PRELIMINARY SCREENING OF PLANT-BASED APHRODISIACS ON THE ZAMBIAN MARKET

BY

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School of Medicine
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DECLARATION

I hereby declare that this dissertation is a product of my own work and that all the sources quoted have been indicated in the references. I further declare that this work has not been previously presented elsewhere whether wholly or in part for any other study programme at any university. It has been produced in accordance with the guidelines for Master of Science in Biochemistry dissertation for the University of Zambia.

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CERTIFICATE OF APPROVAL

The University of Zambia has approved this dissertation of DANNY BANDA as partial fulfilment of the requirements for the award of the Degree of Master of Science in Biochemistry.

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Abstract

Male potency has been a talk of many years since humanity existed and the use of various kinds of substances to stimulate sexual desire has been done for many years. Many plant-based concoctions have been released on the Zambian market for consumption without scientifically proven results or effects. Herbalists, Traditional health practitioners (THPs) have put up many advertisements to spread their market base but all the same without any proven results to show to would-be customers to use a particular product. Two local herbal extracts, *Mutimba vula* (*MTV*) and *Mwana apeluke* (*MWN*) were studied for the presence of medicinally active components and for their sexual behaviour effects in male rats. The main objective of this research work was to determine aphrodisiac properties of MTV and MWN aqueous herbal extracts. Phytochemical screening to determine presence of medicinally active components was performed following standard guidelines. Thereafter, 3 g each of dried powder of MTV and MWN were soaked in 250 mL of distilled water for 3 hours for extraction of active ingredients. Two concentrations, high and low doses of the herbal extracts were administered orally to the treatment groups for 21 days followed by sexual behaviour analysis. Concentration of testosterone in blood samples was determined using a Testosterone Enzyme-Linked Immunosorbent Assay (ELISA) test. Herbal extracts showed varying amounts of saponins, tannins, flavonoids, alkaloids and glycosides. The mounting frequency ($p=0.039$), intromission frequency ($p=0.032$) and penile erections increased ($p=0.001$) significantly indicating enhanced sexual activity in animals treated with the plant extracts. The results indicated that there was no dose-dependent relationship between serum Testosterone levels and the treatment groups ($p=0.061$). It was established that oral administration of *Mutimba vula* and *Mwana apeluke* caused increased sexual performance in rats. However, more studies are needed to exploit the possible mode of action.

**KEY WORDS:** Aphrodisiacs, potency, Mutimba vula, Mwana apeluke, Testosterone
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<td>Superoxide dismutase</td>
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DEDICATION

This dissertation is dedicated to my wife Munsaka Mayambu Banda for her unfailing support, encouragement and inspiration, my sons Chrispin Khondwani Banda and Mphatso Hanzhila Banda who are my source of motivation in my life. My mum for the immerse support given to me throughout my academic journey, my brothers and sisters for the support. Last but not the least; I thank my best friends Andrea Manongwa and Faith Mweene for their moral and financial support during my study.
CHAPTER 1 INTRODUCTION

1.1 Background

Traditional Herbal Aphrodisiacs are medicinal plants that are used to arouse sexual instinct, induce desire and increases pleasure and sexual performance (Lampiao et al., 2017). The word ‘aphrodisiac’ is derived from ‘Aphrodite’, the Greek goddess of love. Many natural substances such as Yohimbine and Mandraken plant, as well as ground Rhinoceros horn in Chinese culture and “Spanish fly” have historically been known as aphrodisiacs in Africa and Europe (Yakubu et al., 2005a). Even in today’s culture, certain foods, such as strawberries and raw oysters are used as aphrodisiacs. Chocolate, coffee, and honey are also believed to have aphrodisiac potential. Although these natural items are claimed as aphrodisiacs, there is no or little scientific confirmation supporting these assertions (Kotta et al., 2013).

In Africa, several plants have been used for many years to improve sexual stimulation and performance (Dare et al., 2015). Therefore, the hunt for an effective aphrodisiac has been a constant pursuit throughout history to ensure both male and female potency in the quest to fulfil the moral and religious issue of procreation. In Zambia, Ndubani (1999) reported an increasing interest regarding the practice of traditional healers and their use of indigenous plants to treat illness (Ndubani and Höjer, 1999).

Among the herbs sold on the streets in Lusaka are two herbs Mutimba vula (MTV) and Mwana apeluke (MWN). Mutimba vula is an indigenous plant whose scientific name is Entada abyssinica. E. abyssinica is a low-branching tree with a flat spreading crown and usually grows to about 7-10 metres (Kindt and Coe, 2005). The tree is harvested from the wild for local use as medicine and source of fibre and wood (Arnold and De Wet, 1993). E. abyssinica has been studied extensively for their antimicrobial and antioxidant properties (Mariita et al., 2010, Odalo et al., 2009, Atindehou et al., 2004). All the tree parts of E. abyssinica have been observed to have some medicinal use. The leaves are said to be febrifuge (a medicine used to reduce fever) and tonic, hence are used to make tonic tea and also used for wound healing (Hines and Eckman, 1993). The bark is reported to be abortifacient and is used in the treatment of colds, stomach pains and bronchial problems. However, the
aphrodisiac properties of its extracts have not been documented to have been subjected to any scientific studies. *Mwana apeluke* powder is a mixture of some local herbal aphrodisiacs. The contents of the mixture were however not revealed by the local herbalist.

One reason why many people seek aphrodisiacs apart from sexual pleasure is male infertility, a world-wide medical and social problem. According to Kamatenesi and others, reproductive health care is the second most prevalent health care problem on the African continent (Kamatenesi-Mugisha and Oryem-Origa, 2005). The fact that more than 12 percent couples worldwide are affected by infertility causes for a global reproductive health concern (Inhorn, 2003). In India alone, the figure stands at 30 million and in half of such cases, men are responsible for the situation. Excessive alcoholism, smoking, late marriages and stress are blamed (Wani et al., 2011b). Male infertility is definitely on the rise and the average sperm count has been decreasing. Virility is sexual desire in the mind whereas fertility is the physical ability of reproducing, which can be judged on two parameters: sperm type (sperm count and motility) and the ability to deposit the sperm into the female reproductive tract during a sexual act (Badami et al., 2000).

Therefore, humans are continuously looking for an ultimate aphrodisiac in search of a heightened sexual satisfaction to take care of biological and personal purposes. Despite the availability of well-known synthetic aphrodisiacs with their exact mechanism of action, people are interested in finding out a natural substance believing that it could have less or no side effects (Singh and Singh, 2012). Natural aphrodisiacs are relatively cheap and could also have additional benefits such as providing nutrition and help recover infertility without adverse side effects. The pursuit for more cost effective herbal alternatives to compete against synthetic drugs is of utmost importance as it will grant those with socio-economic constraints, access to treatment (Erasmus et al., 2015). Some of the well-known herbal aphrodisiacs are *Tribulus terresteris, Withania somnifera, Eurycoma longifolia, Avena sativa, Ginko biloba, Mondea whitei, Psoralea coryifolia* and *Rhinoceros horn* (Bella and Shamloul, 2014).

The rise in research work related to natural aphrodisiacs during the last decade is the proof about the curiosity and the significance of this subject. Though several such
substances are known, natural aphrodisiacs which have undergone rigorous research and could be recommended for human use are only few (Dama et al., 2010). In Zambia, the use of herbal preparations purported to be aphrodisiacs on the streets and in various markets have been on the rise as men from various walks of life purchase these substances. The purpose of this study was to determine the aphrodisiac properties of Mutimba vula and Mwana apeluke herbs sold at various markets in Lusaka.

1.2 Problem Statement
Male potency has been a topic of interest in many communities and the use of various kinds of sexual stimulants has been done for many years to address this issue. (Mukherjee and Wahile, 2006, Halberstein, 2005, Sandroni, 2001). Many herbal medicines have been released on the Zambian market for consumption without scientifically proven results or effects. The rampant use of various aphrodisiacs that are not well documented scientifically exposes the general public to many health related dangers including, but not limited to, chronic toxicity, overdose, dependency etc.

Many men have fallen prey to such mixtures and without tangible scientific proof whether the aphrodisiacs work or not, it is difficult to make informed decisions. Herbalists or traditional health practitioners have put up many advertisements to spread their market base but all the same without any proven results to show to would-be customers how to use a particular product. A lot of men have consumed these products, some with devastating effects leading to committing of crimes, others with over doses resulting in elevated blood pressures, and others with prolonged erections lasting hours.
1.3 Study justification

According to (Naik et al., 2011), the use of aphrodisiac herbs has increased in the recent past because of their efficacy, ‘safety’ and lesser side effects. However, Shamloul (2010) argues that there is little evidence from literature to recommend the usage of natural aphrodisiacs for the enhancement of sexual desire and or performance (Shamloul, 2010). Because of this, there is need to do research in this area of natural aphrodisiacs to understand the nature and mode of action of many of the aphrodisiacs used or being sold on the Zambian market, to be particular.

It is for this reason that this study was conducted to establish the fact by questioning the potency of the herbs by experimentation; and find out whether these plant aphrodisiacs are really potent or could it be that the sellers are being smart and playing psychological tricks on the public.
1.4 Research Question
Do herbal extracts of MTV and MWN sold in Lusaka show aphrodisiac properties when administered orally to male albino rats?

1.5 Main objective
To determine aphrodisiac properties of MTV and MWN aqueous herbal extracts sold in Lusaka, Zambia.

1.5.1 Specific Objectives

1. Determine phytochemicals present in MTV and MWN aqueous herbal extracts.

2. To determine aphrodisiac properties of MTV and MWN herbs in male albino rats

3. To compare serum testosterone levels in the Sildenafil and aqueous herbal extracts groups after administration.
CHAPTER 2 LITERATURE REVIEW

2.1 Herbal aphrodisiacs

Traditional herbs have been a revolutionary breakthrough in the management of erectile dysfunction and have become known world-wide as an ‘instant’ treatment (Adimoelja, 2000). In Uganda, about 70-80% of the Ugandan population still rely on traditional healers for their day to day health care (Kamatenesi-Mugisha and Oryem-Origa, 2005). The World Health Organisation (WHO) estimated the usage of traditional medicine in developing countries to be at 80% (Organization, 2002). This high statistic reveals the wide spread use and their importance in African reproductive health care, Zambia inclusive.

According to Malviya and others (2011), aphrodisiacs can be categorised according to their mode of action into three groups; substances that increase libido (i.e. sexual desire, arousal), substances that increase sexual potency (i.e. effectiveness of erection) and substances that increase sexual pleasure (Malviya et al., 2011). The aphrodisiacs highly sought after by men are those that increase sexual potency (i.e. effectiveness of erection). Yuan (2013) reported that past surveys indicated that nearly 50% of men reported some degree of ED and about 65% were not satisfied with the hardness of their erection (Yuan et al., 2013).

The use of aphrodisiacs to treat sexual disorders is a long back history in different systems of medicine and was practiced by different types of vaidyas and traditional healers in almost all the countries in the world, like China, India, Egypt, Rome and Greece (Sandroni, 2001). Patel (2011) reported that sexual desire is controlled and regulated by the central nervous system which integrates tactile, olfactory, auditory, and mental stimuli (Patel et al., 2011). In this regard, aphrodisiac drugs are said to act by altering the level of neurotransmitters or specific sex hormones in the body such as altering testosterone concentration in the body. Significant increase in serum testosterone concentrations in all male albino rats were observed after being exposed to aqueous extracts of Fadogia agrestis stem which led Malviya and his colleagues (2011) to conclude that the aqueous extracts increased the blood testosterone concentrations and hence the mechanism for its aphrodisiac effects and various masculine behaviours (Malviya et al., 2011). In this line, aqueous extracts from its
stem maybe used to modify impaired sexual functions in animals, those arising from hypotestosteronemia (Yakubu et al., 2005b). The sexual drive in both women and men is influenced by testosterone (T). Males have significantly higher mean testosterone levels than females of the same age. (Döhler and Wuttke, 1975, WEISZ and WARD, 1980, Buss, 2003). This implies that, the higher the testosterone levels in an individual, the more intense his sexual urge. Therefore, plant based aphrodisiacs which increase the levels of testosterone potentially increase the sexual urge of the individuals that take them.

Androgens are sex hormones that play an essential role in male reproductive function. They are known to act both centrally and peripherally for the initiation and maintenance of sexual functions. Various stimuli such as anabolic steroids (testosterone) are known to either up regulate or down regulate androgen response (Gauthaman and Adaikan, 2005). Bijlwan and others (2014) further noted that aphrodisiacs are molecular mimics of neurotransmitters and hormones, therefore, structural standards of deriving the chemobiologic entities were selected from two categories namely neurotransmitters (Dopamine) and hormones (androgen and testosterone) (Bijlwan et al., 2014).

The role of T in sexuality has been widely studied over the past years as noted by Jordan and others (Jordan et al., 2011). In their study, entitled “The role of testosterone in sexuality and paraphilia—A neurobiological approach. Part I: Testosterone and sexuality”, they observed that the wide distribution of androgen receptors throughout the brain and their numerous mechanisms demonstrate the ability of androgens to modulate almost every aspect of sexual behaviour. These include emotional, motivational and cognitive aspects. The sexual interest, desire, libido, fantasy, imagination and pleasure are interlocked in the chemistries of neurochemicals, hormones and bioenergetics. Some symptoms of hypogonadism include sexual symptoms such as loss of libido, erectile dysfunction, difficulty achieving orgasm, diminished sexual penile sensation as well as other symptoms such as fatigue, lack of physical strength, impaired cognitive function, and depressed mood (Wald et al., 2006). It has therefore been noted that a decrease in T levels in blood plasma significantly reduces erectile function in male. Conversely, high T
levels in blood plasma have been correlated with increased masculine features which include male potency among other things.

### 2.2 Mechanism of action of plant aphrodisiacs

It has been noted that the mechanism of action of these plant aphrodisiacs varies. Certain plant aphrodisiacs contain active ingredients that inhibit or activate important enzymes involved in relaxation of penile smooth muscle that result in an erection. Some of the most important enzymes that are central to smooth muscle relaxation of penile tissue are Endothelial Nitric Oxide Synthase (eNOS), Guanylate cyclase (GC), Adenylate Cyclase (AC) and Phosphodiesterase-5A (PDE 5A) (Burnett, 2006a). Penile erection is a highly regulated physiologic event that comprises increased arterial inflow and restricted venous outflow from the penis, coordinated with the corpus cavernosal smooth muscle relaxation (Burnett, 1995). The physiology of an erection according to (Rand, 1992) includes the understanding that a non-adrenergic, non-cholinergic (NANC) mechanism is principally involved. Nitric oxide (NO) is synthesised by the enzyme Endothelial Nitric Oxide Synthase (eNOS) in blood vessels and is involved with regulating vascular tone by inhibiting smooth muscle contraction and platelet aggregation. The study done by Kakiailatu (2000) confirmed that potential sources of NO were in the neuron, sinusoidal endothelium and corporal smooth muscle cells of the penis (Kakiailatu, 2000).

According to Misko and others (1993), they showed that NO operates in the vascular system by stimulating the formation of 3’, 5’-cyclic guanosine monophosphate (cGMP) (Misko et al., 1993). Nitric oxide is believed to be the main vasoactive NANC and chemical mediator of penile erection (Burnett, 2006b). Released by nerve and endothelial cells in the corpora cavernosa of the penis, NO activates soluble guanylyl cyclase (sGC), which acts on Guanosine Triphosphate (GTP) breaking it down to cGMP thereby increasing cGMP levels. Acting as a second messenger molecule, cGMP regulates the activity of calcium channels as well as intracellular contractile proteins that affect the relaxation of corpus cavernosum smooth muscle. In turn, cGMP activates Protein Kinase-G which phosphorylates the Myosin Light Chains (MLC) hence initiating relaxation of the smooth muscle via opening of potassium channels and thus diminishing intracellular calcium levels by preventing influx and promoting calcium sequestration within the sarcoplasmic reticulum. The
activation of the enzyme sGC by conformational change as a result of NO binding to the iron atom of its heme moiety is a critical step. Burnett (2006) further noted that impaired NO bioactivity is a major cause of erectile dysfunction. Perhaps, a deeper understanding of NO bioactivity as activated by plant aphrodisiacs would treat most cases of erectile dysfunction emanating from NO inhibition.

Uckert (2006) reported that the discovery of the importance for relaxation of the human cavernous tissue of the NO and cGMP pathway is a landmark for the development of the “modern” pharmacology of ED (Ückert et al., 2006). It has led to the identification of certain drugs that can elevate intracellular levels of cGMP. Among these are the NO donors sodium nitroprusside, nitroglycerine and linsidomine (SIN-1), and selective inhibitors of PDE 5 (Heaton et al., 1990, Truss et al., 1994).

After the introduction of PDE-Is (sildenafil) in 1998, a large number of studies were conducted which demonstrated that oral PDE 5-Is are highly effective and well tolerated for ED patients (Aversa et al., 2006, Ravipati et al., 2007, Blount et al., 2004, Tsertsvadze et al., 2009). More studies in this area could bring to light active ingredients that could prove more effective in treatment of ED than the conventional PDE inhibitors that have side effects which include dizziness, flushing, dyspepsia, nasal congestion or arthritis (Rossi, 2005).
Figure 1 shows the physiologic mechanism of a normal erection. Nitric oxide (NO) is synthesized from the endothelial cells by the enzyme NO synthase (NOS). It diffuses into the smooth muscle and stimulates guanylyl cyclase. An increase in the cyclic GMP (cGMP) concentration stimulates the release of protein kinases. This causes the potassium channels to open and the calcium channels to close, producing hyperpolarization and, ultimately, smooth-muscle relaxation. eNOS, endothelial NOS; GTP, guanosine triphosphate; nNOS, neuronal NOS; PDE, phosphodiesterase enzyme.

The medicinal potential (antimicrobial, antioxidant, anticancer, antimalarial, immunodulatory, etc.) attributed to plants has been linked to the presence of secondary metabolites (Maddila and Hemalatha, 2017). These various chemical components include saponins, alkaloids, volatile oils, flavonoids and anthraquinones. The phytochemicals include polyphenols, phytosterols, alkaloids and saponins. Polyphenols such as flavonoids have potent antioxidant and scavenging activities which are thought to improve sexual activities and are also associated with reduced

1Adapted from the Journal of Andrology 27, Issue 3, pages 335-347, 2 JAN 2013 DOI:10.2164/jandrol.05136
http://onlinelibrary.wiley.com/doi/10.2164/jandrol.05136/full#f1
risks of cardiovascular disease (Pulido et al., 2000). Most alkaloids exhibit pharmacological and toxicological activities (Wink, 2000). In animals, many secondary metabolites (including alkaloids) are known to affect neurotransmission and signal transduction (Wink, 1993). Because of this, a number of alkaloids have been used in the past for medicinal purposes and even at present (Robinson, 2012). Saponins have detergent properties, give stable forms in water, show haemolytic activity and have a bitter taste (Price et al., 1987). Their biological activity is closely related to chemical structures that determine the polarity, hydrophobicity and acidity of compounds (Rao and Sung, 1995). Because of their medicinal properties, presence of phytochemicals is good basis to suspect a plant to have medicinal properties; however their concentrations in the sample extract determines their ultimate effect.

Literature search on studies done on aphrodisiac properties of E. abyssinica (MTV) did not yield any results. Entada abyssinica is a low-branching tree with a flat spreading crown and usually grows to about 7-10 metres (Kindt and Coe, 2005). The tree is harvested from the wild for local use as medicine and source of fibre and wood (Arnold and De Wet, 1993). E. abyssinica has been studied extensively for their antimicrobial and antioxidant properties (Mariita et al., 2010, Odalo et al., 2009, Atindehou et al., 2004). A study carried out by (Fabry et al., 1998) titled, “Antibacterial activity of East African medicinal plants” showed that plant extracts of E. abyssinica showed weak antibacterial activities of the methanol extract. A number of biologically active compounds have been isolated from E. abyssinica such as diterpenes, kolavenol, flavonoids and phytosterol glycosides.

Mwana apeluke is sold as a mixture of plant herbs whose identities were not revealed at the time of purchase hence no literature review on it was done.

This study was therefore, designed to evaluate the possible aphrodisiac properties of aqueous extracts of MTV and MWN in male albino rats.
CHAPTER 3  METHODOLOGY

3.1  Study Design
The study design for this research project was an Interventional Study involving white albino rats’ species.

3.2  Materials and Reagents
Reagents used were Ferric Chloride solution, concentrated Sulphuric acid, Chloroform, fragments of metallic Magnesium, concentrated Hydrochloric acid, glacial acetic acid, copper acetate solution, Estrumate (brand of oestrogen) and Testosterone ELISA Parameter Assay kit from BIOO Scientific Corporation Lot No. 108001051216$60837705129. Other materials used included microplate reader, 10 mL centrifuge tubes (Eppendorf), 10 mL vacutainers (red tops), tube racks, heating mantles, Soxhlet apparatus, distillation apparatus, gloves, dissecting boards, needles, incisors, cotton wool, feeding bottles, electronic beam balance and a suffocating jar.

3.3  Study Site and Sampling
This study was conducted at the University of Zambia, Biological Sciences Department Animal Care Unit, in Lusaka, Zambia.

This study was a single-centre study.

Simple random sampling of the male rats was done. Every second male rat in the sequence was selected for inclusion in the study.

3.4  Sample Size
The sample size was adopted from a similar study done by (Singh and Chaturvedi, 2013), to correlate NO and testicular activity in laboratory mouse, Mus musculus with slight modifications. The modifications done included the reduction in total number of animals used from forty eight to thirty six and extension of the treatment period from seven days to twenty one days. A total of thirty six male rats randomised into six groups of six each were used in the study.
3.5 Inclusion criteria and Exclusion Criteria

Animals that were used in this study were sexually active adult male and female rats. Female rats were brought to oestrous before the tests were done. The males’ weight of between 200-300 g and females’ weighing 250-270 g were included in the study.

All young rats and adult rats with weight more or less than the given ones above were excluded. Sexually inactive males were also excluded (Wani et al., 2011a, Alhowiriny et al., 2013)

3.6 Experimental Design

The sequence of experiments reported here where executed in the following manner: first the plant herbal preparations in powder or root form were purchased locally from traditional health practitioners and pre-heated where necessary at the University of Zambia, Department of Chemistry. Pre-treatment included drying to reduce moisture content and size reduction by homogenisation using the Waring Blender at full speed. The powdered sample was kept at ambient temperature avoiding direct sunlight in self-sealing 500 mL transparent polythene bags.

Phytochemical screening followed using standard methods as described in this report. The next set of experiments involved preparation of various concentrations of the herbal preparation for oral administration. These steps ran concurrently with the conditioning of the rats.

3.7 Herbal Preparation

All phytochemical screening tests were carried out at the University of Zambia, Department of Chemistry. A Soxhlet assembly apparatus and an ethanol-water mixture solvent were used for sample extraction. An ethanol stock solution (95 % ethanol) was diluted to a 70 % ethanol-water mixture which was used as the solvent.

The volume of ethanol to use was calculated using the formula: -

\[ M_1V_1 = M_2V_2 \]
Where $M_1$, was the initial molarity of ethanol (95 % ethanol), $V_1$, was unknown volume of ethanol to be used, $M_2$, was 70% ethanol concentration needed and $V_2$, was the total volume needed (250 mL). The calculated $V_1$ was 184.2 mL.

Therefore, 184.2 mL of 95 % ethanol was placed in a volumetric flask and filled to the mark with distilled water to make up a volume of 250 mL of an ethanol-water mixture. This ethanol-water mixture (70 percent ethanol) was then used as a solvent in the extraction of the active ingredients with each herb undergoing a minimum of ten extraction cycles.

To determine how much herb was used in the extraction procedure, the following steps were used. First the mass of an empty beaker was taken followed by the mass of the beaker plus the thimble and finally the mass of the beaker, thimble and the herbal material together.

The mass of the herbal material was taken as the difference in mass between the mass of the beaker, thimble and herbal material minus the mass of the beaker and thimble. For instance, for herb JNNM1, calculations were done as follows:

\[
\begin{align*}
\text{Mass of empty beaker} &= 90.4 \text{ g} \\
\text{Mass of beaker + thimble} &= 95.0 \text{ g} \\
\text{Mass of beaker + thimble + herbal material} &= 118.5 \text{ g} \\
\text{Mass of herbal material} &= 23.5 \text{ g}
\end{align*}
\]

After the Soxhlet extraction procedure was done, the ethanol was distilled out using simple distillation and the remaining aqueous solution of the herbal extract mixture was left to air dry in a Petri dish. The dry sample containing active ingredients obtained was then used for phytochemical screening purposes.
3.8 Phytochemical screening tests

Phytochemical examinations were carried out for all the extracts as per standard methods outlined in *Phytochemical screening and Extraction; A review* (Tiwari *et al.*, 2011)

1. **Detection of Alkaloids**

On a steam bath, 3 mL of aqueous extracts were stirred with 3ml of 1% Hydrochloric acid after which Mayer’s reagent (Potassium Mercuric Iodide) was added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2. **Test for Tannins**

Equal volumes (2 mL) of the aqueous extract and distilled water were stirred and a few drops of Ferric Chloride (FeCl₃) solution added. The formation of a green precipitate was taken as an indication for the presence of tannins.

3. **Test for Saponins**

Equal volumes (5 mL) of aqueous extract and distilled water was shaken vigorously in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of Saponins.

4. **Detection of Phytosterols**

The Libermann Burchard’s test was used to test for phytosterols. First, the extracts were treated with chloroform and filtered. The filtrates were then treated with few drops of acetic anhydride, boiled and cooled followed by addition of concentrated Sulphuric acid. Formation of brown ring at the interface indicated the presence of phytosterols.

5. **Test for Flavonoids**

To 3 mL of the extract 2 fragments of metallic Magnesium added followed by addition of 0.5 mL of concentrated Hydrochloric acid. Colour observations were made after 5 minutes: red colour indicated presence of flavonols, orange-for flavons, red-violaceous-characteristic of flavanones or green-in case of flavanols.
6. Test for Glycosides

The Keller-Kiliiani test was used to test for presence of glycosides. Two millilitres of the extract was dissolved in an equal volume of glacial acetic acid containing one drop of FeCl$_3$ solution. The mixture was then poured into a test tube containing 1 mL of concentrated sulphuric acid (H$_2$SO$_4$). A brown ring at the interface indicated the presence of a deoxy sugar, characteristic of cardenolides.

7. Detection of Diterpenes

The Copper Acetate Test was used to determine presence of diterpenes. Extracts were dissolved in water and treated with 3 drops of copper acetate solution. Formation of emerald green colour was an indication of the presence of diterpenes.

3.9 Preparation of plant extracts

The tea strain extraction formula was used to extract active ingredients for the treatment groups. This was done in line with the instructions that local herbalists give to their clients regarding herbal formula preparation. Three grams of plant material was placed separately in 250 mL of luke-warm water in a corked flat bottomed flask and left to strain for 3 hours before being administered. The herbal preparation was then decanted through a filter paper to obtain a filtrate. Half of the filtrate (125 mL) was diluted in a volumetric flask to yield 250 mL of a diluted extract solution. The remaining undiluted filtrate was taken to be the 100 percent concentration (High dosage) while the diluted filtrate was taken to be 50 percent concentration (Low dosage). These concentrations were then administered to the treatment groups orally for a period of 21 days. Everyday this procedure was repeated in order to prepare a fresh sample for administering. Any sample that was left after treatment was discarded.

3.10 Conditioning of rats

The rearing and conditioning of the rats was done at the University of Zambia Biological Sciences Department at the Animal Care Unit.

Sexually active male and female rats were used for this study. The rats were maintained at a temperature of 25 ± 2 °C. A reversed twelve-hour light-dark cycle was employed with fluorescent ceiling light. A dim red light was provided during
the dark cycle (Feldman et al., 1994). Conditioning of the rats to adjust to the environment was done two weeks before the start of the experiment. The experiments were then carried out on day 21 after two weeks of conditioning between 9:00 AM-12:00AM (Pednekar et al., 1993) (Hardy and Debold, 1971).

3.11 Preparation of females
Female rats were brought to oestrous state by administration of oestrogen through a single intramuscular injection of Estrumate (estradiol valerate) at a dose of 2 µg/kg, 48 hrs before the test. Estrumate is a product of Schering-Plough (PTY)/(EDMS)BPK 54 Electron Avenue ISANDO 1600, RSA. This drug was purchased from Livestock Services Zambia. The drug however is designed to be used in cows and therefore the dosages were calculated to take into consideration the weights of the rats.

3.12 Training of male rats
Males were trained individually with active female rats in oestrous state in a transparent mating arena for 15 min. A male rat was considered sexually active if it attempted to mount any active female rat introduced in the cage. Only such male rats were included for the subsequent experiments.

3.13 Mating behaviour tests
Thirty-six sexually active male rats were selected for the study. They were divided into six groups with each group having six rats (Zade et al., 2013). Group 1 rats each received 1 mL of distilled water orally as vehicle and served as control group.

Group 2 rats each received Sildenafil citrate at a dose of 25 mg/kg body weight orally and served as the standard reference group.

Two different herbal preparations were used for treatment.

Group 3 and Group 4 rats each received 2 mL of Mutimba vula percent (MTV high dosage) and 2 mL of Mutimba vula 50 percent (Low dosage) herbal extracts respectively.
The Group 5 and Group 6 rats were each treated with 2 mL of *Mwana apeluke* 100 percent (High dosage) and 2 mL of *Mwana apeluke* 50 percent (Low dosage) herbal extracts respectively.

The herbal preparations were administered orally in form of aqueous suspensions for a period of 21 days (Gopumadhavan et al., 2003).

Eight female rats were artificially brought into oestrus (heat) by a method which involved administration of oestrogen through a single intramuscular injection of Estrumate at a dose of 2 µg/kg, 48 hours before the experiment (Szechtman et al., 1981). This was done because the female rats allow mating only during the oestrus phase as they are receptive to males during this period.

### 3.14 Sexual behaviour

Male rats were placed in an observation glass individually in order to acclimatise them with the cage environment. Then a sexually receptive female rat was allowed to enter the test cage silently from a slide door inside the cage. The behavioural observations were then carried out taking into account the following parameter: - Mounts, Intromissions and Penile erections (Sharma et al., 2009).

#### 3.14.1 Mounting behaviour

A mount was seen when one rat places its forequarters on another rat's rump from behind. Mounting is the male copulatory position, and was seen when a male rat mounted a female prior to mating. The parameter of mounting observed was Mounting Frequency (MF) defined as the average number of mount during 15 min observation.

#### 3.14.2 Intromission behaviour

Intromission is the action or process of inserting the penis into the vagina in sexual intercourse. When an intromission had occurred, rats showed a “vigorous jump” which was not observed after a mount (an unsuccessful intromission). Normal mounts were not usually followed by grooming, while intromissions were. The parameter of intromission that was analysed was Intromission Frequency (IF) defined as average number of intromission during a 15 min observation.
3.14.3 Penile erection (PE)

This was determined when the rats bent down to lick their erect penis during the observation period. The number of PE was recorded during the observation period.

3.15 Sample Collection and Preparation

3.15.1 Sex organ parts

After 21 days of treatment, all the male rats were anaesthetised and then sacrificed (killed) by decapitation. The testis, seminal vesicles, epididymis and prostate glands were carefully removed and immediately weighed on an electronic beam balance. After weighing, the sex organ parts were then discarded.

3.15.2 Blood Samples

After male rats were decapitated, about 3-5 mL of blood samples were collected from the Jugular vein of each rat and placed in 10 mL vacutainers (red tops) which were labelled for identification. The samples were then allowed to coagulate under room conditions. After this, the samples were taken to the University of Zambia, School of Veterinary Medicine, Department of Biomedical sciences, where they were centrifuged at 3000 x g for 5 min in order to separate the plasma from blood. The plasma samples were then put in 3 mL yellow vacutainers and stored at -80 °C before assay.

3.15.3 Testosterone (T) Assay Procedure

All reagents and samples were brought to room temperature using a water bath before use. Table 1 shows the Testosterone Kit contents, their amounts and storage conditions prior to performing the Elisa test.
Table 1 Kit Contents, Storage and Shelf Life

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone-coated Microtiter Plate</td>
<td>1 x 96-well Plate (8 wells x 12 strips)</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>Testosterone Standards:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative control (white cap tube)</td>
<td>0.8 mL</td>
<td></td>
</tr>
<tr>
<td>0.05 ng/mL (yellow cap tube)</td>
<td>0.8 mL</td>
<td></td>
</tr>
<tr>
<td>0.15 ng/mL (orange cap tube)</td>
<td>0.8 mL</td>
<td></td>
</tr>
<tr>
<td>0.45 ng/mL (pink cap tube)</td>
<td>0.8 mL</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>1.35 ng/mL (purple cap tube)</td>
<td>0.8 mL</td>
<td></td>
</tr>
<tr>
<td>4.05 ng/mL (blue cap tube)</td>
<td>0.8 mL</td>
<td></td>
</tr>
<tr>
<td>100 ng/mL (spiking, optional, red cap tube)</td>
<td>0.8 mL</td>
<td></td>
</tr>
<tr>
<td>Testosterone Antibody #1</td>
<td>12 mL</td>
<td>2-8 °C *</td>
</tr>
<tr>
<td>100X HRP-Conjugated Antibody #2</td>
<td>250 mL</td>
<td>2-8 °C *</td>
</tr>
<tr>
<td>Antibody #2 Diluent **</td>
<td>20 mL</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>10X Sample Extraction Buffer **</td>
<td>25 mL</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>20X Wash Solution **</td>
<td>28 mL</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>Stop Buffer **</td>
<td>14 mL</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>TMB Substrate **</td>
<td>12 mL</td>
<td>2-8 °C</td>
</tr>
<tr>
<td>Clean Up Mix (optional, for plasma/serum sample only)</td>
<td>28 mL X 2</td>
<td>2-8 °C</td>
</tr>
</tbody>
</table>

Preparation of 1X Sample Extraction Buffer

One (1) mL of 10X Sample Extraction Buffer was mixed with 9 mL of distilled water to make a 1X Sample Extraction Buffer.
Sample Preparation

- Two (2) mL of the plasma sample was mixed with 6 mL of acetonitrile and 0.5 mL of Clean Up Mix. The mixture was vortexed for 3 min at maximum speed manually.

- The sample was then centrifuged for 5 minutes at 4000 x g at room temperature. After this, 3 mL of the acetonitrile supernatant was then transferred into a new vial. A rotary evaporator was then used to dry the sample in a 60-70 °C water bath under reduced pressure.

- A 0.5 mL of 1X Sample Extraction buffer was then added to the dried residue and the mixture vortexed for 1 minute at maximum speed. After this, the sample mixture was again centrifuged for 5 minutes at 4,000 x g at room temperature. The upper hexane layer was then discarded.

- Finally, 50 µL of the lower aqueous layer per well was then used for the assay.

(a) Reagent Preparation

All reagents were brought up to room temperature using a water bath before being used (1 hour at 20 – 25 °C). Solutions were prepared just prior to ELISA test.

Preparation of 1X HRP-Conjugated Antibody #2

The secondary antibody was diluted 1:100

Preparation of 1X Wash Solution

The Max Signal Testosterone ELISA testing protocol was followed.
The ELISA plate layout was prepared as shown in Table 2

### Table 2 Elisa plate layout

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SD1a</td>
<td>SD1b</td>
<td>SD2a</td>
<td>SD2b</td>
<td>SD3a</td>
<td>SD3b</td>
<td>SD4a</td>
<td>SD4b</td>
<td>SD5a</td>
<td>SD5b</td>
<td>SD6a</td>
<td>SD6b</td>
</tr>
<tr>
<td>B</td>
<td>SD7a</td>
<td>SD7b</td>
<td>B0</td>
<td>B1</td>
<td>B2</td>
<td>B3</td>
<td>B4</td>
<td>B5</td>
<td>B6</td>
<td>B7</td>
<td>B8</td>
<td>B9</td>
</tr>
<tr>
<td>C</td>
<td>N1a</td>
<td>N1b</td>
<td>N2a</td>
<td>N2b</td>
<td>N3a</td>
<td>N3b</td>
<td>N4a</td>
<td>N4b</td>
<td>N5a</td>
<td>N5b</td>
<td>N6a</td>
<td>N6b</td>
</tr>
<tr>
<td>D</td>
<td>P1a</td>
<td>P1b</td>
<td>P2a</td>
<td>P2b</td>
<td>P3a</td>
<td>P3b</td>
<td>P4a</td>
<td>P4b</td>
<td>P5a</td>
<td>P5b</td>
<td>P6a</td>
<td>P6b</td>
</tr>
<tr>
<td>E</td>
<td>Mvh1</td>
<td>Mvh1</td>
<td>Mvh2</td>
<td>Mvh2</td>
<td>Mh3</td>
<td>Mvh3</td>
<td>Mvh4</td>
<td>Mvh4</td>
<td>Mhv5</td>
<td>Mhv5</td>
<td>Mhv6</td>
<td>Mhv6</td>
</tr>
<tr>
<td>F</td>
<td>Mvl1</td>
<td>Mvl1</td>
<td>Mvl2</td>
<td>Mvl2</td>
<td>Mvl3</td>
<td>Mvl3</td>
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<td>G</td>
<td>Mh1</td>
<td>Mh1</td>
<td>Mh2</td>
<td>Mh2</td>
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<td>Mh5</td>
<td>Mh5</td>
<td>Mh6</td>
<td>Mh6</td>
</tr>
<tr>
<td>H</td>
<td>Ml1</td>
<td>Ml1</td>
<td>Ml2</td>
<td>Ml2</td>
<td>Ml3</td>
<td>Ml3</td>
<td>Ml4</td>
<td>Ml4</td>
<td>Ml5</td>
<td>Ml5</td>
<td>Ml6</td>
<td>Ml6</td>
</tr>
</tbody>
</table>

**Key**
- **SD** - standard samples
- **N** - negative group
- **P** - sildenafil (positive) group
- **Mvh** - mutimba vula high dosage
- **Mvl** - mutimba vula low dosage
- **Mh** - mwana apeluke high dosage
- **Ml** - mwana apeluke low dosage

Table 2 shows the layout of solutions (controls, standards and unknowns) on the microplate. All controls, standards and samples were assayed in duplicate.

A standard curve was constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

\[
\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample)}}{\text{absorbance zero standard}} \times 100
\]

The mean relative absorbance values for each sample were extrapolated to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.
3.16 Statistical Analysis

A paired t-test was carried out to test for any significant difference in the mean body weight of the rats at the start and at the end of the treatment period. This was then followed by an independent t-test to test for equality of means among groups within the same period. One-way Analysis of variance (ANOVA) was carried out to test for significant variance in the weight of the sex organ parts in the control and treatment groups.

To analyse the serum Testosterone results, One-way ANOVA was used followed by Dunnett’s multiple comparison test. Dunnett’s test was performed to compare each experimental mean of testosterone concentration in the groups and the control mean.

Linear regression was used to determine the correlation that existed between the concentration of the herbal extracts and the serum testosterone levels.

All statistical calculations were carried out using the Statistical Package for Social Sciences (SPSS), version 23 and a Confidence Interval was set at 95% (p ≤ 0.05)

3.17 Ethical Consideration

The permission to carry out this study was sought from the University of Zambia Biomedical Research Ethics Committee (UNZABREC). Ethical clearance was needed because animal subjects were used in the study hence the need to follow standard procedures in the handling of laboratory animals and also because the rats needed to be decapitated after the administration of the drugs to obtain samples (Council, 2010).
CHAPTER 4  RESULTS

4.1  Phytochemical Screening
Herbal preparations of MTV and MWN were analysed for the presence of secondary metabolites through phytochemical screening tests. The results obtained are shown in table 3.

Table 3 Phytochemical Screening results

<table>
<thead>
<tr>
<th>Test</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutimba vula</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Sterols and Triterpenes</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonols</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
</tbody>
</table>

Key
High concentration +++
Moderate concentration ++
Low concentration +
Absent ---

Table 3 shows the relative concentration of the secondary metabolites in the samples which were determined by considering the colour intensity of the positive results of the test result. An intense colour was taken as an indication of a high concentration of the metabolite. The same was done for moderate and low intensity coloration of the positive results.

It was observed that the MTV sample had a very high concentration of tannins, saponins, sterols and alkaloids. A moderate concentration of glycosides and flavanols was also detected.
There were no tannins, saponins or flavonoids in the \textit{MWN} sample. Sterols and alkaloids were however in high concentration while glycosides were in low concentration.

4.2 Sexual behaviour Analysis

4.2.1 Effect of treatment on weight of Sex organ parts

The masses of individual sex organ parts for each male rat in the groups were taken and recorded. The mean weight of each group was then computed and used for further analysis. The weights were then compared between the control groups and the treatments groups to determine if there was any significant variance among the groups using one-way ANOVA.

4.2.1.1 Mass of Testis

Table 4 shows One-Way ANOVA analysis results which revealed that the control group had a testis mean weight of $2.56 \pm 0.14$ greater than the Sildenafil group at $2.33 \pm 0.25$. The \textit{Mutimba vula} 100 percent groups had the highest testis mean weight of $2.78 \pm 0.08$ while the 50 percent concentration group recorded $2.55 \pm 0.11$ representing a difference of 0.23g between the two concentrations of the same herbal preparation. \textit{Mwana apeluke} 100 percent concentration group recorded a $2.74 \pm 0.20$ while the diluted \textit{Mwana apeluke} 50 percent concentration group recorded $2.61 \pm 0.06$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± S.E.M (grams)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.56 ±0.14</td>
<td></td>
</tr>
<tr>
<td>Sildenafil</td>
<td>2.33 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Mutimba vula 100%</td>
<td>2.78 ± 0.08</td>
<td>0.259</td>
</tr>
<tr>
<td>Mutimba vula 50%</td>
<td>2.55 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Mwana apeluke 100%</td>
<td>2.74 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Mwana apeluke 50%</td>
<td>2.61 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>
The one-way Anova \( p \)-value (0.259) indicated that there was no statistically significant variance among the mean weights of testis in the different groups compared to the control.

These results indicate that the herbal preparations had no significant effect on the weight of the sex organ part (testis) in the treated groups overall. However, there was an observable increase in the mean weight of the testis in the *Matimba vula* 100 percent concentration and *Mwana apeluke* 100 percent concentration group.

**Figure 2 Mean mass of Testis**

Values are means ± S.E (n=6). Error bars with Standard Error

Figure 2 shows the mean mass of the testis in the groups indicating that there is no difference in the mean mass of the control group and the treatment groups.

These results showed that herbal preparations of MTV and MWN had no effect on the size of the testis in the treatment groups.

**4.2.1.2 Mass of Seminal Vesicles**

The largest mean weight was observed in the MWN 100 percent group while the smallest mean was recorded in the MTV 50 percent group with an average of 1.41g. The average weights of seminal vesicles in the groups are shown in Table 5.
Table 5 Mass of Seminal vesicles

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Sildenafil</th>
<th>MTV 100%</th>
<th>MTV 50%</th>
<th>MWN 100%</th>
<th>MWN 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>1.42</td>
<td>1.42</td>
<td>1.61</td>
<td>1.41</td>
<td>1.63</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Table 5 shows that the largest mean mass of the seminal vesicles was recorded in the *Mwana apeluke* 100 % group (1.63 ± 0.34g) followed by *Mutimba vula* 100 % group (1.61 ± 0.30g). *Mwana apeluke* 50 % had a mean mass of 1.53 ± 0.31g. *Mutimba vula* 50% had 1.41 ± 0.30g while the control and sildenafil groups had 1.42 ± 0.14g and 1.42 ± 0.07g, respectively.

The mean weights were then compared between the control groups and the treatments groups to determine if there was any significant variance among the groups.

Table 6 One-way Anova analysis results

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.309</td>
<td>5</td>
<td>0.0618</td>
<td>0.69</td>
<td>0.635</td>
<td>2.53</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2.687</td>
<td>30</td>
<td>0.0896</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6 shows that the p-value (0.635) obtained from the one-way ANOVA indicated that there was no significant variance in the mean weights of the seminal vesicles in the control and the treatment groups. This result indicates that the treatment did not have a significant effect on the size of the testis.
Figure 3 shows the mean weight of seminal vesicles in the control compared to the treatment groups. The results obtained indicate that herbal preparations of MTV and MWN did not have an effect on the growth of seminal vesicles of the male rats in the treatment groups.

4.2.1.3 Mass of Epididymis

The mean masses of the Epididymis in the groups are shown in Table 7.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Sildenafil</th>
<th>MTV 100%</th>
<th>MTV 50%</th>
<th>MWN 100%</th>
<th>MWN 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>1.18</td>
<td>1.29</td>
<td>1.33</td>
<td>1.35</td>
<td>1.28</td>
<td>1.34</td>
</tr>
</tbody>
</table>

The control group had a mean weight of 1.18 ± 0.10g lower than all the other groups. Both groups treated with *Mutimba vula* herbal preparations recorded high mean weight values of 1.33 ± 0.08g (for 100% group) and 1.35 ± 0.12g (for 50% group). *Mwana apeluke* 100 percent group had a mean weight of 1.28 ± 0.06g and 1.34 ±
0.07g for *Mwana apeluwe* 50 percent. The sildenafil group had a mean weight of 1.29 ± 0.09g.

The masses of the Epididymis were used to determine whether there was a dose-dependent response of the part to the herbal preparations as compared to the controls. One-way ANOVA was used to determine variance between the groups.

A $p$-value of 0.756 was obtained which indicated that there was no mean weight difference in the treatment groups and the control group. The results showed that the herbal preparations of MTV and MWN did not have an effect on the growth of the Epididymis. Alternatively, this could mean the possible mode of action of these herbal preparations may not include having an effect on the size of the Epididymis.

![Figure 4 Epididymis mean weights](image)

Values are means ± S.E (n=6). Error bars with Standard Error

Figure 4 shows the mean mass of the control compared to the treatment groups. The results obtained showed that oral administration of MTV and MWN does not have an effect on the size of the epididymis of male rats.

### 4.2.1.4 Mass of Prostate Glands

Table 8 shows the computed mean mass of prostate glands of rats in all the groups.
According to Table 8, the largest prostate gland mean weight was recorded from *Mutimba vula* 100% group at 0.55 ± 0.07 g, followed by sildenafil group (0.51 ± 0.04 g), *Mwana apeluwe* 50% (0.50 ± 0.04 g), *Mwana apeluwe* 100% (0.49 ± 0.04 g), Control group (0.45 ± 0.04 g) and the least was *Mutimba vula* 50% (0.39 ± 0.03 g).

The Anova $p$-value for this statistic was 0.248 which indicated that there was no significant statistical difference in the weights of the prostate glands in the control and the treatment groups meaning that the herbs had no effect on the growth of the prostate gland.

**Figure 5 Mean weight of prostate glands**

Values are means ± S.E (n=6). Error bars with Standard Error

Figure 5 shows the variation of the means in the herbal treatment groups compared to the control. The group with the highest mean weight was MTV 100% while the group with the least was MTV 50%. However, as seen from the test statistic, these
differences were not statistically significant indicating that oral administration of MTV and MWN does not have an effect on the size of prostate glands.

4.3 Behavioural studies

The effect of *Mutimba vula* and *Mwana apeluke* treatment on rats’ sexual behaviour was studied. The number of times males mounted females was compared between the control groups and the treatment groups. Similarly, the number of intromissions and the number of times males sniffed behind a female was recorded and compared as above. Mount Frequency (MF) was calculated as the average number of mounts during 15 minutes’ observation period. Intromission Frequency (IF) was defined as the number of intromissions during a 15 minutes’ observation period.

An independent t-test was carried out at 95% confidence level to compare Mounting, Intromission and Penile Erection of each treatment group with the negative control.

4.3.1 Mounts

The number of mounts observed in a 15-minute period was recorded.

**Table 9 Independent t-test analysis results for mounts**

<table>
<thead>
<tr>
<th>Sexual behaviour</th>
<th>Group</th>
<th>Mean diff.</th>
<th>S.E.M</th>
<th>F</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount</td>
<td>Sildenafil</td>
<td>-5.83</td>
<td>3</td>
<td>7.64</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>MTV 100%</td>
<td>-7.17</td>
<td>3.06</td>
<td>3.119</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>MTV 50%</td>
<td>-4.83</td>
<td>1.39</td>
<td>3.58</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>MWN 100%</td>
<td>-4.5</td>
<td>1.99</td>
<td>2.51</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>MWN 50%</td>
<td>-5.33</td>
<td>1.27</td>
<td>3.19</td>
<td>0.002</td>
</tr>
</tbody>
</table>

95% CI, Equal variances assumed

Table 9 shows analysis results for mounts in the treatment groups compared to the control group. The sildenafil group gave a calculated mean difference of -5.83 ± 3.00 with a *p*-value 0.008. The *p*-value obtained indicated that there was a significant difference in the number of mounts between the control group and the sildenafil
The observed difference indicated an increase in mounts of rats in the treatment groups more than in the control.

The group for *Mutimba vula* 100% recorded a \(-7.17 \pm 3.06\) mean difference with a p-value of 0.041. This test statistic result indicated that there was a statistically significant difference in the number of mounts in this group. The rest of groups also recorded low p-values (p<0.05) which indicated that the number of mounts in groups significantly increased in the treatment groups. The mean difference in the various groups was as follows: *Mutimba vula* 50% (\(-4.83 \pm 1.39\)), *Mwana apeluke* 100% (\(-4.50 \pm 1.99\)), and *Mwana apeluke* 50% (\(-5.33 \pm 1.27\)). These results show that herbal preparations of MTV and MWN caused male rats to have increased sexual intent resulting in increased mounts and mounting frequency.

### 4.3.2 Intromissions

The number of intromissions in a 15 min observation period were recorded and analysed by comparing the treatment groups with the control groups.

<table>
<thead>
<tr>
<th>Sexual behaviour</th>
<th>Group</th>
<th>Mean diff.</th>
<th>S.E.M</th>
<th>F</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intromissions</td>
<td>Sildenafil</td>
<td>-3.33</td>
<td>1.26</td>
<td>6.94</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>MTV 100%</td>
<td>-6.83</td>
<td>2.59</td>
<td>42.05</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>MTV 50%</td>
<td>-3.17</td>
<td>0.78</td>
<td>1.42</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>MWN 100%</td>
<td>-2.17</td>
<td>1.13</td>
<td>2.24</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>MWN 50%</td>
<td>-2.67</td>
<td>0.83</td>
<td>3.31</td>
<td>0.009</td>
</tr>
</tbody>
</table>

95% CI, Equal variances assumed.

Table 10 shows the t-test results for the analysis of intromissions in the treatment groups and the control. All the p-values (<0.05) obtained from the analysis of intromission data in the treatment groups indicated that there was a statistical difference in the number of intromissions observed in the treatment groups as
compared to the control group. The difference observed indicated an increase in the number of intromissions in the treatment group as compared to the control group within the 15 min observation period. The most significant result was recorded in the *Mutimba vula* 50% group with a very small p-value of 0.002. The mean number of intromissions were, -3.33 ± 1.26 for sildenafil, -6.83 ± 2.59 for *Mutimba vula* 100%, *Mutimba vula* 50% recorded -3.17 ± 0.78, *Mwana apeluke* 100% (-2.17 ± 1.13) and *Mwana apeluke* 50% group recorded -2.67 ± 0.83.

The observed statistical difference in the treatment groups represented an increase in the number of intromissions observed in the male rats treated with the MTV and MWN herbal preparations indicating that oral administration of the herbal preparations increase number of intromissions.

### 4.3.3 Penile erection

In this study, the positive control group (sildenafil) had a mean difference of -9.50 ± 1.29. The p-value (<0.05) indicated that there was a statistically significant difference in penile erection in the sildenafil group indicating that Sildenafil caused an observable increase in penile erections.

<table>
<thead>
<tr>
<th>Sexual behaviour</th>
<th>Group</th>
<th>Mean diff.</th>
<th>S.E.M</th>
<th>F</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penile erection</td>
<td>Sildenafil</td>
<td>-9.5</td>
<td>1.29</td>
<td>3.62</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MTV 100%</td>
<td>-14.5</td>
<td>1.43</td>
<td>3.19</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MTV 50%</td>
<td>-10.83</td>
<td>1.3</td>
<td>9.45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MWN 100%</td>
<td>-16</td>
<td>2.63</td>
<td>10.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>MWN 50%</td>
<td>-12</td>
<td>1.16</td>
<td>1.45</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 11 Analysis results for penile erection

95% CI, Equal variances assumed.
Table 11 shows results for *Mutimba vula* (100% and 50%) and *Mwana apeluke* groups (100% and 50%) which all gave a p-value less than 0.05. The obtained results indicated that there was a statistically significant increase in penile erections between the control group and the treatment groups. This test statistic provides evidence that the difference in penile erections observed in the treatment groups are not likely due to chance, but are more likely due to administration of the herbal preparations. Both the *MTV* and MWN treated male rats exhibited more frequent anogenital sniffing and licking of females, as compared to the control group (placebo).

The observed difference represents an increase in the frequency of males exhibiting anogenital sniffing and licking of females in the treatment groups. This provided evidence that oral administration of the MTV and MWN herbal extracts increase penile erection compared to the control group.

![Figure 6 Showing mean penile erections in each group](image)

Values are means ± S.E (n=6). Error bars with Standard Error

Figure 6 shows the mean penile erections in the treatment groups and the control. These results indicate significant increase in penile erections due to MTV and MWN herbal treatment.
4.3.4 Mount Frequency

There was generally an increase in the Mount Frequency (MF) in all the treatment groups. The largest increase was observed in the MTV 100% group which recorded a MF of 0.31 ± 0.24. The lowest increase was observed in the group MWN 100% which recorded a MF of 0.22 ± 0.15. A four-fold and five-fold increase in MF in the sildenafil and MTV 100% were recorded while a three-fold increase was observed for the treated groups MTV 50%, MWN 100% and MWN 50%. These results indicate that oral administration of MTV and MWN herbal preparations increase mounting frequency in male rats. A summary of the MF results for all the groups is shown in Table 12. These results show that herbal preparations of MTV and MWN caused significant observable increase in MF in the treatment groups.

<table>
<thead>
<tr>
<th>Table 12 Mount frequency summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Mean MF</td>
</tr>
<tr>
<td>STDEV</td>
</tr>
</tbody>
</table>

The One-Way ANOVA test results shown in Table 13 for the MF among the groups revealed that there was a significant variance in the MF. The high F (2.60) and p value (0.039) indicated that there was a statistically significant variation in the MF amongst the herbal treatment groups under observation an indication that herbal preparations of MTV and MWN caused increase in MF.

<table>
<thead>
<tr>
<th>Table 13 Anova results for mount frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of Variation</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Between Groups</td>
</tr>
<tr>
<td>Within Groups</td>
</tr>
</tbody>
</table>
Figure 4-6 shows the mount frequency plot for the treatment groups and control group. It is evident from the chart that there was a significant increase in the mount frequency in treatment groups compared to the control. The mount frequency of the herbal treatment groups was comparable to the sildenafil group which was the positive control which also revealed increase in MF implying that the herbal preparation had a similar effect to Sildenafil a synthetic drug in increasing MF in male rats.

![Mount Frequency Chart]

**Figure 7 Mount frequency**

Values are means ± S.E (n=6). Error bars with Standard Error

The observed difference represents an increase in the mounting frequency of the male rats in the treatment group. Increase in mounting frequency is an indication for both libido and potency. This therefore, reveals that oral administration of MTV and MWN increases libido and potency.

### 4.3.5 Intromission Frequency

Intromission frequency (IF) is expected to increase if the test herbal drugs are effective. From the results obtained, there was an observed sharp increase in IF in all groups. The largest increase in IF was observed in the MTV 100% group which recorded a 0.250 ± 0.21. MTV 50 % recorded a mean IF of 0.128 ± 0.06 while
Sildenafil group had $0.133 \pm 0.10$. MWN 50% recorded $0.111 \pm 0.06$ while MWN 100% had the lowest IF increase of $0.094 \pm 0.09$. This data is summarized in Table 14. These results show evidence that oral treatment of MTV and MWN herbal extracts cause increase in IF.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sildenafil</th>
<th>MTV 100%</th>
<th>MTV 50%</th>
<th>MWN 100%</th>
<th>MWN 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean IF</td>
<td>0.022</td>
<td>0.133</td>
<td>0.25</td>
<td>0.128</td>
<td>0.094</td>
<td>0.111</td>
</tr>
<tr>
<td>STDEV</td>
<td>0.03</td>
<td>0.1</td>
<td>0.21</td>
<td>0.06</td>
<td>0.09</td>
<td>0.06</td>
</tr>
</tbody>
</table>

After analysis using One-Way ANOVA, a $p$ value of 0.032 was obtained. This test statistic showed that there was a significant variation in the rate of intromissions in the treatment groups compared to the control. The results for the ANOVA analysis are shown in Table 15. These results show that oral administration of MTV and MWN herbal preparations caused an increase the number of intromissions during the observation period, ultimately reducing the time between each intromission. This causes an increase in intromission frequency.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$P$-value</th>
<th>$F$ crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>0.164</td>
<td>5</td>
<td>0.032</td>
<td>2.84</td>
<td>0.032</td>
<td>2.53</td>
</tr>
<tr>
<td>Within Groups</td>
<td>0.346</td>
<td>30</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The IF in all the groups significantly increased and was estimated to have been by a factor of 5 or more compared to the control group.
Figure 8 Intromission frequency

Values are means ± S.E (n=6). Error bars with Standard Error

The MTV 100% treated rats revealed an eleven-fold increase as can be seen from Figure 8 for intromission frequencies. The least increase in IF was in the MWN 100% group which showed a five-fold increase. The Sildenafil and MTV 50% groups showed a seven-fold increase in IF while MWN 50% showed a six-fold increase. These results indicate that there was a statistically significant difference in IF of the control and the herbal treatment groups. The observed difference represents an increase in the intromission frequency of the male rats. Increase in intromission frequency, like increase in MF, is an indication for both libido and potency. These results show evidence that oral administration of MTV and MWN herbal preparations have an effect on sexual characteristics of the male rats by increasing their libido and potency.
4.4 Testosterone Concentration

The highest optical density (OD) for the standard concentrations at 450 nm was 3.028 whilst the lowest recorded was 0.0075. Table 16 shows the OD and their corresponding standard concentrations.

<table>
<thead>
<tr>
<th>Standard concentration (ng/mL)</th>
<th>Mean OD</th>
<th>B/BO</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.289</td>
<td>100</td>
</tr>
<tr>
<td>0.05</td>
<td>3.151</td>
<td>95.8</td>
</tr>
<tr>
<td>0.15</td>
<td>2.598</td>
<td>79</td>
</tr>
<tr>
<td>0.45</td>
<td>1.866</td>
<td>56.7</td>
</tr>
<tr>
<td>1.35</td>
<td>1.308</td>
<td>39.8</td>
</tr>
<tr>
<td>4.05</td>
<td>0.759</td>
<td>23.1</td>
</tr>
<tr>
<td>100</td>
<td>0.124</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*Where B/BO is the relative absorbance, T is Testosterone*

The standard testosterone concentration and the relative absorbance was used to plot the testosterone standard curve shown in Figure 9 using Microsoft excel 2013.
From the standard graph, the formula $y = -16.81 \ln(x) + 45.46$ was used to calculate for the unknown T concentration of the samples using the relative absorbance of each sample. In this formula, $y =$ relative absorbance of the sample and $x =$ unknown T concentration of the sample.
### Table 17 OD readings and Calculated T concentration in samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample OD 450nm</th>
<th>Sample T concentration (ng/mL)</th>
<th>Average T levels/group (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.241</td>
<td>0.519</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.172</td>
<td>0.589</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.023</td>
<td>0.77</td>
<td>0.554</td>
</tr>
<tr>
<td></td>
<td>2.104</td>
<td>0.666</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.454</td>
<td>0.353</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.349</td>
<td>0.427</td>
<td></td>
</tr>
<tr>
<td><strong>Sildenafil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>0.814</td>
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<td>2.107</td>
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<td><strong>MTV 50%</strong></td>
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<td></td>
<td>2.491</td>
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<tr>
<td></td>
<td>2.502</td>
<td>0.324</td>
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<tr>
<td><strong>MWN 100%</strong></td>
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<tr>
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<td>2.467</td>
<td>0.345</td>
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<td>2.497</td>
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<td>2.779</td>
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<tr>
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<td></td>
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<tr>
<td></td>
<td>2.644</td>
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<td>0.336</td>
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<td>2.661</td>
<td>0.243</td>
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<td>2.811</td>
<td>0.185</td>
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</tr>
<tr>
<td></td>
<td>1.901</td>
<td>0.96</td>
<td></td>
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<tr>
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<td>2.834</td>
<td>0.178</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.777</td>
<td>0.197</td>
<td></td>
</tr>
</tbody>
</table>
Table 17 shows the optical densities obtained and their corresponding calculated testosterone concentrations in each of the blood samples that were assayed.

### 4.5 Statistical analysis of Testosterone (T) levels

In this study, the highest serum T concentration was recorded from MTV 100 % group with a concentration range of 1.128 ± 0.090 ng/mL. The lowest was recorded from Mwana apeluke 50 % concentration group with serum T mean levels ranging between 0.178 ± 0.084 ng/mL. The ANOVA univariate test results of between-subject factors revealed that there was no statistically significant variance in the concentration of T among the groups. The Levene’s (F) value for between subject factors was 2.391 indicating that the error variance of the dependent variable (T) was equal among the groups. Levene’s test statistic reveals a direct proportionality to the strength of the alternative hypothesis \( (H_1) \). The recorded \( p=0.061 \) indicated that there was no significant difference in serum T concentrations among the different dosage groups of the different herbal preparations that were used in the study. However, the Sildenafil and MTV 100% groups are the only two groups that showed a noticeable increase in T concentrations in the treatment groups as can be seen from Figure 10. The increase observed in these groups was very small and hence statistically insignificant compared to T levels in the control group. In the other three groups (MTV 50%, MWN 100% and MWN 50%), there was an observed reduction in serum T levels.

The univariate ANOVA test for tests of between-subject effects, done at 95% CI, gave a \( p \) value= 0.123 which indicated that there was no significant difference in serum T levels between the groups. The F statistic was 1.905, \( R \) squared = 0.241 (Adjusted \( R \) squared = 0.114). The small \( R \) value recorded revealed that there was no association that existed between the different dosages in the groups and the mean serum T levels observed. Hence, the effect observed could have been due to other factors not included in the parameters of interest of this study.

Dunnett’s t test, which compared all the other groups to the control group, was performed. In this test, the serum T levels in the control group was expected to be lower than the treatment groups, hence test was, Dunnett’s> control mean. The results of Dunnett’s test revealed that there was no significant difference in the serum T levels of the groups and the control.
All the treatment groups had serum T levels lower than the control groups except for the Sildenafil and MTV 100% groups which showed increased T levels. The Dunnett’s test results are summarized in Table 4.16. These results indicate that MTV and MWN herbal extracts did not have an effect on serum T levels of the treated male rats.

Table 18 Dunnett’s multiple comparison test results

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil</td>
<td>Negative Control</td>
<td>0.006</td>
<td>0.127</td>
<td>0.847</td>
</tr>
<tr>
<td>MTV 100%</td>
<td>Negative Control</td>
<td>0.060</td>
<td>0.127</td>
<td>0.936</td>
</tr>
<tr>
<td>MTV 50%</td>
<td>Negative Control</td>
<td>-0.144</td>
<td>0.127</td>
<td>0.363</td>
</tr>
<tr>
<td>MWN 100%</td>
<td>Negative Control</td>
<td>-0.233</td>
<td>0.127</td>
<td>0.131</td>
</tr>
<tr>
<td>MWN 50%</td>
<td>Negative Control</td>
<td>-0.209</td>
<td>0.127</td>
<td>0.179</td>
</tr>
</tbody>
</table>

Table 18 shows the multiple comparison tests performed between the control and the treatment groups which gave a p value > 0.05 indicating that there was no significant difference in serum T levels between the control and the treatment groups.

This result indicated that the herbal preparations had no effect on the level of testosterone in the blood of the treatment groups.

The marginal means of Testosterone concentration in the groups were used to generate a plot shown in Figure 10.
The highest T levels were recorded from the MTV 100% group with least recorded in the MWN 100% group.

Table 19 shows results of linear regression analysis of the effect of dosage on T levels in blood serum of the rats. The analysis was done at 95 CI to show the strength of association between the independent variable (dosage) and the dependent variable (T).

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2$ (%)</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>23.9</td>
<td>2.43</td>
<td>0.069</td>
</tr>
</tbody>
</table>

The Levene’s statistic (F) is directly proportional to the strength of the alternative hypothesis ($H_1$), P value shows the percentage error and $R^2$ shows the strength of association between independent and dependent variables. The $R^2$ of 23.9%, P = 0.069 showed that the strength of association between dosage administered in the groups and the measured Testosterone in blood samples was very weak. The observed 23.9% of variations in T levels in blood samples could have been largely
due to other factors and not the varied herbal dosage. Because of the weak association of less than 30%, we can conclude that the observed variations in T levels were not influenced by the varied herbal dosages in the treatment groups. Overall, these results indicate that the herbal preparations of MTV and MWN had no effect on the testosterone biosynthetic pathway resulting in the measured testosterone in the treatment groups showing no variation.
CHAPTER 5 DISCUSSION

5.1 Phytochemical screening

Phytochemical screening revealed the presence of tannins, saponins, flavonoids, alkaloids, glycosides, sterols and triterpenes in MTV while only tannins, saponins and flavonoids were absent in MWN herbal extract. The phytochemical analysis results obtained in this study for MTV are consistent with results obtained by (Teke et al., 2011) from qualitative analysis of methanol extract and fractions of *E. abyssinica* which revealed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides. The presence of these phytochemicals in the herbal extracts which show medicinal activity was an indication of the herbal extracts’ ability to exhibit physiological activity (Edeoga et al., 2005). Tannins have structures that are suitable for free radical scavenging activities serving as excellent hydrogen or electron donors to form radicals that are relatively stable due to delocalisation resulting from resonance and unavailability of site for attack by molecules of oxygen (Lima et al., 2005). Tannins have been demonstrated to exhibit anti-inflammatory activity by exerting anti-oxidative properties in reducing $O_2^-$, plasma extravasations and cell migration mainly in leukocytes and potentiates the activity of SOD in radical scavenging (Nardi et al., 2007) (Russo et al., 2005). Substances with antioxidant and anti-inflammatory activity, at least in part, act by a modulation of oxidative stress which is one of the confounders in ED. Therefore, the presence of tannins in the extracts indicates that they have potential to increase sexual function by modulating oxidative stress. Saponins from the extracts could have had several biological effects on the treated male rats that included membrane-permeabilising, immunostimulant, hypercholesterolaemic and anticarcinogenic properties (Francis et al., 2002) which can lead to significantly affect growth, feed intake and reproduction in animals.

The presence of alkaloids in our sample extracts is supported by (Coe and Anderson, 1996) who reported that the majority of plant species they studied contained alkaloids. Presence of alkaloids is associated with medicinal uses and one of their common biological properties is cytotoxicity (Nobori, 1994). Other researchers have reported the analgesic (Yadav and Agarwala, 2011), antispasmodic and antibacterial properties of alkaloids (Njoku and Akumefula 2007). Glycosides on the other hand,
are known to lower the blood pressure according to a lot of reports such as reported by (Okwu and Okwu, 2004).

The presence of tannins and saponins in *E. abyssinica* (Mutimba vula) has also been reported by other researchers (Cibikwa Désiré, 2015). As with other polyphenols, tannins can also bind to some free radical producing enzymes to form an insoluble tannin-protein complex, complex with catalytic metallic ions making it unavailable to initiate oxidation reaction and inhibit lipid peroxidation process. In a study by (Fan et al., 2016), the antioxidant activities of Panax notoginseng Saponins (PNS) were observed and results revealed that PNS improved erectile function in diabetic rats. This improvement was associated with suppressed oxidative stress and restored functions of endothelial cells and smooth muscle cells in the penis. Oxidative stress is an important factor in vascular complications in diabetes and ED (De Andrade et al., 2007). Another type of saponins, Ginsenoside Rg1, was observed to improve copulatory behaviour of male mice by altering both testosterone levels and signal transduction pathway in corpus cavernosum. NO/cGMP pathway appeared to play a key role in mediating the effect of Rg1 on male sexual function (Wang et al., 2010). The presence of saponins in MTV in higher concentration is an indication of the ability of the saponins in the extract played an important role in improving copulatory behaviour of the male rats. In this study, we postulated that presence of flavonoids in MTV is evidence of the ability of the saponins and tannins in the extract to act as good antioxidants, free scavenging, and inhibition of peroxidation properties (Tunalier et al., 2007, Rauha et al.). These findings are in agreement with a study done by (Arts and Hollman, 2005) who reported that these groups of phytochemicals are known to play some beneficial roles in the prevention of many oxidative and inflammatory diseases inhibiting oxidative and inflammatory enzymes (Middleton et al., 2000). This notion that extracts could be potential inhibitors of oxidative stress, which is one of the causes of ED in males is supported by the work done by (Barassi et al., 2009) whose findings provided important support for an antioxidant therapy to try and correct oxidative stress in arteriogenic ED patients. The presence of saponins and tannins is evidence of the role they played in improving copulatory behaviour of the treated male rats.
The effects observed in this study could also be attributed to the activities of flavonoid constituents in MTV extract. Flavonoids are reported to have useful pharmacological properties that include anti-inflammatory, antiallergic, enzyme inhibitors, antiviral, antispasmodic and antimicrobial activity (YUE and WANG, 2004). They are also known antioxidants and enhance the oxidation status. However, flavonoids have been implicated as antifertility agents (Iranloye and Owokunle, 2008) and observations from this study showed that MTV flavonoids reduced T levels (Aravindakshan et al., 1985). The effects of low T levels in the MWN groups in this study could be attributed to absence of saponins in the extract. Studies have implicated the saponin component of plants in enhancing aphrodisiac properties due to their stimulatory effect of androgen production (Gauthaman et al., 2002). Saponins are reported to increase levels of T and Luteinizing hormone (LH). The decrease in level of serum T indicates and correlates to the absence of saponins in the MWN extracts. Notable from the results, is that, though not significant, the serum T levels is higher in MTV extract which contained saponins findings which are in tandem with those of (Koumanov et al., 1982) suggesting that saponins increase endogenous T levels.

These phytochemical results led us to conclude that the observed aphrodisiac activity of MTV and MWN extracts was due to the presence of saponins, sterols, tannins, alkaloids, flavonoids and glycosides because some compounds belonging to these phytochemical groups isolated or found to be present in other plant species had been previously reported to possess aphrodisiac properties at different extents (Clark et al., 1984, Pare et al., 2014). Other researchers also reported some natural products to possess aphrodisiac properties examples include pyran-flavones (Drewes et al., 2002), flavones (Estrada-Reyes et al., 2013) and xanthones (Rakuambo et al., 2006).

5.2 Sexual behaviour
The effect on sexual potency was assessed by testing the action of MTV and MWN aqueous extracts on mount frequency (MF) and intromission frequency (IF). Results from Table 13 and Table 15 indicate that oral administration of the extracts at both 100 % and 50 % doses produced significant increase of these parameters in the treated animals compared to the negative controls demonstrating the extract’s potency effect in treated animals (Dabhadkar and Zade, 2013).
The effect of copulatory efficiency was assessed by testing the action of MTV and MWN aqueous extracts on penile erection. Penile erection is important for evaluating the effect of administered sample on erectile function. Results obtained (Table 11) indicated that oral administration of the extracts produced significant increase of the sexual parameter suggesting a better sexual performance (Thakur et al., 2009).

5.3 Testosterone levels

The animal model used in this study has been used by other researchers to assess effects obtained from medicinal plants on reproductive functions in male (Mohammad et al., 2009). The traditional claims attributed to these herbs for their use as aphrodisiacs have generated immense commercial activity on these drugs.

Many medicinal plants are reported to be effective as aphrodisiac agents through mechanisms such as vasodilation, generation of nitric oxide, gonadotropins and elevation of androgens (Cimanga Kanyanga et al., 2016). According to (Chauhan et al., 2007), the androgenic properties of the plant extracts are responsible for increased T levels since androgens possessed anabolic activity. This view was reported also by (Vogel, 2002) that the androgens affect development of secondary sex organs in the male as the growth of the ventral prostate and seminal vesical is dependent on the presence of male sexual hormones. Findings by (Thakur et al., 2009) suggest that improvement in body weight is generally attributed to steroid genesis and is a biological indicator for effectiveness of the herbal drugs in improving the genesis of steroidal hormones.

The results from this study revealed a different trend from the one observed by these other researchers despite the presence of steroidal saponins in the MTV extract. Results in Table 18 show analysis results for T levels and the obtained p values indicate that there was no significant difference in T concentration in the herbal groups compared to the standard reference group Sildenafil. The observed results are similar to results reported by (De Andrade et al., 2007) who reported that there was no significant difference in testosterone levels in the placebo and the treated groups. However, they recorded a significant increase in sexual performances in the groups treated with Korean Ginseng. Because there was no statistically significant difference in the testosterone levels between the placebo and the treated groups, this suggests
that the beneficial effects of MTV and MWN on erectile function were not related to testosterone levels.

This observation suggests that the mode of action of the aphrodisiacs under study could be through another biological pathway that enhanced sexual performance and not through increased T levels. One such pathway is the action of acetylcholine pathway which causes the discharge of sensory impulses from the skin (Douglas and Gray, 1953) or through soluble guanylate cyclase (sGC). Soluble guanylate cyclase is an important enzyme in corpus cavernosum smooth muscle cells since it regulates the synthesis of cGMP. It is expressed in most cells of the cardiovascular system and in many other cell types (Carvajal et al., 2000). The levels of sGC mRNA and protein often change in response to different physiological conditions and development stages. By formation of cGMP as a second messenger, sGC plays an important role in different physiological processes and is considered the key enzyme mediating vascular relaxation induced by NO and NO-releasing agents (Bush et al., 1992). According to (Flesch et al., 1997), carbon monoxide (CO), another physiological activator of sGC, is also capable of binding to the heme group of sGC and converts GTP to cGMP after activation.

Furthermore, it was observed by (Chauhan et al., 2007) that drug induced changes in neurotransmitters levels or their action at cellular level could also change sexual behaviour. This stands to support our claim that an alternative pathway to testosterone could have been responsible for the observed sexual improvement in the MTV and MWN-treated male rats.

However, it is also likely that the extracts help in the secretion of testosterone and make it better available to gonads. The high saponin content in MTV could have contributed to the steroidal biogenesis and ultimately high testosterone concentration observed in the group. The production of T has been reported to be as a result of gonadotropic activity as well as increased availability of precursors in the form of steroidal components (Haren et al., 2002). Although from the present investigations no direct correlation to an increase in endogenous testosterone production can be made, still it is quite probable that the occurrence of steroidogenic compounds in the extracts under investigation may be responsible for better gonadotropic activity. The secretion of testosterone is responsible for androgenic activity as well as
development of male accessory sexual organs namely prostate glands, seminal vesicles, vas deferens, and epididymis (Thakur et al., 2009). In another study done by (Watcho, 2004), results obtained indicated that the serum and testicular concentrations of testosterone remained unchanged at all-time points during their study. They also noted that as the duration of the treatment was prolonged, the sensitivity of the steroidogenic mechanism to the bioactive molecules present in the plant extracts decreased. This could also have been the case in our study and the 21 days’ duration may have had such an effect on the steroidogenic mechanism or perhaps using a different animal model could yield different results. To our knowledge, this study is the first to be conducted to assess the aphrodisiac properties of MTV and MWN using male rats as an animal model.
CHAPTER 6  CONCLUSION

This study showed there was a significant increase in sexual behaviour parameters studied such as MF, IF and PE due to the presence of phytochemical compounds that possess aphrodisiac properties.

From the results, there was no correlation observed in concentration of T and the dosage groups. Based on these findings, it is likely that the extracts of MTV and MWN improve sexual function via another main mode of action and not directly by increasing the concentration of serum Testosterone in blood.

Thus, this study demonstrates scientifically the aphrodisiac potential of MTV and MWN as being potent to increase sexual action.

6.1 Limitations

This study could not proceed without limitations and these include: -

1. Inadequate finances to fully cover all requirements of the project.

2. Unavailability of a Fluorescent based microplate reader to read a fluorescent based ELISA kit.

6.2 Recommendations

The present study has shown the potency of the extracts of Mutimba vula and Mwana apeluke as sexual enhancers. From the information gathered in this study, we recommend that: -

1. Quality tests on the herbal drugs must be done by Zambia Medicines Regulation Authority so that the public can have this information as they consume these products.

2. Further research must be done to determine the mechanism of action of these herbal products in causing increased sexual activity using a PDE 5A assay Kit.
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APPENDIX A - PLANT IDENTIFICATION RESULTS

THE UNIVERSITY OF ZAMBIA
DEPARTMENT OF BIOLOGICAL SCIENCES

Date: 15th May 2017
Name of client: Danny Banda
Number of specimens: 2
Specimen name(s):
Mulilila – *Ozoroa reticulata* (Bak. F.) R. & A. Fernandes (Anacardiaceae)
Mutimba mvula – *Entada abyssinica* Steud. ex A. Rich (Fabaceae, Mimosoideae)

Identified by: Mrs Florence Nyirenda (MSc.).

Designation: Scientist / Herbarium Assistant

Signature: [Signature]

P.O. Box 32379
Lusaka
Zambia

Your ref:
Our ref: