# THE PROSPECTIVE OF USING PLANTS WITH ANTIFERTILITY EFFECTS IN CONTROLLING THE RODENT PEST, *MASTOMYS NATALENSIS*

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## EXTENDED ABSTRACT

The prolific nature of some rodents has been a significant threat to the livelihood and public health in sub-Saharan Africa escalating the use of rodenticides. Regrettably, rodent pests are increasingly becoming resistant to rodenticides due to bait shyness or resisting death. Also, the rodenticidal poison has been killing other untargeted species of animals. Thus, alternative methods of rodent control including the use of birth control methods are increasingly being explored. So, the current study investigated the antifertility potential of *Acacia nilotica* pods and *Albizzia lebbeck* stem bark extracts in the *Mastomys natalensis*. Specifically, the study was done to investigate the effect of the plant's extracts on the fertility success rate of female and male *M. natalensis*, to evaluate the effect of the plant's extracts on follicular and corpora lutea counts in the female rat's ovaries, to explore the effect of the plant's extracts on the sperm cells parameters and histomorphology of the testes in the male rats and to determine the phytochemical constituents of the two plants.

Pods of *A. nilotica* were collected from Kilwa and stem barks of *A. lebbeck* were collected from Morogoro urban, in Tanzania. The *M. natalensis* were collected from Sokoine University of Agriculture (SUA) owned farms. Experiments and Laboratory works were done at the College of Veterinary Medicine and Biomedical Sciences (CVBMS) at SUA. This thesis contains the research works done in the current study and is divided into three sections. Section one comprises introduction and literature review, problem statement, objectives, the rationale of the study, scope and limitations of the study. Section two encompasses the three manuscripts describing the research work done. The third section consists of the general conclusion and recommendation of the study.

The first manuscript assesses the pathophysiological significance of *A. nilotica* pods aqueous extract and *A. lebbeck* stem bark methanolic extract on the reproductive system of female *M. natalensis*. A total of 60 sexually mature rats were randomized into a 2 x 3 factorial design for treatments (Control *A. nilotica,* and *A. lebbeck*) and treatment duration (7 or 14 days). Control rats consumed basal feed, treated rats consumed feed with 2% w/w of either of the plant extracts. At the end of treatment duration, treated female rats were cohabited with males for 16 days and sacrificed 20 days after the first day of cohabitation. Parameters assessed at necropsy included the pregnancy rates, number of fetal implantations, possible resorption sites, and fetal litter size. Other evaluated parameters included the ovarian weights, follicular and corpora lutea numbers, and general histopathology of ovaries. Results showed that pregnancy percentages, the number of fetal implantations, and fetal litter size were significantly reduced ($p< 0.01$) in rats under the *A. nilotica* and *A. lebbeck* extract treatments relative to the control. The ovarian weights of rats receiving the extracts did not differ significantly from the control ($p > 0.05$). However, the number of corpora lutea of pregnancy was significantly reduced ($p < 0.001$) in the ovaries of rats under extract treatments than in the control. Instead, the ovaries of rats receiving the two extracts contained a larger number of atretic follicles, signifying halted ovulatory and conception activities. In conclusion, the study demonstrated that dietary inclusion of crude extracts from *A. nilotica* pods and *A. lebbeck* stem bark can lead to decreased fertility success rates in *M. natalensis* female rats through suppression of ovulatory activities and induction of follicular atresia.

The second manuscript evaluated the contraceptive potential of the methanolic extracts from *A. nilotica* pods and *A. lebbeck* stem bark in male *M. natalensis*. A total of 90 rats were randomized into a 3 x 3 factorial design for treatment (control, *A. lebbeck* and *A. nilotica*) ($n = 10$) and treatment durations (15, 30, or 60 days). Control rats consumed

plain feed. Treated rats consumed feed with 2% w/w of either of the plant extract. Following treatment, male rats were mated to untreated females before sedation in ether and sacrification. Assessments were done on fertility success rates (number of impregnated females), weights of testes and reproductive glands, sperm cell parameters, and testes histopathology. Results revealed that the fertility success rate was reduced to 0% in the *A. nilotica* treated rats at all the treatment durations and in the *A. lebbeck* treated rats after 60 days of treatment. Moreover, the extract-treated rats revealed a significant reduction in the testes, seminal vesicles, and epididymides weights. Also, sperm cell density and the proportions of live and progressively motile spermatozoa were significantly reduced and there were numerous seminiferous tubules with damaged and thinned germinal epithelium and widened empty lumen in the extract-treated rats. In conclusion, treatment with *A. nilotica* or *A. lebbeck* extract in male rats reduced their fertility success rates through distortion of testicular structure and disruption of spermatogenesis.

The third manuscript evaluated the phytochemical constituents of raw grounded materials and methanolic extracts of *A. nilotica* pods and *A. lebbeck* stem bark extracts and the potential effects the methanolic extracts may have on spermatozoa morphologies in the male *M. natalensis*. A portion of the grounded materials from each plant was extracted in 70% methanol. Both the grounded materials and corresponding methanolic extracts were assessed for the presence of phytochemicals with antifertility potentials. A total of 90 male rats were randomized into 9 groups based on treatment (control, *A. lebbeck* and *A. nilotica*) (n = 10) and extract treatment durations (15, 30, or 60 days). Results indicated that flavonoids, tannins, saponins, steroids, terpenoids and plant phenols were all present in the powdered raw materials and their corresponding extracts of both plants. However, saponin was shown to be more intensely present in the powdered and

methanolic extracts of the *A. lebbeck* compared to those of *A. nilotica* while the reverse was true for flavonoids and tannins. Spermatozoa with normal head-abnormal tail, normal head-tailless, abnormal head-normal tail, both abnormal head and tail spermatozoa were more revealed in extracts treated rats relative to the control. In conclusion, the studied pods of *A. nilotica* and the Stem bark of *A. lebbeck* are rich in flavonoids, tannins, saponins, steroids, terpenoids and plant phenols which possess some antifertility properties explaining the adverse effects of the plant's extracts on spermatozoa morphologies. The research information contained in this thesis contributes significantly to the limited available knowledge on the antifertility efficacies of medicinal herbal extracts in the wild rodents, more particularly the *M. natalensis*.

## DECLARATION

I Lusekelo Msomba Mwangengwa do hereby declare to the senate of Sokoine University of Agriculture that, this thesis is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a degree award.

_____ _____

Lusekelo Msomba Mwangengwa Date
(PhD Candidate)

The above declaration is confirmed by;

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Prof. Robert Max Date
(Supervisor)

_____ _____

Prof. Noel Kanuya Date
(Supervisor)

_____ _____

Dr. Gaymary Bakari Date
(Supervisor)

## COPYRIGHT

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Also in a very special way, I would like to thank my late mother Luthi Sisambi, and father Gideoni Msomba for raising me into whom I am and for educating me. Rest in peace my parents.

Although there have been several contributors enabling me to complete this work, all the views expressed in this thesis are mine and I remain personally responsible for any errors, omissions, inadequacy in the views and results presented, or any other shortfalls herein.

## DEDICATION

I dedicate this PhD work to my daughters Anna and Luth, my son Ezekiel and my late parents Luth Sisambi and Gideoni Msomba wherever they are.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PAPERS

Paper 1: Lusekelo M. Mwangengwa, Gaymary G. Bakari, Noel L. Kanuya and Robert A. Max (2021). Antifertility effects of crude extracts from *Acacia nilotica* pods and *Albizzia lebbeck* stem bark in female multimammate rats, *Mastomys natalensis. Journal of Physiology and Pathophysiology* 12(1): 1-10.

Paper 2: Lusekelo M. Mwangengwa, Gaymary G. Bakari, Noel L. Kanuya and Robert A. Max (2021). Effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the reproductive system of male *Mastomys natalensis*: An anti-fertility studies. *African Journal of Biological Sciences* 4(2): 106-119.

Paper 3: Lusekelo M. Mwangengwa, Gaymary G. Bakari, Noel L. Kanuya and Robert A. Max (2021). *A. nilotica* pods and *Albizia lebbeck* stem bark: Evaluating the plant extract's Phytochemical composition and their effects on spermatozoa morphologies in rats.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| µm | Micrometers |
| $^0$C | Celsius centigrade |
| 10µl | Microliter |
| AChE | Acetylcholinesterase |
| AL | *Albizzia lebbeck* |
| AN | *Acacia nilotica* |
| CASA | Computer Assisted Sperms Analysis |
| CM | Centimeters |
| COSTECH | Tanzanian Commission for Science and Technology |
| CVBMS | College of Veterinary Medicine and Biomedical Sciences |
| FSH | Follicle Stimulating Hormones |
| g | Gram |
| HPLC | High-Performance Liquid Chromatography |
| IRPM | Integrated Rodent Pest Management |
| Kg | Kilograms |
| kHz | Kilohertz |
| L | Liter |
| LDV | Laser doppler velocimetry |
| LFA | Large Folding Aluminum |
| LH | Luteinizing Hormones |
| MEP | Multiple exposure photomicrography |
| Mg | Milligrams |
| Ml | Milliliters |
| PBS | Phosphate Buffer Solution |

| | |
|---|---|
| PH | Logarithm of the reciprocal of hydrogen ion concentration |
| SUA | Sokoine University of Agriculture |
| TEP | Timed exposure photomicrography |
| WHO | World Health Organization |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 General Introduction

One of the reasons for the growing interests in animal contraception includes the need to develop a novel way of controlling the population of rodent pests in the field. The context is, during an outbreak, rodent pests have been a great threat to the livelihood, causing substantial destruction of food crops and properties besides their involvements in the transmission of some zoonotic diseases.

Among the rodent species with significant economic and public health impacts in sub-Saharan Africa includes the *Mastomys natalensis* (multimammate rat). The *M. natalensis* is a small-sized species of rodent of the Muridae family and genus Mastomys. The rodent species is widely distributed in sub-Saharan Africa, covering a wide range of habitats from the West, East, Central to the South African countries (Fichet-Calvet, *et al*., 2008; Colangelo *et al*., 2013; Borremans *et al*., 2015).

Normally, the multimammate rats are highly prolific and their eating behaviors make them compete greatly for grain crops with human beings (Mwanjabe *et al*., 2002; Swanepoel *et al*., 2017). Also, the rodent pests are highly involved in the transmission of some deadly zoonotic disease pathogens (Lecompte *et al*., 2006; Andrianaivoarimanana *et al*., 2013; Mgode *et al*., 2014) increasing the need for their control.

The abundance of preferred foods, mostly grain seeds, is one of the vital factors enabling the *M. natalensis* to reproduce rapidly in large numbers in their habitats (Mulungu *et al*., 2013). As a result, during an outbreak the numbers of multimammate rats are normally

large enough to inflict significant damage to livelihood and public health (Mwanjabe *et al*., 2002; Lecompte *et al*., 2006).

Anticoagulant rodenticides have been the most reliable tool for rodent pest management in sub-Saharan Africa (Mulungu *et al*., 2010). Unfortunately, at the peak of the rodent's reproductive periods, the population of some rodent species including the *Mastomys natalensis* can be large enough to overcome even the most effective anticoagulant rodenticides (Mulungu *et al*., 2010; 2013). Moreover, on top of killing the rodents of targets, rodenticides can kill other species of animals which were not the target in the environment (Brakes and Smith, 2005; Thomas *et al*., 2011; Smith and Shore, 2015; Elliott *et al*., 2016).

Furthermore, rodent pests are increasingly showing resistance to rodenticides by avoiding eating the familiar poisoned baits ("bait shyness") (Saxena, 2014), or resisting being killed by the consumed rodenticides (Philipe *et al*., 2018; Marquez *et al*., 2019). That behavior is of great concern as it risks evolving a generation of rodent pests that will become resistant to most of the rodenticides available in the future. Moreover, some anticoagulant rodenticides kill after inflicting prolonged suffering on rodents, contravening animal welfare requirements (Watt *et al*., 2005; Fisher *et al*., 2019).

All the above information, entices the need to search for some innovative methods of rodent control that are both effective and environmentally friendly. Among the methods worth exploring could be those that make rodent pests reproduce less (Contraception). The science behind contraception in rodent pests is supported by the fact that rats or mice are easily adversely affected by any bad influences on their reproductive system (Cheng and Mruk, 2002). That means any pathological insults of natural or artificial

sources in the reproductive tracts of rats or mice could easily contribute to infertility (Davis *et al*., 2001; Asuquo, 2012; Reddy *et al*., 2015). Among the chemical or biochemical agents with some reported antifertility actions in rats or mice include certain synthetic chemical hormones (Zhao *et al*., 2007; Su *et al*., 2016), certain immunological agents (Paterson *et al*., 2000; Massei *et al*., 2020) and some plant extractives (Devi *et al*., 2015).

While both the immunocontraception and synthetic steroid hormone have been very effective in rodents' fertility supression in Laboratory studies (Massawe *et al*., 2018; Massei *et al*., 2020), none of the methods have been applied in the real field situation of rodents' control. There are no known application methods of the immunocontraceptives to the animals of targets in the field situation delaying their deployments (Barlow, 2000). Moreover, there are worries that any accumulation of synthetic steroid hormones in the environment could spark widespread damage to other untargeted species of animals (Smith and Shore, 2015; Elliott *et al*., 2016; Shore and Shemesh, 2016; Adeel *et al*., 2017; Houtman *et al*., 2018). Thus, efforts are still in high demand to probe for new, effective, and safe contraceptive sources of rodent pests as an alternative to the environmentally unfriendly chemical rodenticides and steroid hormones. In that context, medicinal herbs with antifertility properties could be among the potential alternative sources of contraceptives for rodent pests. So far, extensive research works have been done in the field of medicinal plant research and the antifertility actions of several plant species have been revealed in both sexes of some mammals (Devi *et al*., 2015). In male mammals, the herbal product have been shown to perturb the hypothalamus-pituitary-testis hormonal secretory axis (Joshi *et al*., 2011; Asuquo, 2012) hence inducing some structural and functional disorganizations in the testis and accessory reproductive glands (Verma *et al*., 2002; Gupta *et al*., 2004; Raji *et al*., 2006; Asuquo, 2012; Lampiao, 2013).

In female mammals, the plant extracts antifertility actions involve disruption of the estrous cycles, perturbation of folliculogenesis, and stimulation of follicular atresia (Circosta *et al*., 2001; Raj *et al*., 2011; Ngadjui *et al*., 2013). Other plant antifertility mechanisms of action in female animals include suppression of ovulation (Malashetty and Patil, 2007; Raj *et al*., 2011) anti-embryo implantation (Raj *et al*., 2011), disruption of hormonal synthesis, release, and functions (Dokhanchi *et al*., 2013).

Furthermore, once are proven to be effective, medicinal plants could probably serve as an environmentally friendlier source of rodents' birth control pills. The reason is that medicinal herbs are part of the nature making them decompose more rapidly in the environment. Also, medicinal herbs are plenty in the wildlife and they can be made available in large amounts at cheap costs through cultivation in gardens or farms (Silori and Badola, 2000).

More importantly, species of medicinal herbs with antifertility potential are not that scarce in sub-Saharan Africa. Among the locally available antifertility plant species in the region include *Acacia nilotica* (Lampiao, 2013), commonly known as gum Arabic tree, babul, thorn mimosa, Egyptian *acacia*, or thorny *acacia* (Verma, 2016). In traditional medicine, various plant components of *A. nilotica* have been used for the treatment of various ailments (Verma, 2016). Another plant is *Albizzia lebbeck*, also grouped as Fabaceae. The plant species is native and widely distributed in the tropical regions of Africa, Asia, and Northern Australia (Gupta *et al*., 2004; Verma *et al*., 2013). Besides their uses in traditional medication, the extractives from *Acacia nilotica* and *Albizzia lebbeck* have been reported to have some antifertility properties (Gupta *et al*., 2004; Lampiao, 2013). It is therefore of most importance to know whether the plant

species with antifertility actions could be efficacious enough to work as a contraceptive source for rodent pests.

## 1.2 Description of *M. natalensis*

*Mastomys natalensis,* also known as multimammate rats, the common African rats, or the African soft-furred mouse, is a rodent species in the Muridae family and genus *Mastomys*. The rodent species has a body length of 10 to 15 cm and a tail length of 8-15 cm. The adult rat's body colour of furs and eyes is darkish brown. Females *M. natalensis* has many mammary glands ranging from 8 to 24, hence the name multimammate rat (Meester, 1960). In captivity, *M. natalensis* has been shown to live up to 3 years (Donnelly *et al*., 2015) and at birth, the young rats may have up to 2.2g of mean body weight with the adult's body weight ranging from 20 to 80 grams (Meester, 1960).

**Figure 1.1: Description of the multimammate rat (*Mastomys natalensis*)**

### 1.3 Reproductive Behavior of *M. natalensis*

The *M. natalensis* are naturally highly prolific ranking them among the most copious and rampant rodent pests in sub-Saharan Africa (Dlamini *et al*., 2011; Swanepoel *et al*., 2017; Mayamba *et al*., 2019). The population of *M. natalensis* shows the tendency of varying with seasons (Mulungu *et al*., 2010; 2013). The changing population density of the rodent pests has been associated with the changing breeding patterns largely influenced by rainfall intensities and the farming spells of grain crops (Makundi *et al*., 2007; Mulungu *et al*., 2010 ; 2013).

Normally, female *M. natalensis* are the key determinants of the pest species generation succession. Because of that, the reproductive behavior of the female *M. natalensis* has been well studied in captivity by some Zoologists (Johnston and Oliff, 1954; Meester 1960). The female rodent pests' high prolific nature is due to their reproductive behavior including their short estrous cycle length of 8.8±0.4 days (Johnston and Oliff, 1954; Meester, 1960). Also, a short gestation length of 23 ± 10.7 days, and a large number of pups averaging 8 with a maximum of 17 pups being reported in some births (Johnston and Oliff, 1954; Meester, 1960). The rodent’s age at the perforation of the vulva is 76±5.9 days (Johnston and Oliff, 1954; Meester, 1960).

Moreover, weaning of mothering *M. natalensis* occurs mostly from 21 days post-delivery, the littering interval is 33.1 days (Johnston and Oliff, 1954; Meester, 1960) and post-partum estrous occurs after 2.6±0.52 days (Johnston and Oliff, 1954; Meester, 1960). The mean age of female *M. natalensis* at first estrous is 104±9.2 days and their average age at first birth is 130 days (Johnston and Oliff, 1954; Meester, 1960).

## 1.4 Habitat Range, Distribution and Feeding Habits of *M. natalensis*

The distribution of *M. natalensis* in sub-Saharan Africa is vast covering almost the whole sub-continent from the West, East, Central to the South African countries (Colangelo *et al*., 2013) (Fig. 2). Moreover, the *M. natalensis* prefer living close to human settlements in its habitat. However, a large number of the rodent pests have been reported in the subtropical and tropical dry forests, subtropical and tropical moist lowland forests, dry and wet savanna (Granjon *et al*., 1997; Lalis *et al*., 2006; Colangelo *et al*., 2013). Likewise, the dry or moist shrublands of the subtropical and tropical or arable land, pastureland, rural gardens, urban areas, irrigated land, and seasonally flooded agricultural landscapes are good habitats of *M. natalensis* (Granjon *et al*., 1997).

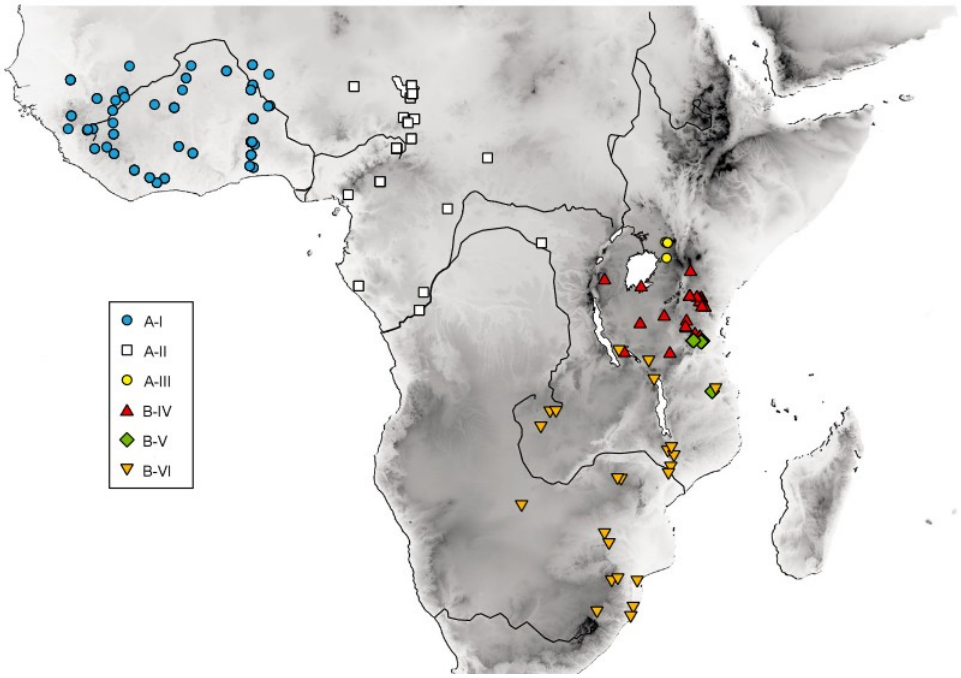


**Figure 1.2: Distribution by phylogroups (A-I, A-II, A-III, B-IV, B-V, B-VI) of the multimammate rats (*M. natalensis*) in sub-Saharan Africa (Colangelo *et al*., 2013).**

More importantly, the population of *M. natalensis* is very dynamic. In East African countries, the rodent population has been reported to start increasing in numbers from early March or April coinciding well with the maturation of grain crops (Mulungu *et al*., 2013; Dlamini *et al*., 2011). The rodent pest populations have been reported to attain pickiness during the mid dry season in July to August or September, in which most of the grain crops have been harvested (Makundi *et al*., 2007; Dlamini *et al*., 2011; Mulungu *et al*., 2013). Therefore, it certainly shows that, feeds types and availability plays a great role to influence the dynamicity of the *M. natalensis* population in their natural habitats (Mulungu *et al*., 2013).

Normally, the *M. natalensis* prefer to eat grain seeds of grasses, millet, corn, maize, and rice as the energy source foods besides their hunt for insects to supplement protein requirements (Iwuala *et al*., 1980; Massawe *et al*., 2011). As a result, the *M. natalensis* are normally found in abundance in the glass lands close to the grain seed crops and farmlands with grain crops raising a great competition for food with human beings (Mulungu *et al*., 2013).

### 1.5 *M. natalensis* in Crop Losses and Transmission of Zoonosis

Outbreaks of *M. natalensis* have been a consistently great threat to public health and household income in sub-Saharan Africa. The perspective is, a large number of rodent pests do occur in the period of population explosions. At large numbers, the rodent pests have been shown to inflict great economic loss on farmers by destroying their food crops, clothing, and building materials, plus the costs involved in controlling the rodents (Skonhoft *et al*., 2006; Mdangi *et al*., 2013; Makundi *et al*., 1991). For instance, some recent estimations of rodents-caused crop destruction in some African countries indicate a rodent caused loss of over 46% of grain crops during the seedling, 15% before harvest,

and 7.9% in stores (Makundi *et al*., 1991; Mdangi *et al*., 2013; Swanepoel *et al*., 2017). Further estimation done in Tanzania indicated a rodent caused yield loss of maize alone of 5 to 15%, amounting to an average of nearly 412.500 tons of maize loss per year (Makundi *et al*., 2010).

Moreover, the *M. natalensis* outbreaks have been contributing greatly to the public health instability due to the rodent pest's involvement in hosting and transmitting some deadly zoonotic disease pathogens. The rodent's transmitted pathogens include those which cause Lassa fever (Lecompte *et al*., 2006), plague (Ziwa *et al*., 2013) and leptospirosis (Mgode *et al*., 2014). Lassa fever is a rodent transmitted hemorrhagic disease with a morbidity rate of up to 26% and a mortality rate of up to 2%, as reported in some Western African countries (McCormick *et al*., 1987). Lassa fever is caused by Arenaviruses (Lecompte *et al*., 2006) and is transmitted from rodents to human beings through direct contact with the blood, urine, and fecal materials of the infected animals (Lecompte *et al*., 2006; Bonwitt *et al*., 2017).

Leptospirosis is another deadly rodent-borne bacterial disease that affects human beings and animals (Mgode *et al*., 2014; Schoonman and Swai, 2009). Leptospirosis is caused by some pathogenic Leptospiral species which infect human beings through direct contact with the urine of the infected animals or in a urine-contaminated environment (Mgode *et al*., 2014). Leptospirosis is still a burden in some sub-Saharan African countries (Mgode *et al*., 2014) and a prevalence of over 15% in the screened human population has been reported in some areas in Tanzania (Schoonman and Swai, 2009; Mgode *et al*., 2019). In endemic areas, the magnitude of Leptospirosis shows a pattern of increasing sharply after every heavy rainfall yearly coinciding well with an increasing population of rodent pests (GhizzoFilho *et al*., 2018).

Plague is another rodent-borne zoonotic disease caused by *Yersinia pestis*. The disease is normally transmitted to human beings through a bite by fleas carried by rodent pests (Ziwa *et al*., 2013). In Tanzania, the disease has been endemic in Handen and Karatu (Ziwa *et al*., 2013) where some deaths have been reported every time there is an outbreak. Several rodent species, including the *M. natalensis*, have been the potential hosts of the fleas which transmit plague to human beings (Ziwa *et al*., 2013).

### 1.6 Control Measures of M. natalensis in Sub-Saharan Africa

Chemical rodenticides and traps have been the backbone of rodent pest control techniques in sub-Saharan Africa. Other methods such as biological, ecological, and integrated rodent pest management strategies are available but rarely employed in the real practices of rodents population management in the region (Mulungu *et al*., 2010).

Chemical rodenticides are synthetic chemical poisons that can kill various rodent species, including rats, mice, squirrels, woodchucks, chipmunks, porcupines, nutria, and beavers. Anticoagulant rodenticides are the most commonly used chemical rodenticides in sub-Saharan Africa (Mulungu *et al*., 2010). Anticoagulant rodenticides are of two categories; the first and second generation (Fisher *et al*., 2019; Frankova *et al*., 2019). The first generation includes the long-acting anticoagulant rodenticides requiring multiple doses to kill the rodents (Watt *et al*., 2005). Long-acting anticoagulant rodenticides include; warfarin, pindone, and coumatetralyl (Watt *et al*., 2005). Second generation anticoagulants rodenticides require only a single dosage to cause the acute toxic effect in the animals and include; difenacoum, bromadiolone, brodifacoum, and flocoumafen (Watt *et al*., 2005). Anticoagulant rodenticides kill the animals by obstructing the hemostatic process, leading to internal bleeding and death (Watt *et al*., 2005).

Despite their importance in rodents' population management in sub-Saharan Africa, anticoagulant rodenticides have been with several undesirable shortfalls as well. Long-acting anticoagulant rodenticides tend to kill brutally by inflicting prolonged suffering on rodents before their death (Watt *et al*., 2005; Nattrass *et al*., 2019). Also, rodent pests are increasingly avoiding eating familiar (previously encountered) poisoned baits (bait shyness) hence impeding the efficiency of rodenticides (Saxena, 2014). Moreover, some rodent pests are gradually becoming rodenticide resistant (Marquez *et al*., 2019). That is a major concern as it risks evolving a rodenticide-resistant pest generation that could potentially pass its genes to subsequent generations (Phillipe *et al*., 2018; Marquez *et al*., 2019). Moreover, any contamination of chemical rodenticides in the environment could easily harm or kill other untargeted species of animals (Brakes and Smith, 2005; Smith and Shore, 2015).

The biological control methods of rodent pests include the use of pathogens, cats, dogs, and predatory birds to control the intended pest animals. However, only cats have been practically used to control rats and mice in some household settings in sub-Saharan Africa (Nattrass *et al*., 2019). Nevertheless, using cats in large rodent pest-infested fields could be practically impossible.

Under integrated rodent pest management (IRPM), several rodent pest management practices are normally applied collectively to achieve better results in rodents population management than when those methods are used separately (Desoky, 2018). The ecologically-based management strategy of rodent pests is a technique that involves gathering some key preliminary ecological information on the behavior, population dynamics, and distribution of rodent pests before deciding on the appropriate control measures (Singleton *et al*., 2004; Makundi *et al*., 2006). When implemented correctly, the

integrated rodent pest management (IRPM) and ecologically-based rodent control strategies are rated to be very effective in controlling the rodent pests population (Singleton *et al*., 2004; Makundi *et al*., 2006). However, the two methods require a high level of knowledge which is mostly unavailable to farmers, especially in developing countries.

### 1.7 Potential Alternative Methods of Rodent Pests Managements

The prolific and abundant nature of *M. natalensis* species has been a tool giving them a survival advantage against even the most effective control measures (Mulungu *et al*., 2010). Therefore, recent research efforts are geared to consider exploring the techniques that will make rodent pests reproduce less (Stenseth *et al*., 2001). Examples of the contraceptive methods that are still under investigation include the use of immunocontraception (Paterson *et al*., 2000; Massei *et al*., 2020), synthetic steroid hormones (Massawe *et al*., 2018), and plant extractives with antifertility properties (Tran and Hinds, 2013).

In immunocontraception, certain immune-contraceptives are administered in animals to destroy specific membrane proteins on the spermatozoa or oocytes, leading to infertility (Paterson *et al*., 2000; Massei *et al*., 2020). Despite being an effective antifertility technique in laboratory studies (Massei *et al*., 2020), the immunocontraception approach has never been applicable in real field situations of rodent's control. The reason is that there are no known delivery methods of the immune vaccines to the animals of targets in the field environment (Cooper and Larsen, 2006).

The antifertility potential of synthetic steroid hormones, such as levonorgestrel, mifepristone, quinestrol, and diethylstilbestrol, has been well studied in rodent pests and results have been promising (Zhao *et al*., 2007; Su *et al*., 2016; Massawe *et al*., 2018). However, the endocrine disruptive nature of synthetic steroid hormones has been a major concern constraining its application in the environment. It is well argued that any accumulation of synthetic steroid hormones in the environment could easily cause some harm to other untargeted species of animals including human beings (Shore and Shemesh, 2016; Adeel *et al.*, 2017; Houtman *et al*., 2018). However, very recent studies indicate that when properly managed, remnants of synthetic steroid hormones are easily degradable hence have less adverse effects in the environments (Zhang *et al*., 2020; Chiang *et al*., 2020). Yet, still, the technology involving synthetic steroid hormone could probably be costly and unaffordable to many of the end users in the lower income countries due to the likely high costs of production.

## 1.8 The Antifertility Potential of Medicinal Plant Preparations

Medicinal plant preparations are increasingly gaining popularity in the modern world of medicine to supplement the available conventional drugs and chemicals used in humans and animals. For instance, medicinal herbs with antifertility properties are increasingly being considered to supplement or replace conventional drugs for birth control pills in human beings (Devi *et al*., 2015). Therefore, the antifertility effects of several plant species have been well studied in some animal models including rabbits, rats, and mice aiming to study their contraceptive potential (Tran and Hinds, 2013; Devi *et al*., 2015). Examples of antifertility plants, their extractives, and antifertility mechanism of action have been outlined below.

### 1.8.1 Medicinal herbal extracts with anti-ovulatory actions

Some plant preparations cause anti-ovulatory activities as their antifertility mechanism of action when they are treated in female rats or mice (Raj *et al*., 2011; Jain *et al*., 2015). Such plant extracts are reported to contain phytoestrogen and other phytocompounds capable of disrupting the oestrous cycle and blocking the ovulatory process when treated in female mammals (Raj *et al*., 2011; Jain *et al*., 2015). Examples of plant extractives with anti-ovulatory activities include the following:

- Petroleum ether extracts of *Polygonum hydropiper* (Hazarika and Sarma, 2007),
- Aqueous extracts of *Calotropis procera* roots (Circosta *et al*., 2001),
- Ethanolic and benzene extracts of *Momordica charantia* seeds (Sharanabasappa *et al.*, 2002),
- Ethanolic extracts of *Azadirachta indica* flower (Gbotolorun *et al*., 2008),
- Ethanolic extracts of *Crotalaria juncea* seeds (Malashetty and Patil, 2007),
- Methanolic leaf extract of *Cissampelos pareira* (Ganguly *et al*., 2007),
- Aqueous extracts of *Garcinia kola* seeds (Abu *et al*., 2013),
- Aqueous extracts of *Aspilia africana* leaves (Oyesola *et al*., 2010),
- Aqueous extracts of *Cnidoscolous aconitifolius* leaves (Adeleke *et al*., 2016),
- Ether extract of *Plumbago rosea* stems (Sheeja *et al*., 2008), the petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of *Plumbago zeylanica* (Edwin *et al*., 2009) have anti-ovulatory actions.

### 1.8.2 Medicinal plants with anti-implantation activities

Some herbal extracts have anti-implantation effects as a mechanism that leads to infertility when they are treated in female rats or mice (Raj *et al*., 2011; Shaik *et al*., 2017). A few examples outlined here include;

- Benzene extracts of *Hibiscus rosa sinensis* roots (Vasudeva and Sharma, 2008),
- Extracts of *Butea monosperma* roots (Sharm *et al*., 2012),
- Aqueous extract of *Ocimum sanctum* leaves (Poli and Challa, 2019),
- Ethanolic, aqueous, and chloroform extracts of *Striga orobanchioides* (Hiremath *et al*., 1994),
- Aqueous and ethanolic leaves extracts of *Adhatoda vasica* (Nath *et al*., 1992),
- Ethanolic extracts of *Ailanthus excels* leaves, stem, and barks (Tamboli and Konadawar, 2013),
- Ethanolic extracts of *Momordica cymbalaria* roots (Koneri *et al*., 2006; 2007)
- Ethanolic extract of *Calotropis procera* roots (Circosta *et al*., 2001), the *Crotalaria juncea* seeds extracts (Malashetty, 2004; Malashetty and Patil, 2007)
- Ethanolic extracts of *Lawsonia inermis* leaves (Agunu *et al*., 2011),
- Ethanolic extracts of *Mentha arvensis* leaves (Sharma and Jocob, 2001),
- The methanolic extracts of *Polygonum hydropiper* roots (Hazarika and Sharma, 2007) and the petroleum Ether Extract of *Cassia fistula* seeds (Yadav and Jain, 2009).

### 1.8.3 Medicinal plants with antispermatogenic activities

The extracts of some plant species execute their antifertility actions by disrupting the process of spermatogenesis in male rats or mice. A few examples of such plant extractives include;

- Methanolic roots extract of *Plumbago zeylanica* (Purohit *et al*., 2008),
- Aqueous extracts of *Piper nigrum* fruits or black pepper (Mishra and Singh, 2009a).
- Ethanolic *extracts of Azadirachta indica* seeds and leaves (Bansal *et al*., 2010),
- Methanolic extracts of *Baleria prionitis* roots (Singh and Gupta, 2016),

- Methanolic extracts of *Tinospora cordifolia* stem (Gupta and Sharma, 2003),
- Aqueous extract of *Leptadenia hastata* (Bayala *et al.*, 2011),
- Methanolic extract of *Dendrophthoe falcata* stem (Gupta *et al*., 2008),
- Ethanolic extract of *Cannabis sativa* seeds (Sailani and Moeini, 2007),
- Ethanolic extract of *Curcuma longa* rhizome (Mishra and Singh, 2009b),
- Ethanolic extract *Mondia whitei* root (Watcho *et al*., 2001),
- Ethanolic extract of *Enicostemma axillare* leaves (Dhanapal *et al*., 2012),
- Methanolic extracts of *Ricinus communis* seeds (Maurya *et al*., 2004),
- Ethanolic extract of *Spondias mombin* leaf (Asuquo, 2012),
- Ethanolic extracts of *Sarcostemma acidum* (Verma *et al*., 2002).

### 1.8.4 Herbal products with adverse effects on reproductive hormones

Some plants have high levels of phytoestrogens and other plant sterols with antifertility actions (Burton and Wells, 2002; Qasimi *et al.*, 2017). When consumed by animals, phytoestrogens work by mimicking the function of body steroids (Burton and Wells, 2002; Qasimi *et al.*, 2017). Phytoestrogen can bind to the same receptors aimed for the natural body's estrogen thereby causing multiple oestrogenic effects including a disruption in the secretory axis of the hypothalamus, pituitary glands, and the gonads (Burton and Wells, 2002; Qasimi *et al.*, 2017). Suppression of the pituitary-gonadal-hormonal axis can in turn contribute to several reproductive abnormalities including disruption of gametogenesis (Burton and Wells, 2002; Qasimi *et al.*, 2017). Moreover, several research works indicate that phytoestrogens may cause infertility by blocking the process of ovulation, altering the rhythm of the oestrous cycle, or inducing some functional and morphological defects in the gonads (Sharanabasappa *et al*., 2002; Hazarika and Sarma, 2007; Jerald *et al*., 2012). Examples of the plants and plant preparations with anti-oestrogenic activities include;

- Methanolic extracts of Q*uassia amara* (Faisal *et al.,* 2006),
- The crude extracts of *Barleria prionitis* roots (Singh and Gupta, 2016),
- Methanolic extract of *Ricinus communis* seed (Raji *et al.,* 2006),
- Ethanolic extract *Damask rose* petals (Jahromi *et al.,* 2016),
- Aqueous extract of the heart of palm (Palmito) (Dokhanchi *et al.,* 2013),
- Aqueous extract of *Garcinia kola* seed (Abu *et al.,* 2013).
- Ether extract of C*assia fistula* Seeds (Yadav and Jain, 2009),
- Ethanolic extracts of *Hibiscus rosa-sinensis* roots (Vasudeva and Sharma, 2008),
- Petroleum ether, benzene, chloroform, and alcohol extracts of *Momordica charantia* seed (Sharanabasappa *et al.,* 2002).
- Methanolic extracts of *Cuminum cyminum* seed (Shirke and Jagtap, 2009),
- Ethanolic extract of *Nelumbo nucifera* seeds (Mutreju *et al.,* 2008).

### 1.8.5 Medicinal plant extracts with abortifacient actions

Some herbal extracts work by causing abortions as their antifertility mechanism of action when they are treated in successfully mated female mammals (Rajeswari and Rani, 2014). Examples of such abortifacient plant preparations include;

- Ethanolic extract of *Alstonia scholaris leaves* (Choudhary *et al*., 2017),
- Ethanolic extracts of *Spondias mombin's* leaves (Asuquo *et al*., 2013),
- Aqueous extracts of *Coriandrum sativum* seeds (Al-Said *et al*., 1987),
- Ethanolic extracts of *Moringa oleifera* leaves (Sethi *et al*., 1988),
- Ethanolic extracts *Marsdenia tinctoria* (Chowdhury *et al*., 1994),
- Methanolic extract of *Anthocephalus cadamba* stem bark (Shaikh *et al*., 2015),
- Ethanolic extracts of *Alangium salviifolium* stem bark (Murugan *et al*., 2000),
- Ethanolic extracts of *Derris brevipes* root powder (Badami *et al*., 2003),
- Ethanolic extract of *Momordica cymbalaria* roots (Koneri *et al*., 2006; 2007),

- Methanolic leaves extract of *Achyranthes aspera* (Shibeshi *et al*., 2006),
- Methanolic root extract of *Plumbago rosea* (Zubaid *et al*., 2007).

## 1.9 Morphological Description and Medicinal Use of *Albizzia lebbeck* and *Acacia nilotica*

*Acacia nilotica* and *Albizzia lebbeck* are among the tree species largely used in traditional medicine (Rajvaidhya *et al*., 2012; Dorostkar, 2015; Verma, 2016).


**Figure 1.3: Picture of *A. nilotica* and *A. lebbeck***

*Acacia nilotica* is a plant species in the Fabaceae family that is widely distributed in the tropical and subtropical regions (Rajvaidhya *et al*., 2012; Dorostkar, 2015; Verma, 2016). *A. nilotica* is a perennial tree with a height ranging from 2 to 20 m. The plant has a thick spherical crown with black coloured stems and branches and grey to pinkish slash fissured bark, exuding reddish, low-quality gum. Moreover, the plant has straight, light, thin, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without thorns (Rajvaidhya *et al*., 2012).

The medicinal value of *A. nilotica* is due to their anti-inflammatory, antioxidant, antidiarrhoea, antihypertensive and antispasmodic, antibacterial, anthelmintic, antiplatelet aggregatory, anticancer, and acetylcholinesterase (AChE) inhibitor properties making them useful in traditional medicines (Rajvaidhya *et al*., 2012; Dorostkar, 2015; Saeedi *et al*., 2020). The active phytochemical constituents in the *A. nilotica* include tannins, Saponin, flavonoids, alkaloids, fatty acids, and polysaccharides (gums) (Rajvaidhya *et al*., 2012).

*A. lebbeck* is a deciduous, perennial and medium-sized legume tree native to Asia, Northern Australia, and Western Africa (Orwa *et al*., 2009; Lado *et al*., 2020). *A. lebbeck* is a large tree that can grow to a height ranging from 3 to15 m in plantations and up to 30 m in open spaces (Orwa *et al*., 2009). Moreover, the plant has a dense shade-producing crown, which can be as large as 30 m in diameter (Orwa *et al*., 2009). Leaves of *A. lebbeck* are bipinnate with 3-11 pairs of bright green, oblong leaflets, 1.5-6.5 cm long x 0.5-3.5 cm broad. Also, the plants have globular clusters of about 15-40 white fragrant inflorescences flowers (Orwa *et al*., 2009). The fruits of *A. lebbeck* are 10-30 cm long x 3-6 cm broad, reddish-brown pods that contain 5-15 flat rounded, free moving seeds (Orwa *et al*., 2009; Verma *et al*., 2013).

The pharmacological potential of *A. lebbeck* is due to their anti-asthmatic, anti-inflammatory, antifertility, anti-diarrhoeal, antiseptic, anti-dysenteric, and antitubercular properties (Verma *et al*., 2013) making them highly useful in traditional medicine. The reported pharmacologically active phytochemical constituents in the *A. lebbeck* are melacacidin, D-catechin, β-sitosterol, *Albizzia*hexoside, betulnic acid, saponins, and echinocystic acid glycosides (Verma *et al*., 2013; Kokila *et al*., 2013; Lawan *et al*., 2018).

Moreover, the antifertility properties of *A. nilotica* and *A. lebbeck* have been revealed in some documented studies after feeding the extractives from the two plants in the Wister rats (Gupta *et al*., 2004 and 2005; Lampiao, 2013). However, whether the antifertility potential of *A. nilotica* and *A. lebbeck* extracts could be efficacious enough to control the fertility of the highly prolific rodent pests need to be investigated.

### 1.10 Preparation of Plant Extracts in Medicinal Plant Researches

Depending on the plant components (roots, stems, shoot, leaves, flowers, pods, or seeds) harvested for extraction and the end products (crude or pure extracts) required, various protocols of plant extract preparation are available (Azwanida, 2015; Altemimi *et al*., 2017; Rabiu and Haque, 2020). Generally, the initial step in plant extraction involves collecting the plant components of interest followed by washing, drying, and grinding them into smaller-sized particles (Altemimi *et al*., 2017; Rabiu and Haque, 2020). Grinding of the plant materials is done to increase the surface area for easy extraction by the solvents. However, before extraction the plant materials are normally either shed dried, oven-dried, microwave dried, or freeze-dried and not sun-dried, to preserve the plant active compounds from ultra-violet rays degradation (Azwanida, 2015).

Also, the plant extraction process may use any of the several available techniques depending on the types of plant materials available, end products required, and the resources available in the laboratory (Altemimi *et al*., 2017; Rabiu and Haque, 2020). The most common and widely employed plant extraction methods in medicinal plant research include maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extractions (Azwanida, 2015; Altemimi *et al*., 2017; Rabiu and Haque, 2020).

Maceration is the commonest and simplest plant extraction method whereby the powdered plant materials aimed for extraction are normally added in a stoppered solvent containing container and then the mixture is left to stand at room temperature for at least 72 hours while frequently shaken to hasten the extraction process (Azwanida, 2015; Rabiu and Haque; 2020). The mixture is then filtered to get the filtrate and the residues are discarded. This method is useful and suitable for thermolabile plant material (Azwanida, 2015; Rabiu and Haque, 2020).

Infusion is a process of plant extraction whereby the ground plant materials to be extracted are placed inside a clean container. The extraction solvent, hot or cold, is then poured on top of the material, soaked, and kept for a short period before filtration. This method is closely related to maceration however it is suitable for extracting readily soluble bioactive constituents (Azwanida, 2015; Rabiu and Haque, 2020).

Digestion is the plant extraction process involving moderate heating to facilitate the extraction process (Azwanida, 2015; Rabiu and Haque, 2020). The method begins with grinding the plant material of interest. The solvent of choice is then poured into a clean container where the powdered plant materials are added for extraction.
The mixture is then placed over a water bath or in an oven for heating. Heat is usually applied throughout the extraction process to decrease the extraction solvent's viscosity and enhance secondary metabolites' removal. This method is normally used when dealing with readily soluble plant materials (Azwanida, 2015; Rabiu and Haque, 2020).

Another method for plant extraction is decoction. The procedures in decoction involve placing dried and ground plant materials into a clean container, followed by the addition of water and stirring. Heat is then applied throughout the process to hasten the extraction

(Azwanida, 2015; Rabiu and Haque, 2020). Normally, the heating and stirring process lasts for about 15 min and the ratio of solvent to the powdered plant materials is 4:1 or 16:1. The method is suitable for the water-soluble and heats stable plant material (Azwanida, 2015; Rabiu and Haque, 2020).

Percolation is another plants extraction method that uses an apparatus called a percolator. The extraction process starts by moistening the dried, ground, and finely powdered plant material with the solvent of choice in a clean container (Azwanida, 2015; Rabiu and Haque, 2020). The mixture is then kept for 4 hours, after which the content is transferred into a percolator with the lower end closed, allowing the mixture to stand for a period of 24 h. The solvent of choice is then poured from the percolator's top until the powdered material is completely saturated. After that, the lower part of the percolator is opened, and the liquid is allowed to drip slowly. At the same time, some quantities of solvent are usually added continuously and left to percolate downward by gravitation through the powdered plant material to facilitate the extraction. The addition of solvent is normally stopped when the volume of solvent added reaches 75% of the entire preparations' intended quantity. The resulting mixture of the materials is then filtered and decanted to obtain the extract (Azwanida, 2015; Rabiu and Haque, 2020).

Soxhlet extraction is the extraction method of plant material using the Soxhlet apparatus. In this method, the finely ground plant material is placed in a porous bag or “thimble” made of strong filter paper placed in the Soxhlet apparatus's extraction chamber. The extracting solvent in the heating flask is heated, and its vapors condense in the attached condenser. The condensed extractant passes into the thimble containing the powdered plant material to facilitate the extraction. When the liquid level in the extraction chamber rises to the top of the siphon tube, the liquid contents of the extraction chamber siphon into the heating flask, and the process is continued until no drops of solvent from

the siphon tube leave a residue when evaporated. Compared to other methods described above, Soxhlet extraction gives a large yield of extracts using a much smaller quantity of solvent (Azwanida, 2015; Rabiu and Haque, 2020).

Microwave-assisted extraction is one of the advanced extraction procedures in the preparation of medicinal plants. The technique uses the mechanism of dipole rotation and ionic transfer by displacement of charged ions present in the solvent and drug material. This method is suitable for the extraction of flavonoids (Azwanida, 2015; Rabiu and Haque, 2020).

Under the Ultrasound-assisted extraction method of plants, very high-frequency sound energy, normally greater than 20 kHz, is normally applied to disrupt plant cells and increase the surface area of plant materials for solvent penetration to enhance the release of secondary metabolites. In this method, the dried plant materials are usually ground into fine powders and then sieved properly. The sieved powdered materials are then mixed with the appropriate solvent for extraction and packed into the ultrasonic extractor. The high sound energy is then applied to hasten the extraction process by reducing the heat requirements. The ultrasound-assisted extraction has several advantages in that it applies to the small sample, reduces the time of extraction and amount of solvent used, and maximizes the yield. However, the method is difficult to be reproduced. Also, a high amount of energy applied may degrade the phytochemical by producing free radicals (Altemimi *et al*., 2017; Rabiu and Haque, 2020).

The choice of a solvent to use in the extraction process largely depends on the polarity of the phyto-compounds in the involved plants (Azwanida, 2015; Altemimi *et al*., 2017; Rabiu and Haque, 2020). Hydrophilic plant compounds require polar solvents such as

distilled water, methanol, ethanol, or ethyl-acetate for the extraction process (Rabiu and Haque, 2020). However, more lipophilic compounds require non-polar solvents such as dichloromethane or a mixture of dichloromethane/methanol in the ratio of 1:1, Acetone, and n-Butanol as the solvent of choice for extraction (Rabiu and Haque, 2020). However, the frequently used solvents in the plant extraction process include Water, Ethyl acetate, Chloroform, n-Hexane, Diethyl ether, Ethanol, Methanol, Petroleum ether, Acetone, and n-Butanol (Azwanida, 2015; Altemimi *et al*., 2017; Rabiu and Haque, 2020).

### 1.11 Plant Extract Treatment Methods in Experimental Animals

When studying the effects of antifertility plants in rats or mice, the plant extract of interest has been normally administered orally by gastric gavage. Special feeding needles with smooth bulbed ends have been the frequently used tool to drop the intended amount of extracts through the mouth and esophagus directly into the animal's stomach (Murugan *et al*., 2000; Zubaid *et al*., 2007; Yadav and Jain, 2009). Nevertheless, some studies have used experimented plant extracts through administering in rats or mice by intraperitoneal injection (Faisal *et al*., 2006).

### 1.12 Duration of Plant Extracts Treatments during Fertility Studies

In many fertility studies investigating the effects of plant extract, the duration of extract treatments to the experimental rats or mice has been surprisingly different even in studies working on related objectives. Treatment durations of plant extracts adopted by various studies during fertility experiments in rats and mice have been revealed (Tables 1.1a and 1.1b).

**Table 1.1a: The plants extract treatment duration in antifertility studies involving rats or mice**

| Plant extracts | Treatment duration (days) | Study animal | Activities | References |
|---|---|---|---|---|
| Ethanolic extract of *Alstonia scholaris* | 1 to 7 days of pregnancy | Wistar rats | Abortifacient | (Choudhary *et al*., 2017) |
| *Ethanolic extract of Spondias mombin's* leaves | 1 to 7 days of pregnancy | Wistar rats | Abortifacient | (Badami *et al*., 2003) |
| Aqueous extract of *Coriandrum sativum* seeds | 8 to day-12 | Wistar rats | Abortifacient | (Al-Said *et al*., 1987) |
| Methanolic root extract of *Plumbago rosea* | 10 days from day 10 of gestation | Wistar rats | Abortifacient | (Zubaid *et al*., 2007) |
| Ethanolic roots extract of *Plumbago zeylanica* | 60 days | Wistar rats | Antispermatogenic | (Purohit *et al*., 2008) |
| *Sarcostemma acidum ethanolic roots extract* | 60 days | Wistar rats | Antispermatogenic | (Verma *et al*., 2002) |
| Methanolic extract of *Baleria prionitis* roots | 60 days | Wistar rats | Antispermatogenic | (Singh and Gupta, 2016) |
| Methanolic extract of *Tinospora cordifolia* stem | 60 days | Wistar rats | antispermatogenic | (Bayala *et al*., 2011) |
| Methanolic extract of *Dendrophthoe falcate* stem | 60 days | Wistar rats | antispermatogenic | (Gupta *et al*., 2008) |
| *Leptadenia hastate* aqueous extract | 60 days | Wistar rats | antispermatogenic | (Verma *et al*., 2002) |
| Ethanolic extract of *Enicostemma axillare* leaves | 55 days | Wistar rats | antispermatogenic | (Dhanapal *et al*., 2012), |
| Ethanolic extract of *Mondia whitei* root bark | 55 days | Wistar rats | antispermatogenic | (Watcho *et al*., 2001), |
| Methanolic extract of *Ricinus communis* seeds | 30 days | Wistar rats | antispermatogenic | (Maurya *et al*., 2004) |
| Aqueous extracts of *Piper nigrum* fruits | 20 or 90 days | Wistar rats | antispermatogenic | (Mishra and Singh 2009a) |
| C*assia fistula* Seeds' ether extracts | 5 days | Wistar rats | oestrogenic | (Yadav and Jain, 2009) |

**Table 1.1b: The plants extract treatment duration in antifertility studies involving rats or mice**

| Plant extracts | Treatment duration | Study animal | Activities | References |
|---|---|---|---|---|
| Ethanolic extract of *Nelumbo nucifera* seeds | 40 days | Wistar rats | oestrogenic | (Mutreju *et al*., 2008) |
| Benzene extracts of *Hibiscus rosa sinensis* roots | 7days | Wistar rats | ant-implantation | (Vasudeva and Sharma, 2008) |
| Extracts of *Striga orobanchioides* | 7days | Wistar rats | ant-implantation | (Hiremath *et al*., 1994) |
| Aqueous extracts of *Ailanthus excels* leaves, stem, and barks | 7days | Wistar rats | ant-implantation | (Tamboli and Konadawar, 2013) |
| Extracts of *Butea monosperma* roots | 30 days | Wistar rats | ant-implantation | (Sharm *et al*., 2012), |
| *Crotalaria juncea* seeds extracts | 30 days | Wistar rats | ant-implantation | (Malashetty, 2004), |
| Aqueous extracts of *Ocimum sanctum* leaves | 15 days | Wistar rats | ant-implantation | (Poli and Challa, 2019) |
| Petroleum Ether Extract of *Cassia fistula* (Seeds) | 5 days | Wistar rats | ant-implantation | (Yadav and Jain, 2009) |
| Benzene extract of *Momordica charantia* seeds | 30 days | Wistar rats | anti-ovulatory | (Sharanabasappa *et al*., 2002) |
| Ethanolic extract of *Crotalaria juncea* seeds | 30 days | Wistar rats | anti-ovulatory | (Malashetty and Patil, 2007) |
| Aqueous extract of *Garcinia kola* seeds | 30 days | Wistar rats | anti-ovulatory | (Abu *et al*., 2013) |
| Petroleum ether extracts of *Polygonum hydropiper* | 12 days | Wistar rats | anti-ovulatory | (Hazarika and Sarma, 2007) |
| Ethanolic extract of *Azadirachta indica* flower | 21 days | Wistar rats | anti-ovulatory | (Gbotolorun *et al*., 2008) |
| Aqueous extract of *Cnidoscolous aconitifolius* leaves | 7 days | Wistar rats | anti-ovulatory | (Adeleke *et al*., 2016) |
| Ether extract of *Plumbago rosea* stems | 5 days | Wistar rats | anti-ovulatory | (Sheeja *et al*., 2008) |

## 1.13 Plants Extract Treatment Dosage during Fertility Studies in Rats or Mice

During fertility studies involving medicinal herbs in rats or mice, the dosage of plant extracts given to the treated group of animals has been different even in studies dealing with related objectives. The treatment dosages adopted by various studies using similar or different medicinal plants are shown in Tables 1.2a and 1.2b bellows.

**Table 1.2a: Dosage of plant extracts in antifertility studies involving rats or mice**

| Plant extracts | Daily dosage of extracts | Study animal | Activities | References |
|---|---|---|---|---|
| *Alstonia scholaris extracts* | 250 and 500 mg/kg | Wistar rats | Abortifacient | (Choudhary *et al*., 2017) |
| *Spondias mombin's* leaves extracts | 250, 350 and 500 mg/kg body weight | Wistar rats | Abortifacient | (Asuquo*et al*., 2013) |
| *Coriandrum sativum* seeds aqueous extracts | 250 and 500 mg/kg bodyweight | Wistar rats | Abortifacient | (Al-Said*et al*., 1987) |
| *Moringa oleifera* leaf extracts | 175 mg/kg body weight | Wistar rats | Abortifacient | (Sethi *et al*., 1988) |
| Ethanolic extracts of *Marsdenia tinctoria* | 300,200 or 100 mg/kg body weight | Wistar rats | Abortifacient | (Chowdhury *et al*., 1994) |

**Table 1.2b: Dosage of plant extract in antifertility studies involving rats or mice**

| Plant extracts | Daily dosage of extracts | Study animal | Activities | References |
|---|---|---|---|---|
| Root extracts of *Plumbago rosea* | 400 and 800 mg/ kg bodyweight | Wistar rats | Abortifacient | (Zubaid *et al*., 2007) |
| C*assia fistula* Seeds ether extracts | 100, 200 and 500mg/kgbodyweight | Wistar rats | Anti-oestrogenic | (Yadav and Jain, 2009) |
| *Hibiscus rosa-sinensis* roots ethanolic extracts | 2000 or 4000 mg/kg body weight | Wistar rats | Anti-oestrogenic | (Vasudeva and Sharma, 2008) |
| *Plumbago zeylanica* roots extracts | 150 mg/kg body weight | Swiss albino rats | Anti-spermatogenic | (Purohit *et al*., 2008) |
| *Piper nigrum* aqueous fruits extracts | 25 or 100 mg/kg body weight | Albino mice | Anti-spermatogenic | (Mishra and Singh, 2009a) |
| *Baleria prionitis* roots extracts | 100 mg/kg body weight | Wistar rats | Anti-spermatogenic | (Singh and Gupta, 2016) |
| *Tinospora cordifolia stem* extracts | 100 mg/kg body weight | Wistar rats | Anti-spermatogenic | (GuptaandSharma,2003) |
| *Dendrophthoe falcate* stem extracts | 100 mg/kg body weight | Wistar rats | Anti-spermatogenic | (Gupta *et al*., 2008) |
| *Leptadenia hastate* aqueous extract | 100, 200, 400 and 800 mg/ body weight | Wistar rats | Anti-spermatogenic | (Bayala *et al*., 2011) |
| *Cannabis sativa* seeds extracts | 20 mg/day per rat | Wistar rats | Anti-spermatogenic | (Bajaj and Gupta, 2013) |
| *Ricinus communis* seed methanolic extracts | 20 or 40 mg/kg body weight | Wistar rats | Anti-spermatogenic | (Maurya *et al*., 2004) |
| *Spondias mombin* leaf ethanolic extract | 250 and 500mg/kg body weight | Wistar rats | Anti-spermatogenic | (Asuquo, 2012) |
| *Sarcostemma acidum* extracts | 50 and 100 mg/kg body weight | Wistar rats | Anti-spermatogenic | (Verma *et al*., 2002) |

## 1.14 Potential Affected Tissues, Organs by the Infertility Inducing Plants in Mammals

Based on several documented research works, the most targeted tissues by the fertility disrupting agents of plant origin in mammals include the hypothalamus and anterior pituitary gland (Hassan *et al*., 2017; Yakubu *et al*., 2008; Altınterim, 2014). Others include the ovary, the oviduct, uterus, and vagina in females and the testes, epididymides, prostate gland, and seminal vesicles in male animals (Devi *et al*., 2015; Ruben *et al*., 2015; Shaik *et al*., 2017).

## 1.15 Parameters and Tissues Assessed During Fertility Studies in Male Animals

The white albino rats, mice, and Rabbits have frequently been experimented with as animal models during fertility studies involving the plant extracts (Devi *et al*., 2015; Jain *et al*., 2015; Shaik *et al*., 2017). Moreover, the testes, epididymis, seminal vesicles, and prostate glands have been the most frequently assessed tissues in male mammals to determine the medicinal plant antifertility actions (Raji *et al*., 2006; Mishra and Singh, 2009 a and b). The testes play a vital role in gametogenesis and hormone production. The epididymides serve as the storage and maturation site for sperm cells. Seminal vesicles secrete seminal fluids, which nourish sperm cells with fructose and add volume to the semen. The prostate gland produces prostate fluids, which add volume to the semen. Therefore any significant damage to these tissues could easily contribute to infertility. Investigations in antifertility studies have also been assessing the alteration in levels of reproductive hormones, mainly the follicle-stimulating hormones (FSH), Luteinizing hormones (LH) and testosterone (Gupta *et al*., 2016)

### 1.16 Parameters and Tissues Assessed During Fertility Studies in Female Animals

The estrous cycle's length and ovulatory activities are frequently assessed parameters when studying the antifertility effects of plant extract in female rats or mice (Malashetty and Patil, 2007; Monima *et al*., 2019). Other studied parameters in the female animals include fertility performances based on the number of fetal implantations, fetal resorption sites, abortions, fetal litter size, pup's number, and fetal birth weights following plant extract treatments (Sewani-rusike, 2013; Ainehchi and Zahedi, 2014).

Furthermore, the ovaries have also been frequently assessed histopathologically for any potential alterations in the follicular and corpora lutea appearance and numbers following plant extracts treatment (Malashetty and Patil, 2007; Gadelha *et al*., 2014). Investigations have also been assessing the reproductive hormones, mainly the follicle-stimulating hormones (FSH), Luteinizing hormones (LH), and oestrogen (Circosta *et al*., 2001; Malashetty and Patil 2007; Dokhanchi *et al*., 2013; Jahromi *et al*., 2016) for any potential changes in levels following plant extract treatments. Moreover, the anatomy and histology of the oviducts and uterus are also frequently assessed to dig into the potentially toxic effects of the plant extract in the treated animals during fertility studies (Gadelha *et al*., 2014; Mary, *et al*., 2017; Monima *et al*., 2019).

### 1.17 Sperm Cells Collection and Analysis in Rats or Mice

The frequently used methods to collect sperm cells from the epididymides during sperm cells analysis in rats or mice include the diffusion and aspiration methods (Chapin *et al*., 1992). Under the diffusion methods, the caudal part of the epididymis is normally excised and minced by a scissor in a petri dish with warm (37 $^{0}$C) physiological saline to release the sperm cells (Chapin *et al*., 1992; Seed *et al*., 1996). On the other way, several cuts can

be made on the caudal part of the epididymis while the tissue is immersed in a physiological buffered solution for a specific time allowing diffusion of sperm cells into the physiological buffered solution (Raji *et al*., 2006; Ramesh and Kumaran, 2013). The aspiratory methods of sperm cells collection involve making a small cut in the caudal epididymis where a capillary tube is inserted to aspirate sperm fluids from the tissue (Chapin *et al*., 1992; Singh and Gupta, 2016). The most common parameters assessed from the epididymal sperm cell samples during fertility studies include sperm cell volume, motility, morphology, density, and viability (WHO, 2010).

### 1.17.1 Analytical methods of sperm cell's motility in rats or mice

The methods for sperm cell motility analysis in rats and mice have been well-reviewed in several documented studies (Working and Hurtt, 1987; Talarczyk-desole *et al*., 2017; Chaurasia and Upahdyay, 2016). The techniques range from the traditional manual methods employing visual analysis of sperm cells on the light microscope to the semi-automated or automatic methods using computerized video images (WHO, 2010; Talarczyk-desole *et al*., 2017).

#### 1.17.1.1 Manual methods of sperm cells motility analysis

The manual technique is the simplest procedure for sperm cells motility assessments. The easiest approach in the manual technique is done by counting all the non-motile sperm cells on a wet slide followed by fixing the slide and then counting the total sperm cells from which the percentage of motile sperm cells is determined (Chapin *et al*., 1992). In other manual techniques, some video clips of specific time lengths showing sperm cell movements can be recorded and then assessed later by tracing individual sperm cell movements (Linder *et al.*, 1990).

In the other manual methods, the motility of sperm cells is scored by observing under the microscope the number of spermatozoa crossing a specific defined line or area on the field of view in a given period (Mortimer, 1997). Other approaches include the use of timed-exposure photomicrography (TEP), Multiple exposure photomicrography (MEP), Turbidimetry and nephelometry methods, and Laser doppler velocimetry (LDV) (Mortimer, 1997). Under the TEP, spermatozoa are visualized microscopically using darkfield optics, and the movement is recorded by exposing sperm cells to a single frame of photographic film for a given time length (Mortimer, 1997). Track analysis is then made on the photo-micrography allowing estimation of sperm cell subpopulation percentages in a preparation (Mortimer, 1997).

Under multiple exposure photomicrography (MEP), a still camera is normally used to record images of spermatozoa in a microscope field (Mortimer, 1997). Images of motile spermatozoa appear as six-ringed chains the length and shape of which describe the distance travelled by each spermatozoon in one second, while immobile spermatozoa are overexposed (Mortimer, 1997).

The turbidimetry and nephelometry methods involve measuring the rate of sperm migration out of semen into a prepared artificial medium, normally calculated as a function of the increase in optical density of the artificial medium (Mortimer, 1997).

The Laser Doppler velocimetry (LDV) method uses a monochromatic laser beam under the principle that the population of motile spermatozoa will scatter a monochromatic laser beam proportionally to sperm cells movement (Mortimer, 1997).

### 1.17.1.2 Semi-automated analytical methods of sperm cell motility

The semi-automated methods of sperm cell motility analysis include the timed-videomicrography (Chapin *et al*., 1992). Under the timed video micrography, some videotapes of motile spermatozoa are recorded and replayed to analyze the motion, net displacement, and distance traveled by spermatozoa over one second (Chapin *et al*., 1992). The protocol uses semi-automated analysis methods for the analysis of video images of spermatozoa (Aitken *et al*., 1982; Mortimer, 1997).

### 1.17.1.3 Automated analytical methods of sperm cell's motility

The automated systems of sperm cell motility analysis employ computerized video micrographs. The most popular automated techniques include the Computer-Assisted Sperm cell Analysis (CASA) system and other automated video systems (Working and Hurtt, 1987; Chaurasia *et al*., 2016; Talarczyk-desole *et al*., 2017). The CASA is a computer-assisted system that uses a high-resolution camera connected to a phase-contrast microscope to record and analyze sperm cells' motion. With CASA analysis, progressive motility is defined as the percentage of motile sperm with a linear index, often combining minimum values for Average Path Velocity and Straightness (Talarczyk-desole *et al*., 2017).

The advantage of the CASA is that it provides a rapid assessment method of sperm cell motility, giving more reproducible (less variable) results than the manual microscopic methods (Centola, 1996). However, the CASA system is a new technology not available in many laboratories. Also, it is well argued that when all the needed procedures are properly followed and done correctly the manual method of sperm cell analysis can still yield more reliable results at cheaper costs than the CASA system (Mahony *et al*., 1988).

### 1.17.2 Analysis of sperm cell's morphology in rats or mice

The World Health Organization 2010 guidelines have been the standard procedures followed in many laboratories when analyzing human sperm cells (WHO 2010) and rats or mice spermatozoa (Yotarlai *et al*., 2011). Based on the WHO 2010 guidlines, the analysis of sperm cell morphology is normally done from the dry preparations of sperm cell samples. The whole process involves adding an aliquot of sperm cell suspension on a slide followed by air drying it then staining it normally with Eosin Y (Wyrobek and Bruce, 1975) or a combination of eosin and nigrosin (Lucio *et al*., 2013; Oliveira *et al*., 2015) or with the Walls and Ewa's stain (Raji *et al*., 2006). In other dry preparations, methylene blue and basic fuchsin (Yotarlai *et al*., 2011) or the Indian ink stain (Uslu *et al*., 2012) have been used as staining techniques of choice together with Papanicolaou staining, Diff-Quik rapid staining kit, and Shorr staining during spermatozoa morphology analysis (WHO, 2010).

#### 1.17.2.1 Classification of sperm cell morphologies in rats or mice

While there are no specified classification criteria for sperm cell morphology during sperm cell analysis in rats or mice, two common grouping criteria have been described in some studies (Kempinas and Lamano-Carvalho, 1988; Ahmed *et al*., 2013). In the first criterion of spermatozoa morphology classification, the sperm cells are normally categorized based on the shape of the head and flagellum (Kempinas and Lamano-Carvalho, 1988; Ahmed *et al*., 2013).

In the second classification criterion, sperm cells are sorted based on the appearance of the head, neck, midpiece, and tail of sperm cells (Kempinas and Lamano-Carvalho, 1988; Ahmed *et al*., 2013). Based on the shapes of the head and flagellum, sperm cells can be

classified as; (a) normal shaped, (b) normally shaped head separated from flagellum, (c) misshapen head separated from flagellum, (d) misshapen head with normal flagellum, (e) misshapen head with abnormal flagellum, (f) degenerative flagellar defect(s) with normal head, and (g) other flagellar defects with the normal head (Kempinas *et al*., 1998; Ahmed *et al*., 2013).

Based on the appearance of the head, neck, midpiece, and tail of spermatozoa, a normal-shaped spermatozoon is identified by a sickle-shaped head with an elongated straight tail. Sperm cell's head abnormalities may classically involve the blunt hooked head, banana-head, amorphous, bent head, pin-head, small head, and two-head (Ahmed *et al*., 2013). Neck and mid-piece abnormalities may involve a bent neck or bent mid-piece. Tail abnormalities may involve a coiled tail, broken tail, serrated tail, double tail, and bent tail (Ahmed *et al*., 2013).

### 1.17.3 Sperm cell counts in rats or mice

When counting the rats or mice spermatozoa, the samples of sperm cells are normally collected from the cauda, head, or entire epididymis. However, it is the cauda part that has been the preferred sampling site of sperm cells when counting matured spermatozoa (Gupta., 2006; Mishra and Singh, 2009; Lucio *et al*., 2013) because of its role as the storage site of matured spermatozoa in mammals. Therefore, many studies have been targeting the cauda part of epididymides to sample the spermatozoa for sperm cell counts in rats. The process is done by mincing the epididymal tissue portion while it is immersed in a certain amount of physiological saline to allow sperm cell release (Kempinas and Lamano-Carvalho, 1988; Raji *et al*., 2006; Uslu *et al*., 2012).

In another easier way, the epididymis can be processed by lacerating its cauda part with a razor or surgical blade while the tissue portion is immersed in a known amount of warm (37 $^{0}$C) physiological saline (1-10ml) to release sperm cells and prepare the sperm cells suspension (Raji *et al*., 2006; Uslu *et al*., 2012). Also, sperm cells can be collected from the epididymis by completely homogenizing the whole tissue or its cauda part to allow sperm cells release into the prepared buffered solution (Kempinas and Lamano-Carvalho, 1988).

**1.17.3.1 Sperm cells dilution for density evaluation in rats or mice**

During sperm cell counts, a dilution factor of 1:100 for sperm samples and a diluent solution, have been frequently used when preparing the sperm cell suspension (Kempinas and Lamano-Carvalho 1988; Ramesh and Kumaran, 2013; Oliveira *et al*., 2015), as recommended by the WHO (2010) guidelines. However, modifications to the dilution factors have been common in some documented research works. For instance, in a study by Wang (2002), they used a dilution of 0.5 ml of sperm suspension and 2 ml of PBS when doing sperm cell counts in rats. Moreover, the WHO, (2010) methodology, recommends a diluent solution composed of 50 g of sodium bicarbonate, 10 ml 35% of formalin, and 0.25 g of trypan blue or 5 ml of saturated (>4 mg/ml) gentian violet, making a final volume of 1 L with distilled water in a volumetric flask for sperm cell dilution. The inclusion of trypan blue or gentian violet in the solution is done technically to stain the sperm cell's heads for increased visibility (WHO, 2010).

However, different diluent fluids have also been employed for sperm cell dilution in various fertility research works. For instance, Uslu *et al*. (2012) used a diluent solution composed of 5g sodium bicarbonate, 1 ml of 35 % formalin and 25 mg eosin per 100 ml

of distilled water to dilute the rabbit's sperm cells. Another study by Oliveira and his colleagues used a diluent composed of paraformaldehyde and sodium citrate only (Oliveira *et al*., 2015). Also, a solution consisting of proteolytic enzymes, mostly trypsin has been a diluent solution of choice during sperm cell density determination in some fertility assessments (Kempinas and Lamano-Carvalho, 1988). Moreover, in some studies, normal saline has been used as a diluent solution of choice for sperm samples when doing sperm cells counts in rats (Hammodi, 2011).

#### 1.17.3.2 Counting of sperm cells on a neubauer haemocytometer

The Neubauer haemocytometer has been the standard instrument used in many laboratories to determine the concentration of spermatozoa in a given sample of sperm cells. The WHO (2010) guidelines recommend the 100µm-deep Neubauer haemocytometer as the standard chamber for sperm cell number determination. Just briefly, the process of sperm cells counting involves fixing the microscopic coverslip on the cleaned haemocytometer by pressing it firmly onto its moistened chamber pillars. The lower edges of the chambers are then each charged with certain amounts normally 10µl of the diluted sperm suspension and left for some minutes to allow the settling down of sperm cells. The counting is usually done by bright field microscopy at 200x magnification by focusing the hemocytometer's center squires, usually used for red blood cell counts (WHO, 2010). The total count of sperm cells is usually multiplied by a dilution factor per a particular volume of sperm cell suspension (WHO, 2010).

### 1.17.4 Determination of sperm cells vitality in rats or mice

The vitality (live or dead) of sperm cells is one of the routinely assessed sperm cell parameters during fertility studies in rats or mice. The vitality of sperm cells is normally

assessed by the procedures that involve the mixing of an equal amount of sperm suspension and staining (nigrosine-eosin) solution to form a preparation from which dry smears are prepared and examined (WHO 2010, Raji *et al*., 2006, Ramesh and Kumaran, 2013, Hammondi, 2011). The nigrosin-eosin solution is used to dye the sperm cells head and tail making it easier for recognition of both the live sperm cells which remain unstained hence appear whitish and the dead or non-viable ones that appear reddish or pinkish after picking the stain (Raji *et al*., 2006; WHO, 2010; Hammondi, 2011; Ramesh and Kumaran, 2013).

### 1.18 Fertility Performance Test during Fertility Studies in Rats or Mice

The fertility performance test is one of the frequently assessed parameters during antifertility studies in rats or mice. During the fertility performance test, the treated group of female or male rats are normally mated with the untreated fertile counterpart males or females, followed by assessments of various fertility-related parameters (Hyacinth *et al*., 2011; Ainehchi and Zahedi, 2014; Massawe *et al*., 2018).

The process starts by allowing the mating pair (female to male) to cohabit in a cage normally at a ratio of 1:1, 1:2, or 1:3 for a particular duration to facilitate mating (Watcho *et al*., 2009; Hyacinth *et al*., 2011; Ainehchi and Zahedi, 2014). In some studies, the mating pair has been allowed to cohabit for just one night (Hyacinth *et al*., 2011; Ainehchi and Zahedi, 2014) or 5 days (Watcho *et al*., 2009). Yet, in other studies, the male and female animals have been allowed to cohabit for up to 10 days (Massawe *et al*., 2018) or 15 days (Hammondi, 2011) to facilitate mating. Successful mating is normally confirmed by assessing the presence of the copulation plugs on the vulva or sperm cells in the vagina smears of female rats (Watcho *et al*., 2009; Hyacinth *et al*., 2011, Ainehchi and Zahedi, 2014).

Once successful mating is confirmed, the female animals are normally closely monitored for pregnancy by palpation of the lower abdomen and monitoring of body weight changes (Massawe *et al*., 2018,). Pregnancy outcomes are also assessed at necropsy to determine the number of fetal implants, fetal resorption sites, potential abortions, fetal litter size, and the pup's weights (Hyacinth *et al*., 2011; Sewani-rusike, 2013; Ainehchi and Zahedi, 2014; Massawe *et al*., 2018).

### 1.19 Histopathology of Gonads

During antifertility studies, the histopathology of gonads (ovaries and testes) has been an important assessment to reveal the reproductive toxic potential of the plant's preparations. The standard procedures in gonadal histopathology involve tissue harvesting and fixation, tissue embedding, sectioning, mounting, staining, and microscopic examination.

#### 1.19.1 Tissue fixation for reproductive tissues

The importance of tissue fixation in histology is to preserve the natural state of a particular tissue and cells until the processing time. Fixation of the reproductive tissues or organs is done on fresh tissues at necropsy. For instance, after opening the abdomen of rats or mice, the testis or ovaries are normally trimmed out and fixed either in formalin (10%) (Fraser, 1985; Raji *et al*., 2006; Gadelha *et al*., 2014; Ellenburg *et al*., 2020), Glutaraldehyde (3-5%) (Hess and Moore, 2019), Bouin fluids (Ellenburg *et al*., 2020) and other fixatives such as the Modified Davidson's fluid (Lanning *et al*., 2002) and 4% paraformaldehyde (Lihui, 2020; Mayo, 2001). However, some studies have been using a mixture of more than one fixative to preserve the reproductive tissues before histological processing for improved tissue sectioning (Howroy *et al*., 2005). For instance, there are studies where gonads of rats have been fixed firstly in Bouin fluid for 24 hours then in absolute ethanol until the processing time (Howroy *et al*., 2005; Oliveira *et al*., 2015).

### 1.19.2 Embedding of reproductive tissues for histology

Fixed reproductive tissues are normally embedded to facilitate sectioning, staining and visualization. The procedures involve tissues dehydration in graded ethanol followed by clearing them in ether, benzene, or chloroform then embedding the cleared tissues in paraffin wax (Mishra and Singh, 2009a and 2009b; Gadelha *et al*., 2014; Hess and Moore. 2019; Monima *et al*., 2019).

### 1.19.3 Sectioning and staining of the reproductive tissues for histology

For a detailed assessment of tissues on a microscope, the embedded reproductive tissues are normally serially sectioned and stained to increase tissue visibility. A calibrated microtome has been the standard instrument used for tissue sectioning. The instrument is normally adjusted to prepare tissue of specific sizes, usually 4 to 5µM, for histological staining (Raji *et al*., 2006; Mishra and Singh, 2009a and 2009b; Gadelha *et al*., 2014; Monima *et al*., 2019). The staining process is normally carried out on the tissue section while mounted on the microscopic slides.

The common staining solution used to dye the reproductive tissue sections for histological viewing includes a combination of haematoxyline and eosin (Mayo, 2001; Howroy *et al*., 2005; Oliveira *et al*., 2015; Mary *et al*., 2017; Hess and Moore, 2019). Though, in some cases, the periodic acid-Schiff stain has been used as the staining solution of choice especially when the objective was to detect polysaccharides in the reproductive tissues (Hess and Moore, 2019).

### 1.19.4 Microscopic evaluation of the gonads

The bright-field microscopy has been the most popular simplest instrument used to evaluate the Haematoxyline and Eosine and the periodic acid-Schiff stained histological sections of the rats and mice gonads (Bolon *et al*., 1997; Malashetty and Patil, 2007;

Gadelha *et al*., 2014; Hess and Moore, 2019). Additionally, some microscopes are normally equipped with a calibrated micrometer in the eyepiece allowing for histometric measurements of cells or tissues for detailed assessment of their sizes in toxicological studies (Hess and Moore, 2019). For instance, there are studies where bright-field microscopy has been used to evaluate the number and sizes of spermatogonium, spermatocytes, spermatids, Sertoli cells, and Leydig cells (Hess and Moore, 2019) in the male's testes and follicles and corpora lutea in the female rats or mice (Bolon *et al.*, 1997; Malashetty and Patil, 2007; Bernal *et al*., 2010; Gadelha *et al*., 2014).

### 1.19.5 Possible actions of plant extracts for testicular and follicular damages

Seminiferous Tubules (ST) is a functional unit of the testes made up of Sertoli cells, germ cells and Leydig cells (Cheng and Mruk, 2002). In male rats, smooth interaction between Sertoli and germ cells enabled by different cellular junctions is vital for a healthy Seminiferous Tubule (ST) (Cheng and Mruk, 2002). Together with the hormones from the hypothalamus-pituitary-testicular axis, cellular junctions regulate the biochemical, molecular, and cellular events pertinent to spermatogenesis in the seminiferous tubule’s epithelium (Cheng and Mruk, 2002). Therefore, anything (toxins of chemical or plant extracts, hormones, etc) disturbing the Hypothalamic-Pituitary axis, the cellular junctions or both could certainly lead to functional and cellular disorganization in the ST perturbing the spermatogenic process in the testes (Cheng and Mruk, 2002). The cellular junctional types controlling the intimacy between Sertoli and germ cells in the rat’s testis include the occluding, anchoring, and communicating gap junctions (Cheng and Mruk, 2002).

Likewise, some plants and environmental toxins may prompt pathological follicular atresia by inducing granulosa cell apoptosis or necrosis (Gadelha *et al*., 2014). For instance, Gossypol, one of the plant extracts has been shown to promote the

degeneration of follicles through induction of granulosa cell's apoptosis in the extract treated Wistar rats (Gadelha *et al*., 2014). However, follicular atresia is a normal physiological process in the female rat's ovaries to regulate the number of developing follicles, and therefore, the involvement of toxins from plants or the environments in follicular atresia can be revealed through a careful comparison between the control and the toxins exposed rats.

### 1.20 Study Area

Pods of *Acacia nilotica* were collected from Nangurukuru in Kilwa, Lindi region in the Southern-East of Tanzania (8°56′0″S39°30′45″E). Lindi region is situated in Southern Tanzania between latitudes 70 55' and 100 50' south of the equator and longitudes 360 51' to 400 East ( Kyalangalilwa *et al*., 2013; Length, 2015). The region shares its borders with Pwani region to the North, the Indian Ocean to the East, the Mtwara region to the South, the Morogoro region to the West, and the Ruvuma region on the South-West. The dominant climate in the Lindi region is hot and humid with a normal ambient temperature range of 24.5 $^{0}$C to 27 $^{0}$C throughout the year (Length, 2015; Kyalangalilwa *et al*., 2013). However, some areas of the region have air temperatures varying from 22.2 $^{0}$C in July to 27.70 $^{0}$C in March and April, with the humidity averaging 87% mmHg (Kyalangalilwa *et al*., 2013; Length, 2015).

The soil of low land areas in the Lindi region is characterized by having deep, leached sandy soils derived from terrestrial sands, gravels, calcretes, and laterites of the Miocene to Pleistocene age supporting the growth of various vegetation (Kyalangalilwa *et al*., 2013; Length, 2015). Soils in low land areas support the growth of various vegetation and trees including the Acacia tree species (Kyalangalilwa *et al*., 2013; Length, 2015). The ridges have a mixture of the ancient coral rag and sandy loam and clay soils carrying

different types of shrubs, grasslands, and tree species including those from the Fabaceae family (Length, 2015).

The stem barks of *Albizzia lebbeck* were collected from the Morogoro region around the Sokoine University of Agriculture (SUA), Main campus. Morogoro region is one of the regions in the Tanzania Mainland located on the eastern side of the country. Morogoro region lies between latitudes 5º58' and 10º00' South of the Equator and between longitudes 35º25' and 38º30' East of Greenwich. The region is bordered by seven regions. In the north are Tanga and Manyara while on the eastern side are the Pwani and Lindi regions. On the western, there are Dodoma and Iringa Regions while Ruvuma is located on the southern side of the Region (Kimambo *et al*., 2019).

The climate of the Morogoro region is well known for its moderate rainfall and temperature with heavy rainfall being experienced between November and May yearly (Ernest *et al*., 2017; Kimambo *et al*., 2019). The Region has an ambient temperature ranging between 18 to 30 $^{0}$C, averaging 25 $^{0}$C mostly all year round (Ernest *et al*., 2017; Kimambo *et al*., 2019). The average annual rainfall intensity in the Morogoro region varies between 600mm and 1800mm and the varying topographical nature of different areas influences greatly the patterns of the rainfall (Kimambo *et al*., 2019).

Different topographical and ecological zones of the Morogoro region are possessed by different soil types (Msanya *et al*., 2001). The common soil type in the mountainous and hilly areas is mainly oxisols generally low in nitrogen and phosphorus (Msanya *et al*., 2001). The soil types in the valley and low lands are generally characterized by fertile alluvial soils (Msanya *et al*., 2001) allowing the growth of various vegetation including

different Albizzia species (Ndemanisho *et al*., 2006; Ngomuo, 2010). Sandy and clay soils are common in woodlands and grasslands (Ndemanisho *et al*., 2006).

The topography of the Morogoro region is characterized by mountainous and hilly areas which include the Ukaguru Mountains, and the Uluguru and Nguru mountain ranges (Msanya *et al*., 2001). Others include Mahenge and Udzungwa Mountain ranges (Msanya *et al*., 2001). The second feature that characterizes the region's topography is the lowlands especially the Kilombero valley and the northern parts of the region (Msanya *et al*., 2001).

Authentication of *A.nilotica* and *A. lebbeck* was carried out by an experienced University botanist from the Department of Ecosystems and Conservation at SUA. The experiments were conducted at the Small Animal Research Unit (SARU) in the College of Veterinary Medicine and Biomedical Sciences (CVMBS), SUA in the Morogoro region, Tanzania.

### 1.21 Study Subjects

The experimental animals involved in the current study were the males and females *Mastomys natalensis*. The animals were captured alive from the SUA owned farms after permission, using the Sherman LFA aluminium traps. The captured rats were housed singly in plastic cages and left to adapt to the standard conditions of 12/12 light-dark cycle, 25±5ºC, and 35%−60% relative humidity for 2 weeks while feeding on broiler finisher and *adlib* water.

### 1.22 Sample Size Determination of the Study Subjects

The sample size for female rats was determined using G-power 3.1.9.4 under the assumptions that;

Number of animal groups = 6

Effect size = 0.40

Power (1-β) = 0.80

Significance level α =0.05

The sample size for females 111

Number per group =18 however we used 10 rats per group which is the acceptable number based on the law of diminishing return (Charan and Kantharia, 2013)

Likewise, the male rat's sample size was determined using G-power 3.1.9.4 under the assumption that;

Number of animal groups = 9

Effect size = 0.40

Power (1-β) = 0.80

Significance level α =0.05

Sample size for females 111

Number per group =18 however we used 10 rats per group which is the acceptable number based on the law of diminishing return (Charan and Kantharia, 2013). Out of the 220 collected male's *M. natalensis*, 90 rats were selected for this study. And out of the 190 captured female rats, 60 were used in experiments for this study. The criterion for inclusion was the bodyweight category of 25-50 g for sexually mature rats (Lalis *et al*., 2006) and a healthy body condition.

### 1.23 Problem Statement and Justification of the Study

The high fertility rate of *M. natalensis* has been a significant threat to the livelihood and public health in sub-Saharan Africa due to the rodent pest's involvement in food crops

destruction and transmission of zoonotic diseases. As a result rodenticides, mostly anticoagulant rodenticides have been used extensively to manage their population by killing the rodents which consume the poisons. However, several unbearable shortfalls associated with rodenticide use have been the drivers of an increasing need for some new techniques. Moreover, most rodent killing poisons can also hurt or kill other untargeted species of animals when they contaminate the environment (Brakes and Smith, 2005). Furthermore, rodent pests are increasingly becoming resistant to rodenticides by avoiding eating the poisoned baits (Prescott *et al*., 1992; Saxena, 2014) or by resisting being killed by the poison they consume (Philippe *et al*., 2018; Marquez *et al*., 2019). Additionally, the prolific nature of *M. natalensis* has been a significant tool enabling the rodents to thrive well even in the presence of the most effective anticoagulant rodenticides (Mulungu *et al*., 2013). Therefore, there is increasing need to develop some new and more sustainable methods of rodent control with more emphasis being on those approaches that will make rodent pests reproduce less.

### 1.24 Rationale of the Study

There is a growing research interest in animal contraception as a potential alternative to the environmentally unfriendly chemical rodenticides in rodent pest population management (Cooper and Larsen, 2006; Zhao *et al*., 2007; Massawe *et al*., 2018). Present efforts in rodent's fertility research have been focusing on identifying effective contraceptive techniques that will help to manage the population of rodent pests (Cooper and Larsen, 2006; Zhao *et al*., 2007; Massawe *et al*., 2018). For instance, there are ongoing research activities on synthetic steroid hormones (Zhao *et al*., 2007; Massawe *et al*., 2018) and immunocontraceptives (Peterson *et al*., 2000; Cooper and Larsen, 2006) as potential birth control pills for rodent pests. However, despite the excellent results yielded

from laboratory studies involving synthetic steroid hormones and immunocontraception, the technologies have been merely experimental. The two tactics have never been in real practice in the field emvironment of rodent pest's management (Peterson *et al*., 2000; Massei *et al*., 2020; Massawe *et al*., 2018).

The lack of appropriate methods to administer the immune contraceptives to the targeted animals has been delaying deployments of the immunocontraceptive in the field situation. Moreover, there are concerns related to synthetic steroid hormonal use due to their persistence in the environment and endocrine disruptive properties hence becoming a threat to other untargeted species. However, animal contraception remains to be among the better options to be adopted for sustainable rodent pest's management. Medicinal herbs with antifertility properties could be among the potential alternative sources of birth control pills for rodent pests. That is because several investigated plant species have been showing significant antifertility effects in both sexes of the studied animal model (Devi *et al*., 2015).

Some of the plant preparations with reported antifertility effects include the extracts of *Acacia nilotica* and *Albizzia lebbeck* (Gupta *et al*., 2006; Lampiao, 2013). However, it remains greatly important to know whether medicinal herbs with antifertility properties could be effective enough to reduce the fertility of the most prolific rodent pests as a way to control their population in the field.

### 1.25 Hypothesis of the Study

The study hypothesis states that the feed incorporated extracts of *A. nilotica* pods or *A. lebbeck* stem bark at 2% w/w will cause some antifertility-related morphological and

functional alterations in the reproductive system when fed to *M. natalensis* (multimammate rats).

### 1.26 Scope and Limitation of the Study

The antifertility potential of various plant species has been well-reviewed in several documented reports (Joshi *et al*., 2011; Devi *et al*., 2015). However, not all the plant species have antifertility properties. That is because, different or similar plant species may have the same or differing medicinal properties due to the varying levels and composition of the pharmacologically active phytocompounds (Liu *et al*., 2016; Cirak and Radusiene, 2019).

There are several environmental factors including the varying climatic condition, topography, light intensities, temperatures, radiations, and soil properties (salinity, PH, water content), influencing greatly the composition and levels of the plant's active compounds (Liu *et al*., 2016; Cirak and Radusiene, 2019). Thus, the differences in levels and compositions of the pharmacologically active phytocompounds can happen even in plants from similar species (Liu *et al*., 2016; Cirak and Radusiene; 2019).

Therefore, it is important to note that, the results produced in the current study involved the plants collected from one location in the country and the plants were harvested in the dry season of the year. Hence, the antifertility efficacies of *A. nilotica* pods and *A. lebbeck* stem bark extract in *M. natalensis* revealed in the current study may not necessarily represent what could have happened had the plants been harvested in different geographic regions or at a different season of the year.

Moreover, most of the plant species golbally do produce secondary metabolites (Phyto-compounds) (Dai and Mumper, 2010; Cheynier, 2012) with different biological functions in the plants. Some plants have phytocompounds with antimicrobial, antioxidants, anticancer, antihyperglycemic, anti-inflammatory agents as their pharmacological activities when treated in animals (Dai and Mumper, 2010; Cheynier, 2012).

However, as mentioned before, the levels and composition of Phyto-compounds are the key determinants of the medicinal, toxic or nutritional potential of various plant species (Dai and Mumper, 2010; Cheynier, 2012). For instance, the stem barks of *A. lebbeck* are rich in melacacidin, D-catechin, β-sitosterol, *Albizzia*hexoside, betulnic acid, saponins, echinocystic acid glycosides (Kokila *et al*., 2013) while pods of *A. nilotica* are rich in tannins, saponins, flavonoids, alkaloids, fatty acids, and polysaccharides (gums) among their pharmacological agents (Rajvaidhya *et al*., 2012). Several studies indicates that β-sitosterol, saponins, phytoestrogen, and other plant sterols have some antifertility properties (Gupta *et al*., 2005; Singh and Gupta, 2016). Therefore, the abundance of β-sitosterol, saponins, phytoestrogen, tri-tarpens and other plant sterols in the *A. nilotica* pods and *A. lebbeck* stem bark (Kokila *et al*., 2013; Sawant *et al*., 2014) could be explaining the reported antifertility properties of the two plant species (Gupta *et al*., 2005; Singh and Gupta, 2016).

Nevertheless, the current study did not isolate and study any individual active plant compounds responsible for the antifertility actions. Whether isolating the active principle compounds from the studied plants could have produced different results from that of the crude extracts remains to be answered. Nevertheless, isolation of the pure active

compounds from plant crude extracts requires advanced techniques and equipment, including the High-Performance Liquid Chromatography (HPLC) (Gupta *et al*., 2005; Singh and Gupta, 2016) and such equipment were not available in the current study set up.

However, in logical sense, the crude extracts are very likely be more efficacious in causing the desired effects than the single isolated active compound due to the potential synergistic effects of various active ingredients in the crude extracts. Also, mixing of the crude extracts with stiff porridge was done manually using hands leaving room for an uneven distribution of the extracts in the baits. However, the pelletizer machine was used to mix further the extracts and stiff porridge.

### 1.27 Research Permit

The research permit (Permit No 2019-225-NA-2019-47) was granted by the Tanzanian Commission for Science and Technology (COSTECH) before the study's commencement. Guidelines for caring and the use of laboratory animals were followed adequately during the rat's handling and restraints.

### 1.28 Objective of the Study

#### 1.28.1 Overall objective

To investigate the antifertility potential of *Acacia nilotica* pods and *Albizzia lebbeck* stem bark crude extracts in the Multimammate rat's (*Mastomys natalensis*).

#### 1.28.2 Specific objectives

i. To investigate the effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the fertility success rate of the female and male *M. natalensis*,

ii. To evaluate the effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the ovarian follicular and corpora lutea counts in the female *M. natalensis*,

iii. To explore the effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the sperm cells parameters of *M. natalensis* male rats,

iv. To determine the effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the histomorphology of the *M. natalensis*, gonads (testes and ovaries),

v. To determine the phytochemical constituents of the *A. nilotica* pods and *A. lebbeck* stem bark.

### 1.29 Organization of the Thesis

This thesis is organized into three chapters preceded by an extended abstract summarizing the objectives, methodologies, key research findings, and conclusion of the study. Chapter one covers an introduction and literature review, problem statement and justification of the study, study area, study subjects, study objectives, and rationale of the study. Chapter two is presenting the results generated from the specific objectives which are synthesized into either published papers or manuscripts submitted for publication in peer-reviewed scientific journals. The format and writing style of each paper followed the requirements of the respective journals. Chapter three consists of the overall conclusion of the study and recommendations.

# CHAPTER TWO

## Paper One

## Antifertility effects of crude extracts from *Acacia nilotica* pods and *Albizia lebbeck* stem bark in female multimammate rats, *Mastomys natalensis*

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**Paper Two**

# Effect of *A. nilotica* pods and *A. lebbeck* stem bark extracts on the reproductive system of male *Mastomys natalensis*: An Anti-fertility studies

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**Paper Three**

# 2.0 *Acacia nilotica* Pods and *Albizia lebbeck* Stem Bark: Evaluating the Plant Extract's Phytochemical Composition and the Effects of Methanolic Extract on Spermatozoa Morphologies in Rats

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## 2.1 Abstract

The study evaluated the phytochemical constituents of the raw grounded materials and methanolic extracts of *A. nilotica* pods and *A. lebbeck* stem bark extracts and the potential effects the methanolic extracts may have on spermatozoa morphologies in the male *M. natalensis*. A portion of the grounded materials from each plant was extracted in 70% methanol. Both the grounded materials and corresponding methanolic extracts were assessed for the presence of phytochemicals with antifertility potentials. A total of 90 male rats were randomized into 9 groups based on treatment (control *A. lebbeck* and *A. nilotica*) (n = 10) and treatment durations (15, 30, or 60 days). Results indicated that flavonoids, tannins, saponins, steroids, terpenoids and plant phenols were all present in the powdered raw materials and their corresponding extracts of both plants. Judged by the intensity of colour change after a chemical reaction, saponin was shown to be more intensely present in the powdered and methanolic extracts of the *A. lebbeck* stem barks compared to those of *A. nilotica* pods while the reverse was true for flavonoids and tannins. Spermatozoa with normal head-abnormal tail, normal head-tailless, abnormal head-normal tail, both abnormal head and tail spermatozoa were more revealed in rats treated with either of the plants methanolic extract relative to the control. In conclusion, the studied pods of *A. nilotica* and the Stem bark of *A. lebbeck* are rich in flavonoids, tannins, saponins, steroids, terpenoids and plant phenols that possess some antifertility properties hence explaining well the adverse effects of the plant's methanolic extracts on spermatozoa morphologies.

Keywords: *Phytochemical analysis, Antifertility, A. nilotica, A. lebbeck, spermatozoa, morphologies*.

## 2.2 Introduction

Most plant species on Earth do produce secondary metabolites (Phyto-compounds) serving a diverse range of biological functions in the plants and animals (Dai and Mumper, 2010; Cheynier, 2012). However, among the medicinal herb's vital characteristics include their phytocompounds to differ in levels and composition depending on factors, such as climate, habitat, soil nutrients, time of harvest, stress and physiological age of plants affecting their pharmacological properties (Liu *et al*., 2016; Cirak and Radusiene, 2019).

Thus, medicinal plants with antifertility, antimicrobial and toxic actions have a high level of compounds with antifertility, antimicrobial and toxic properties respectively. That means, it is the level and composition of the phytochemicals in plants that determine the nutritional, toxic or medicinal potential of the plant's extractives (Samtiya *et al*., 2020). Examples of the well-known phytochemicals in plants include plant Phenolics, Terpenoids, and Alkaloids (Cheynier, 2012).

Plant phenolics are plant compounds that have a benzene ring ($C_6$) and one or more hydroxyl groups (Cheynier, 2012; Lin *et al*., 2016). Plant phenolics include flavonoids, phenolic acids, tannins, lignin, Salicylic acid (Dai and Mumper, 2010; Cheynier, 2012). Plant phenolics play some important roles in plant development, particularly in lignin and pigment biosynthesis (Dai and Mumper, 2010; Cheynier, 2012). They also provide protection, structural integrity and scaffolding support to plants (Dai and Mumper, 2010; Cheynier, 2012).

In animals, some plant phenolics offers significant health benefits as potent antioxidants by scavenging the Reactive Oxygen Species (Halliwell *et al*., 2002), reducing the risk of type 2

diabetes by slowing the absorption of glucose in the gastro intestinal tract (Sales *et al*., 2012). However, some phenolic compounds have antifertility properties and they include total flavonoids which showed some anti-implantation and abortifacient effects (Nayaka *et al*., 2014), Isoflavonoids with some potent antispermatogenic activities (Moersch *et al*., 1967) and tannin with some spermicidal effects (Benhong, 2012).

Terpenoids are plant compounds derived from the 5-carbon compound isoprene and the isoprene polymers called terpenes (Hollman, 2001; Panche *et al*., 2016). Terpenoids include mono-terpenes (eg aromatic oils), diterpenes, triterpenes (eg saponins glycoside), Meroterpenes and sesqui-terpenes (eg rubber), hemi-terpenes, b-Sitosterol, resins, waxes, steroids and carotenoids. Most terpenoids are active compounds with various pharmacological functions (Hollman, 2001; Panche *et al*., 2016). Some of the terpenoids such as the Monoterpenes, Diterpenoids, Sesquiterpenes, Triterpenes, possess some potent antimicrobial and anticancer activities (Hollman, 2001; Panche *et al*., 2016). Other terpenoids including triterpenes (Chaudhary *et al*., 2007), saponins (Shu *et al*., 2015) and b-Sitosterol (Singh and Gupta, 2016) have some antispermatogenic and spermicidal effects.

Alkaloids are a group of organic nitrogen-containing basic compounds with a diverse range of biological effects on human beings and animals (Dai and Mumper, 2010; Cheynier, 2012). Most of the plant's alkaloids are toxins and they include morphine, strychnine, quinine, ephedrine, and nicotine, caffeine, cocaine, capsaicin and cyanide (Dai and Mumper, 2010; Cheynier, 2012). Others include coronaridine, voacangine, ibogamine, 19-oxocoronaridine, and the pseudoindoxyl of voacangine (Meyer *et al*., 1973), Quinolizidine (Olayemi and Raji, 2011) just to mention a few.

Besides their toxic potential in animals, some alkaloids have antifertility properties and they include Coronaridine with anticonception activities in female rats (Meyer *et al*., 1973). Others include Quinolizidine which in one study caused a significant reduction in sperm counts, motility, viability, and defects in sperm cells morphology and plasma levels of testosterone, luteinizing hormone and follicle-stimulating hormone when treated orally to rats (Olayemi and Raji, 2020).

*Albizia lebbeck* or *Acacia nilotica* are among the tree species with some medicinal properties. *A. lebbeck* is a deciduous, perennial and medium-sized legume tree native to Asia, Northern Australia, and Western Africa (Orwa *et al*., 2009). Studies done elsewhere indicate the Phytochemical constituents in *A. lebbeck* to include cinnamic and benzoic acids, flavonoids, alkaloids, glycosides, saponins, tannins, steroids and various terpenoids (Lawan *et al*., 2018) which are responsible for its medicinal and antifertility properties (Gupta *et al*., 2005 and2006).

*A. nilotica* belongs to the family Fabaceae and is a tree species widely distributed in tropical and subtropical regions globally, including sub-Saharan Africa (Rajvaidhya *et al*., 2012). Documented studies indicate the presence of tannins, saponins, flavonoids, steroids alkaloids, fatty acids, and polysaccharides (gums) in *A. nilotica* (Rajvaidhya *et al*., 2012), which are responsible for its medicinal, toxic and antifertility properties (Burton and Wells, 2002; Atif *et al*., 2012; Singh and Gupta, 2016).

As mentioned above, medicinal plants may have similar or differing compositions and levels of phytochemicals which defines their pharmacological potential. Therefore, the current study evaluated the phytochemical constituents of *A. lebbeck* stem back and *A.*

*nilotica* pods extract collected from the eastern regions of Tanzania. Moreover, our previous publication reported the contraceptive action of *A. lebbeck* and *A. nilotica* extracts in females *M. natalensis* also the adverse effects caused by the plant's extracts on seminiferous tubules histopathology, sperm cell's motility, vitality and concentration in male's *M. natalensis* (Mwangengwa *et al*., 2021a and b). However, the effects of the plant extracts on spermatozoa morphologies were not presented. Therefore, the current work is also presenting the potential effects of *A. lebbeck and A. nilotica* extracts treatments on the morphology of the *M. natalensis* spermatozoa.

## 2.3 Methodology

### 2.3.1 Study Area

*Acacia nilotica* pods were collected from Nangurukuru in Kilwa, Lindi Region in southeastern Tanzania (8° 56′0″S, 39°30′45″E), whereas stem barks of *Albizia lebbeck* were collected in Morogoro Urban, Tanzania (6° 50' 42.66" S, 37° 39' 29.14"E). Authentication of the two trees was carried out by a botanist from the Department of Ecosystems and Conservation, at Sokoine University of Agriculture (SUA).

### 2.3.2 Processing of the plants

Processing of the plants for phytochemical analysis was carried out at the Toxicology laboratory in the College of Veterinary Medicine and Biomedical Sciences (CVMBS) at SUA. A total of 10 kg each of *A. lebbeck* stem barks and fresh matured pods of *A. nilotica*, were collected in August and transported in bags to the laboratory, where they were dried under the shed until they were breakable.

The dried plant materials were then chopped into smaller pieces using a knife before being ground into fine particles (1 mm) using an electric grinder. Extraction involved the soaking of 500 g of powdered materials of each plant in 1.5 L of 70% methanol (Gupta *et al.*, 2004)

for 72 hours. The crude extracts from *A. lebbeck* and *A. nilotica* were obtained by filtering the methanolic extract through gauze and then cotton wool. Methanol was removed from the filtrates using a vacuumed rotary evaporator at $80^0$C. The resultant filtrates were further concentrated using a water bath at $40^0$C until they were a solid mass.

### 2.3.3 Phytochemical analysis

Evaluation of phytochemical constituents was carried out as per Edeoga *et al*. (2005) methodology with some minor modifications. Briefly, a total of 5 g of the dried powdered samples of *A. lebbeck* stem bark and *A. nilotica* pods were each boiled in 200 ml of distilled water in beakers and then filtered to prepare stock solutions. Similarly, 5 g of the crude extracts of *A. lebbeck* stem bark and *A. nilotica* pods were separately dissolved into 200 ml of distilled water and then filtered to prepare stock solutions.

#### 2.3.3.1 Test for flavonoids (alkaline reagent test)

A total of 5 ml of dilute $NH_3$ solution was added to 3 ml of the stock solution of *A. lebbeck* stem bark or *A. nilotica* pods extracts followed by the addition of 2ml of concentrated $H_2SO_4$. The formation of yellow coloration that disappeared on standing was an indication of the presence of flavonoids.

#### 2.3.3.2 Test for saponins (frothing tests)

About 1 mil of stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added separately in test tubes followed by the addition of 5 mils of distilled water. The mixture was then shaken vigorously for 5 minutes then allowed to settle for 30 minutes. The appearance of honeycomb froth was an indication of the presence of saponin.

**2.3.3.3 Test for steroids (libermann burchard's test)**

A total of 2ml of stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added with 2.5 ml of chloroform followed by slow addition of 2.5 ml of concentrated $H_2SO_4$. A colour change from violet to blue or green indicated the presence of steroids.

**2.3.3.4 Test for triterpenoids (salkowski's test)**

About 5 ml of the stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were mixed with 2 ml of chloroform. Then 3 ml of concentrated $H_2SO_4$ was added slowly and carefully. The formation of a reddish-brown colouration indicated the presence of triterpenoids.

**2.3.3.5 Test for tannins (Ferric chloride test)**

A total of 2 ml of stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added to the test tube followed by the addition of 3 ml of 10% ferric chloride solution. A brownish-green or blue-black colouration indicated the presence of tannins.

**2.3.3.6 Test for phenol (Gelatin test)**

About 2 ml of the stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added to the test tube followed by the addition of 2 ml of gelatin solution. The formation of white precipitate indicated the presence of phenolic compounds.

**2.3.4 Preparation of the plain and extract containing test feed**

The extracts of *A. lebbeck* and *A. nilotica* were treated in the *M.natalensis* rats through feed baits. Details describing the preparation of the plain and extracts containing taste feed have been presented in our previous publication (Mwangengwa *et al*. (2021). Just

briefly a stiff porridge was prepared by boiling and stirring a mixture of 10 kg of maize flour, 6 kg of roughly crushed maize, 250 g of fish meal, and 1 kg of cane sugar for 20 min.

A standard pelletizer machine (KENWOOD, type MG51, designed at Hampshire, PO 9NH in the UK, made in China) was employed to process a portion of the stiff porridge mixture to prepare the basal feed pellets. The remaining stiff porridge mixture was spiked with the extract of either *A. nilotica* pods or *A. lebbeck* stem barks before pelleting. Spiking was done in such a way that the treated feed contained 2% (w/w) of either of the two crude extracts.

The 2%w/w was equivalent to 0.2g (200mg) of the extract added to 9.8g (9 800 mg) of feed making 10g (10 000mg) of the feed extract mixture. The assumption was that rats may consume up to 10 g/100g body weight per meal per day (Krishnakumari *et al*., 1979). A dosage of 200mg of the extract used in the current study considered the dosage range of 100 to 200 mg/ kg body weight of rats which have been used in other antifertility studies involving *A. lebbeck* (Gupta *et al*., 2004; 2006) and *A. nilotica* (Lampiao, 2013).

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**2.3.5 Experiment setup and treatments**

The detail of the experimental setup has been explained elsewhere (Mwangengwa *et al*., 2001). Briefly, a total of 90 male rats were stratified on a bodyweight basis and randomized into 9 groups (Table 2.1). Treatment was done in parallel for 15 (to assess immediate effects), 30 (intermediate effects), and 60 days (to evaluate for potential chronic effects) (Table 2.1). The amount of test feed supplied daily for each rat was 10 g, assuming that rats consume up to 10g/100g bodyweight meals per day (Krishnakumari *et al*., 1979). Set up of

experiments was done at the Small Animal Research Unit (SARU) in the College of Veterinary Medicine and Biomedical Sciences (CVMBS), at SUA.

The *M. natalensis* were collected from the SUA farms by using Sherman LFA aluminium traps. Out of the 220 collected male rats, 90 of them were selected for this study. The selected rats were housed singly in plastic cages and left to adapt to the standard conditions of a 12/12 light-dark cycle, 25±5*ºC*, and 35%−60% relative humidity for two weeks while feeding on broiler finisher and *adlib* water.

The criterion for inclusion and exclusion in the experiments was the bodyweight category of 25-50 g for sexually mature rats (Lalis *et al*., 2006) and a sound body condition. The Tanzanian Commission for Science and Technology (COSTECH) granted the research permit (Permit No 2019-225-NA-2019-47) before the study's commencement. Guidelines for caring and using laboratory animals were properly followed during the handling and restraints of the experimental rats.

**Table 2.1: Experimental design to evaluate the contraceptive potential of *A. nilotica* pods and *A. lebbeck* stem bark methanolic extracts in the male *M. natalensis***

| Group (n=10) | Treatment allocation | Trial duration |
|---|---|---|
| Control (C) | 10 g of plain pellets per rat/day | 15 (days) |
| *A. lebbeck* (AL) | 10 g of pellets with 2 % of *A. lebbeck* stem barks extract per rat/day | |
| *A. nilotica*(AN) | 10 g of pellets with 2 % of *A. nilotica* pods extract per rat/day | |
| Control (C) | 10 g of plain pellets per rat/day | 30 (days) |
| *A. lebbeck* (AL) | 10 g of pellets with 2 % of *A. lebbeck* stem barks extract per rat/day | |
| *A. nilotica* (AN) | 10 g of pellets with 2 % of *A. nilotica* pods extract per rat/day | |
| Control (C) | 10 g of plain pellets per rat/day | 60 (days) |
| *A. lebbeck* (AL) | 10 g of pellets with 2 % of *A. lebbeck* stem barks extract per rat/day | |
| *A. nilotica*(AN) | 10 g of pellets with 2 % of *A. nilotica* pods extract per rat/day | |

### 2.3.6 Determination of sperm cell morphologies

The effects of *A. lebbeck* and *A. nilotica* extracts on sperm cell counts, motility and vitality have been presented in our previous publication (Mwangengwa *et al*., 2021b). The current study presents the effects of the plant's extracts on sperm cell morphologies. The morphology of spermatozoa was determined as per Lucio *et al*. (2013) methodologies. The rat's testes' caudal epididymides were dissected out and homogenized into 1 mL of warm (37 $^{0}$C) normal saline in a small beaker using a dissection scissor to make a sperm cell suspension. One drop of the sperm cell suspension was added on a microscopic slide followed by one drop of nigrosine-eosine and mixed with a toothpick. Then several slides of thin smears were prepared and were air-dried before being examined by a bright-field microscope at x 200 magnification.

The percentages of normal shaped sperm cells with sickle-shaped heads and large straight elongated tails versus the abnormal sperm cells with weird heads, tails or tailless were determined from the total counts of 200 sperm cells from five different fields of the haematohxyline and eosin-stained microscopic slides (Lucio *et al*., 2013).

Sperm cell morphology was classified based on the shape of the head and flagellum as described by Kempinas and Lamano-Carvalho (1988) and Ahmed *et al*. (2013). Normal-shaped spermatozoa had an intact head and flagellum. Abnormal shaped sperm cells were classified as follows (a) normal head separated from flagellum, (b) misshapen head separated from flagellum, (c) misshapen head with normal flagellum, (d) misshapen head with abnormal flagellum, (e) degenerative flagella defect(s) with normal head and (f) other flagella defects with the normal head.

### 2.3.7 Data analysis

IBM SPSS statistics 20 was used for data analysis to analyze the proportion of sperm cells with normal and abnormal morphologies. Statistical association related to treatment, treatment duration and sperm cell morphologies were assessed by Chi-squared test using the multinomial logistic regression model. Statistical significant differences and associations were considered at $p< 0.05$).

## 2.4 Results

The intensity of colour change during the chemical reaction in phytochemical analysis indicated that flavonoids, tannins, saponins, steroids, terpenoids and plant phenols were all present in the powdered raw materials and their corresponding methanolic extracts of both *A. nilotica* pods and *A. lebbeck* stem bark (Table 3.2 and 3.3). While the presence of saponin was more intense in the powdered and methanolic extracts of *A. lebbeck* stem barks compared to those of *A. nilotica* pods the reverse was true for Tannins (Tables 2.2 and 2.3).

The existence of flavonoids was less intense in raw plants powder than in corresponding extracts of both *A. lebbeck* and *A. nilotica*. Other phytocompounds including plant phenolics, terpenoids and plant steroids were equally highly present in both the powdered raw materials and methanolic extracts of both *A. nilotica* pods and *A. lebbeck* stem bark.

Morphological analysis of spermatozoa of the rats indicated a significant reduction of the normal-shaped sperm cells in the *A. nilotica* pods and *A. lebbeck* stem barks extract-treated rats relative to their control counterparts at all the treatment duration of 15, 30 and 60 days (Fig. 2.1). Comparison among the extract-treated rats revealed that normal shaped sperm cells were significantly more reduced in the *A. nilotica* than *A. lebbeck* treated rats at all the treatment duration except in the 15 days treated groups (Fig. 2.1).

Spermatozoa with normal head and abnormal tail were significantly ($p<0.05$) more revealed in the *A. lebbeck* and *A. nilotica* extracts treated than in the control rats across all the treatment durations of 15, 30, and 60 days (Figure 2.2). However, the treatment duration of 15 days indicated a large proportion of sperm cells with normal head and abnormal tail in the *A. lebbeck* stem bark than *A. nilotica* extracts treated rats (Figure 2.2). Also, the 30 days and 60 days treated groups of rats revealed a large proportion of spermatozoa with normal heads and abnormal tails in the *A. nilotica* extracts treated relative to the *A. lebbeck* extracts treated rats (Figure 2.2).

Sperm cells with a normal head but tailless (Figure 2.3) and those with an abnormal head but normal tail (Figure 2.4) were significantly more displayed in the *A. lebbeck* and *A. nilotica* extracts treated rats relative to the control counterparts. Moreover, the treatment duration of 15 and 30 days indicated a non-significantly differing proportion ($p > 0.05$) of normal head-

tailless and that of abnormal head-normal tail spermatozoa between the *A. lebbeck* and *A. nilotica* extracts treated rats (Figure 2.3 and 2.4). However, in the 60 days treated groups of rats, sperm cells with normal head-tailless and those with an abnormal head-normal tail were significantly ($p < 0.05$) more revealed in the *A. nilotica* extract-treated relative to the *A. lebbeck* treated animals (Figure 2.3 and 2.4).

Sperm cells with abnormalities on both the head and tail were significantly ($p < 0.05$) present in the *A. lebbeck* and *A. nilotica* extracts treated rats across all the treatment duration of 15, 30, and 60 days (Figure 2.5). The treatment made for 15 days indicated the proportion of sperm cells with both head and tail defects not differing significantly ($P > 0.05$) between the *A. lebbeck* and *A. nilotica* extracts treated rats (Figure 2.5). However, in the 30 days treated groups, there was a high percentage of sperm cells with both head and tail abnormalities in the *A. nilotica* extract treated rats compared to the *A. lebbeck* extracts treated rats (Figure 2.5). Nevertheless, the 60 days treated groups displayed more sperm cells with head and tail defects in the *A. lebbeck* extract-treated relative to the *A. nilotica* extract treated rats (Figure 2.5).

Histomorphological pictures of spermatozoa are presented in Figure 2.6. The micrographic pictures of spermatozoa from the 15, 30 and 60 days treated rats indicate sperm cells in the control groups of rats to be dominated by those with normal morphologies. However, the pictures from the *A. lebbeck* and *A.nilotica* treated rats spermatozoa were dominated by the sperm cells with abnormalities on the head, tail or both tail and head (Figure 2.6).

**Table 2.2: Phytochemical constituents of *A. lebbeck* stem bark's raw powder and the 70% methanolic extracts**

| Compound tested | Test methods | Colour intensity, in a stock solution of *A. lebbeck* stem bark, raw powder | Colour intensity, in a stock solution of *A. lebbeck* stem bark, crude extracts |
|---|---|---|---|
| Flavonoids | Alkaline reagent test | ++ | +++ |
| Saponins | Frothing tests | +++ | +++ |
| Steroids | Libermann Burchard's test | +++ | ++ |
| Tannins | Ferric chloride test | ++ | ++ |
| Terpenoids | Salkowski's test | +++ | +++ |
| Phenols | Gelatin test | +++ | +++ |

+++: highly present, ++: moderately present, +: Low, -: absent.

**Table 2.3: Phytochemical constituents in raw powder and the 70% methanolic extracts of A.nilotica pod's**

| Compound tested | Test methods | Colour intensity, in a stock solution of *A. nilotica* pod's raw powder | Colour intensity, in a stock solution of *A. nilotica* pod's crude extracts |
|---|---|---|---|
| Flavonoids | Alkaline reagent test | +++ | +++ |
| Saponins | Frothing tests | + | + |
| Steroids | Libermann Burchard's test | +++ | +++ |
| Tannins | Ferric chloride test | +++ | +++ |
| Terpenoids | Salkowski's test | +++ | +++ |
| Phenols | Gelatin test | +++ | +++ |

+++: highly present, ++: moderately present, +: Low, -: absent.

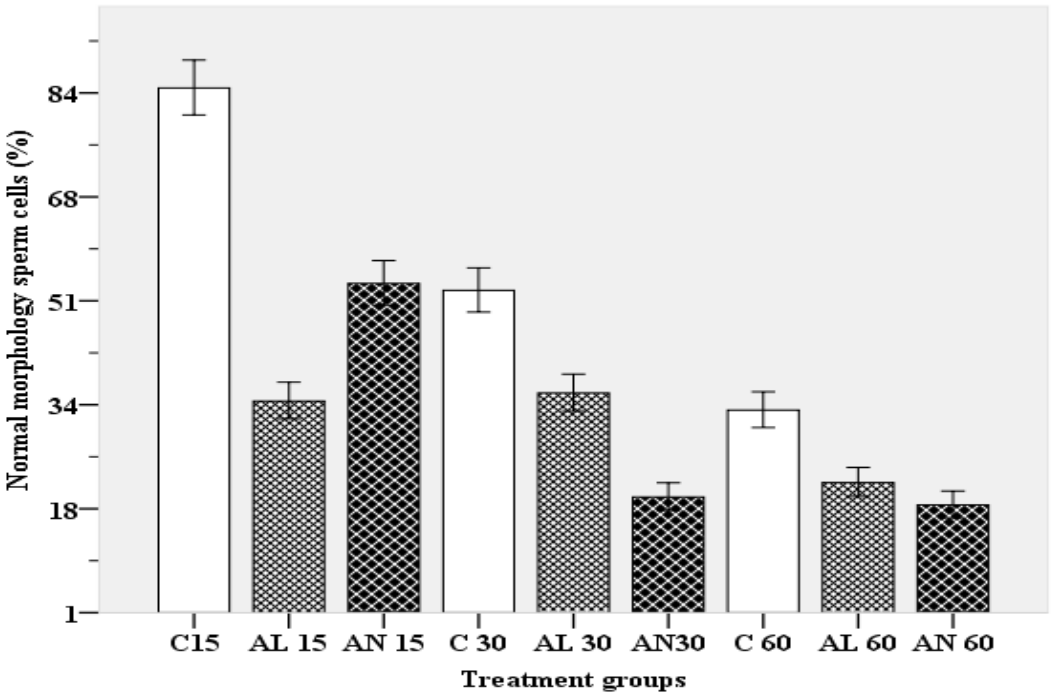


**Figure 2.1: Effect of crude plant extract in feed on the percentage of spermatozoa with normal morphology following its treatment for 15, 30, or 60 days in the *M. natalensis* male rats.**

C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods aqueous extract.

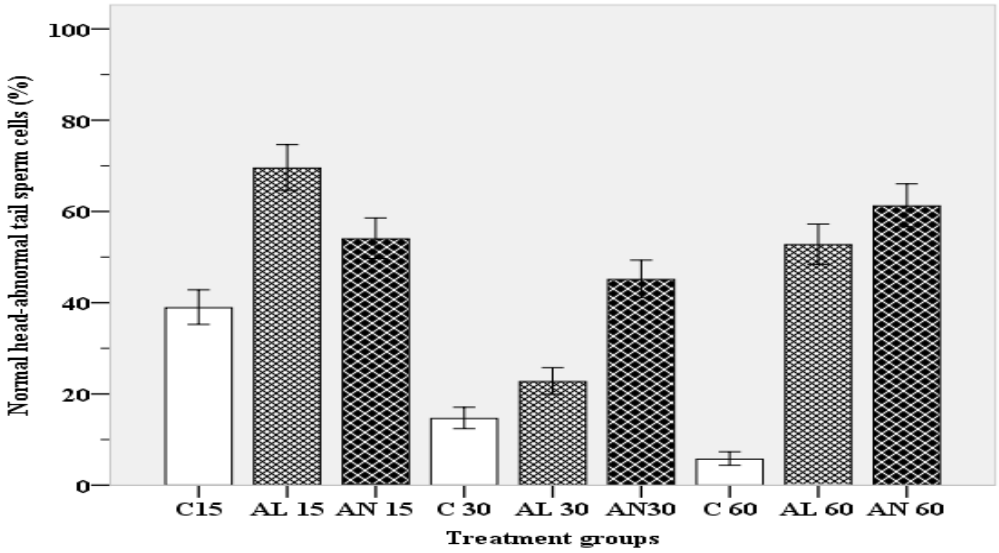


**Figure 2.2: Effect of crude plant extract in feed on the percentage of spermatozoa with the normal head-abnormal tail following its treatment for 15, 30, or 60 days in the M. natalensis male rats.**

C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods aqueous extract.

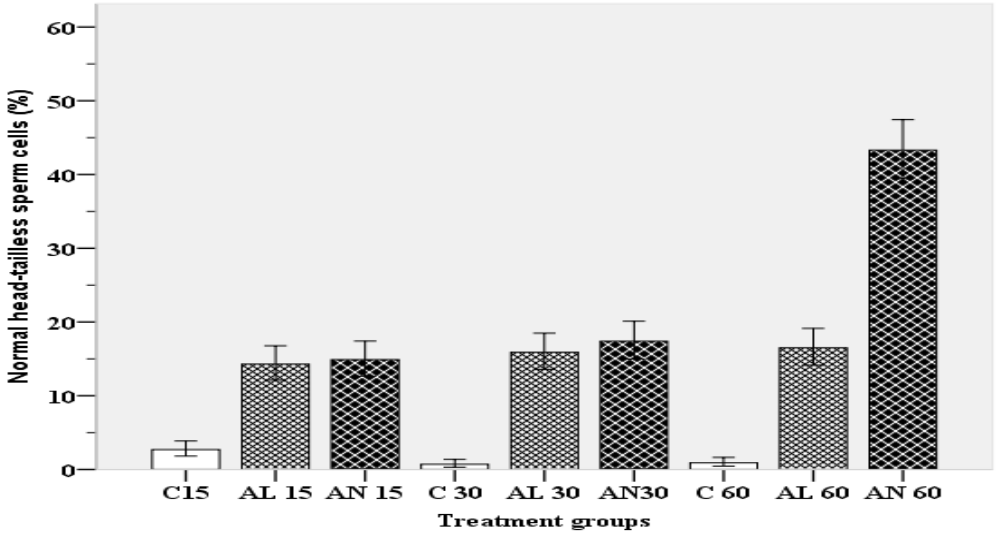


**Figure 2.3: Effect of crude plant extract in feed on the percentage of spermatozoa with normal head- tailless following its treatment for 15, 30, or 60 days in the M. natalensis male rats.**

C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods aqueous extract.

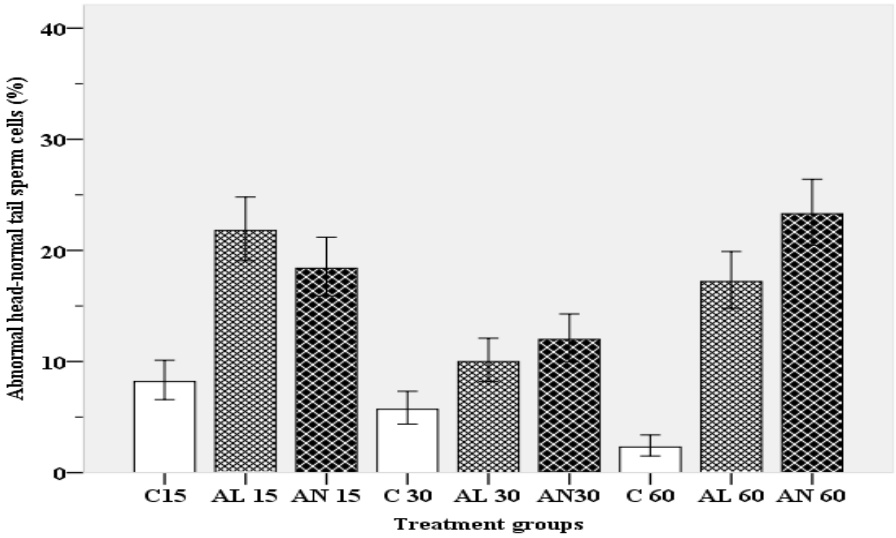


**Figure 2.4: Effect of crude plant extract in feed on the percentage of spermatozoa with abnormal head- normal tail following its treatment for 15, 30, or 60 days in the M. natalensis male rats.**

C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods aqueous extract.

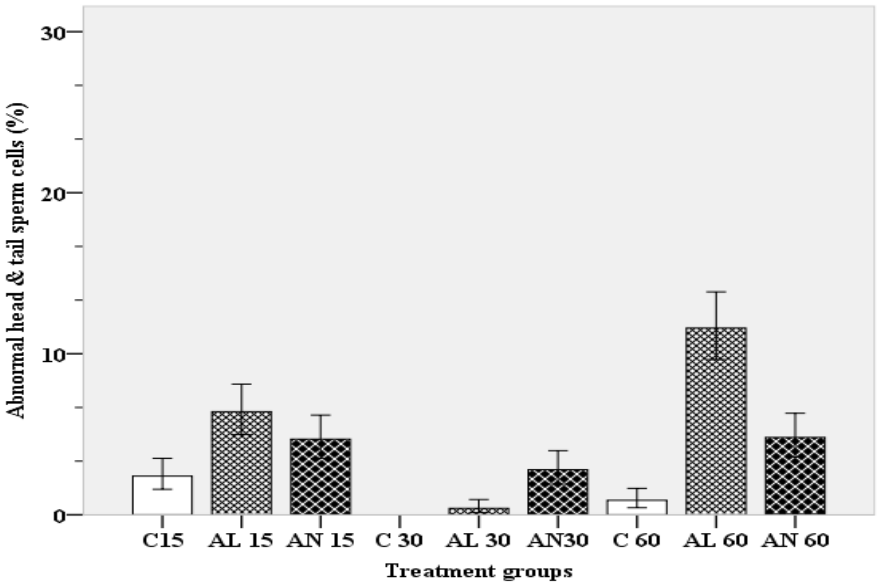


**Figure 2.5: Effect of crude plant extract in feed on the percentage of spermatozoa with defects on both head and tail following its treatment for 15, 30, or 60 days in the M. natalensis male rats.**

C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods aqueous extract.

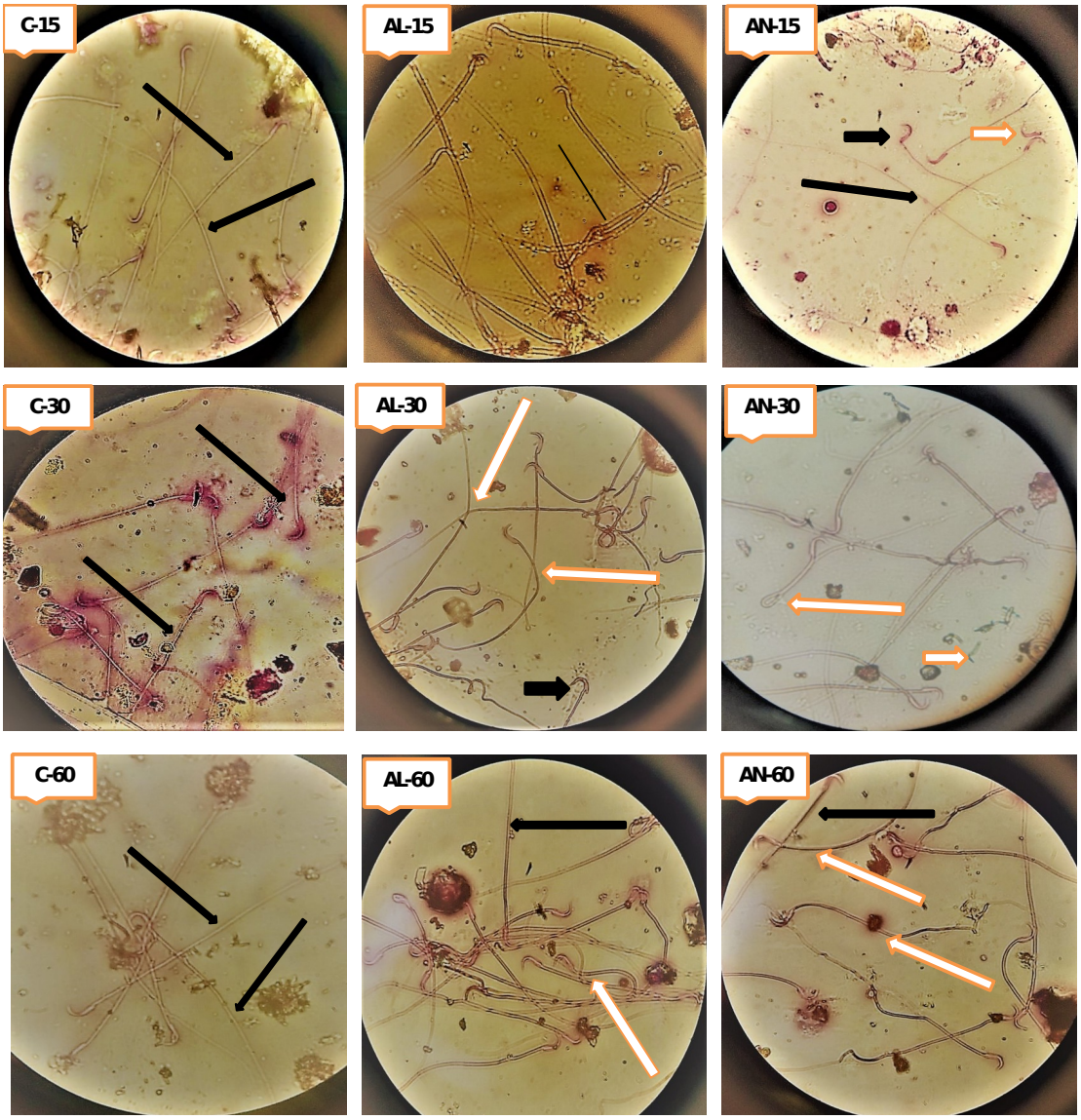


**Figure 2.6: Micro-graphic pictures of sperm cells at x200. Shows the effect of crude plant extract in feed on the morphology of head and tail of spermatozoa following its treatment for 15, 30, or 60 days in the *M. natalensis* male rats.**

C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods aqueous extract. A long black arrow shows normal spermatozoa, a long whitish arrow shows sperm cells with a bent tail, a short white arrow shows tailless sperm cells, short black arrow shows sperm cells with bent head.

**2.5 Discussion**

In this study, the phytochemical analysis indicated that flavonoids, tannins, saponins, steroids, terpenoids and plant phenols were all present in both the powdered raw materials and in the corresponding methanolic extracts of both *A. nilotica* pods and *A. lebbeck* stem bark. Judged by the intensity of colour change after a chemical reaction, plant phenols, terpenoids and plant steroids were equally intensely present in both the powdered raw materials and the methanolic extracts of both *A. nilotica* pods and *A. lebbeck* stem bark.

Our results are consistent with the results of Auwal *et al.* (2014) who analyzed the phytochemical constituents of *A. nilotica* pods, Okoro *et al*. (2014) who analyzed the phytochemical composition in leaves, stem bark and root extracts of *A. nilotica* and Sawant *et al.* (2014) who analyzed the phytochemical constituents of *A. nilotica* stem bark. Our results were also agreeable with the results of Elshiekh *et al*. (2020) who assessed the phyto compounds present in *A. lebbeck* seeds, Lawan *et al*. (2018) who revealed the presence of all phytochemicals except steroids and phylobotannins in *A. lebbeck* leaf extracts and El-Ghany *et al*. (2015) who carried out a phytochemical and biological study on the *A. lebbeck* stem bark. In all those studies flavonoids, tannins, saponins, steroids, terpenoids and phenolic compounds were shown to be among the major secondary metabolites highly present in the plant extracts.

Flavonoids are a group of plant compounds with variable phenolic structures found mostly in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine (Panche *et al*., 2016). Based on the carbon of the C ring on which the B rings are attached, flavonoids are classified as flavones, flavonols, flavanones, flavanonols, flavanols or catechins, iso-flavanoids, anthocyanins and chalcones (Panche *et al*., 2016). In plants, flavonoids play

some key biological roles in plant pigmentation and protection. In animals, flavonoids have been shown to play some key roles as anti-oxidants, anti-inflammatory and anti-microbial biochemical agents (Cushnie and Lamb, 2005; Panche *et al*., 2016). Yet, some flavonoids such as the iso-flavanoids are phytoestrogen meaning that they bind to similar oestrogenic receptors used by body estrogen in animals thereby exerting multiple oestrogenic effects including perturbation of reproductive activities (Wocławek-Potocka *et al*., 2013).

Tannins are water-soluble polyphenols that are present in the bark of trees, wood, leaves, buds, stems, fruits, seeds and roots of several plants (Chung *et al*., 1998). In many plant species, tannins offer structural and protection functions (Cuong *et al*., 2019). In animals, tannins have no known useful role besides their anti-nutritional properties once they are consumed by animals (Chung *et al*., 1998). However, some studies indicate tannins to have some spermicidal effects when tested in-vitro and in vivo in rabbits, indicating their potential antifertility properties (Benhong *et al*., 2012).

Saponins are naturally occurring phytochemicals consisting of triterpenoid or steroidal aglycones linked to oligosaccharide moieties that are widely distributed in various plants (Mugford and Osbourn, 2013; Faizal and Geelen, 2013). In plants, saponins serve a diversity of biological activities ranging from plant protection to amphipathic properties (Mugford and Osbourn, 2013). In some animals, saponins have been shown to cause some antifertility activities (Gupta *et al*., 2005; Shu *et al*., 2015).

Plant steroids are groups of hormones derived from *S*-squalene-2,3-epoxide via the acetate-mevalonate pathway (Gunaherath *et al*., 2020). Plant steroids have a list of medicinal benefits in animals including their significant hypolipidemic, anti-inflammatory, anticancer, anti-ulcer, anti-fungal, anti-oxidants and anti-atherogenic

capabilities when consumed (Grattan, 2013; Gunaherath *et al*., 2020). However, several other studies indicate that the antifertility properties of some medicinal herbs are partly attributable to their high content of phytoestrogen (Gebrie *et al*., 2005; Taur and Patil, 2011; Qasimi *et al*., 2017). In mammals, phytoestrogens cause infertility by mimicking the body's estrogens. They normally bind to the same intracellular estrogenic receptors used by body estrogen thereby exerting multiple estrogenic activities, such as uterotropic effect, sterility, or disruption of normal reproductive processes (Burton and Wells, 2002).

Plant Phenolics are secondary metabolites produced through the pentose phosphate through phenylpropanoid metabolization in plants (Randhir *et al.*, 2004; Panche *et al*., 2016). In animals, plant phenols offer strong antioxidant effects protecting couples of cellular biomolecules such as DNA, lipids and proteins from oxidative damage (Hollman, 2001). Moreover, some phenolic compounds such as isoflavones may mimici the function of bodily oestrogens affecting the animal’s reproductive function (Panche *et al*., 2016; Dai and Mumper, 2010a). In some studies, plant phenolics have been clearly shown to cause some antifertility activities in the treated rats (Shehab and Abu-Gharbieh, 2014). Therefore, the study concludes that pods of *A. nilotica* and Stem bark of *A. lebbeck* collected during the dry season in the eastern regions of Tanzania are rich in flavonoids, tannins, saponins, steroids, terpenoids and plant phenolic which could be responsible for the antifertility properties including their adverse effects on sperm cell morphologies in the *M. natalensis* rats.

Also in the current study, the abundance of sperm cells with morphological defects was observed in the plant extract treated rats mostly reflected the *A. lebbeck* stem bark and *A. nilotica* pod extract’s spermicidal potential. The dominant morphological defects of spermatozoa were the secondary abnormalities mostly dominated by the spermatozoa with

normal head-abnormal tails followed by those with normal head-tailless and those with abnormal head-normal tails.

Furthermore, there were few spermatozoa with deformities on both head and tail in rats under the extracts of *A. lebbeck* stem bark or *A. nilotica* pods. Results on sperm cell morphologies obtained in this study were related to the results of Saba *et al*. (2009) who revealed a significantly large proportion of sperm cells with secondary deformities in the *Lagenaria breviflora* fruit extract treated male Wister rats. Also, Oridupa *et al*. (2018) reported related results to the current study where a high percentage of spermatozoa with secondary morphological defects were revealed in male Wister rats exposed to the acetone extracts of *Combretum sordidum*.

**2.6 Conflict of Interests**

The authors have not declared any conflict of interest.

**2.7 Acknowledgments**

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# CHAPTER THREE

## 3.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

### 3.1 GENERAL DISCUSSION

An outbreak of rodent pests such as the multimammate rat (*Mastomys natalensis*) have been a significant threat to the livelihood and public health in many places in the sub-Saharan Africa (Mwanjabe *et al*., 2002; Swanepoel *et al*., 2017). The rodent pest's involvements in destruction of crop seeds, house properties and transmission of some deadly zoonotic diseases have been causing a non-ending conflict with human beings. Consequently, rodenticides have been in large use in sub-Saharan Africa to mitigate the rodent past's population by killing the rodents that consume the poison (Mulungu *et al*., 2010). However, there are several studies reporting increasing resistance of rodent pests resistances against many of the currently used rodenticides (Saxena, 2014). Also, the risks for collateral damages associated with rodenticide use have been another worrying issue (Brakes and Smith, 2005. All the above information has been among the drivers for increased need of some novel, sustainable methods of rodent's control. Among the highly explored new techniques of rodents control includes those aiming to reduce the fertility rate of rodents.

The science behind contraception in rodent pests is supported by the fact that rats or mice are easily adversely affected by any bad influences on their reproductive system (Cheng and Mruk, 2002). That means any pathological insults of natural or artificial sources gaining access to the reproductive tracts of rats or mice could easily contribute to infertility (Davis *et al*., 2001; Asuquo, 2012; Reddy *et al*., 2015). Among the chemical or biochemical agents with some reported antifertility actions in rats or mice include certain synthetic chemical hormones (Zhao *et al*., 2007; Su *et al*., 2016), certain immunological agents (Paterson *et al*., 2000; Massei *et al*., 2020) and some plant extractives (Devi *et al*., 2015).

However, despite showing excellent efficacies in laboratory studies, both the immunocontraception and synthetic steroid hormone have been not in use in the field environments of rodents control (Massawe *et al*., 2018; Massei *et al*., 2020). There are no known application methods of the immunocontraceptives to the animals of targets in the field situation delaying their deployments (Barlow, 2000). Also, due to its endocrine disruptive properties there great concern related to use of synthetic steroid hormones in the environments constraining its application in rodents management (Smith and Shore, 2015; Elliott *et al*., 2016; Shore and Shemesh, 2016; Adeel *et al*., 2017; Houtman *et al*., 2018). As a result, natural products including the use of medicinal plants are increasingly being explored as potential alternative rodent's fertility control sources.

In the current study aiming to evaluate the contraceptive potential of *A. nilotica* and *A. lebbeck* in the Multimammate rats (*Mastomys natalensis*), crude extracts of the two plants indicated clearly the role medicinal plants can play to reduce fertility and the population of rodent pests and thus be useful in rodent's population managements. The crude extracts from *A. nilotica* and *A. lebbeck* in *Mastomys natalensis* revealed fertility reduction in both sexes of the rodent pests. In the female rats, the plant extract caused disruption of ovulation leading to follicular degeneration and a reduction in fertility success rate, conception and fetal implantation activities.

In the male rats, the plant extracts caused significant damages in the seminiferous tubules of the rat's testis leading to disrupted spermatogenesis and a complete reduction of fertility success rate after 60 days of treatments with *A. lebbeck* extracts while in the *A. nilotica* treated male rats a complete suppression of fertility was observed at all the treatment duration of 15, 30 and 60 days.

Furthermore, it was shown in the current study during phytochemical analysis that the powdered raw materials and their corresponding extracts of both *A. nilotica* and stem bark of *A. lebbeck* collected during the dry season in the eastern regions of Tanzania, were rich in flavonoids, tannins, saponins, steroids, terpenoids, and plant phenols. At high concentration some compounds types including some flavonoids types such as the iso-flavanoids (Wocławek-Potocka *et al*., 2013), tannins (Benhong *et al*., 2012), saponins (Gupta *et al*., 2005, Shu *et al*., 2015), phytoestrogen (Burton and Wells 2002) and some plant phenolics (Shehab and Abu-Gharbieh, 2014) are said to have antifertility effects. Therefore, the presence of flavonoids, tannins, saponins, steroids, terpenoids and plant phenolic in the pods of *A. nilotica* and Stem bark of *A. lebbeck* could explain the antifertility actions of the plant extracts in the *M. natalensis* rats as were observed in the current study.

## 3.2 CONCLUSIONS

The crude extracts from *A. nilotica* and *A. lebbeck* caused a significant reduction of fertility success rate in both sexes of the rodent pests *Mastomys natalensis*. In female rats the extracts interrupted the ovulatory process leading to follicular degeneration, hence reduced implantation and pregnancy percentages. In male rats, the extracts caused significant damages in the seminiferous tubules of the extracts treated rats testis leading to perturbed spermatogenesis and a reduction of fertility success rate. In both cases the extracts from *A. nilotica* were more effective in causing the observed effects than those from *A. lebbeck*.

## 3.3 RECOMMENDATIONS

i. Elucidate the biochemical and molecular mechanism of the plant extracts leading to ovulatory failure, follicular atresia in the female rats and seminiferous tubule epithelial damage in the male rats.

ii. Investigate whether the antifertility effects of *A. lebbeck* stem bark and *A. niotica* pod's extracts revealed in the current study are revisable or not.

iii. Evaluate the shelf life of the *A. lebbeck* stem bark and *A. niotica* pod's extracts containing baits as this could be the convenient treatment approach once the plants extract are proven to be efficacious enough.

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