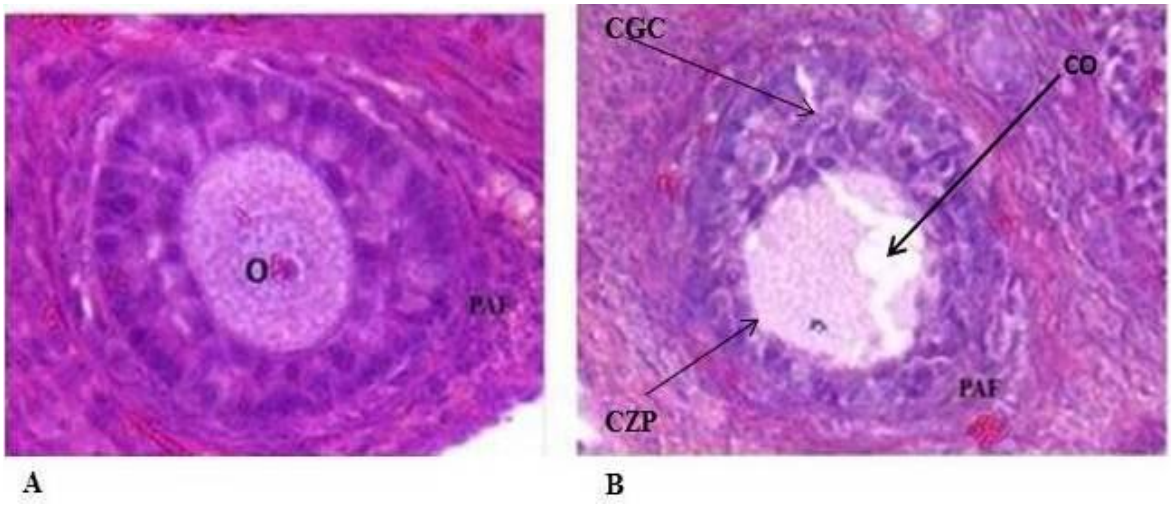


Figure 24: The effect of Croton menyharthii on an early pre-antral follicle

24A: Control, intact oocyte and zona pellucida, intact structural intergrity of granulosa cells and theca cells. 24B: Pre antral follicle: Structural intergrity of granulosa cell layer disrupted, oocyte compromised, ooplasm shrinkage, zona pellucida and theca cells disrupted.

Magnification × 400. Key: CO-compromised oocyte PAF- preantral follicle CGC- compromised granulosa cells CZP- compromised zona pelucida.

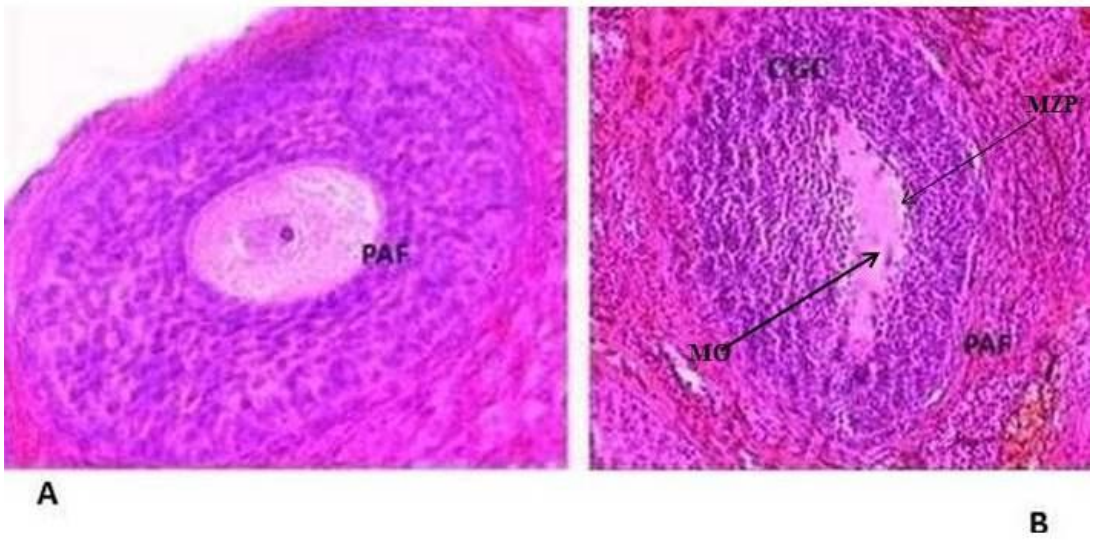


Figure 25: The effect of C*roton menyharthii* on pre-antral follicle**.**

25A: Control, the photomicrograph shows a pre antral follicle. Structural intergrity of granulosa cells intact, presence of viable oocyte and zona pellucida. 25B: Atretic pre-antral follicle, lack of oocyte and zona pelucida. Granulosa cells condensed with loss of cytoplasm. Presence of pyknotic cells in the granulosa cell inner lining and disrupted theca cell layer.
Magnification × 400. Keys: PAF- preantral follicle CGC-condensed granulosa cell layer MZP-missing zona pelucida MO-missing oocyte

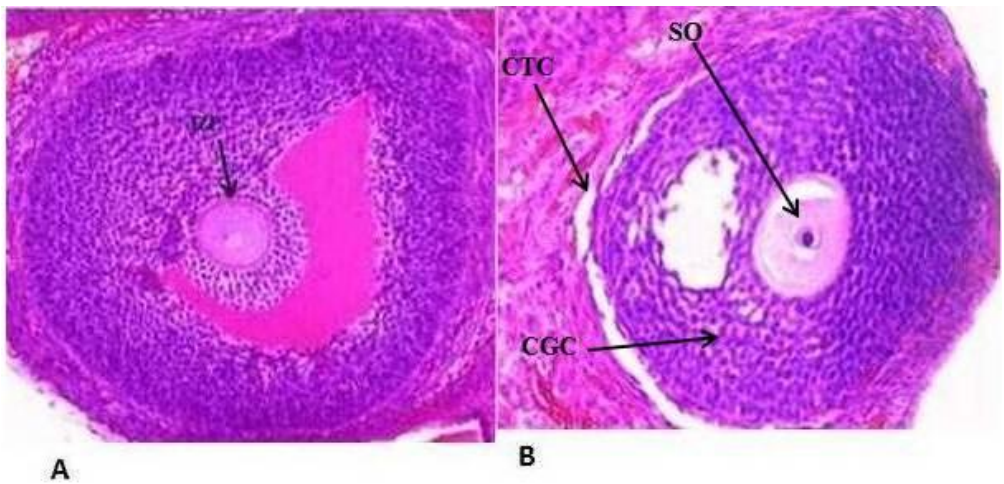


Figure 26: The effect of *Croton menyharthii* on an antral follicle

26A: Control, the Figure shows an antral follicle with an intact oocyte and well defined zona pelucida. 26B: degenerating antral follicle, ooplasm shrunken, oocyte compromised, pyknotic cells in granulosa cell layer, granulosa and theca cell layer structural intergrity compromised. Magnification × 400.
Keys: CGC-condensed granulosa cell layer SO-shrunken ooplasm CTC-compromised theca cells

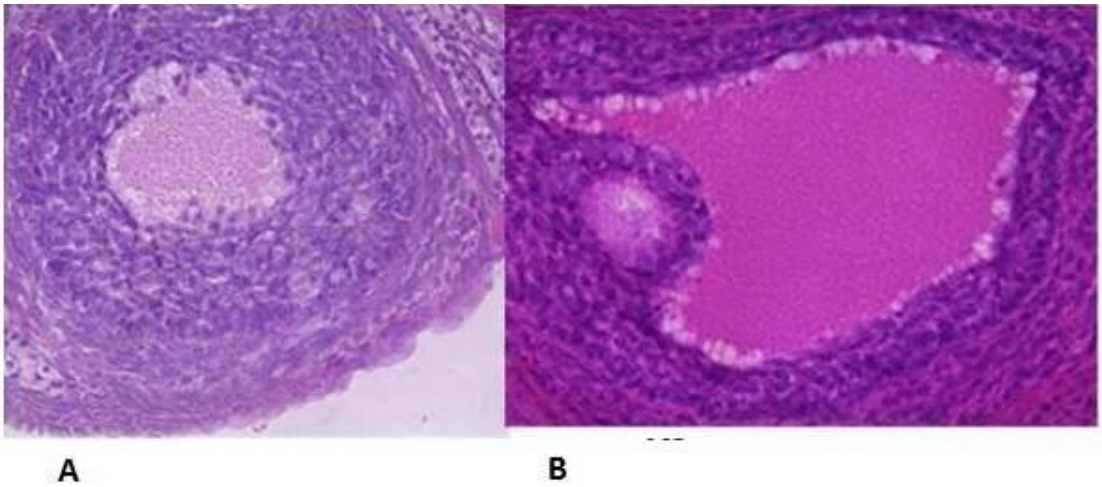


Figure 27: The effect of *Croton menyharthii* on an antral follicle

27A: degenerating pre antral follicle showing a compromised granulosa cell layer, oocyte missing and absence of zona pellucida. 27B: degenerating antral follicle; zona pelucida missing, oocyte compromised, condensed granulosa cell layer. Magnification × 400.

5.3.1.2 Effect of *Uvariodendron kirkiii* extract on follicles

Figures 28B to 33B are a selection of slides that show the effect of *Uvariodendron kirkii* on ovarian tissue. Figure 28B shows the presence of a degenerating preantral follicle, fewer or missing oocytes as well as fewer primary and primordial follicles (Fig 28B) compared to control (28A) which shows the presence of structurally intact pre antral follicles. Fig**.** 29B shows the effect of *Uvariodendron kirkii* on preantral follicles. The follicles are degenerating. The most significant finding being; missing oocytes and zona pelucida. The granulosa cell layer is also condensed compared to the negative control (Fig. 29A). Fig.30B shows a degenerating antral follicle. The most significant finding is the loss of the oocyte, shrunken ooplasm, loss of zona pellucida and presence of pyknotic cells within granulosa cell layer. The granulosa cells structural intergrity is compromised compared to the control (Fig. 30A) which had an intact oocyte, and zona pelucida, intact structural intergrity of granulosa cell layer and an intact theca interna and externa cell layer. Fig. 31B shows a degenerating antral follicle, though the oocyte was present it had shrunken ooplasm and was missing the zona pelucida. There was loss of granulosa and theca cell layer structural intergrity and presence of pyknotic cells within the granulosa cell layer compared to the control (Fig. 31A) a maturing antral follicle with an intact oocyte, zona pelucida and a well defined granulosa and theca cell layer. Fig. 32B was a degenerating secondary follicle; a significant finding being the missing oocyte and zona pelucida. The granulosa cell layer structural intregrity was compromised compared to the control (Fig. 32A) with a viable oocyte, zona pelucida, granulosa and theca cell layers. Fig. 33B shows degenerating primordial follicles, the structural intergrity of a group of primordial follicles was compromised when compared to the control Fig. 33A).

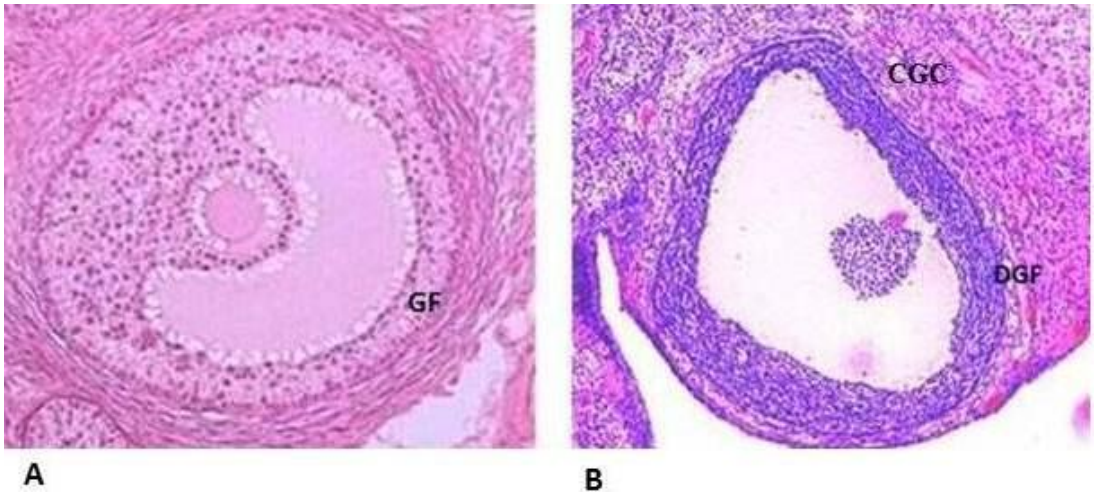


Figure 28: The effect of *Uvariodendron kirkii* on an antral follicle

28A: Control, the photomicrograph shows an antral follicle with a well-defined oocyte. Granulosa cell, theca interna and externa cell layer have intact structural intergrity. 28B: Atretic antral follicle with loss of oocyte and condensed granulosa cells (loss of cytoplasm). Compromised theca cell layer compared to the control. Magnification × 400.

Keys: GF- graffian follicle DGF-degenerating graffian follicle CGC-condensed granulosa cell layer

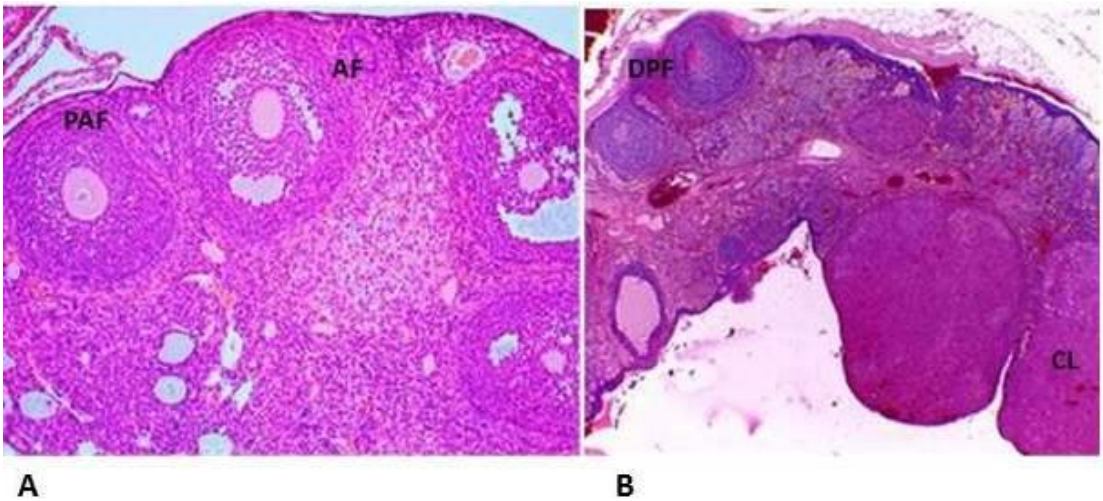


Figure 29: The effect of *Uvariodendron kirkii* on a pre antral follicle

29A: Control, the photomicrograph shows a pre and antral follicle. The structural intergrity of both follicles is intact. The micrograph shows an intact oocyte, zona pelucida and intact structural intergrity of granulosa and theca interna layer. 29B: The photomicrograph shows a degenerating preantral follicles, with missing oocytes and zona pelucida. The granulosa cell layer is condensed. Magnification ×100.

Keys: PAF preantral follicle AF- antral follicle DPF-degenerating preantral follicle CL-corpus luteum

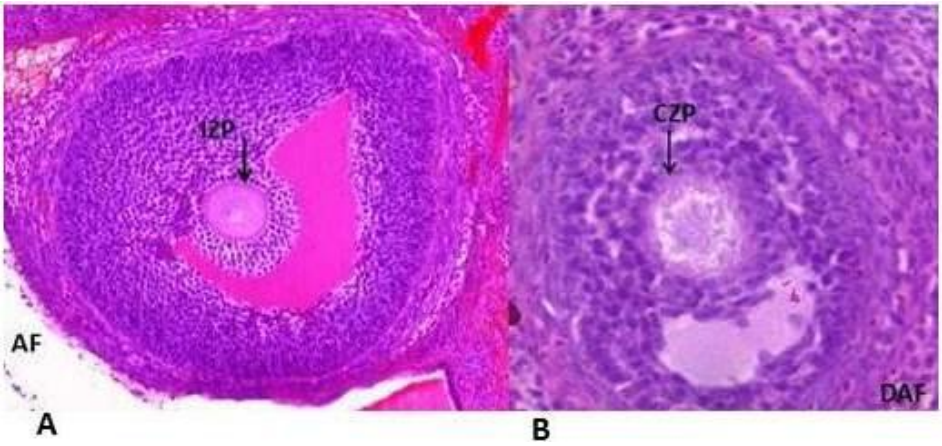


Figure 30: The effect of *Uvariodendron kirkii* on an antral follicle

30A: The photomicrograph shows an antral follicle with an intact zona pelucida, presence of oocyte, cumulus oophorus structural intergrity intact. 30B: the photomicrograph shows a degenerating antral follicle, with loss of an oocyte, shrunken ooplasm, loss of zona pellucida and pyknotic cells within granulosa cell layer. Magnification × 400.

Keys: AF-Antral follicle; DAF-degenerating antral follicle; CZP-compromised zona pelucida IZP- intact zona pelucida

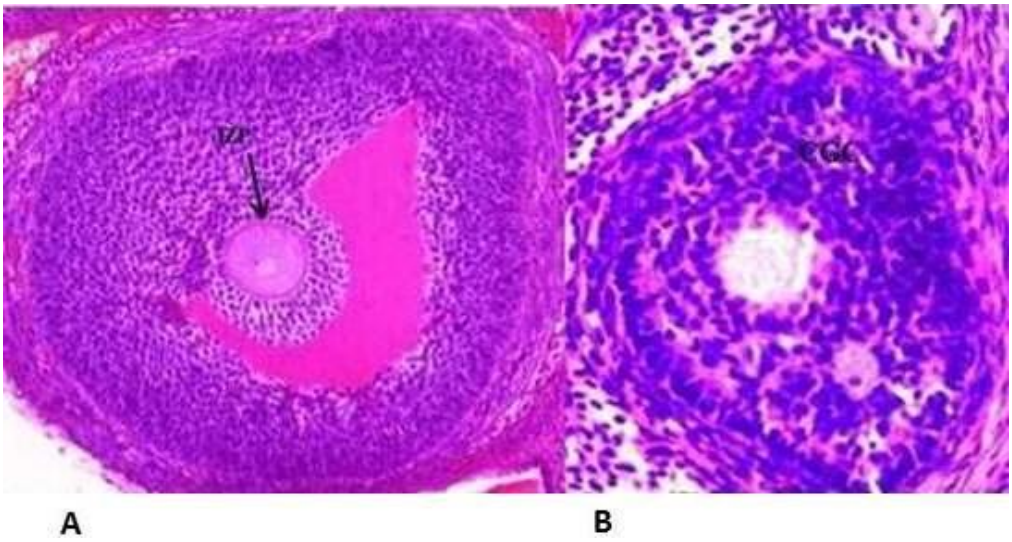


Figure 31: The effect of *Uvariodendron kirkii* on an antral follicle.

31A: Control, the figure shows an antral follicle with an intact oocyte and well defined zona pelucida. 31B: The figure shows a degenerating antral follicle, with shrunken ooplasm, pyknotic cells in granulosa cell layer, loss of granulosa and theca cell layer structural intergrity, zona pellucida is missing. Magnification × 400.

Keys: IZP-intact zona pelucida CGC- compromised granulosa cell layer.

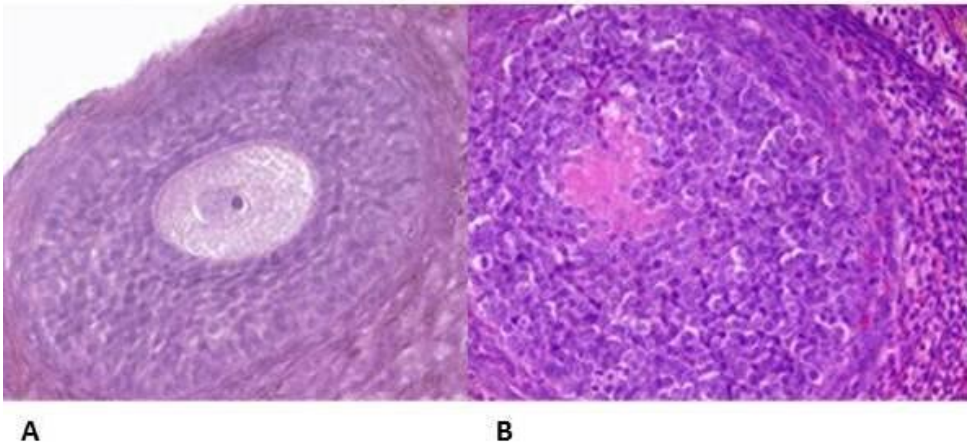


Figure 32: The effect of *Uvariodendron kirkii* on a secondary follicle

32A: Control: Secondary follicle, intact structural intergrity of oocyte, zona pelucida, granulosa and theca cell layer. 32B: Degenerating secondary follicle, oocyte and zona pelucida missing with a compromised granulosa cell layer. Magnification × 400.

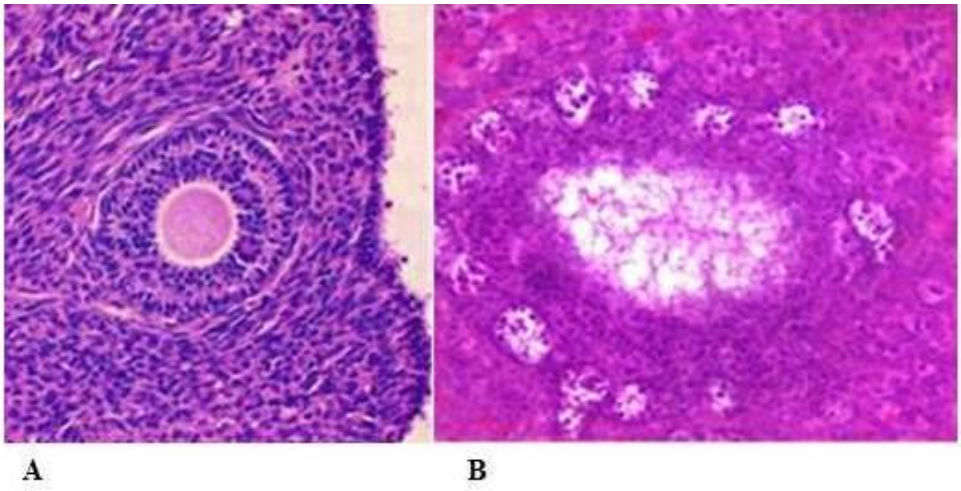


Figure 33: The effect of *Uvariodendron kirkii* on primordial follicles

33A: Control: intact oocyte, presence of zona pelucida. Structural intergrity of granulosa cells intact. 33B: Degenerating primordial follicles. Structural intergrity of follicular cells compromised.
Magnification × 400.

5.3.1.3 Effect of *Croton menyharthii* and *Uvariodendron kirkiii* extract on corpus luteum

Figures 34B to 36B are representative photomicrographs showing the effect of *Croton menyharthii* extracts on corpus luteum. The ovaries were harvested at metestrus (34A and 35A) and Diestrus (36A to 39A). Fig 34B shows degenerating corpora lutea though the numbers were not significantly different from control 34A. Fig.35B shows significant increase in corpora lutea numbers and a decline in size compared to the control 35A. At 800mg/kg *Croton menyharthii* extract (Fig. 35B and 36B) compromised the structural intergrity of the corpora lutea compared to the control 35A. Fig. 36B shows a disruption of the corpora lutea structural intergrity, condensed cytoplasm and presence of pyknotic cells in corpora lutea compared to the control 36A. The number of corpora lutea are however not significantly different from the control 36A. Figures 37B to 39B are representative photomicrographs showing the effect of *Uvariodendron kirkii* extracts on corpus luteum. 37B shows a few of the corpora lutea undergoing hypertrophy, condensed cytoplasm and presence of pyknotic cells in corpora lutea compared to control 37A. There was a significant increase in number of corpora lutea compared to the control 37A. Fig.38B shows a significant increase in number of corpus lutea compared to the control 38A. The ovarian stroma is mostly occupied by corpora lutea and some atretic ovarian follicles compared to the control 38A. At 800mg/Kg *Uvariodendron kirkii* extracts caused a disruption of corpora lutea structure (Fig. 39B), condensed the cytoplasm and there was presence of pyknotic cells. One of the corpora lutea on the right side of the figure had undergone hypertrophy. The ovarian stroma structure is disrupted and calcified. Uvariodendron kirkii caused a significant reduction in corpora lutea number compared to the control 39A.

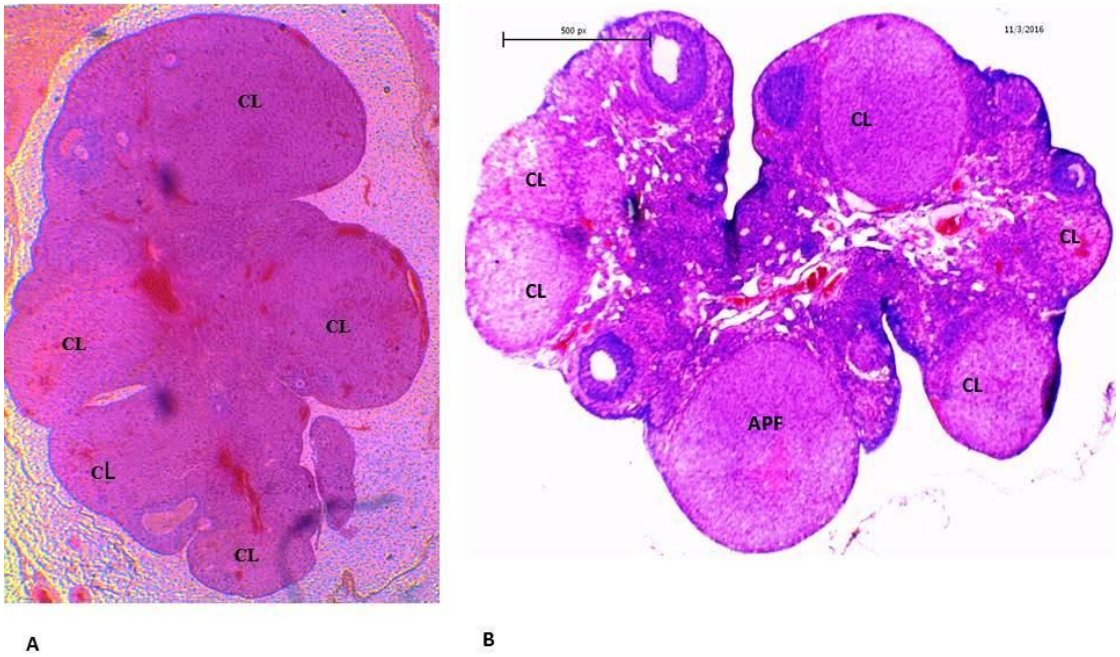


Figure 34 : The effect of 500mg/Kg *Croton menyharthii* on corpus luteum

34A: control- intact structural intergrity of corpus luteum, no signs of vacuoles. 34B: the photomicrograph shows the presence of various stages of ovarian follicles. The number and size of corpus luteum is not significantly different from control 34A. However the corpus luteum on the left of photomicrograph were atretic showing signs of vacuolation. Magnification × 100.

Key- CL-corpus luteum

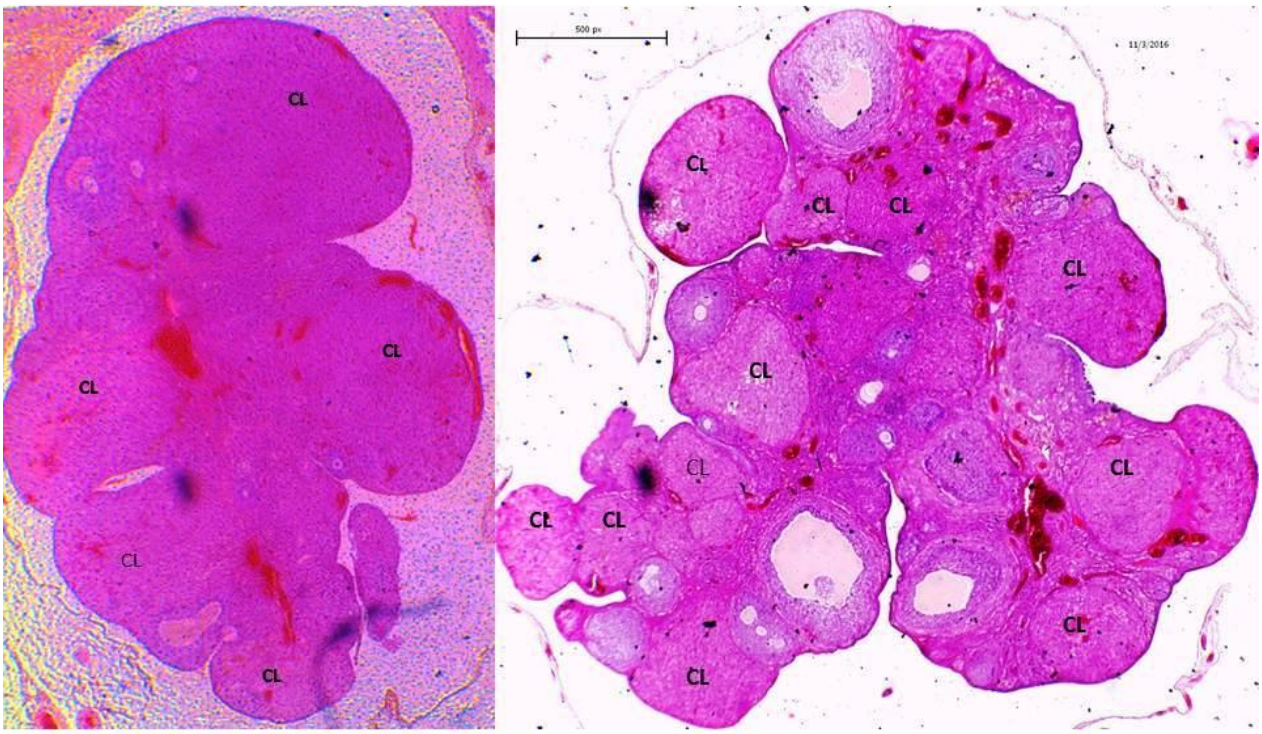


Figure 35: The effect of 800mg/Kg *Croton menyharthii* on corpus luteum

35A: control- intact structural intergrity of corpus luteum, no signs of vacuoles. 35B: At 800mg/kg the photomicrograph shows the extract caused a significant increase of corpora lutea numbers but a decline in corpora lutea sizes compared to the control 35A. Structural intergrity of the corpora lutea was compromised compared to the control 35A. Magnification × 100.

Key- CL-corpus luteum

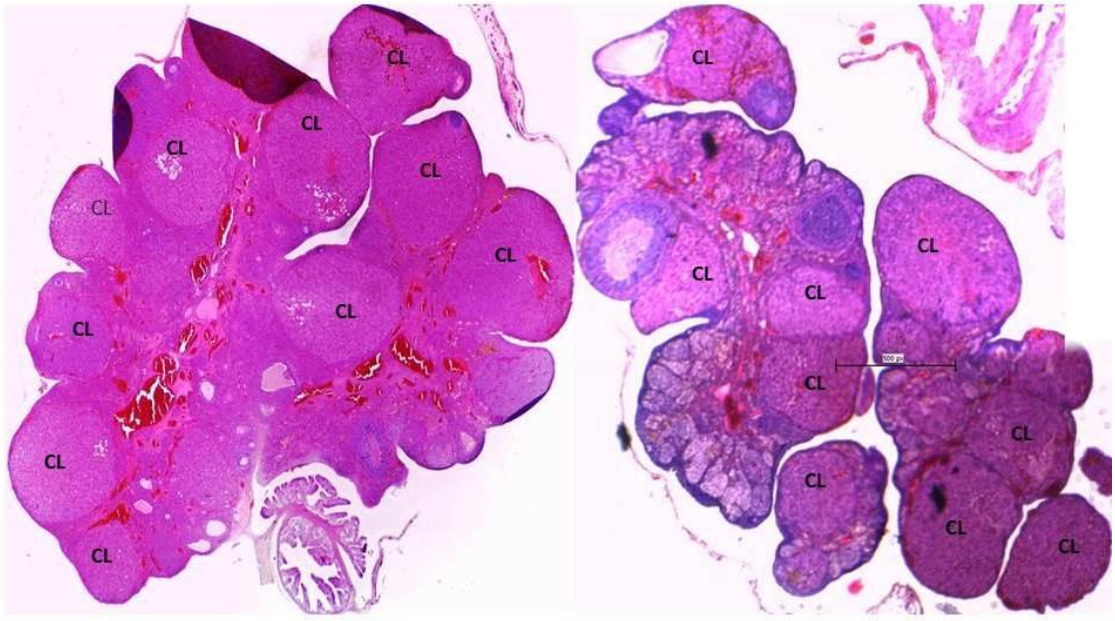


Figure 36: The effect of 800mg/Kg *Croton menyharthii* on corpus luteum

The ovary was harvested at diestrus phase of the cycle. 36A: Control photomicrograph shows intact structural intergrity of the corpora lutea. 36B: photomicrograph shows a disruption of the corpora lutea structural intergrity. Condensed cytoplasm and presence of pyknotic cells in corpora lutea. The number of corpora lutea are not significantly different from the control 36A.

Magnification × 100.

Key- CL-corpus luteum

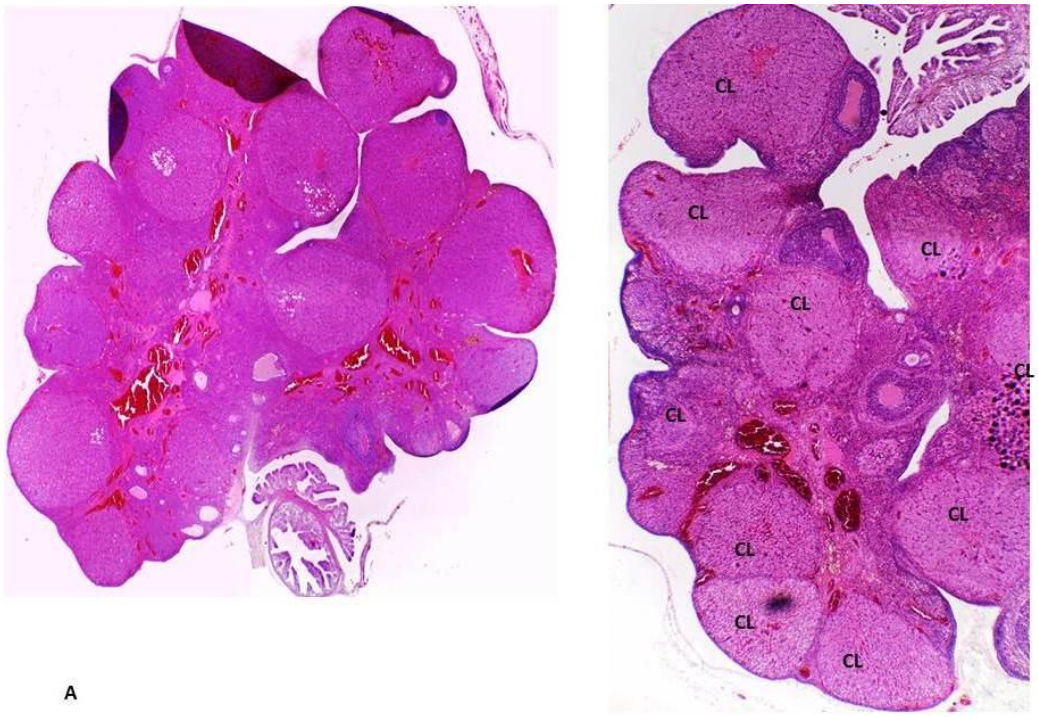


Figure 37: The effect of 500mg/Kg *Uvariodendron kirkii* on corpus luteum

37A: Control photomicrograph shows intact structural intergrity of the corpora lutea. 37B: A few of the corpus lutea have undergone hypertrophy, condensed cytoplasm and presence of pyknotic cells in corpus lutea. There is a significant increase in number of corpora lutea compared to the control

Magnification × 100. Key- CL-corpus luteum

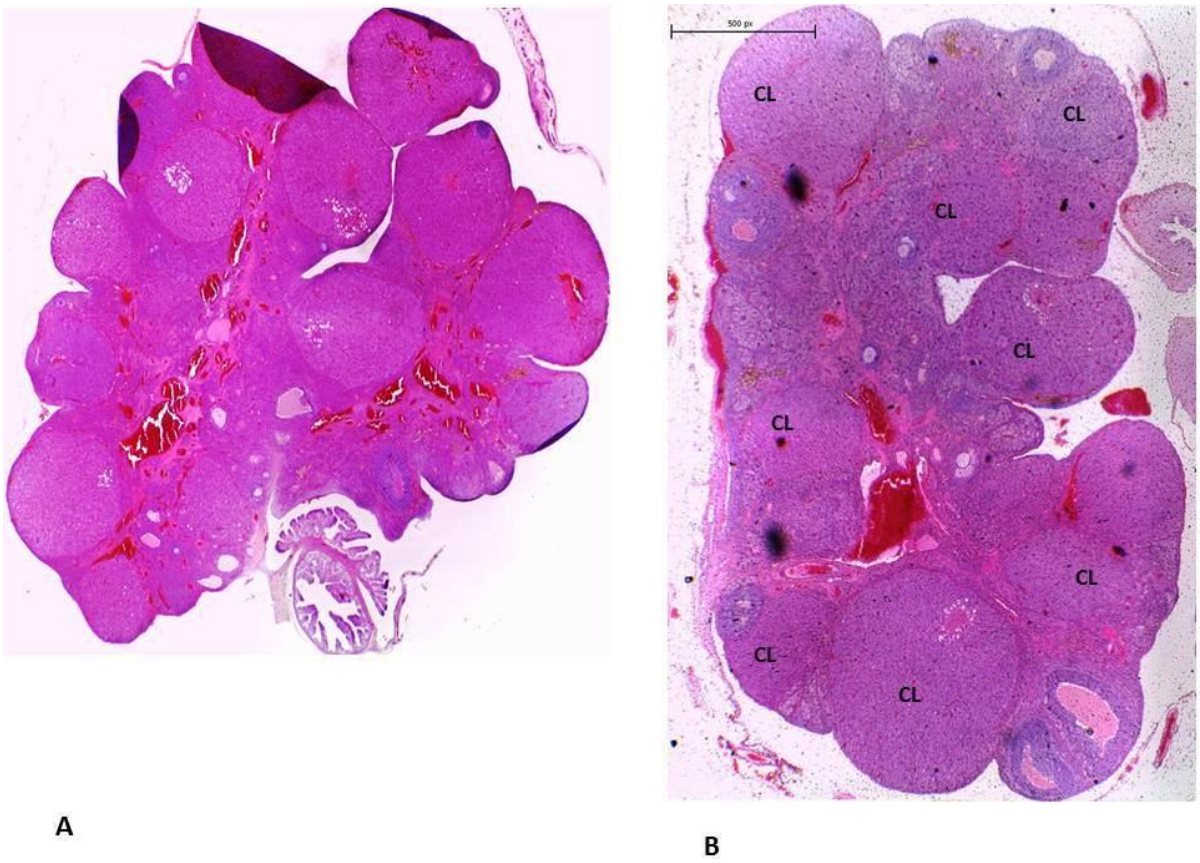


Figure 38: The Figure shows the effect of 800mg/Kg *Uvariodendron kirkii* on corpus luteum

38A: Control photomicrograph shows intact structural intergrity of the corpus lutea. 38B: Some of the corpus lutea have undergone hypertrophy, condensed cytoplasm and presence of pyknotic cells. There is a significant increase in number of corpus lutea compared to the control 38A. The ovarian stroma is mostly occupied by corpus lutea and some atretic ovarian follicles.

Magnification × 100 Key- CL-corpus luteum

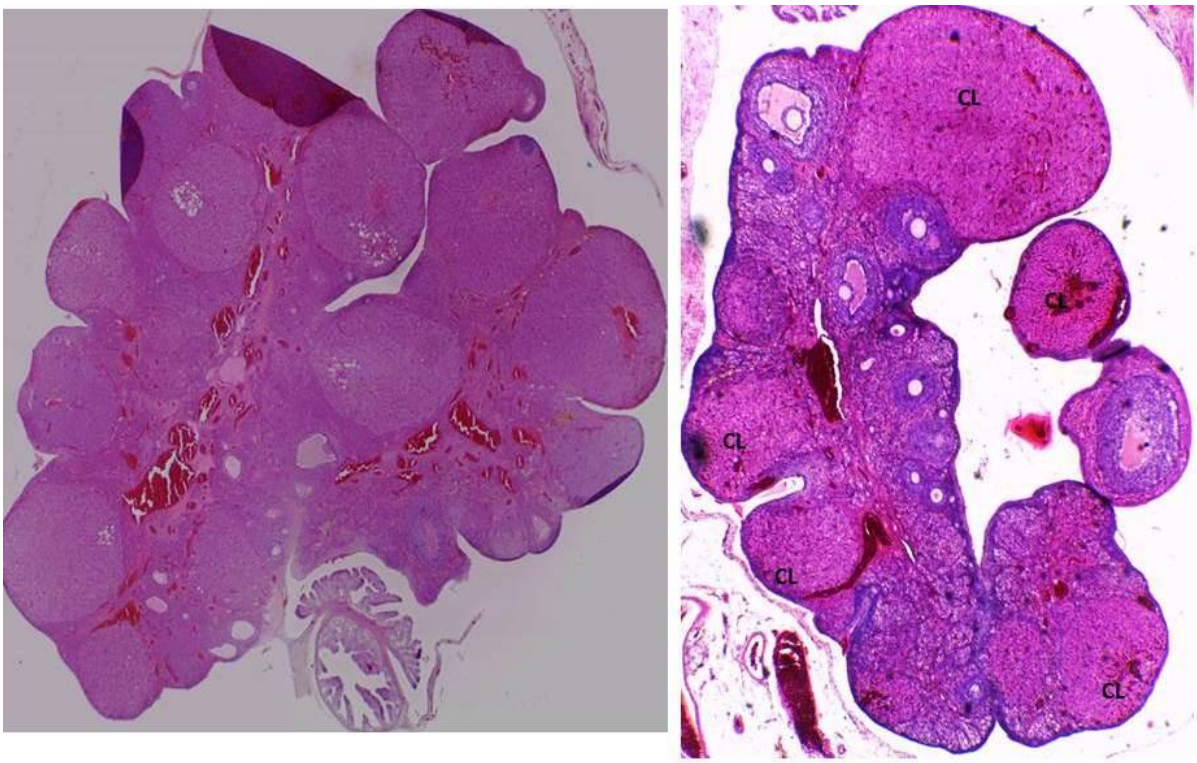


Figure 39: The Figure shows the effect of 800mg/Kg *Uvariodendron kirkii* on corpus luteum

The ovary was harvested at diestrus phase of the cycle. 39A: control intact structural intergrity of the corpora lutea and ovarian stroma. 39B: Disrupted corpora lutea structure, condensed cytoplasm and presence of pyknotic cells. One of the corpus lutum on the right sie of photomicrograph has undergone hypertrophy. The ovarian stroma structure is disrupted and calcified. The number of corpora lutea is significantly reduced compared to the control 39A.

5.3.2 Effect of *Croton menyharthii* and *Uvariodendron kirkii* extract on uterine lining.

Figures 40, 41, 42 and 43 are representative slides showing the effect of the two plants on the endometrium. *Croton menyharthii* caused a disruption of endometrial structural intergrity (Fig. 40B and 41B); loss of endothelial villi (40B); presence of pyknotic cells within uterine stroma (Fig. 41B) compared to the controls (Fig. 40A, 41A) with an intact endothelial lining structural intergrity. *Uvariodendron kirkii* aqueous extract caused a significant uterine gland vacuolation within stroma (Fig. 42B) and a thickened endometrial lining (Fig. 42B, 43B) compared to the negative controls (Fig. 42A and 43A).

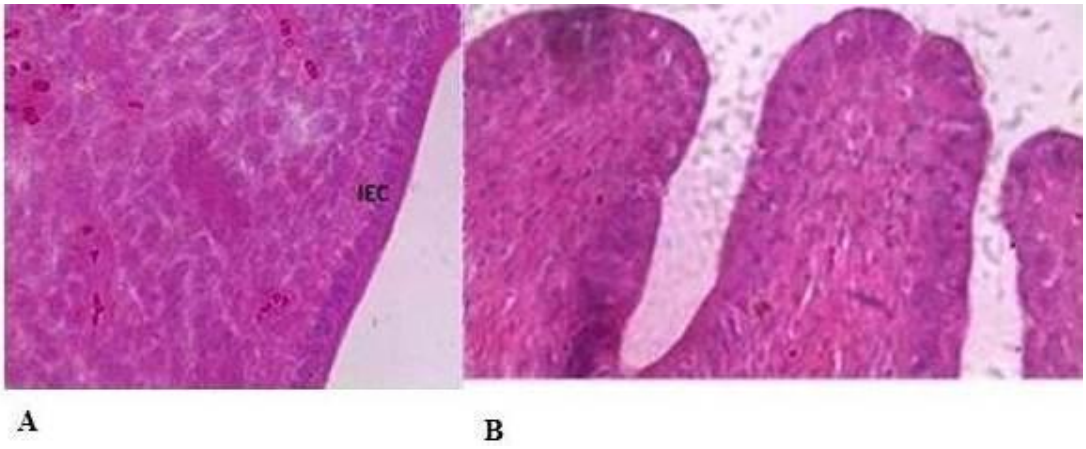


Figure 40: The effect of *Croton menyharthii* on uterine endometrium.

34A: Control: simple columnar endothelial cells, intact structural intergrity. 34B: endothelial loss of structural intergrity and villi, loss of invagination of endothelial lining. Compromised endothelium, stroma cells compromised. Magnification × 400.

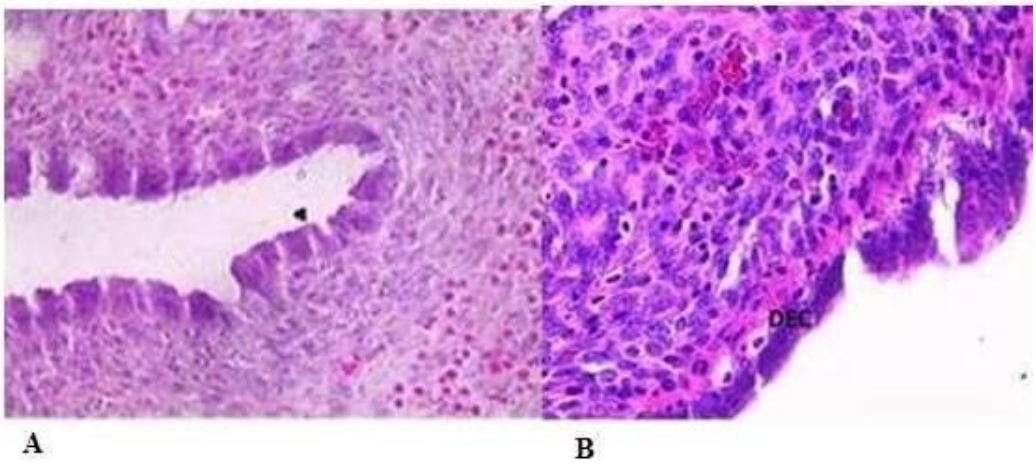


Figure 41: The effect of *Croton menyharthii* on uterine endometrium.

35A: Control: intact simple columnar endothelial cells. 35B: Endothelial cells structural intergrity disrupted and presence of pyknotic cells within uterine stroma. Magnification × 400.

IEC: Intact endothelial cell layer DEC: disrupted endothelial cell layer

Figure 42: The effect of *Uvariodendron kirkii* on uterine endometrium.

36A: Control, intact endothelial columnar cells and presence of villi 36B: *Uvariodendron kirkii* disrupted endothelial cell layer. The extract caused vacuolation of uterine glands within the stroma. DEC: Disrupted endothelial cell layer. Magnification × 400.

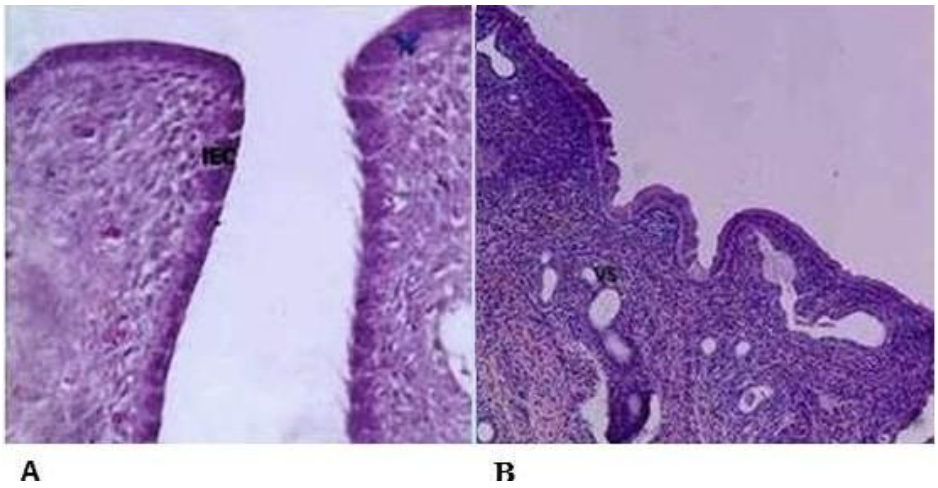


Figure 43: The effect of *Uvariodendron kirkii* on uterine endometrium

37A: Control, intact endothelial columnar cells and presence of villi 37B: *Uvariodendron kirkii* disrupted endothelial cell layer. The extract caused vacuolation of uterine glands within the stroma. DEC: Disrupted endothelial cell layer. Magnification × 400.

5.4 DISCUSSION

This study was undertaken to evaluate the morphological effects of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on the ovaries and endometrium of the/ rat. There has been no study documenting the same so far. Histological studies of ovaries in the control group showed the various types of follicles at all stages of folliculogenesis (primordial, primary, secondary and mature follicles) (Figure 23A). *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract at 500 and 800 mg/Kg caused a significant increase of atretic pre antral follicles (24B, 25B, 29B, 32B) and a significant reduction in primary follicles numbers. This suggests a compromised-folliculogenesis and may explain traditional consumption to regulate fertility. The significant reduction in the number of follicles in the presence of *Croton menyharthii* and *Uvariodendron kirkii* at 500 and 800 mg / Kg supports the findings of chapter 6 of this study on hormonal analysis where a reduction in estradiol and FSH was observed. The reduction in estradiol levels could be due to the compromised granulosa and theca cellular layer as exemplified in figures 24B, 25B, 26B, 27B, 28B, 29B, 30B, 32B which play an active role in androgen and estradiol synthesis. The reduction in estradiol could also be due to a direct effect of both plant extract on the pituitary gland. An optimal blood level of FSH is a pre requisite for initiation and maintenance of normal ovarian folliculogenesis. Therefore the present histological finding suggests a hypothalamic-pituitary gonadal axis dysfunction after treatment with both plant extracts. Similar studies have reported corroborating results (Dinesh et al., 2012; Daniyal and Akram 2015; Devi et al., 2015; Akpantah et al., 2011; Talukder et al., 2014; Sakila et al., 2009; Modaresi et al., 2012; Jyoti et al., 2010; Neetesh et al., 2016; Zhang et al., 2013; Adaay and Mosa, 2012; Yakubu et al., 2008; Thakur et al., 2009 and Krishnamoorthy et al., 2013). The number of primordial ovarian follicles was not affected by *Croton menyharthii* and

Uvariodendron kirkii aqueous extract treatment at 500 mg/Kg but was affected at 800mg/Kg. This may be due to the fact that formation, growth and development of primordial follicles to early primary follicular stages are accomplished during perinatal period. Therefore *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract may not have any effect on the population of these follicles especially at lower concentrations. As shown in this study ovarian follicle atresia might have occurred due to the diminution in serum FSH levels which is in line with the present hormonal results. The zona pellucida (ZP) is a relatively thick extra cellular coat that surrounds the plasma membrane of the oocyte. The ZP is laid down during the final stages of oogenesis when growing oocytes enter their growth phase. As oocytes increase diameter (15-80 μm) the ZP also increases in thickness. The ZP is constructed by three glycoproteins referred to as mZP1, mZP2 and mZP3 that are synthesized and secreted by the growing oocyte. Studies (Kidder and Vandenhyden, 2010) have shown that a disruption of mZP1 leads to a severe reduction of both secondary and Graffian follicles thereby leading to a reduction in ovulated oocytes and litter size. Fully grown oocytes and ovulated eggs from rats without mZP2 and mZP3 glycoprotein lack a ZP and are infertile. mZP2 and mZP3 knock-out mice do not construct ZP resulting in deleterious effects on folliculogenesis leading to a reduced number of antral follicles in ovaries and severely reduced number of ovulated eggs in oviducts. Although this study did not scrutinize the ZP in similar details, it however demonstrated a disruption of the ZP in several photomicrographs (Fig. 24B, 27B, 29B, 30B, 31B, 32B compared to intact ZP in Fig. 31B. Ovarian morphology from mZP2 and mZP3 null females suggests that growing and fully grown oocytes are less intimately associated with follicular and cumulus cells in particular than oocytes from intact females. Notably cells of corona radiata from mZP2 and mZP3 knock-out females are less orderly arrayed around growing oocytes than the control. As stated in the

introduction it is possible that a disruption of this bi-directional communication between oocyte and surrounding granulosa cells is responsible for compromised folliculogenesis and oogenesis especially where the structural intergrity of granulosa cells and theca cells was compromised (Figure 24B, 25B, 29B, 30B and 31B). Through gap junctions, cumulus cells supply nutrients and energy substrates to the oocyte, including amino acids, glucose, and ribonucleosides (Gilchrist et al., 2008). Oocytes are unable to metabolize glucose and can only generate ATP through oxidative phosphorylation. However, cumulus cells consume glucose via aerobic glycolysis, the product pyruvate sent to the oocyte for oxidative phosphorylation via gap junctions and other modes of communication such as paracrine signaling cumulus cells provide developmental assistance to the oocyte in several ways. Paracrine signaling between oocytes and the cells of the follicle is bidirectional and essential to development. In general terms, it has been demonstrated that the disruption of paracrine signaling between mouse oocytes and their cumulus cells *in vitro* reduces oocyte competence (Kidder and Vandenhyden 2010) and compromises fertility. This may be particularly important during pre-ovulatory development, as the rate of pyruvate consumption in maturing metaphase-I oocytes is significantly higher than that in immature oocytes. Cumulus cells also help the oocyte take in amino acids. As follicular cells are recruited in each estrus cycle for growth and maturation, simultaneously the oocytes grow and resume meiosis. This complex process involves a close interaction between the oocyte, surrounding granulosa and theca cells. In this study the results Figures 24B, 25B, 26B, 29B, 30B and 31B showed a disruption of the structural intergrity of oocyte and surrounding cellular cells. Suggests a possible cause of infertility as being due to compromised folliculogenesis and oogenesis.

Croton menyharthii extracts caused a degeneration of corpus luteum (Fig.34B) though the corpus lutea numbers were not significantly affected compared to control 34A. At 800mg/kg *Croton menyharthii* caused a significant increase in corpus lutea numbers (Fig. 35B and 36B) and a decline in size compared to the control 35A. *Uvariodendron kirkii* caused a significant increase in number of corpus lutea (Fig.37B) compared to the control (Fig. 37A). In Fig. 39B a few of the corpus lutea were undergoing hypertrophy. The corpus luteum secretes progesterone hormone. Progesterone in turn plays a crucial role in preparing the endometrium for possible implantation. An increase in number and /or hypertrophied corpora lutea possibly lead to disrupted estradiol progesterone ratio therefore possibly compromising the window of implantation. Progesterone is key in pregnancy maintainance. Near term; levels of progesterone go down triggering other hormonal and molecular changes that terminates gestation. An elevation of progesterone hormone level will possibly lead to an extended gestation.

Structural intergrity of uterine endothelial lining is essential for successful implantation and establishment of gestation. In this study the endometrium was compromised Figures 40B, 41B, 42B and 43B. Endothelium growth and differentiation during each estrus cycle is modulated by estradiol and progesterone. A disrupton of the hormonal balance impedes implantation (Neetesh et al., 2016; Vijay et al., 2004). Significant reduction of estradiol and progesterone (Chapter 6) supports the uterine lining histomorphology results.The results are further supported by the significant reduction in implantation index (Chapter 4). It is possible that endometrial receptivity was compromised leading to failed implantation.

5.5 CONCLUSION

This morphological study has revealed a disruption of the structural intergrity of the follicular cells, granulosa cells, theca cells and a disruption of ZP at both dose levels of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract. This strongly points to interference with folliculogenesis and oogenesis that would lead to a compromised fertility of the female. This might therefore explain the traditional consumption of crude plant extract to cause infertility as a method of contraception desired by the women in Tana River County.

The study in this chapter has successfully evaluated the effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on ovarian and uterine lining histomorphology. A manuscript has been submitted for peer review in the *Journal of Anatomy*.

Kaingu C.K., Oduma J.A., Mbaria J.M. and **Kiama S.G. (2016).** The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on ovarian and uterine histomorphology of female winstar rats.

CHAPTER 6

6.0 THE EFFECT OF C*ROTON MENYHARTHII* AND *UVARIODENDRON KIRKII* AQUEOUS EXTRACT ON FEMALE REPRODUCTIVE HORMONES.

6.1 INTRODUCTION

The estrus cycle is regulated by pituitary gonadotropins (FSH and LH). Serum levels of both gonadotropins are in turn regulated by ovarian steroids (estradiol and progesterone). Therefore a disruption of any of these hormones will compromise folliculogenesis and oogenesis and lead to infertility. Antral follicle functions are governed by FSH levels. Androgen production by theca cells is dependent on LH levels. Granulosa cells convert the androgens to produce estradiol. A dominantly estrogenic environment is correlated with oocyte competence. Uterine receptivity for embryo implantation is regulated by the ovarian hormones estradiol and progesterone. Successful implantation requires coordinated interactions between the blastocyst and uterus. Therefore a disruption of the reproductive hormones compromises fertility. A possible outcome could be implantation failure; and can be explored as a novel contraceptive option. *Croton menyharthii* and *Uvariodendron kirkii* are indigenous in Tana River County and are readily available. The effect of both plants on female reproductive hormones has not been carried out so far. The chapter evaluates the effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), 17β estradiol (ES) and progesterone (P4) hormones.

6.2 MATERIALS AND METHODS

6.2.1 Laboratory animals and experimental protocol

Whole blood was collected from all sacrificed animals in Chapter 5; section 5.2 via cardiac puncture using sterile needles and syringes into plain tubes and allowed to clot for two hours. The clotted blood was centrifuged at 3000rpm for 10 minutes. After centrifugation the serum samples were stored at -20 °C until assayed for LH, FSH, 17β estradiol and progesterone using ELISA.

6.2.2 Determination of 17β Estradiol levels

Twenty five µl of standards, control and extract samples were dispensed into their respective wells. 200 µl conjugate was added to each well. Well A1 was left for substrate blank. The wells were covered with foil. The wells were incubated for 2 hour at 37º C. After 1 hour the foil was removed and well contents aspirated. The wells were washed 3 times with 300 µl diluted wash solution. The soak time between each wash cycle was more than 5 seconds. The remaining fluid was carefully removed by tapping the strips on tissue paper. 100 µl TMB substrate solution was added into all wells. The wells were incubated for 30 minutes at room temperature in the dark. 100 µl stop solution was dispensed into all wells in same order and same rate as for the substrate. The absorbance of the specimen was read at 450nm within 30 minutes after addition of stop solution. The ELISA kit product number was DNOV003 (NovaTec Immunodiagnostica GmbH), Germany.

6.2.3 Determination of Progesterone levels

Twenty µl of standards, control and extract samples were dispensed into their respective wells. 200 µl Progesterone-HRP conjugate was added to each well. Substrate blank was dispensed into

well A1. The wells were covered with foil. The wells were incubated for 1 hour at 37º C. After 1 hour the foil was removed and well contents aspirated. The wells were washed 3 times with 300 µl diluted wash solution. The soak time between each wash cycle was more than 5 seconds. The remaining fluid was carefully removed by tapping the strips on tissue paper. 100 µl TMB substrate solution was added into all wells. The wells were incubated for 15 minutes at room temperature in the dark. 100 µl stop solution was dispensed into all wells in same order and same rate as for the substrate. The absorbance of the specimen was read at 450nm within 30 minutes after addition of stop solution. The ELISA kit product used was DNOV006, (NovaTec Immunodiagnostica GmbH), Germany.

6.2.4 Determination of Follicle Stimulating Hormone

Fifty µl of standards and extract samples were dispensed into their respective wells. 100 µl conjugate was added to each well. Substrate blank was dispensed into well A1. The wells were covered with foil. The wells were incubated for 1 hour at room temperature (22-28º C). After 1 hour the foil was removed and well contents aspirated. The wells were washed 3 times with 300 µl diluted wash solution. The soak time between each wash cycle was more than 5 seconds. The remaining fluid was carefully removed by tapping the strips on tissue paper. 100 µl TMB substrate solution was added into all wells. The wells were incubated for 15 minutes at room temperature in the dark. 100 µl stop solution was dispensed into all wells in same order and same rate as for the substrate. The absorbance of the specimen was read at 450nm The ELISA kit product number was DNOV031 (NovaTec Immunodiagnostica GmbH), Germany.

6.2.5 Determination of Luteinizing Hormone

Twenty µl of standards, control and extract samples were dispensed into thir respective wells in duplicates. 100 µl conjugate was added to each well. Substrate blank was dispensed into well A1. The wells were left to incubate for 1 hour at room temperature (22-28º C). Contents of wells were aspirated after 1 hour and the wells were washed 3 times using 300 µl diluted wash solution. The remaining fluid was carefully removed by tapping the strips on tissue paper. 100 µl TMB substrate solution was added into all wells. The wells were incubated for 15 minutes at room temperature in the dark. 100 µl stop solution was dispensed into all wells in same order and same rate as for the substrate. The absorbance of the specimen was done at 450nm. The ELISA kit product number was DNOV030 (NovaTec Immunodiagnostica GmbH), Germany.

6.2.6 Statistical Analysis

Follicle stimulating hormone, luteinizing hormone, progesterone and 17β estradiol measurements are presented as mean ± SEM. One way Analysis of Variance (ANOVA) was used to analyze the data * ($P<0.05$), ** ($P< 0.01$), ***($P<0.001$).

6.3 RESULTS

Progesterone levels significantly increased (Fig. 45) in treated groups compared to the negative control (0.886 ± 0.03) in a dose dependent manner. Ranging from 0.906 ± 0.02 ($P< 0.01$) at 500mg/Kg of *Croton menyharthii* to 0.911 ± 0.02 ($P<0.001$) at 800 mg/Kg. Similarly, *Uvariodendron kirkii* aqueous extract also caused a significant increase in serum progesterone levels when compared to the negative control; ranging from 0.931 ± 0.04 ($P<0.05$) at 500 mg/Kg to 0.899 ± 0.01 ng/ml ($P< 0.01$) at 800 mg/Kg. Luteinizing hormone levels (Fig. 46) significantly increased in *Croton menyharthii* treated groups compared to the negative controls (0.053 ± 0.01 IU/ml). At 500 mg/ Kg *Croton menyharthii* significantly increased LH levels to 0.069 ± 0.04 ($P< 0.01$) compared to the negative control (0.053 ± 0.01), whereas at 800 mg/ Kg *Croton menyharthii*, the levels increased to 0.059 ± 0.03 ($P<0.05$) compared to the negative control. *Uvariodendron kirkii* aqueous extract on the other hand, caused a reduction in the levels of luteinizing hormone (Fig. 46). The reduction was however not significant when compared to the negative control (0.053 ± 0.01). Values ranged from 0.0443 ± 0.01at 500 mg / Kg to 0.048 ± 0.02 at 800 mg/Kg. Follicle stimulating hormone levels were significantly reduced (Fig. 47) in treated groups compared to the negative control (2.87 ± 0.05) in a dose dependent manner, ranging from 2.56 ± 0.04 ($P<0.05$) at 500 mg/ Kg of *Croton menyharthii* to 2.11 ± 0.02 ($P<0.01$) at 800 mg/Kg. *Uvariodendron kirkii* also caused a significant reduction in serum Follicle stimulating hormone levels compared to the control, ranging from 2.64 ± 0.02 ($P<0.05$) at 500 mg/Kg to 1.98 ± 0.03 ($P<0.01$) at 800 mg/Kg. Estradiol 17β serum levels were significantly reduced (Fig. 48) in treated groups compared to negative control (44.80 ±0.03 pg/ml) in a dose dependent manner. Ranging from 38.20 ± 0.01 ($P< 0.05$) at 500mg/Kg of *Croton menyharthii* to

37.70 ± 0.05 (P< 0.01) at 800 mg/ Kg. *Uvariodendron kirkii* aqueous extract also caused a significant reduction in serum Estradiol 17β levels; ranging from 35.61 ± 0.01 (P<0.01) at 500 mg/Kg to 33.50 ± 0.05 (P<0.001) at 800 mg/Kg compared to negative control.

Table 15: The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extracts on female reproductive hormones and percentage loss of ovum

Treatment	Dose (mg/gram BW)	Progesterone (ng/ml)	LH (IU/ml)	FSH (IU/ml)	Estradiol (pg/ml)	% loss of ovum
Positive Control		1.042 ± 0.01	0.379 ± 0.01	4.11 ± 0.02	67.14 ± 0.03	
Negative Control	0.5ml DW	0.886 ± 0.03	0.053 ± 0.01	2.87 ± 0.05	44.80 ± 0.03	0
Croton menyharthii	500	0.906 ± 0.02*	0.069 ± 0.04**	2.56 ± 0.04*	38.20 ± 0.01*	18
Croton menyharthii	800	0.911 ± 0.012*	0.059 ± 0.03*	2.11 ± 0.021**	37.70± 0.05*	48
Uvariodendron kirkii	500	0.931 ± 0.04**	0.0443 ± 0.01	2.64 ± 0.02*	35.61 ± 0.01**	39
Uvariodendron kirkii	800	0.899 ± 0.01*	0.048 ± 0.02	1.98 ± 0.03**	33.50 ± 0.05***	67

The Table shows the effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on serum progesterone, luteinizing hormone, follicle stimulating hormone and estradiol levels. The extracts significantly reduced 17β estradiol and FSH serum levels ($P<0.05$ to $P<0.001$). Both plant extracts significantly increased progesterone levels ($P<0.05$ to $P<0.01$). *Uvariodendron kirkii* extract caused a non significant reduction in LH levels where as *Croton menyharthii* significantly ($P<0.01$, $P<0.05$) increased LH serum levels. The values are mean ± SEM.

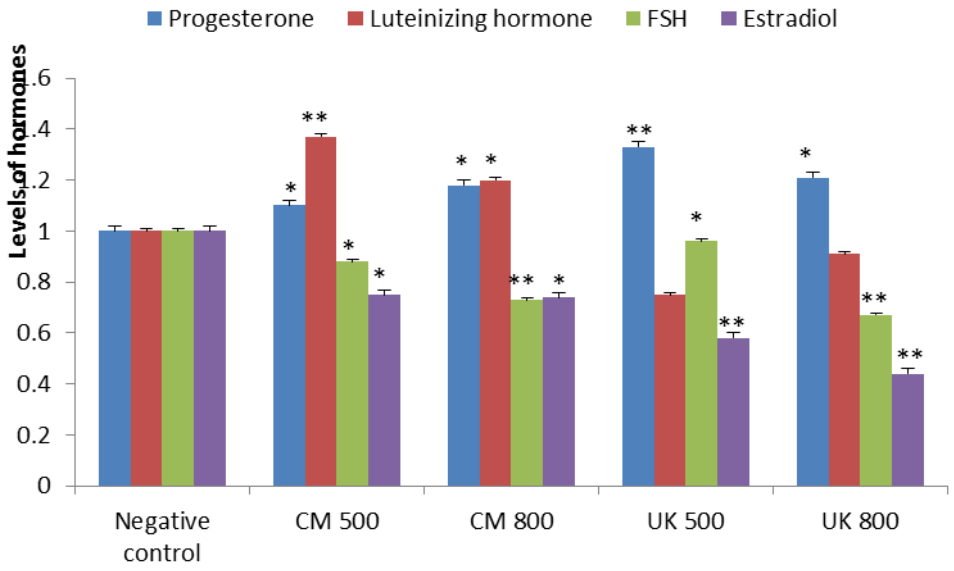


Figure 44: The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on reproductive hormones.

Figure 44 shows the effect of both plant extracts on reproductive hormones. There was a signifiant dose dependent reduction in Estradiol and FSH by both plant extracts compared to the negative control ($P<0.05$ to $P<0.001$). There was a significant increase in LH by *Croton menyharhtii* and non significant decrease in LH by *Uvariodendron kirkii* compared to the negative control. For progesterone, there was a significant ($P<0.05$) increase by *Croton menyharhtii* at 800mg/Kg and *Uvariodendron kirkii* at 500 and 800 mg/Kg compared to the negative control. The negative control data has been normalized. The values are mean ± SEM. *P<0.05**P< 0.01 ***P<0.001.

CM-Croton menyharthii UK-Uvariodendron kirkii

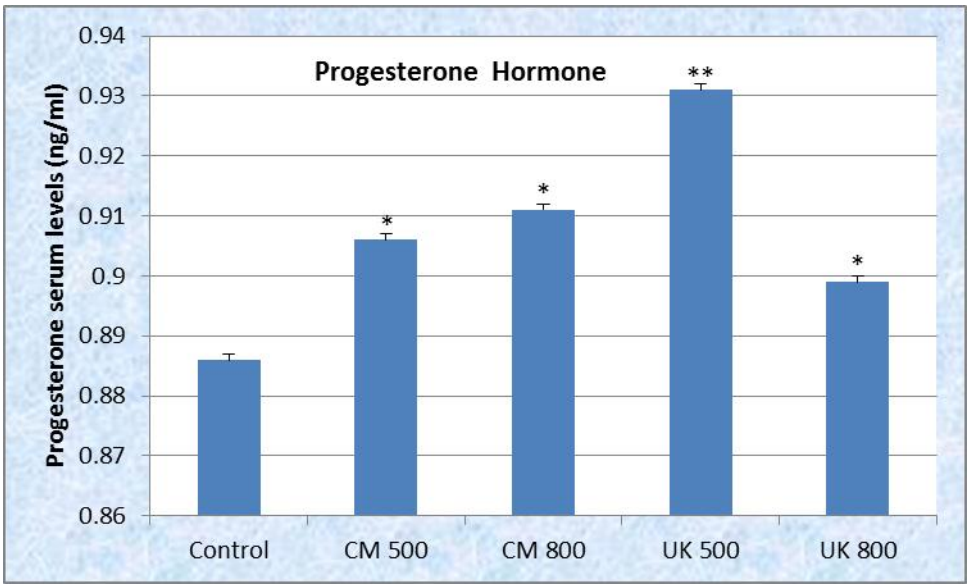


Figure 45: Effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on serum progesterone levels.

Figure 45 shows a significant increase in progesterone serum levels ($P<0.05$) at 500 and 800 mg/Kg *Croton menyharthii*. The levels were also significantly increased ($P<0.01$) at 500 mg/kg and ($P<0.05$) at 800 mg/Kg *Uvariodendron kirkii*. The values are mean ± SEM. ** $P<0.01$ *$P<0.05$. Keys: CM-Croton menyharthii UK-Uvariodendron kirkii.

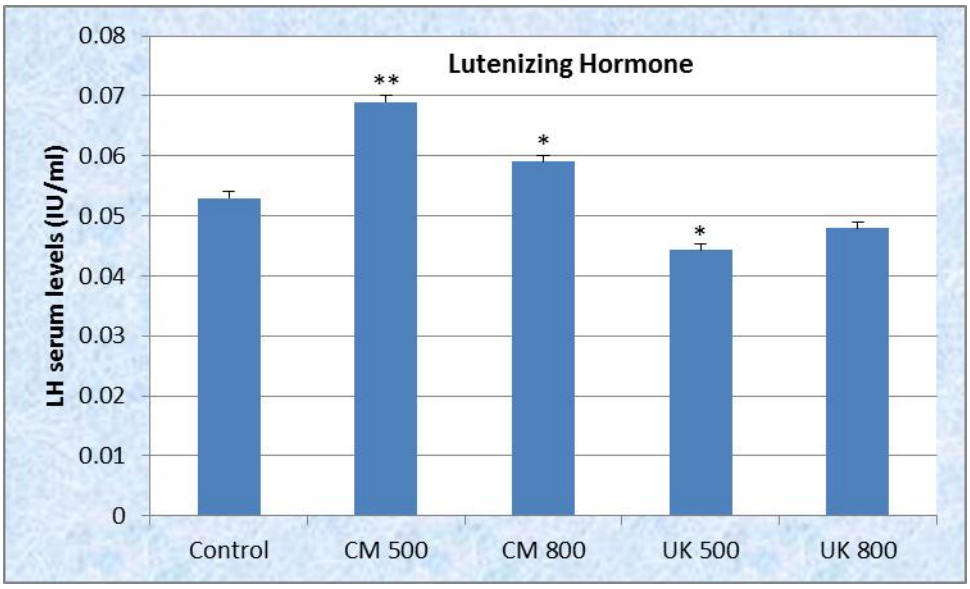


Figure 46: Effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on serum Luteininzing hormone levels.

Figure 46 shows a significant increase in LH serum levels (P<0.01) at 500 mg/Kg and (P<0.05) at 800mg/Kg *Croton menyharthii* compared to the negative control. *Uvariodendron kirkii* reduced LH serum levels significantly (P<0.05) at 500mg/Kg compared to the negative control.

The values are mean ± SEM. ** P<0.01 *P<0.05. Keys: CM-Croton menyharthii UK-Uvariodendron kirkii.

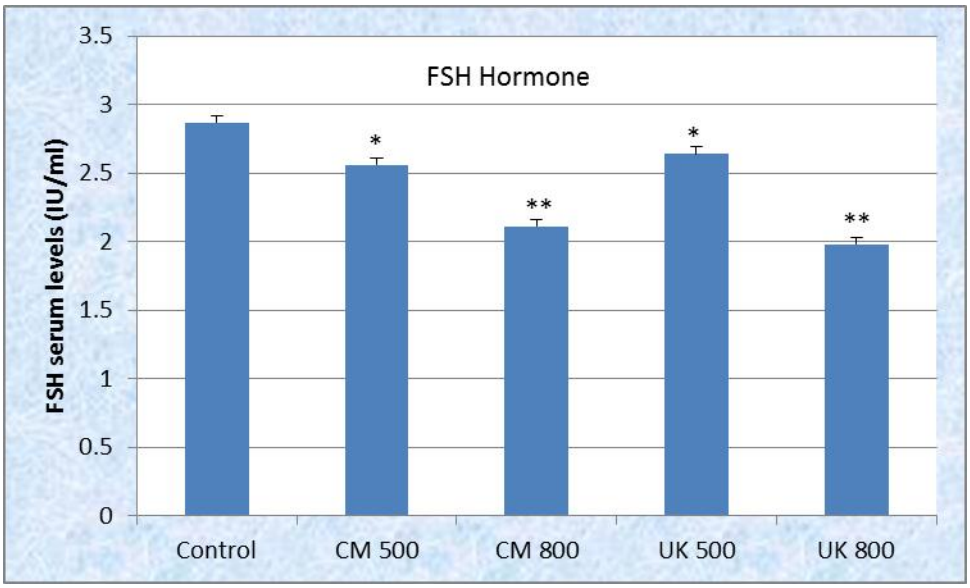


Figure 47: Effect of *Croton menyharthii* and *Uvariodendron kirkii* extract on serum Follicle Stimulating Hormone (FSH) levels.

Figure 47 shows significant reduction in FSH serum levels ($P<0.01$) at 800 mg/Kg for both plant extracts and ($P<0.05$) at 500 mg/Kg for both plant extracts compared to the negative control.

The values are mean ± SEM. ** $P<0.01$ *$P<0.05$. Keys: CM-*Croton menyharthii* UK-*Uvariodendron kirkii*

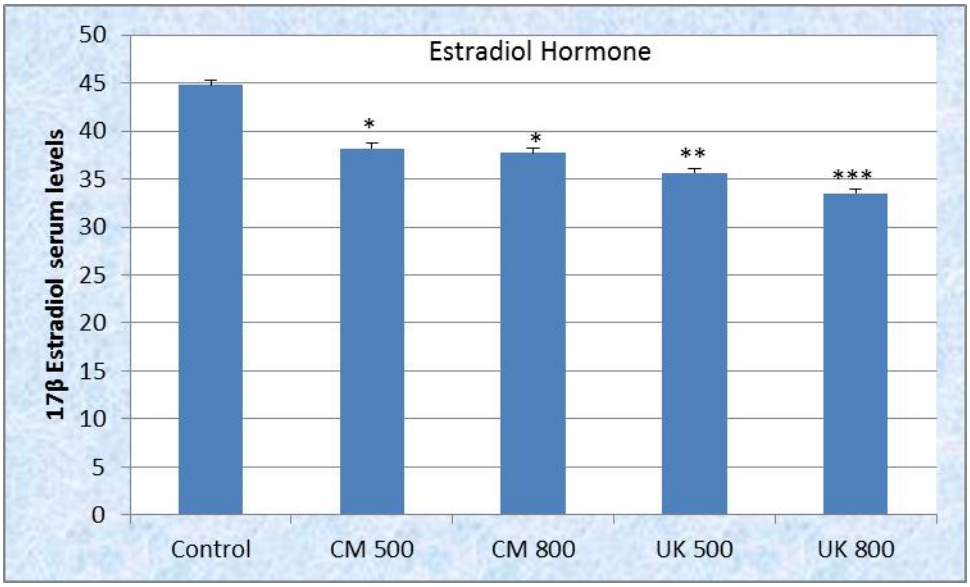


Figure 48: Effect of *Croton menyharthii* and *Uvariodendron kirkii* extract on serum 17β Estradiol levels.

Figure 48 shows a significant reduction of 17β Estradiol serum levels ($P<0.001$) at 800 mg /kg and ($P<0.01$) at 500mg/Kg Uvariodendron kirkii. 17β Estradiol serum levels were also significantly reduced ($P<0.05$) by *Croton menyharthothii* at both dose levels.

The values are mean ± SEM. *** $P<0.001$ ** $P<0.01$ *$P<0.05$.

Keys: CM-*Croton menyharthii* UK-*Uvariodendron kirkii*

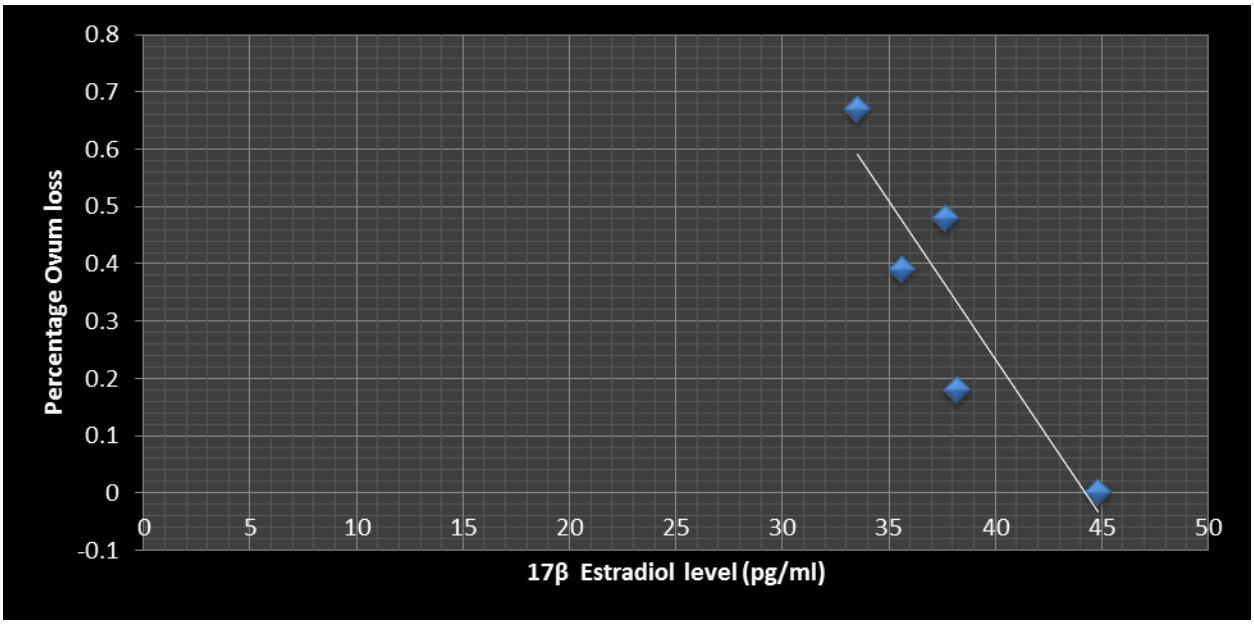


Figure 49: A regression between 17β Estradiol serum levels and percentage loss of ovum at varied doses of *Croton menyharthii* and *Uvariodendron kirkii* extracts.

Figure 49 shows a comparison between 17β Estradiol serum levels and percentage loss of ovum.As levels of estradiol decreased there was a corresponding increase in percentage loss of ovum compared to the control (0% loss of ovum). *Croton menyharthii* at 500mg/Kg caused a reduction of 6.6 pg/ml Estradiol with a corresponding 18% loss of ovum compared to the negative control (Table 15). *Croton menyharthii* at 800mg/Kg caused a reduction of 7.1 pg/ml Estradiol with a corresponding 48% loss of ovum; *Uvariodendron kirkii* at 500mg/Kg caused a reduction of 9.19 pg/ml Estradiol with a corresponding 39% loss of ovum; *Uvariodendron kirkii* at 800mg/Kg caused a reduction of 11.30 pg/ml Estradiol with a corresponding 67% loss of ovum compared to the negative control.

6.4 DISCUSSION

Successful mammalian reproduction requires a closely regulated and balanced hormonal interplay involving hypothalamic GnRh, pituitary gonadotrophins, and ovarian steroids. *Croton menyharthii* and *Uvariodendron kirkii* aqueous extracts at 500 and 800 mg/ kg caused a significant reduction in serum FSH and estradiol levels (Table 15). These findings together with a significant reduction in number of primary, secondary and antral follicles in Chapter 5; indicate a direct effect of *Croton menyharthii* and *Uvariodendron kirkii* on the ovarian structure in the rats. Estradiol is responsible for multiple functions in the female; such as causing the secretory cells lining the lumen of the oviduct to synthesize and secrete glycoproteins that play a key role in the nourishment of the embryo. Differentiation and ciliation of the oviduct inner most layer are induced by estradiol. In this study a reduction of estradiol levels possibly compromised oviduct intergrity and might have interfered with fertilization, early embryonic growth and movement of the fertilized embryo towards the uterus. Estrogen through a feedback mechanism influences the production of FSH from the pituitary gland. When FSH in synergy with LH reach a critical concentration the growing antral follicles start synthesizing and secreting estradiol. Estradiol is key in endometrium priming in preparation for implantation. Reduced estradiol levels seen in this study could have interfered with endometrial priming thereby compromising implantation. Reduced FSH levels as seen in the present study (Table 15) can alter estradiol profiles leading to increased atretic follicles as reported in chapter 5 (Fig 23B). This in turn would lead to significant reduction in fertility index compared to the control (Figure 15). Thakur et al., (2009) reported similar findings when rats exposed to aqueous and ethanolic extracts of *Carum carvi* and *Curcuma longa*, showed a significant antifertility activity, and significantly

decreased FSH and LH. In contrast to this study, they reported enhanced estradiol levels. In this study, whereas *Croton menyharthii* aqueous extract significantly increased LH levels ($P<0.01$) and ($P<0.05$) at 500mg/Kg and 800mg/Kg respectively (Table 15), Uvariodendron kirkii at both doses reduced levels of LH be it unsignificantly (Fig. 39). Peak LH levels are key in ovulation induction of the dominant antral follicle. LH is responsible for ovarian production of both estradiol and progesterone. LH in synergy with FSH stimulates normal follicular growth and ovulation. Low levels of LH lead to impaired follicular growth, impaired oocyte maturation and in adequate androgen and estrogen synthesis. It is possible the reduced levels of FSH and LH seen in this study led to implantation failure. Our findings are in line with Yakubu et al., 2011; Sharangouda and Saraswati, 2013; Udoh et al., 2009; Udoh et al., 2005b; Kage et al., 2009; Thakur et al., 2009; Jyoti et al., 2010; Zhang et al., 2013; Akpantah et al., 2011; Talukder et al., 2014; Sakila et al., 2009; Sharangouda and Patil 2007; 2008 and Monsefi et al., 2012 who corroborate this study by reporting significant reduction in FSH, LH, estradiol and progesterone serum levels due to *Cnidoscolous aconitifolius, Caricapryl seeds, trichosanthes cucumerina, Ocimum sanctum, Radix astragali, Azadirachta indica, Abrus precatorius, Andrographis paniculata*; *Anethum graveolens Carum carvi* and *Curcuma longa* plant extracts respectively in rats. In contrast to this study, Thakur et al., (2009) reported increased levels of estradiol. Reports by Krishnamoorthy et al., (2013) were also in contrast to this study in that *Andrographis paniculata* caused a significant increase in serum levels of FSH, LH, estradiol and progesterone levels. Modaresi et al., (2012) reported mixed results where *Fenugreek* seeds caused a significant reduction in FSH and LH but an increase of estradiol and progesterone levels. In this study the administration of the aqueous extract of both plants at 500 and 800 mg/Kg resulted in a significant reduction in estradiol but increase in progesterone (Fig. 45). The slightly enhanced

progesterone might be due to the prolonged diestrus phase that is seen in chapter 4. At 800mg/kg *Croton menyharthii* and *Uvariodendron kirkii* caused a significant increase in corpus lutea numbers (Fig. 35B, 36B, 37B and 39B) compared to the control 35A, 36A, 37A and 39A).. Probably the significant increase in numbers and hypertrophy of corpus lutea seen in chapter 5 led to the high serum levels of progesterone (Fig. 45). The corpus luteum secretes progesterone hormone. Progesterone in turn plays a crucial role in preparing the endometrium for possible implantation. An increase in number of corpus lutea possibly leads to higher levels of progesterone hormone leading to a disrupted estradiol progesterone ratio. A disturbance in serum ratio of the two sex hormones compromises the implantation window. Progesterone hormone is key in maintainance of pregnancy. Near term; progesterone levels decline triggering other hormonal and molecular changes that terminates gestation. A sustained higher concentration of progesterone hormone probably led to prolonged gestation seen in chapter 4.

The marked decrease in LH and FSH (Fig. 37) reported here could explain the ovulation and estrous cycle disruption by the plant extract (Chapter 4). Several authors demonstrated that the LH release surges at the proestrus stage are responsible for ovulation. All substances able to inhibit this release could provoke an ovulation disruption by decreasing the number of pre ovulatory follicles or disrupt estrous cycle. The molecular mechanism by which *Croton menyharthii* and *Uvariodendron kirkii* causes a decline in pituitary gonadotropins is unclear. Additional studies are therefore still required. Estradiol is synonymous with fertility. A disruption of estradiol levels impacts fertility in rats, mice and other vertebrates. In this study the levels of estradiol were disrupted in a dose dependent manner. We have also reported a dose dependent loss of ovum (Table 15). Ovarian function is driven by pituitary gonadotropins (FSH and LH). Follicles are recruited on cyclic bases to grow and mature. Once they reach the antral

stage, LH influences the theca cells to produce androgens which are converted by an aromatase within granulosa cells to estradiol under the influence of FSH. Estradiol is responsible for the growth and proliferation of endometrial layer in preparation for implantation and an enhanced expression of ER and PR nuclear receptors. It therefore follows that a disruption of estradiol disrupts the ovarian signaling pathways and function thereby imparting fertility.

6.5 CONCLUSION

The aqueous extract of the root bark of *Croton menyharthii* and *Uvariodendron kirkii* reduced the serum levels of estradiol hormone but enhanced progesterone levels. This hormonal disruption might have affected folliculogenesis and steroidogenesis in the ovary. The hormonal findings may be due to hypothalamic-pituitary-gonadal axis dysfunction after treatment with the plant extracts since FSH and LH were also affected. *Croton menyharthii* and *Uvariodendron kirkii* have potential as anti-fertility agents and research should be undertaken to exploit their antifertility effect. Qualitative and quantitative analysis of the bio active compounds of both plants should be carried out to determine the compound responsible for the anti-implantation effect.

In this chapter, the study has successfully evaluated the effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on female reproductive hormones. A manuscript has been submitted for peer review in the *International Journal of Medicinal Plants* (PHOTON).

Kaingu C.K., Oduma J.A., Mbaria J.M. and **Kiama S.G. (2016).** The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on Follicle Stimulating Hormone, Luteinizing hormone, Estrogen, Progesterone serum levels in female Wister rats**.**

CHAPTER 7

7.0 PHYTOCHEMICAL SCREENING, BRINE SHRIMP LETHALITY AND TOXICOLOGICAL EFFECTS OF *CROTON MENYHARTHII* AND *UVARIODENDRON KIRKII* EXTRACTS

7.1 INTRODUCTION

Majority of today's drugs are derived from plant resources. Since time in memory, medicinal plants have been used to manage illnesses, as plant derived medicines have made large contributions to the health and well-being of humans. According to the World Health Organization (WHO), 80% of the world's population relies on plants for their primary health care1. The medicinal value of plants is due to the substances that it contains which produce a physiological action on the body. Some examples of these substances are alkaloids, essential oils, tannins, resins, and many others. Bioactive chemicals in plants have been associated with management of many chronic diseases in humans. Saponins exhibit abortifacients, anti-zygotic and anti-implantation properties. Some have caused sterility in mice (Francis et al., 2002). Alkaloids from Mussaenda pubescens extract terminated pregnancy in rats and the plant is used as a contraceptive in China (Quin and Xu, 1998). Alkaloids are used to manage many illnesses including cancer, malaria, as analgesics, hypertension and central nervous system disorder. Among plant derived constituents; abridine (*Abrus preactorius*), saponins (*Achyranthes bidentata*), Butin (*Butea monosperma*), Embelin (*Embelia ribes Burm*) momorcharins, β sitosterol (*Ananas comosus*), Vicorides among others have shown potential antifertility effect (Dinesh et al., 2012). Secondary metabolites of *Croton menyharthii* and *Uvariodendron kirkii* should be exploited as potential novel contraceptive compounds. Brine shrimp lethality assay is

useful in assessing and monitoring the bioactivity of the plant extracts whereas the acute toxicity study pharmacologically assesses the *in-vivo* toxicity of compounds.

The study determines the phytochemical compounds, brine shrimp lethality and acute toxicity of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract.

7.2 MATERIAL AND METHODS

7.2.1 Phytochemical screening of *Croton menyharthii* and *Uvariodendron kirkii* extracts.

Phytochemical screening of *Croton menyharthii* and *Uvariodendron kirkii* extracts was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of saponins, steroids, tannins, alkaloids, phenols, flavanoids, quinones and terpenoids.

7.2.1.1 Saponins

To test for saponins, 1 gram of *Croton menyharthii* aqueous extract was boiled with 5ml distilled water and filtered. Three millilitres of distilled water was added to the filtrate and shaken vigorously for about 5 minutes. Persistent frothing on warming was an indication of the presence of saponins. The procedure was repeated using *Uvariodendron kirkii* aqueous extract.

7.2.1.2 Sterols

Salkowaski test was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of sterols. Ten milligrams of *Croton menyharthii* aqueous extract was dissolved in 2ml of chloroform after which 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for a few minutes. The development of red color in chloroform layer indicated the presence of sterols. The procedure was repeated for *Uvariodendron kirkii* aqueous extract.

7.2.1.3 Alkaloids

The protocol was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of alkaloids. Ten mg of *Croton menyharthii* aqueous extract was dissolved in 5 ml of hydrochloric acid (1.5% v/v) and filtered. The filtrate was used to test for alkaloids using Dragendorff's test. Dragendorff's reagent composition was 1.7grams Bismuth sub-nitrate, 20ml glacial acetic acid, 80ml distilled water and 100ml of 50% Potassium iodide solution. The mixture made a stock solution.

Two ml Dragendorff's reagent was added into 2ml of the filtrate. The formation of orange-brown precipitate indicated the presence of alkaloids. The procedure was repeated using *Uvariodendron kirkii* aqueous extract and results were recorded.

7.2.1.4 Tannins

The protocol was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of tannins. Five hundred milligrams (0.5 grams) of *Croton menyharthii* aqueous extract was stirred with 10 ml distilled water, warmed and then filtered. 5 ml of the filtrate was allowed to react with 1ml of 5% ferric chloride solution. Dark green or deep blue color indicated the presence of tannin. The procedure was repeated using *Uvariodendron kirkii* aqueous extract.

7.2.1.5 Phenols

The protocol was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of phenols. To test for the presence of phenol phytochemical compound, 2 ml of distilled water was added to a test tube containing 1ml of *Croton menyharthii* aqueous extract followed by a few drops of 10% ferric chloride ($FeCl_3$).

Appearance of blue or green color indicated the presence of phenols. The procedure was repeated using *Uvariodendron kirkii* aqueous extract.

7.2.1.6 Flavonoids

The protocol was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of flavonoids. A few drops of dilute sodium hydroxide (NaOH) were added to a test tube containing 3ml of *Croton menyharthii* aqueous extract filtrate. The presence of an intense yellow color which later became colorless on addition of a few drops of dilute hydrochloric acid indicated the presence of flavonoids. The procedure was repeated using *Uvariodendron kirkii* aqueous extract.

7.2.1.7 Quinones

The protocol was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of quinones. One ml of *Croton menyharthii* aqueousextract filtrate was placed in a test tube followed by 1 ml of concentrated sulphuric acid (H_2SO_4). The formation of red color indicated the presence of quinones. The procedure was repeated using *Uvariodendron kirkii* aqueous extract.

7.2.1.8 Terpenoids

The protocol was carried out as per the method and test used by Ashokkumar et al. (2010) to decipher the presence and quantities of terpenoids. Two ml of chloroform was added to 5 ml of aqueous extract of *Croton menyharhii* filtrate in a test tube followed by 3ml of concentrated sulphuric acid (H_2SO_4). A reddish brown precipitate at the interface indicated the presence of terpenoids.

The procedure was repeated using *Uvariodendron kirkii* aqueous extract.

7.2.2 Brine shrimp lethality bioassay of *Croton menyharthii* and *Uvariodendron kirkii* extract.

Brine shrimps (Artemia salina) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N sodium hydroxide under constant aeration for 48 hours. After hatching, active nauplii free from egg shells were collected from the brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml. of the plant extract was added to 4.5 ml of brine solution and maintained at room temperature for 24 hours under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations (1-5000 µg/ml) of the test substances in a set of three tubes per dose. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC50 values were obtained from the best-fit line plotted concentration verses percentage lethality. Etoposide and Biocristine were used as positive controls in the bioassay.

7.2.3 Acute oral toxicity study of *Croton menyharthii* and *Uvariodendron kirkii* extract.

The acute toxicity study protocol was carried out as per the OECD guidelines number 423 using 3 female rats (weighing between 170-200grams) per step at any of the defined dose levels. Depending on the mortality rate 3 but never more than 6 rats were used per dose level. The result of each step determined if further testing was needed for 3 additional animals at same dose level or 3 additional at the next lower dose level. Food was withheld overnight but water was provided *ad libitum*. The animals were weighed just before extract was administered through intra-

abdominal gavage. Food was withheld for a further 3-4 hours after extract administration. The extract dose range was 2000 to 50mg/Kg starting at the highest level. Clinical symptoms were recorded. The observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic nervous system, central nervous systems, behavior pattern and death. Other observations included: tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

7.3 RESULTS

7.3.1 Phytochemical compounds

Phytochemical screening of the two plants revealed the presence of alkaloids, flavonoids, sterols, phenols, terpenoids, quinones, saponins and tannins in *Uvariodendron kirkii* and *Croton menyharthii* extracts at various concentrations (Table 16). The aqueous extract of *Croton menyharthii* revealed high concentrations of flavonoids, quinones and terpenoids with mild concentrations of sterols, phenols, saponins and alkaloids but no tannins. *Uvariodendron kirkii* aqueous extract had high concentration of flavonoids with mild concentrations of sterols, phenols, saponins, alkaloids, quinones and terpenoids.

Table 16: Phytochemical compounds of C*roton menyharthii* and *Uvariodendron kirkii*

	Sterols	Saponins	Alkaloids	Tannins	Phenols	Flavonoids	Quinones	Terpenoids
U.K	++	++	++	+	++	+++	++	++
C.M	++	++	++	-	+	+++	+++	+++

Table 16 shows phytochemical compounds of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract. Both plant extracts had higher concentrations of flavonoids, sterols, saponins, alkaloids, phenols, quinones and terpenoids

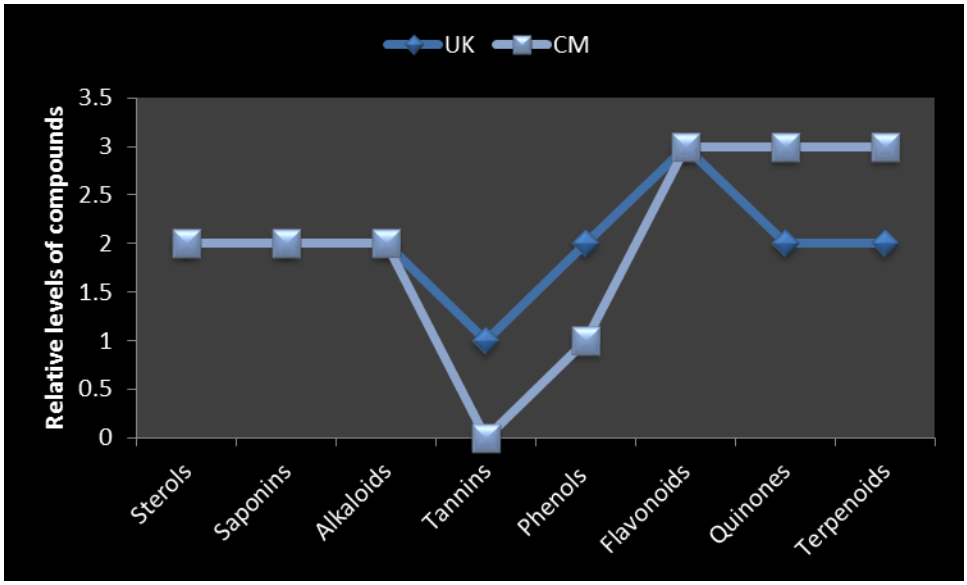


Figure 50: Phytochemical compounds of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract. CM-*Croton menyharthii* UK-*Uvariodendron kirkii*

Figure 50 shows phytochemical compounds in *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract. Both plant extracts had higher concentrations of flavonoids, sterols, saponins, alkaloids, phenols, quinones and terpenoids.

7.3.2 Brine shrimp lethality test

Croton menyharthii and *Uvariodendron kirkii* aqueous extracts have shown moderate brine shrimp lethality and the LC_{50} values were found to be lower than 100 as noted in Table 17. The degree of lethality was directly proportional to the concentration of the extract. Maximum mortalities took place at a concentration of 1000 μg/ml whereas least mortalities were at 10 μg/ml concentration. Maximum mortalities (100%) were observed at a concentration of 1000 ppm in both *Croton menyharthii* and *Uvariodendron kirkii* extracts at 58 and 97% nauplii mortality. Based on the results, the brine shrimp lethality of the 2 plant extracts were found to be concentration-dependent. The observed lethality of the two plant extracts to brine shrimps indicated the presence of potent cytotoxic compounds. According to Meyer et al 1982; crude plant extract is toxic (active) if it has an LC50 value of less than 100 μg/mL while non-toxic (inactive) if it is greater than 1000 μg/ml.

Table 17: The Table shows the number of shrimp nauplii that survived after exposure to *Croton menyhathii* and *Uvariodendron kirkii* extracts and the percentage mortality of shrimp nauplii.

	Concentration (ppm or µg/mL)	Number of surviving nauplii after 24 hours		Total number of survivors	% mortality (Aqueous extract)
		Aqueous	Organic		
Negative control	10	10	10	All survived	0
	100	10	10	All survived	0
	1000	10	10	All survived	0
Etoposide	10	9.6	0	9.6	4%
	100	0	0	0	100%
	1000	0	0	0	100%
Biocristine	10	9.6	0	9.6	4%
	100	1.8	0	8.2	82%
	1000	0	0	0	100
Croton menyhathii	10	8.5	10	1.5	15
	100	8.1	10	1.9	19
	1000	4.2	9.2	5.8	58
Uvariodendron kirkii	10	9.6	8.2	0.4	4
	100	7	3.4	3	30
	1000	0.3	0.6	9.7	97

7.3.3 Acute oral toxicity study

Both plant extracts did not cause any mortality of rats even at the highest concentration of 2000mg/Kg. *Uvariodendron kirkii* at 2000mg/kg showed tremors and lethargy within 30 minutes of treatment. The rats had minimal activity for about 3 hours and then became active. None of the rats died in 24 hours. *Croton menyharthii* at 2000 mg/Kg showed lethargy and minimal activity, tremors were not observed. No mortality was observed in 24 hours. The acute toxicity when repeated at next lower level (300mg/Kg) no signs of toxicity were observed.

7.4 DISCUSSION

Croton menyharthii and *Uvariodendron kirkii* crude extracts are traditionally used as fertility regulators in Tana River County, Kenya. Acute toxicity studies carried out using extracts from *Croton menyharthii* and *Uvariodendron kirkii* plants did not cause any mortality even at the highest dose of 2000 mg/kg. Preliminary phyto-chemistry established the presence of alkaloids, flavanoids, quinones, terpenoids, saponins, sterols, phenols in both plant extracts (Table 16) and presence of tannins in *Uvariodendron kirkii* aqueous extract (Fig. 42). Several studies have reported the effect of alkaloids as fertility regulators. *Abrus precatorius* indole alkaloid completely blocked ovulation and disrupted the estrus cycle in female rats (Okoko, 2010). *Acalypha indica pyranoquinoline* alkaloid had a post coital antifertility effect. Several authors, (Elumalai, 2009; Sasmal, 2011; Saravanan et al., 2012; Balakrisman 2011; Vijayalaxmi et al., 2011; Musa and Bimbo, 2009; Mcneil et al., 2003; and Bhargava et al., 2012) all reported on abortive effect of alkaloids from ; *Achyranthes aspera, Aerva lanata, Alangium salvifolium, Amaranthus spinosus, Annona squamosal, Bambusa vulgaris, Gloriosa superba, Ricinus communis* and *Zingiber officinalis*. Several other studies (Saravanan et al., 2012; Joyti et al.,

2010; Ibrahim and Fulya, 2013; Circosta et al., 2001) also reported on anti-ovulatory properties of alkaloids from *Ailanthus excella, Arecha catechu, Curcuma longa, Papaver sominiferum and Calotropis procera* plants. Endocrine disruption effect of alkaloids from *Citrus bergamia, Cuscuta reflexa Roxb, Datura metei Linn, Derris brevipes, Dloscora pentaphylla Linn, Duckesia verrucosa, Ehretia cymosa Thonn, Eriosema crinitum, Ficus religosa, Ficus wassa, Huperzia saururus, Hymenaea stigonocarpa, Indigofera linnaei, Justicia simplex, Mentha arvensis, Mentha longifolia Linn, Mouriri pusa, nardostachys gradiflora, Persea Americana, Petroseltnum crispum, Ricinus communis, Phoradendron macrophyllum, Pouzolzia hypoleuca , Senecto aureus Linn, Solanum incanum, Trichosanthes tricuspidata* has also been reported (Saravaran et al., 2012; Panda et al., 2011; Soni et al., 2012; Ankush et al., 2011). *Tinospora cordifolia, Sesbania sesban, Mentha arvensis, Hibiscus rosasinensis, Daucus carota, Crataeva nurvala, Cassia fistula, Carum carvi, Azaridachta indica, Antiaris toxicaria, Allium cepa* had antifertility, anti-implantation, contraceptive, caused a resoption of embryos and estrus cycle disruption due to the presence of alkaloids (Dinesh et al., 2012). In this study alkaloids, along with other compounds were present in both plant extracts. It is possible that the antifertility effect in female rats seen in this study could partially be attributed to the presence of the alkaloids, as seen in studies mentioned above. Studies by Francis et al., (2002) indicated anti fertility effect in female rats as possibly being due to the presence of saponins and flavonoids. They further reported on the saponins as having abortifacients, anti-zygotic and anti-implantation effects. These findings are closely corroborated by the findings of this present study. The results of this study are also consistent with those of Londonkar et al., (2009) who showed similar results in rats following treatment with crude *sida acuta* extract. Their report attributed the antifertility effect as being due to the presence of flavonoids which had antizygotic, blastocytotoxic and anti-

implantation activity. Sasmita. (2014), working on *Piper betel,* suggested that possibly flavonoids and saponins from from this plant were responsible for the significant disruption of the estrus cycle leading to infertility in their study. Abrin and abridin compounds from *Abrus precatorius* have been suggested by several authors (Pillai et al., 1982; Bhargava, 1984; Susan et al., 1985; Kong et al., 1989; Hiremath and Rao, 1990; Yuan et al., 1991and Alam et al., 1992a,b) as being responsible for anti-implantation activity in female rats. This is corroborated by Modaresi et al., (2012) who reported on phytochemical compounds exhibiting antifertility activity. Zhu and Che, 1987; Hu et al., 1984 and Bhargava and Dixit,1985 reported on saponins, vicolide D; embelin, methyl aristolate, yuanhuacine, yuanhuatine, momorcochin and plumbagin causing abortion in female rats. On the other hand studies done by Mats et al 1982; Mats et al., 1984; Singh et al., 1985 and Prakash et al., 1991 suggested that contraceptive effect in rats were due to vicolide D, triterpene glycoside, lithospermic acid, cirantine and ferujol phytochemical compounds.

In this study *Croton menyharthii* and *Uvariodendron kirkii* phytochemical analysis showed the presence of several phytochemical compounds (Table 16) in the aqueous extract. Probably the anti- fertility effect of both plants as claimed by the TMPs could be due to the presence of these compounds especially flavonoids, saponins and alkaloids as has been reported also by several authors above. The indole alkaloid, *trans*-*N*-(*p*-coumaroyl) serotonin (4) present in *Croton menyharthii* has shown promising fertility inhibitory effect at a very low dose.

Croton menyharthii and *Uvariodendron kirkii* have shown moderate brine shrimp lethality. The LC_{50} values were found to be lower than 100 at 91 and 77µg/ml respectively. Probably the

biological activity of both plants will be higher when other forms of extraction are explored for example; organic extraction.

7.5 CONCLUSION

The presence of alkaloids, saponins and flavonoids might be responsible for the anti-fertility activity of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract. Further anti fertility, anti-ovulatory and anti-implantation studies on the indole alkaloid, *trans*-*N*-(*p*-coumaroyl) serotonin (4) isolated from *Croton menyharthii* should be explored.

CHAPTER 8

8.0 GENERAL DISCUSSION AND CONCLUSION

World attention is increasingly turning towards the use of local remedies including herbal medicines to alleviate health problems especially in developing countries. Sexual dysfunction and fertility issues in males are creating adverse problems in families all over the world, particularly among the middle to old age men (Kamatenesi- Mugisha and Oryem-Origa, 2005). Indigenous medicinal knowledge has proved useful and has been used for centuries in developing countries where modern medicine is still out of reach of many. When proper research and verification of efficacy of these herbs is done, then their use can be mainstreamed into the healthcare system to the benefit of many inhabitants. This will eventually bring healthcare closer to the people in line with Millennium Development Goals.

8.1.1 Specific Objective 1: Ethnobotanical Survey

The present study established that in Tana River County, the custodians of traditional knowledge, including reproductive health knowledge, were all elderly men and women aged over fifty years with long years of practice. Considering that their knowledge was acquired through inheritance from practicing relatives, coupled with the migration of youth to major towns (according to practicing parents and grandparents), there is danger of this knowledge not being passed on to the younger generations for posterity (Kamatenesi-Mugisha and Oryem-Origa, 2005). Reproductive dysfunction is a major obstacle to social-economic development amongst the inhabitants of Tana River County. Tana River has a pool of TMPs with a wealth of

indigenous knowledge that needs to be exploited. The commonest ailments were pregnancy and related complications, menstrual problems, infertility and fertility regulation.Threatened abortion was the most commonly mentioned pregnancy related problem in the community. The plants used to treat dysmenorrhea for example may be important analgesic agents that need further investigation. Eleven plants were identified as fertility regulators in the ethnobotanical study. In this study; only two have undergone in vivo tests. These plants are being reported for the first time as female fertility regulators. Such species therefore need further investigation to establish their effect on female reproductive parameters, hormonal profiles and ovarian histology. The ethnobotanical study also reported on male sexual dysfunction and infertility iisues that are a concern in Tana River. The plant remedies described in this study could prove useful as treatment for male sexual dysfunction and infertility and needs further investigation.

8.1.2 Specific Objective 2: Effect of both plant extracts on reproductive parameters

Croton menyharthii and Uvariodendron kirkii are indigenous in Tana River County and are readily available. The effect of both plants on the female reproductive system has not been carried out so far. This study has shown that both plant extracts significantly disrupted the estrus cycle, with a significant increase in frequency of appearance of metestrus and Diestrus and a subsequency reduction in frequency of appearance of estrus and proestrus phases. An optimal blood level of FSH is a pre requisite for initiation and maintenance of normal ovarian folliculogenesis. The estrus cycle is driven by both pituitary gonadotropins and ovarian steroid hormones. A disruption / disturbance of hormonal balance especially estradiol disrupts the estrus cycle. This study has shown that both plant extracts significantly disrupted the estrus cycle, with a significant increase in frequency of appearance of metestrus and diestrus and a subsequent reduction in frequency of appearance of estrus and proestrus phases. In this study, pre mating

extract administration of *Croton menyharthii* and *Uvariodendron kirkii* caused a significant reduction in fertility index. The fertility index was also significantly reduced by the pre and post mating (Group 3) treatment regime. In every estrus cycle; a group of follicles are recruited to grow and mature. As much as there might have been an estrus cycle disruption as shown in Table 9 and 11; some of the follicles were still able to undergo ovulation and those rats successfully mated. Female fertility is determined by the developmental competence of oocyte; in its ability to be fertilized and give rise to a viable embryo and for that embryo to successfully implant. It is possible that ovulation occurred but implantation was disrupted as shown by the significant reduction in fertility index. In this study; *Uvariodendron kirkii* had the most significant anti fertility activity (Table 14) compared to the control.

In this study both plant extracts caused significant anti implantation effect in Group 1 and 3 of the treatment regime. Estradiol and progesterone are key hormones in preparation of the endometrium for implantation. A disruption of the sex hormones interferes with the uterine milieu and impacts implantation. Establishment of pregnancy is influenced by progesterone hormone levels. In this study gestation length was prolonged in comparison to the control (Group 1). It is possible that the plant extracts might have disrupted the sex hormones and interfered with gestation length. The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on reproductive parameters provides a pointer towards possible anti fertility properties of the extracts. Since 80 to 100% of the rats were successfully mated after exposure to the premating extract administration, it shows that estrous cycles and ovulations were still occurring. The presence of spermatozoa plugs was evidence that mating and therefore fertilization could occur. The effects of *Croton menyharthii* and *Uvariodendron kirkii* in this study therefore point to anti implantation properties of both extracts.

The results showed that when extract administration was done before and after mating the effect was most effective. In Tana River the treatment for contraceptive effect is carried out throughout one menstrual cycle and repeated every 6 months. Daniyal and Akram, (2015) and Dinesh et al., (2012) support our results by reporting on anti-ovulatory and anti-implantation properties of *Acalypha indica, Ailanthus excelsa, Aristolochia bracteolate, Azadirachta indica, Bambusa vulgaris, Butea monosperma, Citrus medica, Dalbergia saxatilis Linn, Vicoa indica, Plumbago zaylanica, Nelumbo nucifera, Hibiscus rosa-sinensis, Heliotropium indicum, Gloriosa superba, Ferula hermonis, Polygonum hydropiper Linn, Ocimum sanctum, Striga orobanchioides, Ricinus communis, punica granatum, Calotropis procera, Mentha arvensis, Lawsonia inermis, Juniperus communis, Hagenia abyssinica* and *Cicer arietinum* plants. The effect of *Croton menyharthii* and *Uvariodendron kirkii* on anti-implantation activity further supports the theory suggesting that the main anti fertility property of both plant extracts was anti-implantation. Anti-implantation effect could in turn be due to lack of uterine priming by estrogen or due to non viability of zygotes or blastocysts.

8.1.3 Specific objective 3: Effect of extracts on histomorphology of ovaries and uterus

Rising estradiol levels play a major role in uterine endometrial preparation for implantation.Therefore the present histological and hormonal findings in this study suggest a hypothalamic-pituitary gonadal axis dysfunction after treatment with the plant extracts. Fertility regulating effects might be at the level of ovary where folliculogenesis and oogenesis are regulated by hypothalamus, pituitary and gonadal hormones. In this study both plant extracts caused a significant reduction in pre and antral follicle numbers that might have led to the significant reduction in estradiol levels (Chapter 6) which in turn compromised endometrial receptivity leading to a significant anti implantation effect seen in chapter 4.

Studies (Kidder and Vandenhyden, 2010) have shown that a disruption of zona pellucida leads to a severe reduction of both secondary and Graffian follicles thereby leading to a reduction in ovulated oocytes and litter size. In this study a disruption of the ZP in several photomicrographs Fig. 24B, 27B, 29B, 30B, 31B and 32B compared to intact ZP in controls was seen. A disruption of the close interaction between oocyte and surrounding granulosa and theca cells was also seen in this study (Figure 24B, 25B, 29B, 30B and 31B). It is possible that a disruption of this bi-directional communication between oocyte and surrounding granulosa cells is responsible for compromised folliculogenesis and oogenesis. In general terms, it has been demonstrated that the disruption of paracrine signaling between mouse oocytes and their cumulus cells *in vitro* reduces oocyte competence (Kidder and Vandenhyden 2010) and compromises fertility. This may be particularly important during pre-ovulatory development, as the rate of pyruvate consumption in maturing metaphase-I oocytes is significantly higher than that in immature oocytes. Cumulus cells also help the oocyte take in amino acids. As follicular cells are recruited in each estrus cycle for growth and maturation, simultaneously the oocytes grow and resume meiosis. This complex process involves a close interaction between the oocyte, surrounding granulosa and theca cells. In this study the results Figures 24B, 25B, 26B, 29B, 30B and 31B showed a disruption of the structural intergrity of oocyte and surrounding cellular cells. This suggests a possible cause of infertility being due to compromised folliculogenesis and oogenesis.

8.1.4 Specific Objective 4: Effect of extracts on reproductive hormones

The estrus cycle is regulated by pituitary gonadotropins (FSH and LH). Serum levels of both gonadotropins are in turn regulated by ovarian steroids. An optimal blood level of FSH is a pre requisite for initiation and maintenance of normal ovarian folliculogenesis. In this study FSH, and Estradiol levels were significantly reduced. When FSH in synergy with LH reach a critical concentration the growing antral follicles start synthesizing and secreting estradiol. Reduced estradiol levels seen in this study could have interfered with endometrial priming thereby compromising implantation. Reduced FSH levels as seen in the present study (Table 15) can alter estradiol profiles leading to increased atretic follicles as reported in chapter 5. This in turn would lead to significant reduction in fertility index compared to the control as reported in Chapter 4. LH is responsible for ovarian production of both estradiol and progesterone. In this study *Uvariodendron kirkii* extract caused a reduction in LH serum levels. The reduction might have led to insufficient androgen synthesis by theca cells; resulting in insufficient estradiol levels as observed in chapter 6. Fertility regulating effects of compounds might be at the level of ovary where folliculogenesis and oogenesis occurs. Both processes are regulated by hypothalamus, pituitary and gonadal hormones. A disruption of hormonal release either at hypothalamic or pituitary level will interfere with folliculogenesis and oogenesis. This might lead to an-ovulation and/or production of non viable oocytes. In this study, the significant reduction in estradiol levels (Table 16) probably led to the disrupted endometrial lining growth seen in Chapter 5 thereby compromising endometrial receptivity leading to failed implantation and the reduced fertility index shown in Table 9 and 11. A disruption of the sex hormones interferes with the uterine milieu and impacts implantation. In this study the number and size of corpus luteum (Fig. 34B to 39B) were increased probably leading to the elevated progesterone serum levels seen in Chapter

6. Establishment of pregnancy is influenced by progesterone hormone levels. It is possible that the elevated progesterone levels might have led to the prolonged gestation length reported in Chapter 4.

8.1.5 Specific objective 5: Brine shrimp assay, phytochemistry and acute oral toxicity of extracts.

Croton menyharthii and *Uvariodendron kirkii* have shown moderate brine shrimp lethality. Probably the biological activity of both plants will be higher when other forms of extraction are explored for example; organic extraction. Acute toxicity studies carried out using extracts from *Croton menyharthii* and *Uvariodendron kirkii* plants did not cause any mortality even at the highest dose of 2000 mg/kg. In this study *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract phytochemical analysis showed the presence of alkaloids, flavanoids, quinones, terpenoids, saponins, sterols and phenols (Table 16). Probably the anti- fertility effect of both plants as claimed by the TMPs could be due to the presence of these compounds especially flavonoids, saponins and alkaloids as has been reported by (Elumalai, 2009; Sasmal, 2011; Saravanan et al., 2012; Balakrisman 2011; Vijayalaxmi et al., 2011; Musa and Bimbo, 2009; Mcneil et al., 2003; Okoko et al., 2010 and Bhargava et al., 2012).

8.2 CONCLUSIONS

The effect of *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract on reproductive parameters provides pointers towards the possible anti fertility properties of the extracts. From our results *Croton menyharthii* and *Uvariodendron kirkii* aqueous extracts have anti implantation effects which should further be explored. The effect might either be at ovarian level as a result of incompetent zygotes/blastocysts or at uterine level due to hormonal imbalances.

CHAPTER 9

9.1 RECOMMENDATIONS

The study covered areas in both male and female reproductive issues and came up with the following recommendations.

1. *Croton menyharthii* and *Uvariodendron kirkii* have potential as anti fertility agents. Such species therefore need further investigation to establish their mechanism of action.
2. Evaluate the effects of concoction of *Croton menyharthii* and *Uvariodendron kirkii* extracts on anti-fertility and anti-implantation index in female winstar rats.
3. Evaluate the reversible fertility regulating effects of *Croton menyharthii* and *Uvariodendron kirkii* extracts in female winstar rats.
4. Evaluate effects of both plant extracts on blood parameters, liver and kidney function tests in winstar rats.
5. Evaluate the effects of *Croton menyharthii* and *Uvariodendron kirkii* extracts on histomorphology of pituitary gland, liver and kidney.
6. Evaluate the *in vivo* fertility regulating effects of both plant extracts in primates.
7. Evaluate the effects of both plant extracts on reproductive hormones in primates.
8. Evaluate the effects of both plant extracts on ovarian and uterine histomorphology in primates.
9. Isolate and characterize phytochemical compounds of *Croton menyharthii* and *Uvariodendron kirkii* extracts.

10. Study fertility regulating effects of individual isolated compounds using *in vivo* animal tests.

11. The plants used to treat dysmenorrhea for example may be important analgesic agents.
12. The plant remedies described in this study could prove useful as treatment for male sexual dysfunction and infertility.

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APPENDIX 1: DATA ACQUISITION QUESTIONNAIRE

A collaborative RISE AFFNET Natural Product Project by The University of Nairobi in Kenya

A QUESTIONNAIRE FOR HERBAL PLANTS USED FOR THE TREATMENT OF REPRODUCTIVE HEALTH AILMENTS IN BOTH MALES AND FEMALES IN TANA RIVER COUNTY, KENYA.

PART ONE: CONSENT

A. RESEARCHER'S DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual proprietary rights of the herbal practitioners.
2. We will at no time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretense.
3. We will be under no obligation to edit or taper with the information provided by the respondents.
4. The information collected will be used for the described research purpose and not any undisclosed intentions.

Researchers:

1) **Dr. Catherine Kaluwa……..**
2) **Prof. Jemimah Oduma……**
3) **Prof. James Mbaria…….**
4) **Prof. Stephen Gitari Kiama…….**

B. RESPONDENTS CONSENT AGREEMENT

I…… hereby agree to participate in this study with my full consent and conscience, and declare that to the best of my knowledge the information that I have provided is true, accurate and complete.

Signature/ Thumb print……..

PART TWO

A. BIODATA

Enumerator (name)……. Date of interview……

Serial No……..Name of Respondent……..

Division……..... Location……..Sub Location…….....

Village……. Telephone……. Gender…….

(**Answer by ticking {√} in the appropriate box**)

1) What is your age?
   a) Below 18 years{ } b) 18-27 { } c) 28-37 { } d) 38-47 years { }
   e) 48-57 years { } f) Over 57 years { }

2) What is your current marital status?
   a) Single{ } b) Married { } c) Separated { } d) Living with someone { }
   e) Divorced { } f) Widowed { }

3) What is your highest level of education?
   a) None { } b) Primary incomplete { } c) Primary complete { }
   d) Secondary incomplete { } e) Secondary complete { } f) College { }
   g) University { }
   e) Other (Please specify)……

4) What is your religion? .....

5) What is your home language?
   a) Pokomo { } b) Orma { } c) Wardei { } Other……..

6) What is your professional training? …….

7) Are you employed? a) Yes { } b) No { }

8) If yes what is the nature of employment? …….

9) What is your major source of income? .....

SECTION B: TO BE ADMINISTERED TO THE HERBALIST/ TBA

EXPERIENCE IN TRADITIONAL MEDICINE/ PRACTICE

10) For how long have you practiced as a traditional herbalist/ TBA? .....

11) Where do you practice as a traditional herbalist/ TBA (Location)? .....

12) How did you acquire your skills as a herbalist/ TBA?
……
……

13) Do you belong to any registered group of herbalists or TBAs?

a) Yes { } b) No { }

14) If yes what is the name of the group? .....

KNOWLEDGE ON TRADITIONAL HERBAL PRACTICE

15) What kind of reproductive health ailments (problems) do you come across?

i)	Frequent miscarriages	{ }
ii)	Contraceptive need (stop pregnancy)	{ }
iii)	Infertility	{ }
iv)	Excessive bleeding	{ }
v)	No menstruation	{ }
vi)	Irregular menses	{ }
vii)	Painful menses	{ }
viii)	Retained placenta	{ }
ix)	Post-partum hemorrhage	{ }
x)	Chango	{ }
xi)	Male reproductive dysfunction	{ }

xii) Any other { }

16) Do you use medical plants to manage **frequent miscarriages**?

a) Yes { } b) No { }

17) If yes,

i) How many cases were treated in the last month? ……

ii) Number of cases treated in the last six months ……

iii) Was the patient referred to you? ……..

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

18) How often do you handle women who want to control their pregnancies or control their fertility (contraceptive need) …….

19) Do the women come with/ without their husbands for the consultations?

……

20) What are their reasons for wanting to control pregnancy

- Have enough children { }
- Have too many children { }
- Cannot care for any more children { }
- Gets sickly during pregnancy { }
- Husband does not want any more children { }
- Husband has passed away { }

- Husband has left/ died { }

21) Do you use medical plants to control (stop) pregnancies?

a) Yes { } b) No { }

22) If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? ……...

iii) Was the patient referred to you? ……..

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

23) At what age after the last baby do you administer the herbs?

……

24) If for suppression of fertility; what time of the menstrual cycle is the herb administered?

a) During menses { } b) Immediately after menses { } c) Mid cycle { }

d) Other (specify) ……..

25) How do you know if the medicine is working?

i) No menses

a) Immediately after treatment { } b) One month after treatment { }

c) Three months after treatment { } d) Six months after treatment { }

e) Other { }

ii) No pregnancy

a) For one year { } b) For two years { }

c) More than two years { } d) Other …….

26) How long does it take after consumption of herbs for contraceptive effect to occur?

.....

27) Is the effect irreversible? Yes { } No { } Other ……

28) If reversible after what duration of use?

i) One year { } ii) Two years { } iii) Other …….

29) If the treatment does not work what do you do?

a. Repeat the treatment { }
b. Change the herb used { }
c. Refer to hospital { }

30) If a patient gets sick from taking the medicine, what do you do?

a. Change the herb used { }
b. Refer to other herbalist { }

31) Is there any other method used traditionally to confer contraceptive status in a woman?

……

32) Do you treat fertility in males, females or both?

Males { }
Females { }
Both { }

33) Do you use medical plants to treat **infertility**?

a) Yes { } b) No { }

If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? …….

iii) Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

34) If the plant is for enhancement of fertility what time of the menstrual cycle in females is the herb administered?

a) During menses { } b) Immediately after menses { } c) Mid cycle { }

d) Other (specify)……

35) How long does it take before the herbs are effective?

1 week { } 2 week { } 1 month { } 2 months { }

Other ……

36) The fertility enhancing effect is for what duration?

i) 3 months { } ii) 6 months { } iii) 9 months { } iv) One year { }

Other ……

37) Do you use medical plants to treat **excessive bleeding**? Yes { }No { }

38) If yes

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? …….

iii) Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

39) Do you use medical plants to treat **lack of menstruation**?

a) Yes { } b) No { }

40) If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? …….

iii) Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

41) Do you use plants to treat **irregular menses**? a) Yes { } b) No { }

42) If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? …….

iii) Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

43) Do you treat/ manage **painful menses**? Yes { } No { }

44) If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? ……...

iii) Was the patient referred to you?.....

Vernacular name	**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1				

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

45) Do you manage **retained after birth**? Yes { } No { }

46) If yes,

- How many cases were treated in the last month? .....
- Number of cases treated in the last six months? ……...
- Was the patient referred to you?.....

Vernacular name	**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1				

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

47) Do you manage **post-partum hemorrhage**? Yes { } No { }

48) If yes,

i. How many cases were treated in the last month? .....

ii. Number of cases treated in the last six months? ……...

iii. Was the patient referred to you?.....

Vernacular name	**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1				

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

49) What is the condition referred to as **chango**?

……

50) Do you treat change using **medical plants**? Yes { }No { }

51) If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? ……...

iii) Was the patient referred to you?.....

Vernacular name	**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)

			water?		
1					
2					
Route of administration (oral, topical, rectal, inhalation, bathed, etc.)		**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

52) Do you treat **male reproductive dysfunction** (upungufu wa nguvu za kiume)?

Yes { }No { }

53) If yes,

i. How many cases were treated in the last month? .....

ii. Number of cases treated in the last six months? ……...

iii. Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					
Route of administration (oral, topical, rectal, inhalation, bathed, etc.)		**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

54) How long can you keep the medicine before it goes bad?

……

55) What problems do you encounter in herbal medicine practice?

……

……

E: MEDICAL PLANTS USED TO TREAT STD'S

56) Do you treat **sexually transmitted infections**? Yes { } No { }

57) If yes, which ones?

i) ……. iv) ……

ii) ...……v) ……

iii) ……. vi) …….

58) For the above named illnesses,

Type of Illness	Signs/ Symptoms	Patients' complaints
a)		
b)		

59) Do you use medical plants to treat STD'S? Yes { }No { }

If yes,

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

60) How long can you keep the medicine before it goes bad?

……

61) How long does the patient take before feeling well? …….....

62) If the patient takes too much medicine, what happens?

……

63) What do you give to such a person?

……...

64) Do you treat **fibroids**?

Yes { }No { }

65) If yes,

i. How many cases were treated in the last month? .....

ii. Number of cases treated in the last six months? ……...

iii. Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

66) Do you treat **vaginal warts**?

Yes { }No { }

67) If yes,

i. How many cases were treated in the last month? .....

ii. Number of cases treated in the last six months? ……...

iii. Was the patient referred to you?.....

Vernacular name	**Plant part** (root, stem, leaves, bark,	**Method of preparation** (powder, boiled	**Form in which it is used** (powder, boiled,	**Habitat** (boundary marker, bush, crop field,

		tuber, fruit, etc.)	single, boiled mixture, soaked in water) How much of plant in how much water?	etc.)	compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

68) Do you treat **inadequate milk letdown**?

Yes { }No { }

69) If yes,

i. How many cases were treated in the last month? .....

ii. Number of cases treated in the last six months? ……...

iii. Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

70) Do you treat **mastitis**?

Yes { }No { }

71) If yes,

iv. How many cases were treated in the last month? .....

v. Number of cases treated in the last six months? ……...

vi. Was the patient referred to you?.....

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					
2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

72) Among the herbs you have mentioned: which one(s) has side effects (toxic)?

……

Plant: i) …….. Toxic signs (symptoms)…….

ii)…….. Toxic signs (symptoms) ……

iii) …….. Toxic signs (symptoms)…….

73) How do you treat the toxic effects?

Vernacular name		**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much water?	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)
1					

2					

Route of administration (oral, topical, rectal, inhalation, bathed, etc.)	**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

74) Do you use medical plants to **treat/ manage painful menses**?

Yes { }No { }

75) If yes,

i) How many cases were treated in the last month? .....

ii) Number of cases treated in the last six months? ……...

iii) Was the patient referred to you?.....

76) What plants have used to treat/ manage painful menses? Mention six in order of priority

i) ……

ii) ……

iii) ……

77) Is the medical plant used alone or as a concoction?

……

……

……

78) What is the method of preparation? **How much of plant in how much water or alcohol**?

……

79) What is the **dose level** and **duration** of medical plant administration?

……

……

80) Amongst the herbs you have mentioned: which one(s) has side effects (toxic)?

……

Plant: i) …….. Toxic signs (symptoms)……

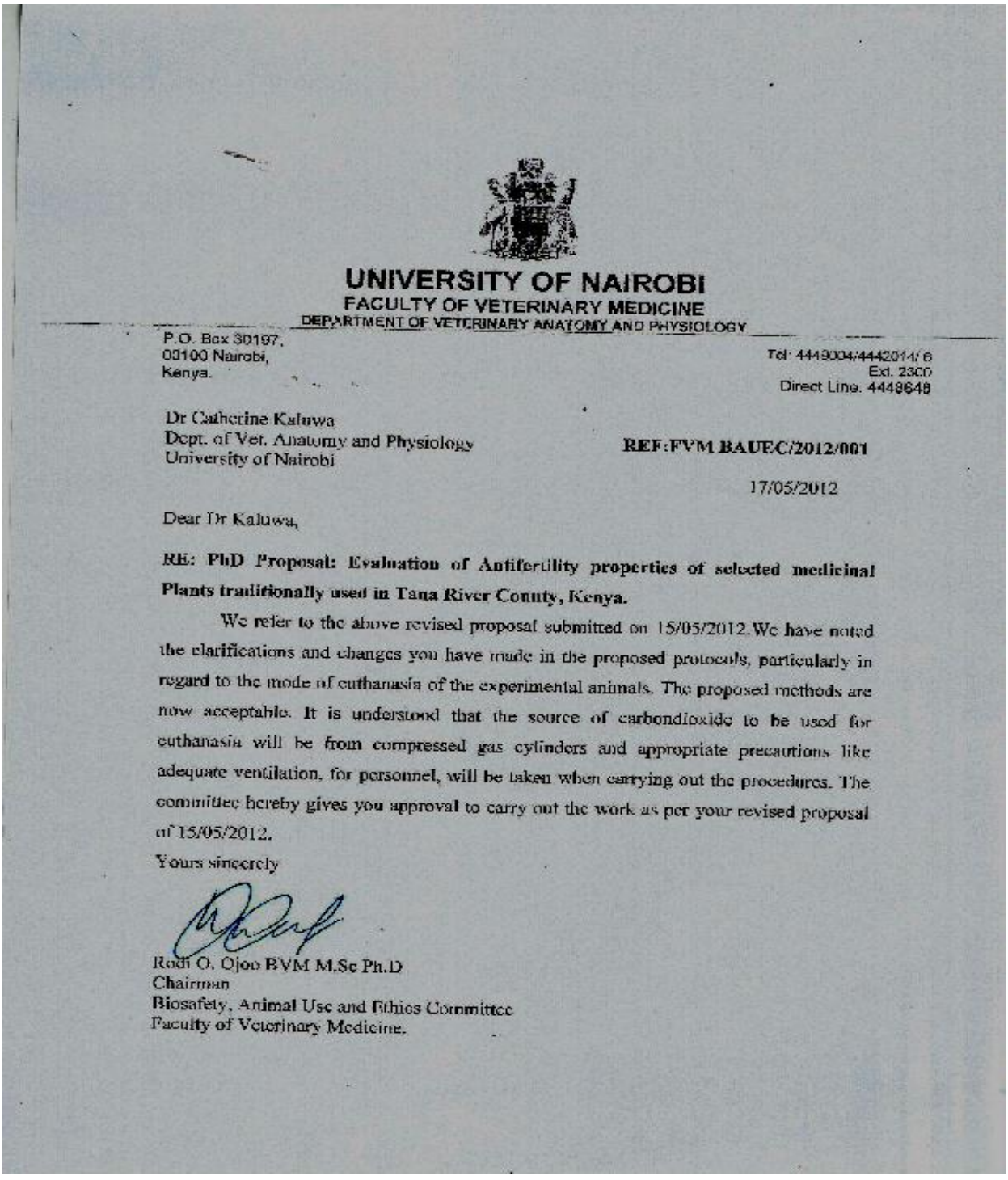
ii)…….. Toxic signs (symptoms) ……

iii) …….. Toxic signs (symptoms)……

Vernacular name	**Plant part** (root, stem, leaves, bark, tuber, fruit, etc.)	**Method of preparation** (powder, boiled single, boiled mixture, soaked in water) How much of plant in how much	**Form in which it is used** (powder, boiled, etc.)	**Habitat** (boundary marker, bush, crop field, compound)

			water?		
1					
2					
Route of administration (oral, topical, rectal, inhalation, bathed, etc.)		**Mixture (concoction) or Single**	**Dosage** (how much is given, after how long, for how long)	**Side effects reported/ Remedy**	**Availability of plant** (readily or not)

APPENDIX 2: BIOSAFETY, ANIMAL USE AND ETHICS CERTIFICATE



UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Dr Catherine Kaluwa
Dept. of Vet. Anatomy and Physiology
University of Nairobi

REF:FVM BAUEC/2012/001

17/05/2012

Dear Dr Kaluwa,

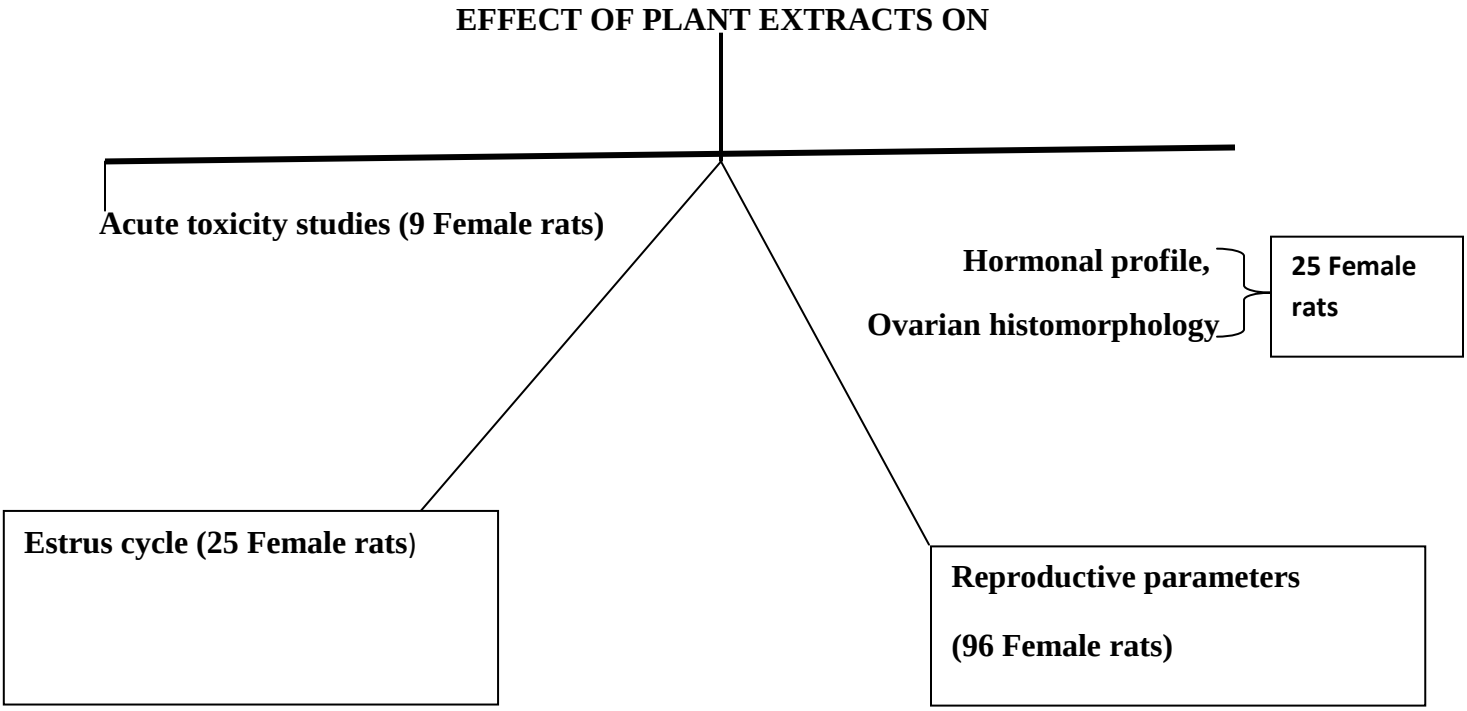
RE: PhD Proposal: Evaluation of Antifertility properties of selected medicinal Plants traditionally used in Tana River County, Kenya.

We refer to the above revised proposal submitted on 15/05/2012. We have noted the clarifications and changes you have made in the proposed protocols, particularly in regard to the mode of euthanasia of the experimental animals. The proposed methods are now acceptable. It is understood that the source of carbondioxide to be used for euthanasia will be from compressed gas cylinders and appropriate precautions like adequate ventilation, for personnel, will be taken when carrying out the procedures. The committee hereby gives you approval to carry out the work as per your revised proposal of 15/05/2012.

Yours sincerely

Rodi O. Ojoo BVM M.Sc Ph.D
Chairman
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

APPENDIX 3: STUDY DESIGN



APPENDIX 4: FEMALE REPRODUCTIVE HEALTH DSYFUNCTIONS PAPER

TANG INTERNATIONAL JOURNAL OF GENUINE TRADITIONAL MEDICINE

Original Article

Medicinal plants traditionally used for the management of female reproductive health dysfunction in Tana River County, Kenya

Catherine Kaluwa Kaingu[1,*], Jemimah Achieng Oduma[1], James Mucunu Mbaria[2], Stephen Gitah Kiama[1]

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Abstract

Reproductive dysfunction is a major health concern amongst the inhabitants of Tana River County. An ethno botanical study was conducted in Garsen, Itsowe and Ngao sub divisions of Tana River County to document the utilization of medicinal plants for the management of female reproductive ailments. The target population was practicing herbalists from Pokomo, Ormo and Giryama communities in the study area. Structured questionnaires and focussed group discussions were used to collect data. Forty eight plant species distributed in 40 genera and 29 families were documented as being important for the management of pregnancy related complications, menstrual disorders, infertility, fibroids and as contraceptives. The species most frequently cited by the herbalists were fourteen. Fifty two percent of the plant species were probably being mentioned for the first time as being useful in reproductive health management. In conclusion, Tana River has a pool of TMPs with a wealth of indigenous knowledge that needs to be exploited. The plants used to treat dysmenorrhea for example may be important analgesic agents that need further investigation while those with anti-fertility properties may contain steroidal phyto chemical compounds. Such species therefore need further investigation to establish their efficacy and mechanism of action.

Keywords medicinal plants, female reproductive ailments, Tana River, Kenya

INTRODUCTION

Herbal medicines have been used for the treatment of human ailments for thousands of years (Yakubu et al., 2007a; Yakubu and Bukoye, 2009). Recently, there has been renewed interest, spearheaded by World Health Organization (WHO), in the use of medicinal plants by traditional healers in Africa. This interest has led to increased research on traditional medicines.

Traditional medicine as practiced among various African societies is based on the concept that the cause of illness and disease or discomfort is sometimes ascribed to forces arising from angered ancestral spirits or evil spirits and witchcraft. Traditional medicine sees the supernatural as the cause of most major illnesses and factors of one's social and economic environment are all considered in diagnosing physical and mental problems in people's lives. Smaller medical issues however are handled with herbal remedies but even this is holistically applied whereby the whole plant, its physical characteristics like its aroma, taste, color and nutrient value, along with the rituals attending to its preparation and administration are just as important as its pharmacological content (Gessler et al., 1995; Okpako, 1999).

Traditional medicinal practitioners (TMPs) by their nature do not keep records and most of the knowledge they have is passed on verbally from generation to generation (Giday et al., 2010). There is therefore need not just to capture this indigenous knowledge but also to study the plants in order to provide credible evidence to support therapeutic efficacy claims by herbalists (Sofowora, 1993).

Reproductive issues and ailments constitute 18% of the global burden of disease for women of reproductive age and are the number one cause of maternal mortality in developing countries (WHO, 2003). Female reproductive ailments range from pregnancy and related complications, fertility issues and menstrual complications. In Tana River County, TMPs are routinely consulted because of their wide indigenous medicinal knowledge base (Swaleh, 1999), a tradition that has persisted in many rural communities due to inequitable health provision.

In Kenya, 75% of health facilities and personnel are concentrated in urban areas (National Policy of Traditional Medicine, 2005). The national doctor patient ratio is 1: 20,000; but in Tana River County with only 57 health facilities, the doctor: patient ratio is 1: 95,500 emphasizing a serious shortage of both health facilities and staff in the County (Tana River District Strategic Plan, 2005 - 2010). On the other hand, the ratio of TMP to patients is 1: 987 (Kenya Housing and Population report, 2009), suggesting that the TMPs are more readily accessible. In General, health sectors including reproductive health face a number of challenges. According to the Tana River District Strategic Plan (2005 - 2010); issues of major concern in reproductive health sector are; unsafe motherhood, high maternal/child mortality rates and inadequate family planning services.

An ethno botanical survey was carried out in Tana River County to identify and document the plants that are used by traditional herbalists for the management of female reproductive ailments and problems. The plant parts, route of administration, method of preparation, dose and whether the plant was administered as a decoction or concoction was documented.

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APPENDIX 5: MALE SEXUAL DYSFUNCTION AND INFERTILITY PAPER

The Journal of Ethnobiology and Traditional Medicine. Photon 119 (2013) 453-463
https://sites.google.com/site/photonfoundationorganization/home/the-journal-of-ethnobiology-and-traditional-medicine
Original Research Article. ISJN: 6642-3194

The Journal of Ethnobiology and Traditional Medicine Photon

Ethnobotanical Survey of Medicinal Plants Used For the Management of Male Sexual Dysfunction and Infertility in Tana River County, Kenya

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Keywords:
Male sexual dysfunction, infertility, Tana-River, Kenya

Abbreviations:
HSD: hypoactive sex drive, ED: erectile dysfunction

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Abstract
Sexual dysfunction afflicts 10% of men of all ages, ethnicities and cultural background. In Tana River County a large percentage of reproductive health ailments are managed by traditional healers. Unfortunately, in traditional medicine practice, there is no documentation and information is passed on verbally from generation to generation. The aim of this study therefore was to identify and document plants that are used for the management of male sexual dysfunctions and infertility in Tana River County. An ethno botanical survey was carried out using structured questionnaires. Nineteen plants belonging to 15 genera and 13 families were reportedly used to treat hypoactive sex drive, manage erectile dysfunction/ impotence and treat male infertility. The plant remedies described and documented in this study represent valuable baseline data on indigenous knowledge, upon which further research can be based. Future scientific research into the efficacy and safe use of the herbs could then prove very useful to herbal medicine practitioners and researchers and will contribute immensely towards future conservation efforts of both the plants and the indigenous knowledge.

Citation:
Kaingu C.K., Oduma J.A., Mbaria J.M., Kiama S.G., 2013. Ethnobotanical Survey of Medicinal Plants Used For the Management of Male Sexual Dysfunction and Infertility in Tana River County, Kenya. The Journal of Ethnobiology and Traditional Medicine. Photon 119, 453-463.

1. Introduction

Reproductive health is one of the most prevalent health care problems in Africa. However, advocates of reproductive health care have been focusing mainly on women and disregarding men. Thus, some diseases such as sexual impotence and erectile dysfunction that deserve mention have not been given due regard, to the detriment of families and societies as a whole (Kamatenesi-Mugisha and Oryem-Origa, 2005).

1.1 Male sexual dysfunction

Sexual dysfunction in males is manifested in several forms and can be classified as (i) arousal disorders; that encompasses erectile dysfuncion (Wagner, 1981), premature, retrograde, retarded or inhibited ejaculation (Metz et al., 1997), and failure of detumescence (prolonged priapism lasting over 4 hours) (Weidner et al., 1997), (ii) disorders of desire (low libido); which is the lack of sexual desire or interest in sex (Benet and Melman, 1995; Schiavi and Segraves,

APPENDIX 6: TRADITIONAL MANAGEMENT OF ILLNESSES PAPER

Asian Journal of Complementary and Alternative Medicine 02 (02); 2014; 01-05.

Asian Journal of Complementary and Alternative Medicine

ISSN: 2347-3894

RESEARCH ARTICLE

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QR Code for Mobile users

Conflict of Interest: None Declared !

Ethnobotanical study of medicinal plants traditionally used in Tana River County for management of illnesses.

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[b]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O Box 30197- 00100, Nairobi, Kenya.

ABSTRACT

Aim of the study: The objective of the study was to identify and document medicinal plants traditionally used by people of Tana River County, Kenya for the management of various ailments.

Materials and methods: The study was conducted in March 2012. Information was gathered from 80 traditional practitioners who lived and practiced in Garsen, Itsowe and Ngao Subdivisions of Tana River using semi-structured questionnaires and focused group discussion. Voucher specimen of cited plants were collected and deposited at the university of Nairobi herbarium.

Results: A total of 31 plants distributed in 25 families were identified. The most popular plant species were eleven and were used for the management of pneumonia, arthritis, kidney problems, fibroids, typhoid, breast cancer, tooth ache, malaria, diabetis, convulsions, stomach ache, constipation, poisoning, cholera, diarrhea, mastitis, migraine, tonsilitis, ulcers, asthma, high blood pressure, urinary incontinence, body warts, milk letdown and as immune boosters. Conclusion: The use of herbs is still very common amongst Tana River inhabitants and the healers still rely largely on naturally growing plant species in their locality. Furthermore, the documented medicinal plants can be used as a basis for future phytochemical and pharmacological studies.

Keywords: *medicinal plants, indigenous management of illnesses Tana River.*

Cite this article as:
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Ethnobotanical study of medicinal plants traditionally used in Tana River County for management of illnesses.
Asian Journal of Complementary and Alternative Medicine 02 (02); 2014; 01-05.

APPENDIX 7: TURNITIN ORIGINALITY REPORT

ANTI-FERTILITY POTENTIAL OF SELECTED MEDICINAL PLANTS OF TANA RIVER COUNTY, KENYA. by **Dr. Catherine Kaingu**

From PhD Thesis (Vet Anatomy and Physiology)

- Processed on 23-Aug-2016 13:20 EAT
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