TOXICITY STUDY OF TWO HERBS USED BY TRADITIONAL HEALERS OF RUKARARWE TO TREAT HERPES ZOSTER IN HIV PATIENTS

BY

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TABLE OF CONTENTS

TABLE OF CONTENTS ii

DECLARATION v

SUPERVISOR'S ENDORSEMENT v

DEDICATION vi

ACKNOWLEDGEMENT vii

LIST OF ABBREVIATIONS viii

ABSTRACT ix

CHAPTER ONE: INTRODUCTION 1

1.1 BACKGROUND 1

1.2 PROBLEM STATEMENT 2

1.3 RESEARCH QUESTIONS 2

1.4 GENERAL OBJECTI VE 2

1.5 SPECIFIC OBJECTIVES 3

1.6 JUSTIFICATION OF THE STUDY 3

CHAPTER TWO: LITERATURE REVIEW 4

2.1 HIV 4

2.2 Herpes zoster 5

2.3 Drosophila melanogaster 7

CHAPTER TREE: MATERIALS AND METHODS 9

3.1 STUDY DESIGN 9

3.2 STUDY SETTING 9

3.3 STUDY POPULATION 9

3.4 SAMPLE SIZE 9

3.4.1 Inclusion criteria 10

3.4.2 Exclusion criteria 10
3.5 SAMPLING TECHNIQUE 10
3.6 DATA COLLECTION PROCEDURES 10
3.6.1 COMPILATION OF KNOWLEDGE 10
3.6.2 ARVs COLLECTION AND PREPARATION 10
3.6.3 PLANT COLLECTION AND PRE-EXTRACTION PREPARATION 11
3.6.4 PLANT EXTRACTION AND PREPARATION 11
3.6.5 PERCENTAGE YIELD DETAMINATION 12
3.6.6 PRELIMINARY PHYTOCHEMICAL SCREENING TESTS 12
3.6.7 FLY STOCK MAINTAINANCE 14
3.6.8 ACUTE TOXICITY TEST 14
3.6.9 CLIMBING ASSAY 14
3.6.10 COPULATION AND MATING ASSAY 15
3.6.11 LONGEVITY ASSAY 16
3.7 OUTCOME MEASURES 16
3.8 DATA ANALYSIS PROCEDURE 16
3.9 ETHICAL CONSIDERATION 16
CHAPTER FOUR: RESULTS 18
4.1 COMPILATION OF KNOWLEDGE 18
4.2 PERCENTAGE YIELD 19
4.3 PRELIMINARY PHYTOCHEMICAL SCREENING TESTS 20
4.4 ACUTE TOXICITY 21
4.5 CLIMBING ASSAY 23
4.6 COPULATION ASSAY 25
4.7 LONGEVITY ASSAY 29

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDETION 31

5.1 DISCUSSION 31

5.2 CONCLUSION AND RECOMMENDETIONS 33

REFERENCES 35

APPENDICES 40

Appendix I 40

Appendix II 41

Appendix III 42

Appendix IV 43

DECLARATION

I AHMAD HALILU do hereby declare that this is my own work and it has never been presented to any university or any other institution for the award of a degree, diploma or any other qualification whatsoever. Where the work of other people has been included, acknowledgment to this has been made in accordance to the text and references.

Signature.....

Date.....27/06/2016.....

SUPERVISOR'S ENDORSEMENT

This proposal has been submitted with my approval as supervisor

Signature:..... Date:.....27 June 2016

SUPERVISOR: MARTA VICENTE CRESPO, PhD

DEDICATION

I dedicated this great work to my beloved parents.

ACKNOWLEDGEMENT

I thank my parents and family for their continued and consistent financial and moral support. I also thank **Dr. Marta Vicente Crespo** for her wonderful supervision from beginning to the end of this project, without her this work cannot be the way it is. Special thanks to Dr. Kim Chung Hyok and the entire Institute of Biomedical Research, Madam Marisa, Madam Hellen Kinyi, Mr. Fred Oginga, Mr. Sanusi Jega, Prof Ahmed Adedeji and Madam Mulkah.

LIST OF ABBREVIATIONS

ARVs	Antiretroviral
AIDS	Acquired Immune Deficiency Syndrome
CD4	Cluster of Differentiation
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IBR	Institute of Biomedical Research
LD50	Lethal Dose in 50% of population
OIs	Opportunistic Infections
PHN	Postherpetic Neuralgia
UNAIDS	United Nations Aids
VZV	Varicella Zoster Virus
WHO	World Health Organization
AZT or ZDV	Zidovudine
3TC	Lamivudine
NVP	Nevirapine
D4T	Stavudine
EFV	Efavirenz
ABC	Abacavir
ddI	Didanosine
LPV	Lopinivir
TDF	Tenofovir
FTC	Emtricitabine
UCG	Uganda clinical guidelines
NRTI	Nucleoside analogue reverse transcriptase inhibitors
NNRTI	Non-nucleoside reverse transcriptase inhibitors
PI	Protease inhibitors

ABSTRACT

Background

Despite the fact that herbal medicines are used by 80% of the World's population (WHO, 2002) and are the main source of health care in the rural communities and the most preferred form of treatment of HIV-related symptoms (Orisatoki and Oguntibeju, 2010), less than 10% of herbal products in the world market are truly standardized to a known active and/or toxic components (Winston *et al.*, 2007). Study carried out in Western Uganda reported 32.8% of same-day use of herbal medicine and pharmaceutical drugs by HIV patients (Deanne *et al.*, 2007). Herpes zoster as a common disease in HIV patients is treated by traditional healers of Rukararwe with herbal preparations without any knowledge on the possible interactions between the treatments and lack of documented evidences to support their claim.

Aim

The aim of the study was to participate in the compilation of their traditional knowledge and make it available for future research, and also to avail the healers with the information on scientific literature. The study also evaluates the toxicity and interaction with ARVs of herbs used by traditional healers of Rukararwe in the treatment of Herpes zoster infection in HIV-positive patients.

Materials and Methods

Different concentrations of aqueous and ethanol extracts of *Warbugia ugandensis* with or without food were fed on *Drosophila melanogaster* and acute toxicity, locomotion assay, longevity study of the flies was done in 3 replicates, together with preliminary phytochemical screening of different extracts. *Sesbania sesban* (1.33µg/100µl) with or without ARVs (AZT, LVD and NVP) were fed on *D. melanogaster* and copulation and mating assay, locomotion assay, longevity study of the flies was done in 3 replicates.

Results and Discussion

Traditional healers mostly used oral formulation (80%), herbs for treating herpes zoster (40%). Steroids, Terpenoids and Glycosides were present in all extracts of *W. ugandensis*. Extracts of *W. ugandensis* were not toxic after 1hour of exposure. Ethanol extract of *W. ugandensis* decreased

negative geotaxis ($P<0.01$) and aqueous extract also ($P<0.05$), *S. sesban* does not affect the performance. Copulation latency of female flies treated with ARVs plus *S. sesban* for 7 days was increased ($P<0.05$). After 14 days of treatment, the flies treated with ARVs plus *S. sesban* shows decreased copulation latency ($P<0.01$) associated with increased copulation index ($P<0.001$), flies treated with *S. sesban* alone shows decreased copulation latency ($P<0.001$) associated with increased copulation duration ($P<0.05$), and finally flies treated with ARVs alone shows increased copulation latency ($P<0.05$) associated with decreased copulation index ($P<0.05$). Finally longevity study has shown toxicity of ethanolic extract of *W. ugandensis* at concentration of 1% and 2%.

Conclusion and Recommendations

These results showed how concomitant administration of ARVs plus *S. sesban* decrease copulation latency and increase copulation duration, especially after exposure for 14 days, at the same time results showed increase copulation latency and decrease copulation durations in flies treated with ARVs alone. My study provides some basic information on interaction between first line ARVs combination in Uganda and a commonly prescribed herb by Rukararwe traditional healers to treat herpes zoster and also give an insight into discovery of new drug or supplement for the improvement of sexual performance.

Further studies should be carried out to find out the mechanisms at which these agents affect copulation behaviors at molecular level. Finally both *S. sesban* and *W. ugandensis* were found to be non-toxic at therapeutic doses on experimental model and they can continue to be used without much fear.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

The World Health Organization (WHO) estimated that 4 billion people (80% of the World's population) use herbal medicines for some phase of primary healthcare (WHO, 2002). Herbal medicine has a long history in African traditional medicine, and its use is still inevitable especially in rural settings. Traditional herbal medicine still remains the main source of health care in the rural communities and the majority of the sub-Sahara African population depends on it as the most preferred form of treatment of HIV-related symptoms (Orisatoki and Oguntibeju, 2010).

A cross-sectional study carried out in Kabarole District, Western Uganda has shown that 63.5% of AIDS patients had used herbal medicine after HIV diagnosis (Deanne *et al.,* 2007). The same study reported 32.8% of same-day use of herbal medicine and pharmaceutical drugs by AIDS patients, which lead to the conclusion that HIV/AIDS outpatients mostly used herbal medicines for the treatment of HIV-related symptoms and to the recommendation of further studies on pharmacological interactions between herbal medicines and ARVs. Despite the popular utilization of many herbal products, less than 10% of herbal products in the world market are truly standardized to known active components and/or toxic constituents (Winston *et al.,* 2007). This is cause for alarm about their safety and the potential consequences of their use.

The human immunodeficiency virus (HIV) is a lentivirus which is subgroup of retrovirus that causes HIV infection and if left without treatment leads to acquired immunodeficiency syndrome (AIDS).

Herpes zoster (shingles or zona) is viral disease caused by varicella zoster virus usually manifested by a painful skin rash with blisters and single dermatomal involvement in a limited area on one side of the body, often in a stripe. Although the rash usually doesn't exceed two to four weeks, some patients experience outstanding nerve pain for months or years, a condition known as postherpetic neuralgia (Stankus, 2000). Varicella zoster virus (VZV) a member of the subfamily Alphaherpesvirinae and it is known to infect humans, causing chicken pox in children and herpes zoster in adults and rarely in children (Steiner *et al.*, 2007). The same article stated that VZV can remain dormant in the nervous system and later in life reactivates to cause shingles

in 10-20% of cases. The exact mechanisms of how VZV remains latent and reactivates itself it's not clear (Johnson, 2003). Incidence of Herpes zoster in the general population was estimated to be between 1.5 to 3 per 1000, and up to 10 to 15 times higher in HIV-positive patients than in the general population (Gebo *et al.*, 2005; Akashdeep and Amanpreet, 2013).Other studies, though, give numbers 15 to 25 higher (Boateng, 2003).

Drosophila melanogaster is a fly of the family Drosophilidae. This specie is also known as the fruit fly or vinegar fly. *D. melanogaster* is widely used in genetics, physiology and pathological studies (Eric, 2001).

1.2 PROBLEM STATEMENT

The Human Immunodeficiency Virus (HIV) infects about 36.9 million people globally (WHO, 2015) and opportunistic infections are the major causes of mortality and morbidity in HIV-positive individuals (Gangadhara and Ramesh, 2014). HIV patients are often under continuous treatment with antiretrovirals (ARVs) and have to combine this with other treatments to manage the opportunistic infections. Different medicinal herbs have been used to treat Herpes zoster. For most of them, the safety profiles and interaction with ARVs are not known.

This study was, therefore, directed towards evaluation of safety profile and interaction study with ARVs of medicinal herbs used in the management of Herpes zoster in HIV-positive individuals

1.3 RESEARCH QUESTIONS

1. Which herbs are used in the treatment of Herpes zoster in HIV-positive patients?
2. Are the herbs safe?
3. Are there any interactions between these 2 herbs and ARVs?

1.4 GENERAL OBJECTI VE

Evaluation of toxicity and interaction with ARVs of herbs used by traditional healers of Rukararwe in the treatment of Herpes zoster infection in HIV-positive patients

1.5 SPECIFIC OBJECTIVES

1. Participate in documentation of knowledge on herbs used in the treatment of opportunistic infections
2. Acute toxicity test of 2 herbs used in the treatment of Herpes zoster
3. To determine the consequences of concomitant use of the 2 herbs together with ARVs on courtship activities

1.6 JUSTIFICATION OF THE STUDY

The Rukararwe group of traditional healers has a long term history and good reputation in the management of many diseases using medicinal herbs and through an established collaboration is ready to relinquish their professional information for the purpose of research or betterment of their patients. They have the knowledge about their practice, but have limited access to any scientific evidence supporting their claims. This study participated in the compilation of their traditional knowledge and making it available for future research, and also availed the healers with the information on scientific literature.

Herpes zoster is common in HIV-positive patients and HIV patients are combining ARVs with traditional remedies to treat this infection without any knowledge on the possible interactions between the treatments. This study provided information on the toxicity profile of the herbs taken alone and in combination with ARVs.

Drosophila melanogaster has been extensively used to study human diseases and is now becoming more and more popular as a tool for drug screen. The field of ethnopharmacology using flies is just as its beginning, with a few successful studies having led to the description of the molecular mechanism of action of some natural products. This study contributed to building a robust body of knowledge on the use of flies in toxicological studies in general and in ethnopharmacology studies in general.

CHAPTER TWO: LITERATURE REVIEW

2.1 HIV

The Human Immunodeficiency Virus (HIV) is a virus that is implicated in the acute and chronic manifestations, neurological disorders and immunological abnormalities linked to acquired immunodeficiency syndrome (AIDs) and it infects 0.6% of the world population (UNAIDS, 2006). HIV like other viruses cannot survive outside the cell or body fluids, and it cannot penetrate uncompromised skin (Tanni *et al.*, 2014). AIDs is a condition which is characterized by weakness of immune system that allows opportunistic infections to emerged, some of which are life-threatening and from 1981 to 2006 it has killed more than 25 million people (UNAIDS, 2006; Douek *et al.*, 2009).

The average survival time after infection with HIV is estimated to be 9 to 11 years, although it strongly depends on the subtype of the virus (UNAIDS, 2007). Transmission of HIV occurs most commonly through sexual intercourse, but also through transfer of blood, mother-to-child transmission during pregnancy or breastfeeding (Tanni *et al.*, 2014). Use of effective chemotherapeutic regimen is the mainstay in the management of opportunistic infections (the major cause of mortality) in HIV-positive individuals and this is true especially if the antiretroviral drugs (ARVs) used are not potent enough to boost immune system (Gangadhara and Ramesh, 2014; Vincent *et al.*, 2000).

HIV infects cells expressing CD4 membrane receptor molecules, such as T helper cells, macrophages, dendritic cells and microglial cells (Cunningham *et al.*, 2010). The normal range of CD4+ T helper cells in a healthy person is 500-1200x10^6cells/L of blood (Bofill et al., 1992). This "CD4 count" decreases gradually with progression of HIV infection rendering the patient susceptible to opportunistic infections (OI). Further depletion of CD4 weakens the immune system and CD4 T cell count reaches below 200cells/μl, risk of OIs increases radically and patient usually progresses to AIDS, CD4 less than 50cells/μl of blood leads to death (Akashdeep and Amanpreet, 2013).

The normal progression of HIV infection involves three stages: acute infection, asymptomatic phase and full blown AIDS. During acute phase, the virus replicates extremely resulting in high viral load and increased risk of transmission. HIV takes 6-12 weeks for a person to become seropositive and this period is known as window period. After seroconversion, the viral load

starts decreasing. The lowest value is called viral set point and determines disease progression. A few patients may not have detectable viral load, remain asymptomatic and this phase lasts for many years depending on HIV strain, host immunity and nutritional factors. The CD4 T helper cell count drops by approximately 50-100cells/μl of blood per year. With gradual decline in CD T cell count, the symptomatic phase, with opportunistic infections manifesting thrush, weight loss and fatigue, starts (Holmes *et al.*, 2003).

The most common opportunistic infections and tumors associated with AIDs includes oral candidiasis (thrush), tuberculosis, reactivation of latent herpes viruses (herpes simplex eruptions and shingles), pneumonia caused by the fungus *Pneumocystis jirovecii,* Epstein-Barr virus-induced B-cell lymphomas and Kaposi's sarcoma (Holmes *et al.*, 2003).

The mainstay of HIV treatment currently is highly active antiretroviral therapy (HAART) (Palella *et al.*, 1998). It has been of significant benefit to many HIV patients since its introduction in 1996. HAART consist of combinations of at least three drugs belonging to at least two different classes of antiretroviral drugs, usually nucleoside analogue reverse transcriptase inhibitors (NRTIs) plus either non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI). The current regimen includes; zidovudine /lamivudine (ZDV/3TC) + nevirapine (NVP) or efavirenze (EFV) (preferred first line regimen in adults), tenofovir plus lamivudine (TDF+3TC) or emitricitabine (FTC) plus NVP or EFV (alternative first line regimen in adults), abacavir/didanosine (ABC/dd)i + lopinovir (LVP) (preferred second line regimen in adults), ZDV plus ddI plus LPV (alternative second line regimen in adults), Stavudine/lamivudine (d4T/3TC) plus NVP or EFV (preferred first line regimen in children), ZDV/3TC plus NVP or EFV (alternative first line regimen in children), ABC/ddI plus LVP or TD (preferred second line regimen in children), d4T/3TC plus ZDV or EFV, or ZDV/3CT plus NVP or EFV (first line regimens in infants), ABC/ddi plus LPV, or ZDV/ddI plus LPV, or ZDV/ABC plus LPV, or ABC/ddI plus LPV (second line regimen in infants)) (UCG, 2010).

2.2 Herpes zoster

Varicella Zoster Virus (VZV) is a neurotropic double stranded DNA virus also known as human herpes virus type 3 (HHV-3) is the causative agent of herpes zoster (Steiner *et al.*, 2007). Immunocompromised and geriatric people are more susceptible to herpes zoster and in HIV

positive patients, incidence of VZV is 15 times higher than in normal people (Akashdeep and Amanpreet, 2013).

Many studies have shown a strong association between HIV and shingle with higher incidence in those with greater HIV-induced immunological suppression (Gebo et al., 2005; Sinayobye *et al.,* 2015). However, despite the relationship between shingle and HIV, studies have different findings between the HIV stage and emergence of shingles (Sinayobye *et al.,* 2015).The impact of HAART (highly active antiretroviral therapy) on the pathogenesis of herpes zoster is not well understood, with some studies suggesting that immune reconstitution after the initiation of ARVs treatment leads to an increased incidence of herpes zoster (Sinayobye *et al.,* 2015). Reactivation of VZV infection may occur at any stage of HIV infection, and may be the first clinical evidence of HIV infection (Colebunders *et al.,* 1988; Boateng, 2003).

Symptoms of herpes zoster include local pain and discomfort, and then progresses to a localized or segmented erythematous, maculopapular eruption along a single dermatome, lesions, pustules, and crusts, which usually remain localized and resolve spontaneously (Stankus *et al.*, 2000). However not all symptoms are seen in one patient and severity depends on the extent of the infection. Blisters and crusts usually last 2-3 weeks, and necrotic lesions may last for up to 6 weeks and result in significant scarring (Colebunders et al., 1988). About Sixty-seven percent (67%) of patients present with single dermatomal involvement, and the thorax is involved in 41%, postherpetic neuralgia as a complication in 18% and other complications such as ocular and visceral involvements accounts for 18% (Gebo et al., 2005). Herpes zoster ophthalmicus comes about when a latent VZV in the trigeminal ganglia involving ophthalmic division (one of the 3 divisions of fifth cranial nerve) of the nerve is affected and this division said to be 20 times more frequently involved than other divisions. And the structures of the eye affected by Herpes zoster ophthalmicus include: eyelid, conjunctiva, cornea (in 65% cases of Herpes zoster ophthalmicus), and sclera. Post Herpetic Neuralgia is more common in Herpes zoster ophthalmicus than any other form of VZV infection and it is characterized transient of sharp pain and itching or pain initiated by usually non-painful stimuli which is attributed to the loss of peripheral sensory neurons (Boateng, 2003).

Drugs used in the management of Herpes Zoster include: Antiviral (e.g. Acyclovir), Antidepressants (e.g. Amitryptyline), Antibiotics (e.g. Cloxaciline) and Steroids. Although the

drug to be used and the dosage form strongly depends on the type of infection, site of the body affected and the severity of the condition (Boateng, 2003).

2.3 Drosophila melanogaster

The developmental period for *Drosophila melanogaster* is affected by different environmental factors and it has a lifecycle of about 9 to 10 days at 25°C under normal environmental conditions (Ashburner and Thompson, 1978; Ashburner *et al.*, 2005). At 25°C female flies lay about 400 eggs, five at a time in average, in a suitable environment (e.g. sucrose agar). The eggs (about 0.5 mm long), hatch after 12–15 hours and the resulting larvae grow for about 4 days, then molting (shedding) take place twice (into 2nd- and 3rd-instar larvae), at time between 24 and 48 h after hatching. During this stage of life it feeds on sugar and microorganisms available. Then the larvae encapsulate in the puparium (process of transforming into pupa) and undergo a 4 day long metamorphosis, after which the adults fly emerge (Ashburner and Thompson, 1978; Ashburner *et al.*, 2005).

Drosophila melanogaster is widely employed in many researches because of its advantages which includes; short lifecycle of about 10 days, easy to handle, ability to reproduce many genetically identical flies in a shorter time and their cost-effectiveness (Eric, 2001; Udai and Charles, 2011).

Researchers have proved that about 75% of disease-related genes in human have a homologue in *D. melanogaster*, making it a suitable candidate model from which findings can easily be extrapolated to human beings (Reiter et al., 2001; Lloyd and Taylor, 2010). The adult fly has structures that perform functions corresponding to those of the heart, gastro-intestinal tract, kidneys and the reproductive tract (Udai and Charles, 2011) making it possible to study the effect of drugs and medicinal remedies in the context of a whole organism. The fly also has other advantages over many models in use since it can be used at any of the four developmental stages of life (embryo, larva, pupa, and adults) depending on the nature of research questions and objectives of the study (Udai and Charles, 2011).

The grade of conserved biology and physiology between fly and human has qualifies *D. melanogaster* as a valuable model in the drug discovery process, especially during screening of

large candidate compounds so that to eliminate and come up with few compounds of higher quality in more cost-effective manner and the research can be continued with mammals at some stage. The embryo is habitually used in the developmental studies, cell fate determination, organogenesis, and neuronal development and axon passageway discovery. The larva, particularly the third instar-larva, is normally used to study developmental and physiological processes in addition to some behaviors such as foraging. The adult fly used in biochemical, genetics and pharmacological studies (Udai and Charles, 2011).

The brain of this fly has more than 100,000 neurons that mediate and control complex behaviors, which includes: copulation, feeding, aggression, grooming, flight navigation, circadian rhythms, sleep, learning and memory. Physiological response to many drugs by this fly has shown to be similar to that of human and this is true especially to centrally-acting drugs. With the aforementioned advantages it becomes possible to use *D. melanogaster* in drug discovery in the following areas: Central nervous system (Alzheimer's disease, Cognitive, Psychosis, Affective disorders, Neurodegeneration, Parkinson's disease, Seizure disorders, Sleep and Triplet repeat expansion diseases), Cancer, Cardiovascular system disorders, Inflammatory disease, infectious disease and Metabolic disorders (Udai and Charles, 2011).

CHAPTER TREE: MATERIALS AND METHODS

3.1 STUDY DESIGN

In this study, two traditional healers belonging to the Rukararwe group were interviewed and their knowledge was recorded. *Drosophila melanogaster* was used as a model to determine the toxicity of two of the plants used by these healers to treat herpes zoster. Acute toxicity test was conducted with eight (8) different concentrations of aqueous and ethanolic extracts of *Warbugia ugandensis*; three (3) independent experiments were conducted each with three (3) replicas. Climbing and longevity assays were also conducted with concentrations below the toxic dose for each of the two herbs (plus ARVs for *S. sesban*). Finally Courtship assay was performed with flies exposed ARVs and/or *S. sesban* plus control in three (3) replicas, each having 10 flies per replica.

3.2 STUDY SETTING

Study was carried out at both Rukararwe which is located in Bushenyi district and Institute of Biomedical Research laboratory which is located within Kampala International University, Western Campus, Ishaka municipality, Bushenyi district.

3.3 STUDY POPULATION

Drosophila melanogaster (fruit flies) w^{1118} strain was used for this study.

The traditional healers associated with Rukararwe are the target population of the systematic compilation of knowledge.

3.4 SAMPLE SIZE

A total of 2 traditional healers were selected for this study. Convenience sampling was used, selecting two of the healers that are closer to the management of Rukararwe. Total of 1,555 *D. melanogaster* were used for this research: acute toxicity (675), climbing assay (420), courtship assay (40) and longevity study (420). A minimum of three (3) replicas with ten (10) flies each was used in each study.

3.4.1 Inclusion criteria

Only traditional healers that are registered members of "Rukararwe Traditional Healers" and only physically fit, mature, virgin and adult fruit flies of strain w^{1118} were used for the study.

3.4.2 Exclusion criteria

Flies with red eye (no w^{1118}), physically damaged and older flies were not included in this study.

3.5 SAMPLING TECHNIQUE

Fruit flies were carefully sampled according to specie, age, sex and physical state using random sampling technique.

3.6 DATA COLLECTION PROCEDURES

3.6.1 COMPILATION OF KNOWLEDGE

Questionnaires of 'Designing a Holistic Approach for the Management of HIV/AIDS' was used for this purpose (Appendix 1 of proposal)

3.6.2 ARVs COLLECTION AND PREPARATION

3TC [4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one] also known as lamivudine, AZT [1-(3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methylpyridine-2,3(1H, 3H)-dione] also known as zidovudine and NVP [11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one] also known as navirapine in a combination having ratio of 150mg:300mg:200mg respectively[the first line ARVs in adult and second line in children in Uganda (UCG, 2014)], manufactured by HETERO LABS LIMITED were obtained from Kampala International University Teaching Hospital (KIUTH). All solutions and dilutions of the drugs were prepared immediately prior to use, where drugs equivalent to human dose (0.0431µg/100µl or/0.0323µg/fly) was prepared, such that each 75µl of food (corn meal agar) contains 0.0323µg. Having it in mind that 1 fly weigh approximately 1mg (Katz and Young, 1975) and a fly eats on average a 75µg (5µg to 150µg) (Ricard *et al.*, 2009).

Up to the dose of 200µg/100µl of AZT and 3TC was found to be non-toxic to third-instar larvae of *D. melanogaster* (Guimaraes *et al.*, 2013).

3.6.3 PLANT COLLECTION AND PRE-EXTRACTION PREPARATION

Sesbania sesban (L.) Merr. and *Warbugia ugandensis Sprague* Plants specimens were collected from Rukararwe Nyakabiri Sub-county, Bushenyi District, western Uganda. Specimens were taken to Mbarara University of Science and Technology, Department of Botany for authentification and issue of collection number.

The leaves of *Sesbania sesban (L.) Merr.* and *Warbugia ugandensis Sprague* were dried under shade for about two to three weeks, then they were pounded using wooden mortar and pestle and sieved to obtain fine powder.

3.6.4 PLANT EXTRACTION AND PREPARATION

(a) *Sesbania sesban (L.) Merr.*

The powdered leaves were macerated in water. Six hundred grams of powdered plant leaves was hot extracted at 60°C for 6 hr using 6 liters of distilled water (Pandhare *et al.*, 2011), which was taken to shaker and allowed to be shaken to complete 24 hours. The fluid obtained was filtered with Whatman No. 1 filter paper and water extract filtrate was poured onto petridishes and dried in oven under low temperature. The process was repeated to obtain higher yield of crude extract. *Sesbania sesban (L.) Merr.* crude extract was prepared at concentration of 1.33µg/100µl/fly (equivalent to 1g/kg) in distilled water, such that each 75µl of food (corn meal agar) contains 0.998µg of the crude extract, up to the dose of 3.33µg/100µ (equivalent to 2.5g/kg) was shown to be non-toxic on acute exposure to rats (Pandhare *et al.*, 2011).

(b) *Warbugia ugandensis Sprague*

The powdered leaves were macerated in ethanol and water. Two hundred and fifty grams of powdered plant leaves was soaked in 2.5 liters of ethanol and three hundred grams of powdered plant leaves was soaked in 3 liters of distilled water, which was divided into different aliquots using conical flasks. The above was taken to a shaker for 24 hours (Olila *et al.*, 2001).The macerated fluid obtained was filtered with Whatman No. 1 filter paper and the filtrate, ethanol was concentrated in a round bottomed flask using a rotary evaporator and water extract filtrate

was poured onto petridishes and dried in oven under low temperature (Olila *et al.*, 2001). The process was repeated to obtain higher yield of crude extract.

Another four hundred and eighty seven grams of powdered plant leaves was soaked in 4.87 liters of distilled water, which was divided into different aliquots using conical flasks. This was taken to a shaker for 24 hours (Olila *et al.*, 2001).The macerated fluid obtained was filtered with Whatman No. 1 filter paper and the filtrate, water was partitioned with chloroform to obtained two different layers (water and chloroform layer). The water extract filtrate was poured onto petridishes and dried in oven under low temperature and the chloroform extract was concentrated in a round bottomed flask using a rotary evaporator to obtain crude extract (Olila *et al.*, 2001).

3.6.5 PERCENTAGE YIELD DETAMINATION

Percentage yield was calculated using the formula W_2-$W_1/W_0 \times 100\%$. Where W_0 is the weight of the initial dried sample, W_1 is the weight of container alone and W_2 is the weight of both extract and container (Anokwuru *et al.*, 2011).

3.6.6 PRELIMINARY PHYTOCHEMICAL SCREENING TESTS

Test for Alkaloids

10mls of the extract was added to 5-10 drops of Wagner's reagent (2g of iodine + 6g of KI in 100mls of distilled water). Formation of opalescent or yellow white precipitate will confirm the presence of alkaloids (Trease and Evans, 2002).

Test for Atnthraquinones (Borntrager's test)

3ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added onto it. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones (Bele and Khale, 2014).

Test for Flavonoids

0.5g of extract was dissolved in sodium hydroxide (NaOH) solution. The appearance of yellow solution that disappears on addition of HCl indicates the presence of flavonoids (Trease and Evans, 2002).

Test for Glycosides

(a)Liebermann's test

2 ml of the organic extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added carefully. A color change from violet to blue to green indicates the presence of a steroidal nucleus (i.e. aglycone portion of glycoside) (Bele and Khale, 2014).

(b)Keller killiani's test

Test solution was treated with few drops of glacial acetic acid and ferric chloride solution and mixed. Concentrated sulphuric acid was added which forms two-layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive result for presence of glycoside (satheesh *et al.*, 2012).

Test for Saponins (Froth test)

5mls of distilled water was added to 0.2g of the extract and shaken vigorously. Froth (foam) that persisted for more than 10 minutes will indicate the presence of saponins (Sofowora, 1993).

Test for Steroids

2 ml of the extract was added in 2 ml of chloroform and 2 ml of concentrated sulphuric acid. A red colour produced in the lower chloroform layer indicates the presence of steroids (Bele and Khale, 2014).

Test for Tannins

10mls of distilled water was added to 0.5mls of each extract and mixed with few drops of ferric Chloride (FeCl3) solution. An immediate visible green precipitate indicates positive test for presence of tannins (Tyler *et al.*, 1981).

Test for Terpenoids

About 0.5g of the extract was dissolved in 1ml of chloroform and 1ml of concentrated H_2SO_4 to form a layer. A reddish brown layer formed at the interface indicates positive result for presence of terpenoids (Trease and Evans 2002).

3.6.7 FLY STOCK MAINTAINANCE

The wild-type flies strain w^{1118} were maintained in cornmeal standard media (containing agar 1.05% w/v, corn meal 7.0% w/v, glucose 7.5% w/v, distilled water 100ml, nipagin 2.33% v/v, propionic acid 0.37% v/v and dried yeast 1.5% w/v) at 25°C under 12hours light-dark cycle. The emerging flies were collected and separated by sex (Hsin-ping, 2012). All experiments were conducted at 25°C under 12hours light/dark.

3.6.8 ACUTE TOXICITY TEST

Eight (8) different concentrations of aqueous and ethanol extracts of *Warbugia ugandensis Sprague* were prepared as 0.0952µg/100µl (equivalent to human dose), 9.52µg/100µl, 20µg/100µl (equivalent to 15g/kg), 1000µg/100µl, 10,000µg/100µl, 75,000µg/100µl, 90,000µg/100µl and 0µg/100µl (control) each with 5% glucose consistently on filter paper (2.5cm by 1cm). Having it in mind that 1 fly weighs approximately 1mg (Katz and Young, 1975) and a fly eats on average a 75µg (5µg to 150µg; Richard *et al.*, 2009).

For each experiment a total of 240 flies (30 flies per groups) were starved for 14 to 16 hours in empty vials, and then fed on the different concentrations of the extracts for 30 minutes. The number of deaths was counted after1 hour of feeding (Leonardo *et al.*, 2011; Udai and Charles, 2011). Three independent experiments were conducted each with three replicas; the last experiment was conducted with 120 flies (15 flies per groups) but 1fly per vial to ensure quality of the results obtained.

3.6.9 CLIMBING ASSAY

(a) *Sesbania sesban (L.) Merr.* aqueous extract

Wild type flies were treated in 4 groups containing: *Sesbania sesban (L.) Merr.* extracts alone; *Sesbania sesban (L.) Merr.* extract plus ARVs; ARVS alone; and control "standard corn meal medium" for 13 days prior to the test. Ten (10) flies from each group were transferred into empty vials, around which a horizontal line 8 cm above the bottom of the vial was drawn as a marker. The flies were allowed to acclimatize for 10 min at room temperature. The procedure entails gently tapping the vials of the flies down to the bottom trice; then after 10 sec the number of flies that crossed the 8cm mark was recorded. Total of 120 wild type flies were used, with three (3)

replicates for each group, and average values was calculated to get the mean (Pendleton *et al.*, 2001). All flies used for this assay were one day old.

(b) *Warbugia ugandensis Sprague*

Aqueous and ethanol extracts of *Warbugia ugandensis Sprague* was separately prepared at dose of 0.0952µg/100µl (equivalent to human dose), 20µg/100µl (equivalent to 15g/kg), 1000µg/100µl, 10,000µg/100µl and 0µg/100µl (control) each formulated in corn meal medium as 0.0000952%, 0.02%, 1%, 10% and 0% respectively. The test was carried out as described in section (a).

3.6.10 COPULATION AND MATING ASSAY

Newly hatched virgin males and females wild type flies were collected in empty vials. Males were separated from females in different vials. Collected flies were kept at 25 °C under 12hours light/dark, while at the same time they were receiving *Sesbania sesban (L.) Merr.* extracts alone, *Sesbania sesban (L.) Merr.* extract plus ARVs, ARVS alone or control "standard corn meal medium" for 7 days (minimum number of days the extract is given in Rukararwe) prior to copulation behavior assay (Hsin-ping, 2012). Then 1 male and 1 female were transferred into copulation assessment chamber using aspiration method, which has shown to not affect copulation behaviors (Trannoy and Kravitz, 2015). All experiments were carried out at 3-6 hours after the light was on; this time is when the females show higher mating activities (Hsin-ping, 2012).

Observation was until successful copulation, the copulation duration (times at which couples initiated and terminated copulation), and the copulation latency (times between which the couples were placed in the chamber and started to copulate) was recorded, all with the help of camera. Courtship index (CI) was calculated by dividing the time spent in copulation divided by the total time until copulation (Charles *et al.*, 2012; Hsin-ping, 2012).

Another set of flies were collected and exactly treated in the aforementioned manner for 14 days (maximum number of days the extract is given in Rukararwe) and copulation assay was also done in similar way.

All experiments were done with four replicates (4 recordings) and the average was taken.

3.6.11 LONGEVITY ASSAY

(a) *Sesbania sesban (L.) Merr.* aqueous extract

Dose at concentration of 1.33µg/100µl/fly (equivalent to 1g/kg) in distilled water was prepared, such that each 75µl of food (corn meal agar) will contain the aforementioned dose, and then 10 wild type virgin flies were raised in a vials containing corn meal medium with *Sesbania sesban (L.) Merr.* extracts alone, *Sesbania sesban (L.) Merr.* extract plus ARVs, ARVS alone or control "standard corn meal medium" for 40 days. Food vial was consistently changed after every 4 days, and dead flies were counted after every 3 days (Hsin-ping, 2012).Three replicates were used (1 for females and 2 for males) each containing 10 flies and all flies used were 1 to 2 days old.

(b) *Warbugia ugandensis Sprague*

Aqueous and ethanol extracts of *Warbugia ugandensis Sprague* was prepared at dose of 0.0952µg/100µl (equivalent to human dose), 20µg/100µl (equivalent to 15g/kg), 1000µg/100µl (, 10,000µg/100µl and 0µg/100µl (control) each formulated in corn meal medium as 0.0000952%, 0.02%, 1%, 10% and 0% respectively. The test was carried out as described in section (a).

3.7 OUTCOME MEASURES

This was basically based on interviews using questionnaires, and also by direct observation of laboratory tests, cameras were used where necessary.

3.8 DATA ANALYSIS PROCEDURE

The data obtained was analyzed using Microsoft excel and PAST software. Descriptive statistical analysis was done together with T-test for comparison. Results obtained were presented in tables and charts.

3.9 ETHICAL CONSIDERATION

Approval to carry out the study was obtained from the management of School of Pharmacy, IBR Kampala International University (KIU) Western Campus, as well as Rukararwe and the traditional healers. The healers were given an informed consent form and the possibility to participate voluntarily or withdraw at any time. The data collected were kept in a locked cabinet

for confidentiality and their use will always be limited to the purpose of the ''Holistic management of HIV'' project.

3.10 LIMITATIONS OF THE STUDY

Relevance to humans always needs to be checked when working with an animal model as great anatomical and physiological variations occurs, this will therefore only give an insight on possible effect of an agent on humans but not 100% extrapolation.

CHAPTER FOUR: RESULTS

4.1 COMPILATION OF KNOWLEDGE

Results from interviewing traditional healers (compilation of knowledge) showed a high use of *Zanthoxylum Gillette* by different traditional healers to treat different ailments (Table 1). The same table showed that herpes zoster (shingles) appears to be the ailment for which there are more herbs available (40% of remedies) followed by diarrhea (20%). The parts of plant used to treat ailments include leaves (66.7%), barks (25%) and seeds (8.3%). An oral formulation appears to be their routine prescribed dosage form in 80% of cases, followed by topical formulations with 20%.

Table 1: Medicinal plants used by the traditional healers of Rukararwe in the management of opportunistic infections in HIV-positive patients.

NO.	SCIENTIFIC NAME	LOCAL NAME (*Ruyankole*)	PART(s) USED	INDICATION	ROUTE OF ADMIN.
01		Engyenyi	Leaves	Diarrhea	Oral
02	*Zanthoxylum Gillette*	Omutatembwa	Bark	Anorexia	Oral
03	*Aloe vera*	Rukaka	Leaves	Malaria	Oral
04	*Phytollacca dodecandra*	Omuhoko	Leaves	Herpes zoster	Topical
05	*Sesbania sesban*	Omungyeganyegye	Leaves	H. zoster	Oral
06	*Zanthoxylum Gillette*	Omutatembwa	Bark	Oral thrush	Oral
07	*Warbugia ugandensis*	Omwiha	Leaves	Herpes zoster	Oral
08	*Mangifera indica*	Omuyemba	Leaves /Bark	Cough	Oral
09	*Psidium guajava*	Ampeera	Leaves	Diarrhea	Oral
10	*Phytollacca dodecandra*	Omuhoko	Seeds /Leaves	Herpes zoster	Topical

4.2 PERCENTAGE YIELD

Percentage yield showed a higher yield with aqueous extract of *S. sesban* (Table 2). The results also showed slightly higher yield with ethanol extract compare to water extract of *W. ugandensis,* with loss of some crude extracts after partitioning aqueous extract with chloroform.

Table2: Results of percentage yield of five different extracts for both W. ugandensis and S. sesban.

MEDICINAL HERB AND SOLVENT USED	**INITIAL POWDER USED (g)**	**PERCENTAGE YIELD (%)**
W. ugandensis, water extract	300g	7.3%
W. ugandensis, ethanol extract	250g	7.6%
W. ugandensis, water-chloroform partitioning (water part)	487g	5.0%
W. ugandensis, water-chloroform partitioning (chloroform part)	487g	1.5%
S. sesban, water extract	600g	8.7%

4.3 PRELIMINARY PHYTOCHEMICAL SCREENING TESTS

Phytochemical screening showed a high level of terpenoids and steroids in both aqueous and ethanolic extracts of *W. ugandensis*, which persist even after partitioning aqueous extract with chloroform (Table 3). Saponins were lost after partitioning aqueous extract with chloroform. And test for glycoside was positive with Keller killiani's test and negative with Lieberman's test.

Table3: Results of preliminary phytochemical screening tests of 4 extracts of W. ugandensis, (+) showing positive test and (—) showing negative test.

PHYTOCHEMICAL TESTS	AQUEOS EXTRACT	ETHANOL EXTRACT	AQUEOUS—CLOROFORM PARTITIONING **(H_20 extract)**	AQUEOUS—CLOROFORM PARTITIONING **($CHCl_3$ extract)**
Alkaloid	—	+	—	—
Anthraquinones	—	—	—	—
Flavonoids	+	—	+	—
Glycosides **Keller killiani's test** **Lieberman's test**	 + —	 + —	 + —	 + —
Saponins	+	+	—	—
Steroids	+	+	+	+
Tannins	+	+	+	—
Terpenoids	+	+	+	+

4.4 ACUTE TOXICITY

Acute toxicity tests for both aqueous and ethanolic extracts of *W. ugandensis* showed that the plant is not toxic after one hour of exposure (Figure 1 and 2).

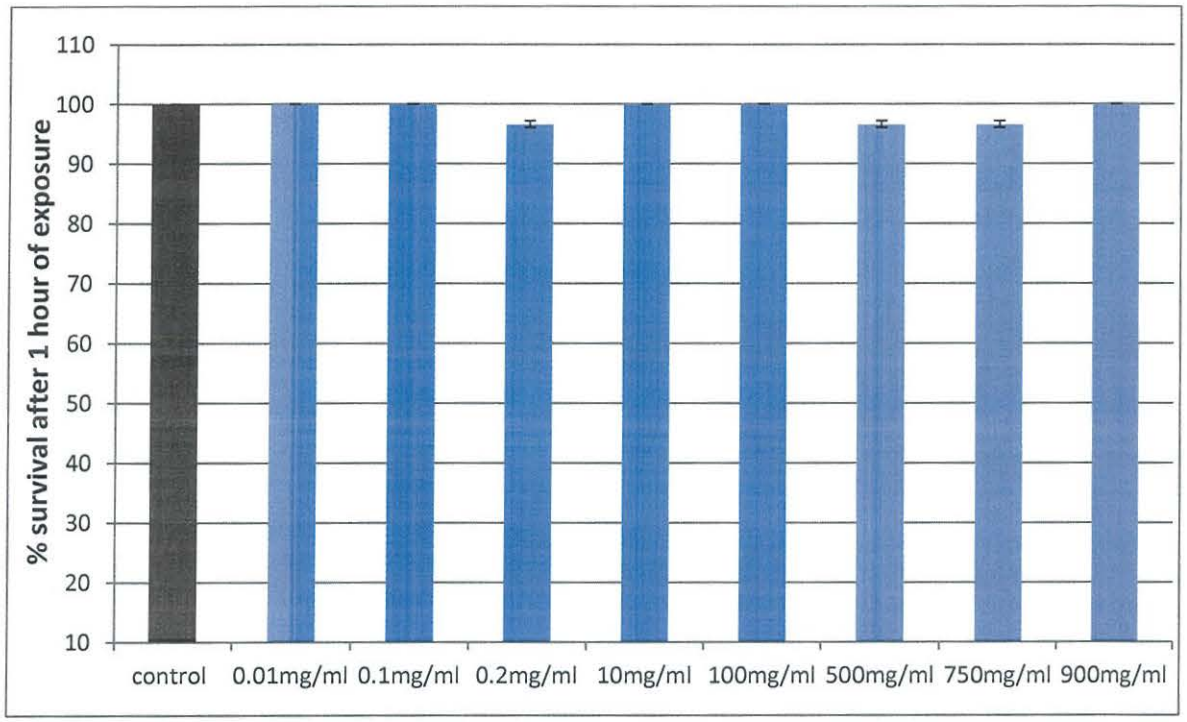

Figure 1: Percentage survival of *D. melanogaster* after one (1) hour of exposure to different concentrations of aqueous extracts of *W. ugandensis*. Standard deviation of 0 to 0.57.

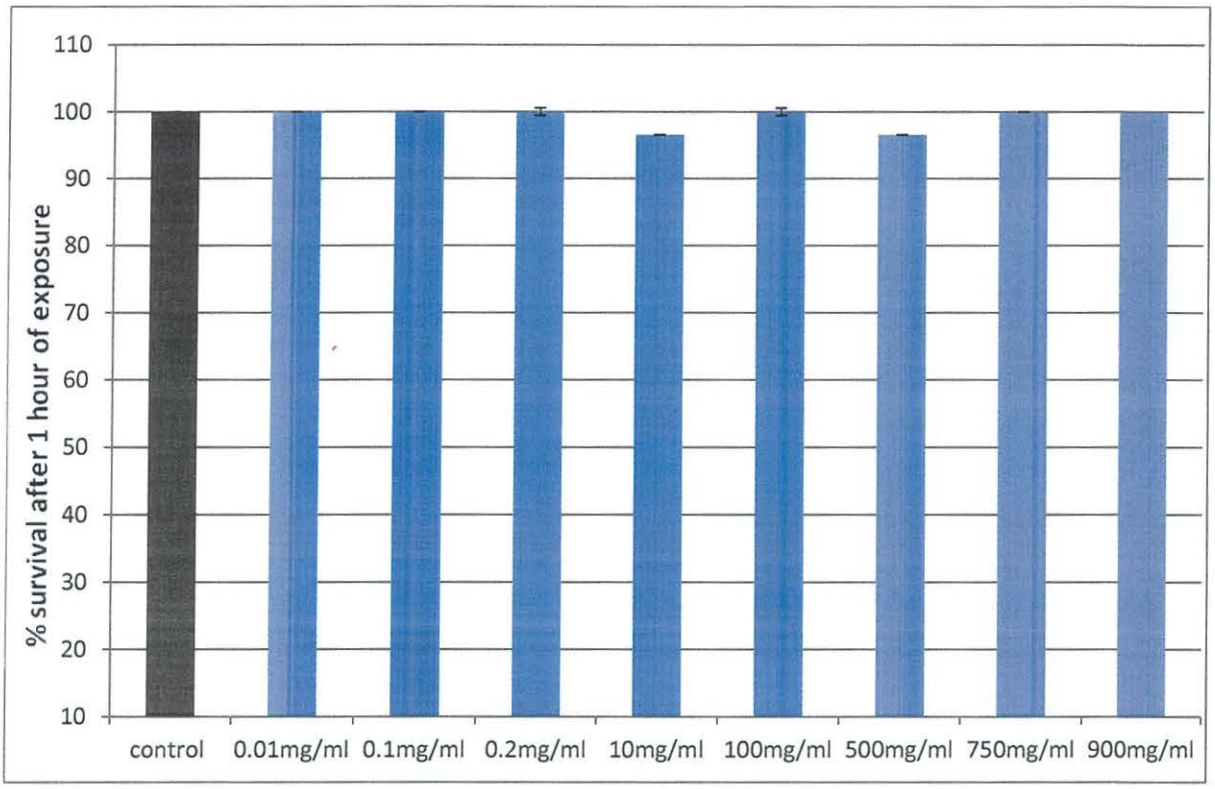

Figure 2: Percentage survival of *D. melanogaster* after one (1) hour of exposure to different concentrations of ethanolic extracts of *W. ugandensis*. Standard deviation of 0 to 0.57.

4.5 CLIMBING ASSAY

Climbing assay for aqueous and ethanolic extracts of *W. ugandensis* and aqueous extract of *S. sesban* with or without ARVs showed a significant difference between control and flies treated with 100mg/ml (10%) of both aqueous and ethanolic extracts of *W. ugandensis* with $P<0.05$ and $P<0.01$ (figure 3,4 and 5) respectively calculated using T-test.

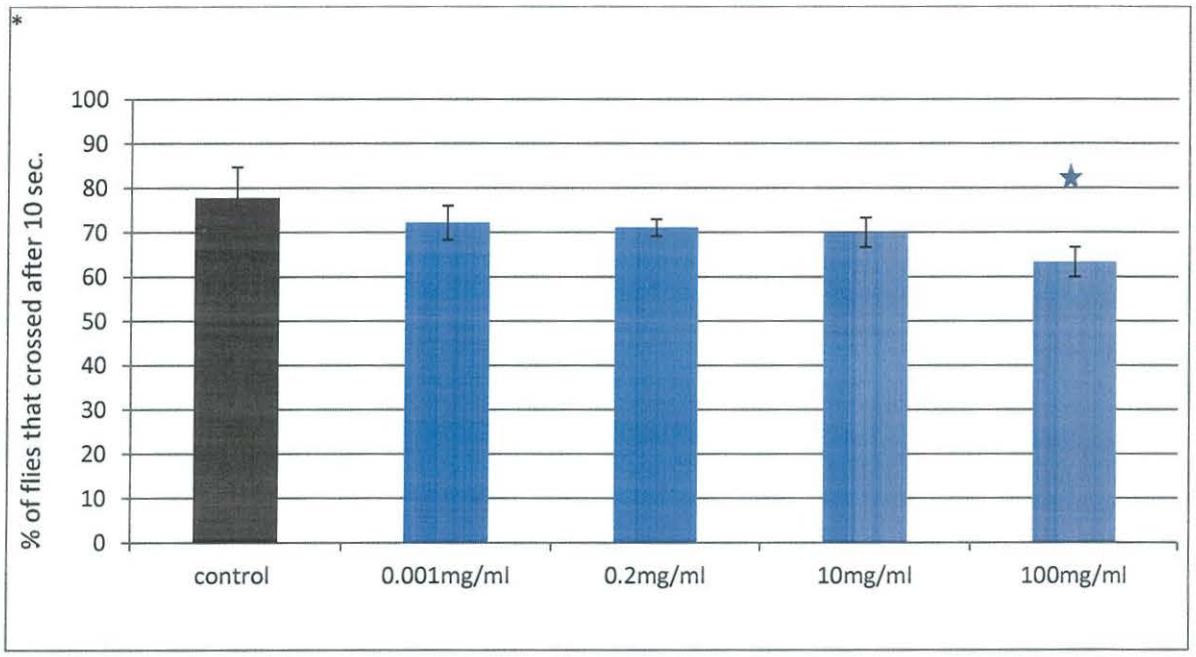

Figure 3: Percentage of flies that crossed 8cm after 13 days treatment with different concentrations of aqueous extract of *W. ugandensis*. Star (★) show significant difference between control and flies treated with 100mg/ml of $P<0.05$ calculated using T-test.

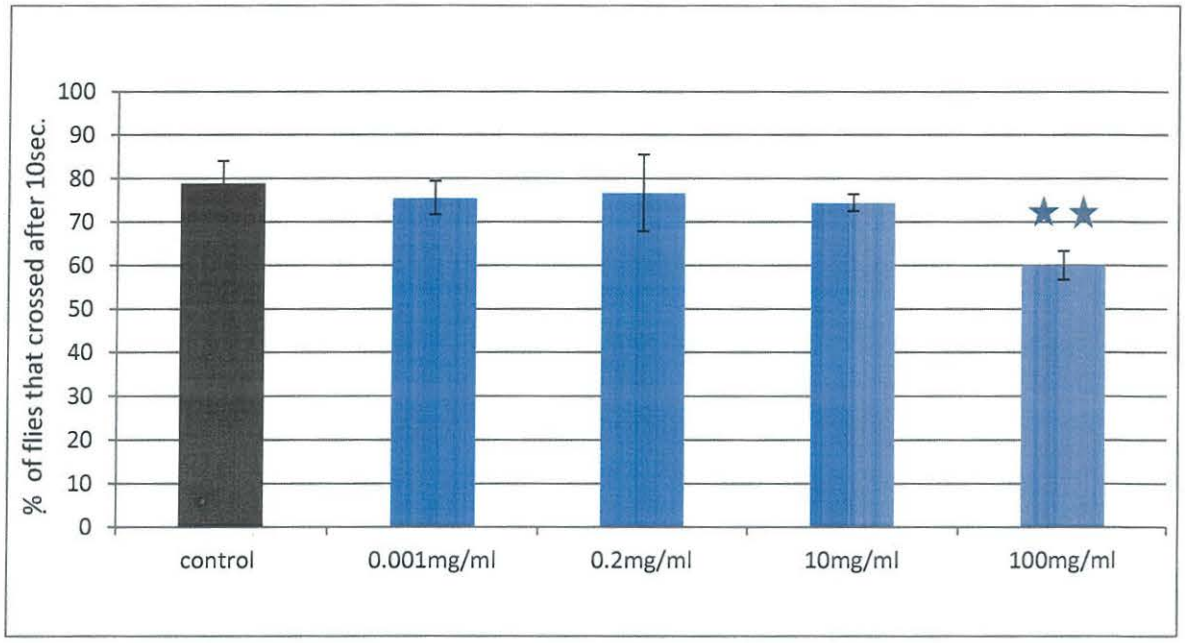

Figure 4: Percentage of flies that crossed 8cm after 13 days treatment with different concentrations of ethanolic extract of *W. ugandensis.* Two stars (★★) show significant difference between control and flies treated with 100mg/ml of $P<0.01$ calculated using T-test.

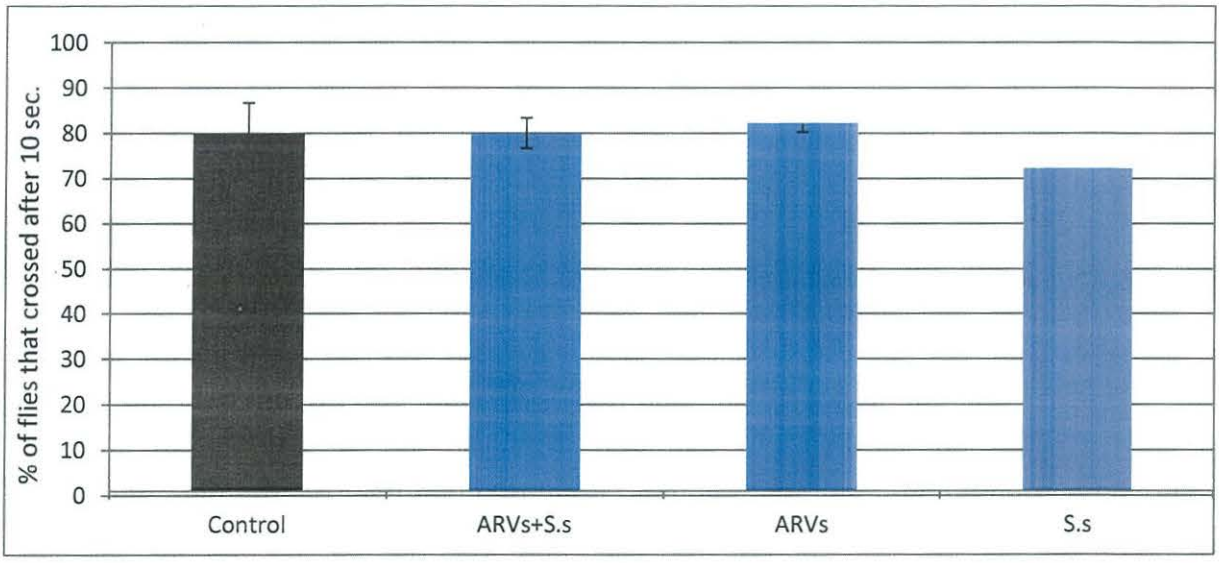

Figure 5: Percentage of flies that crossed 8cm after 13 days treatment with ARVs plus *S. sesban* (ARVs+S.s), ARVs alone *or S. sesban* alone (S.s)

4.6 COPULATION AND MATING ASSAY

Copulation latency, copulation duration and courtship index of flies treated for 7 days showed increase in copulation duration in female flies treated with both ARVs and *S. sesban* of $P<0.05$ (6, 7 and 8) which is not statistically different from when both males and female are treated with ARVs and *S. sesban,* and when only males are treated with similar combination, as calculated using T-test.

Copulation latency, copulation duration and courtship index of flies treated for 14 days showed significant decrease in copulation latency in flies treated with only *S. sesban* or ARVs plus *S. sesban* both having $P<0.001$, and significantly increased in copulation latency in flies treated with ARVs alone with $P<0.05$ (figure 9) calculated using T-test. Significant increase in copulation duration was also seen with flies treated with *S. sesban* ($P<0.05$) (figure 10). Finally courtship index was significantly high compare to control in flies treated with ARVs plus S. sesban ($P<0.001$) and significantly low in flies treated with ARVs alone ($P<0.05$; Figure 11), calculated using T-test.

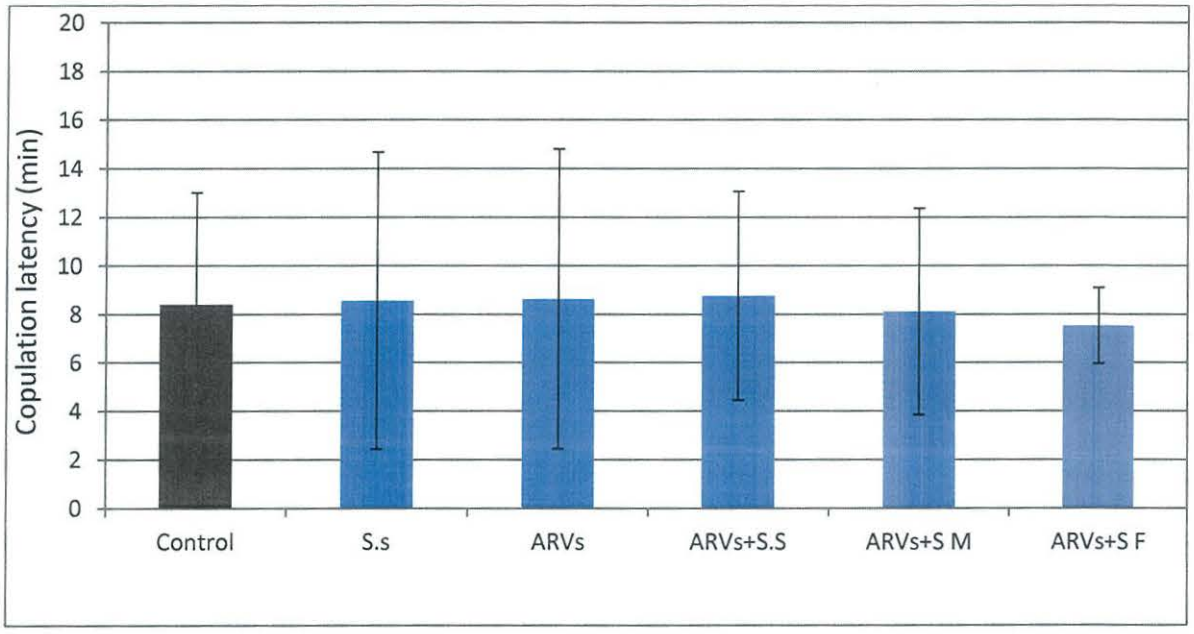

Figure 6: Copulation latency of different groups of flies after 7 days treatment. Flies were treated with *S. sesban* (S.s), ARVs, ARVs plus *S. sesban* (ARVs+S.s), ARVs plus *S. sesban*

where only males were treated and paired with untreated females (ARVs+S.s M) and ARVs plus *S. sesban* where only females were treated and paired with untreated males (ARVs+S.s F).

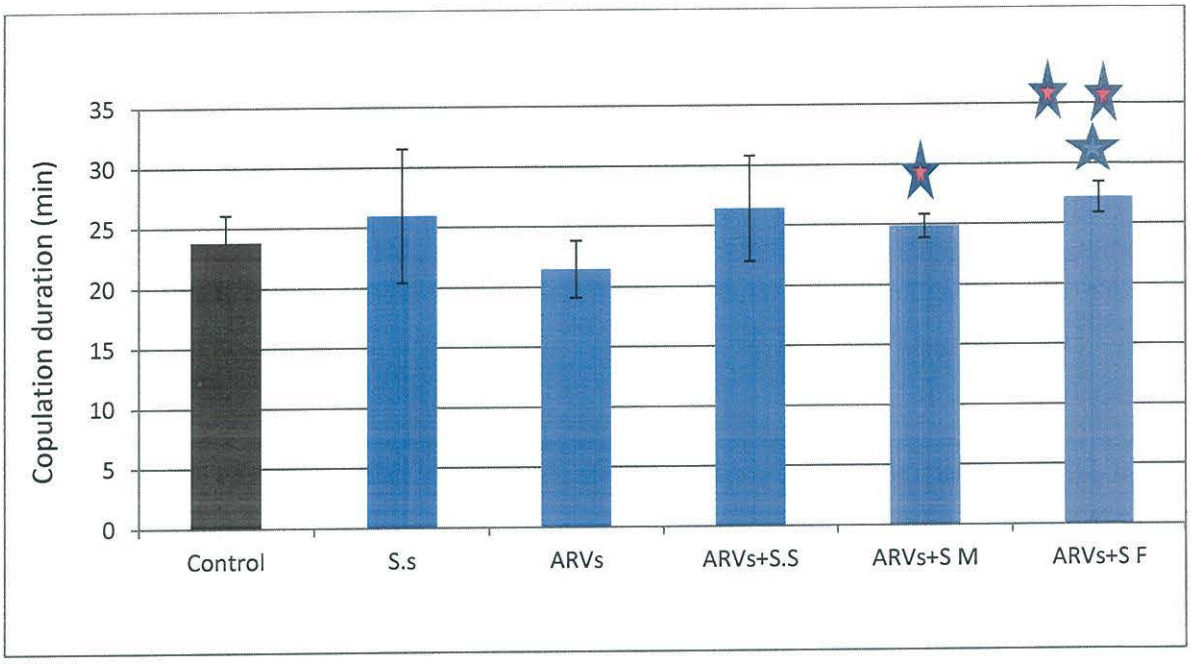

Figure 7: Copulation duration of different groups of flies after 7 days treatment. Flies were treated with *S. sesban* (S.s), ARVs, ARVs plus *S. sesban* (ARVs+S.s), ARVs plus *S. sesban* only males treated and paired with untreated females (ARVs+S.s M) and ARVs plus *S. sesban* only females treated and paired with untreated males (ARVs+S.s F). Blues Star (★) show significant difference between control and female flies treated with both ARVs and *S. sesban* of $P<0.05$ calculated using T-test; and Red Star (★) and stars (★★) show significant difference between female flies exposed to ARVs plus *S. sesban*, males exposed to ARVs plus *S. sesban* and ARVs exposed flies of $P<0.05$ and $P<0.01$ respectively.

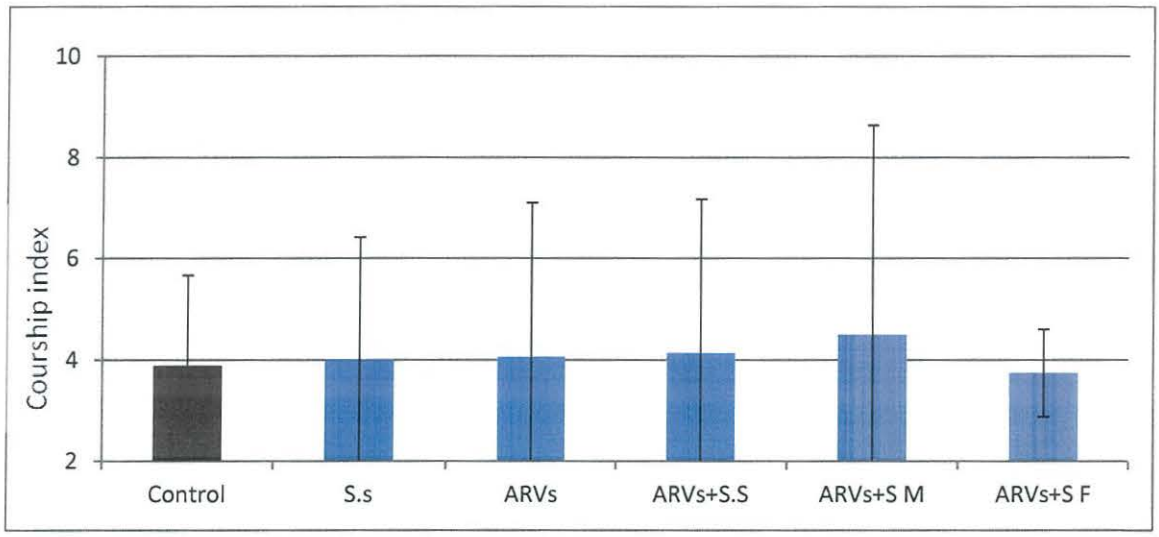

Figure 8: Courtship index of different groups of flies after 7 days treatment. Flies were treated with *S. sesban* (S.s), ARVs, ARVs plus *S. sesban* (ARVs+S.s), ARVs plus *S. sesban* males treated (ARVs+S.s M) and ARVs plus *S. sesban* females treated (ARVs+S.s F).

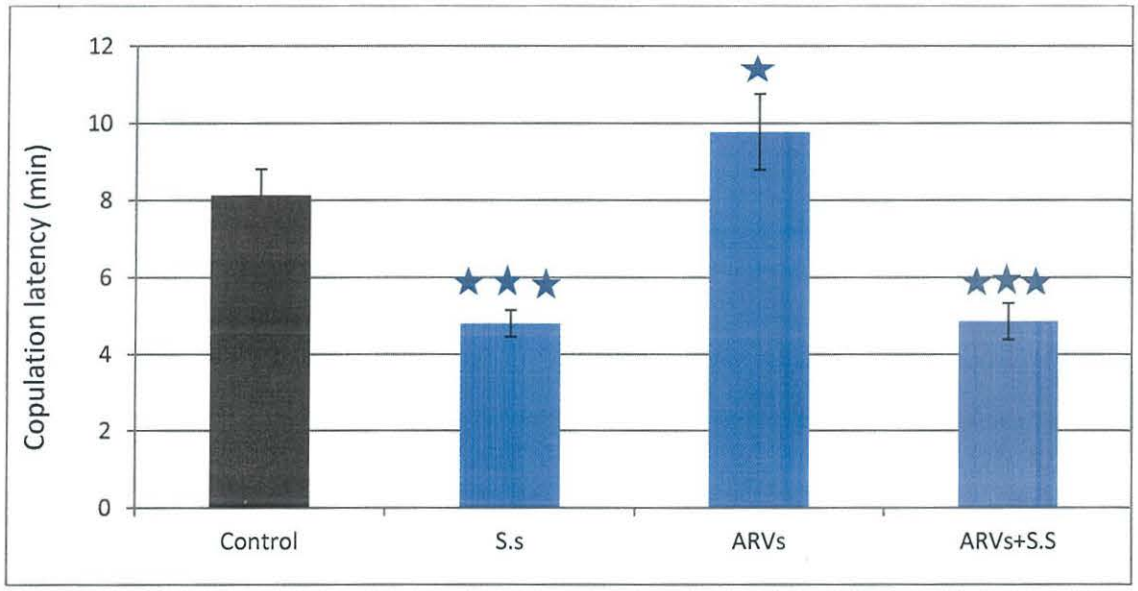

Figure 9: Copulation latency of different groups of flies after 14 days treatment. Flies were treated with *S. sesban* (S.s), ARVs and ARVs plus *S. sesban* (ARVs+S.s). Three stars (★★★), One star (★) and three stars (★★★) show significant difference between control and flies treated with S. sesban, ARVs and ARVs+S.s, of $P<0.001$, $P<0.05$ and $P<0.001$ respectively calculated using T-test.

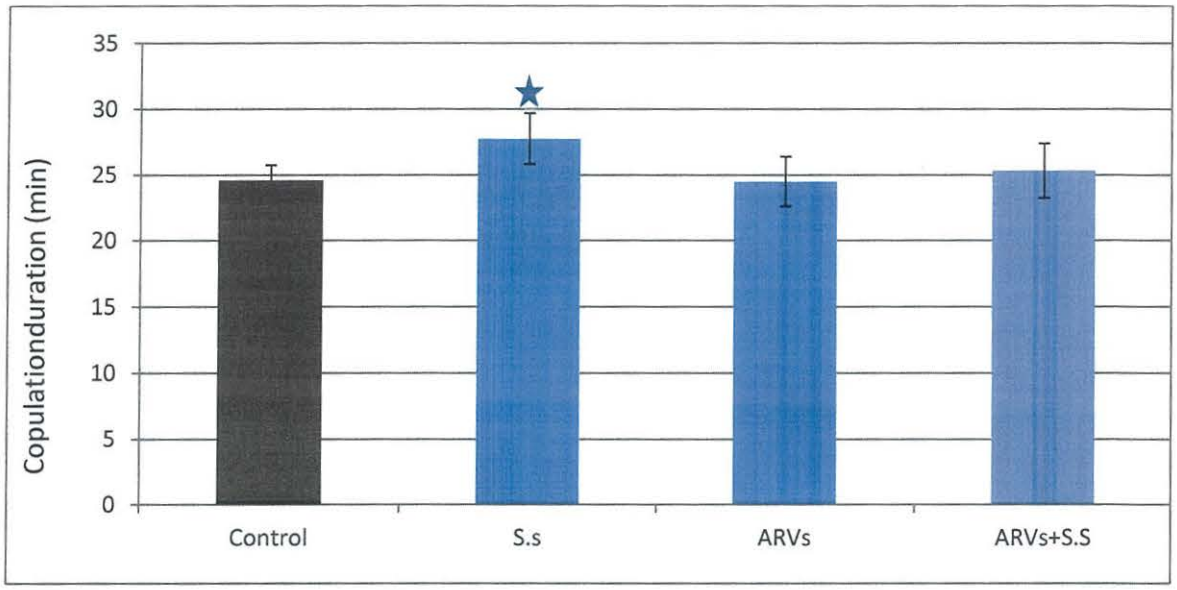

Figure 10: Copulation duration of different groups of flies after 14 days treatment with *S. sesban* (S.s), **ARVs and ARVs plus *S. sesban* (ARVs+S.s).** Star (★) show significant difference between control and flies treated with *S. sesban* of $P<0.05$ calculated using T-test.

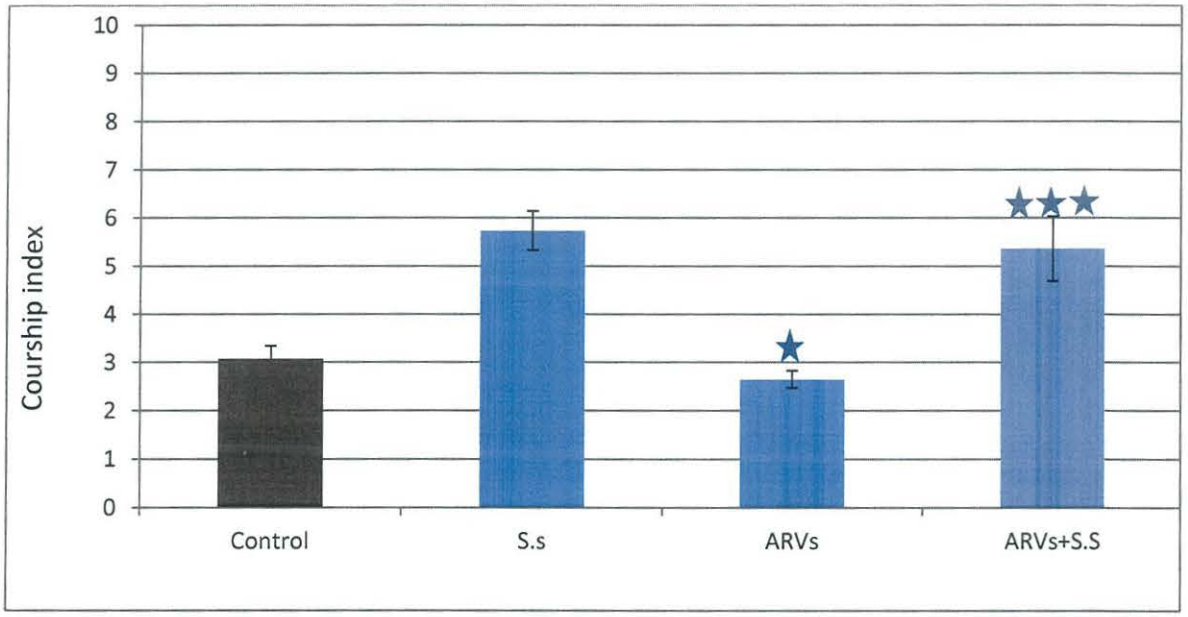

Figure 11: Figure 10: Courtship index of different groups of flies after 14 days treatment with *S. sesban* (S.s), ARVs and ARVs plus *S. sesban* (ARVs+S.s). One star (★) and three stars (★★★) show significant difference between control and flies treated with ARVs and ARVs+S.s, of $P<0.05$ and $P<0.001$ respectively calculated using T-test.

4.7 LONGEVITY ASSAY

Longevity study showed toxicity of ethanolic extract of *W. ugandensis* at high dose (figure 13) and relatively safety of its aqueous counterpart (figure 12). Longevity study for *S. sesban* with ARVs showed safety of the combination (figure 14), better than when the flies were exposed to either of the two separately.

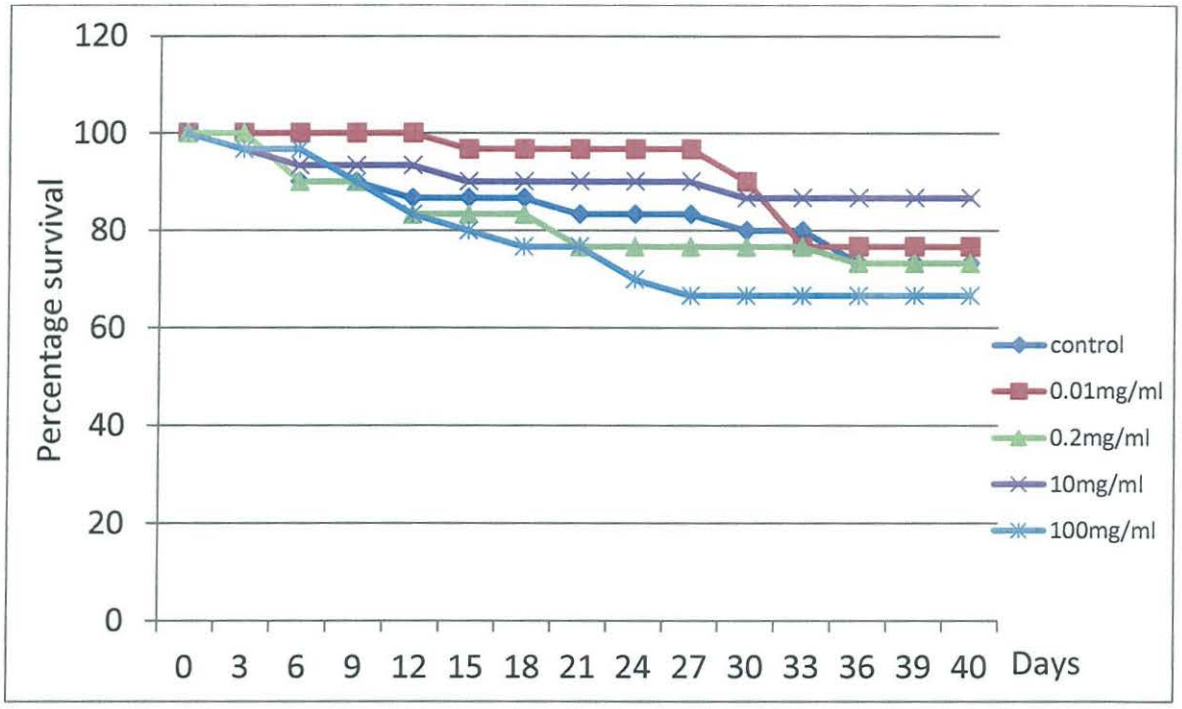

Figure 12: Percentage survival after 40 days of exposure to different concentrations of aqueous extract of *W. ugandensis*.

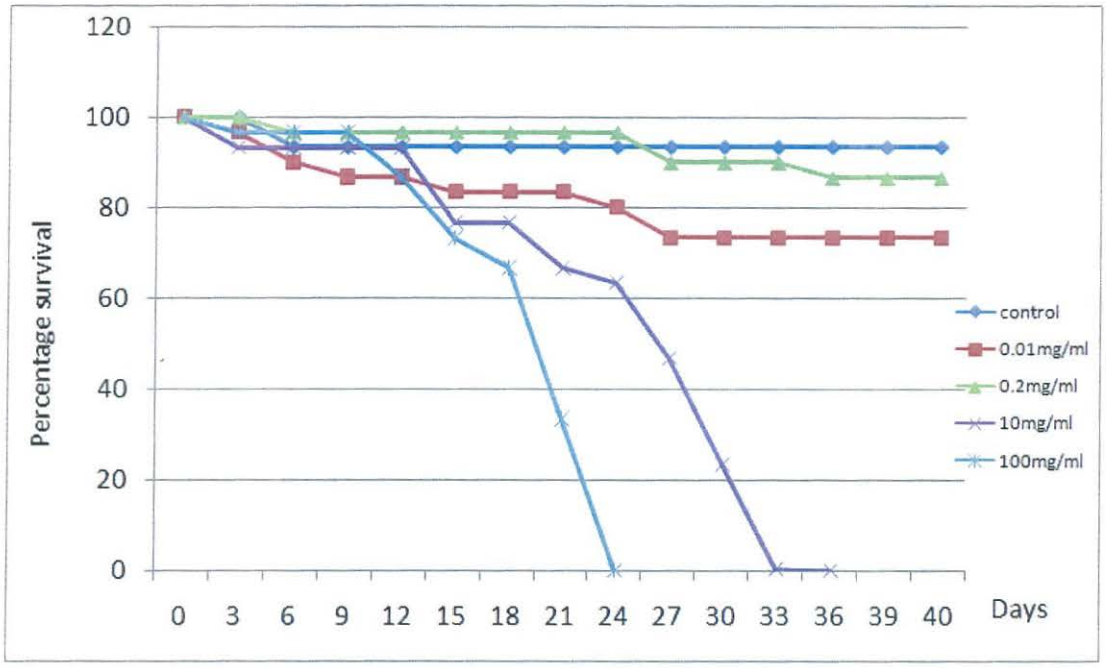

Figure 13: Percentage survival after 40 days of exposure to different concentrations of ethanolic extract of *W. ugandensis*

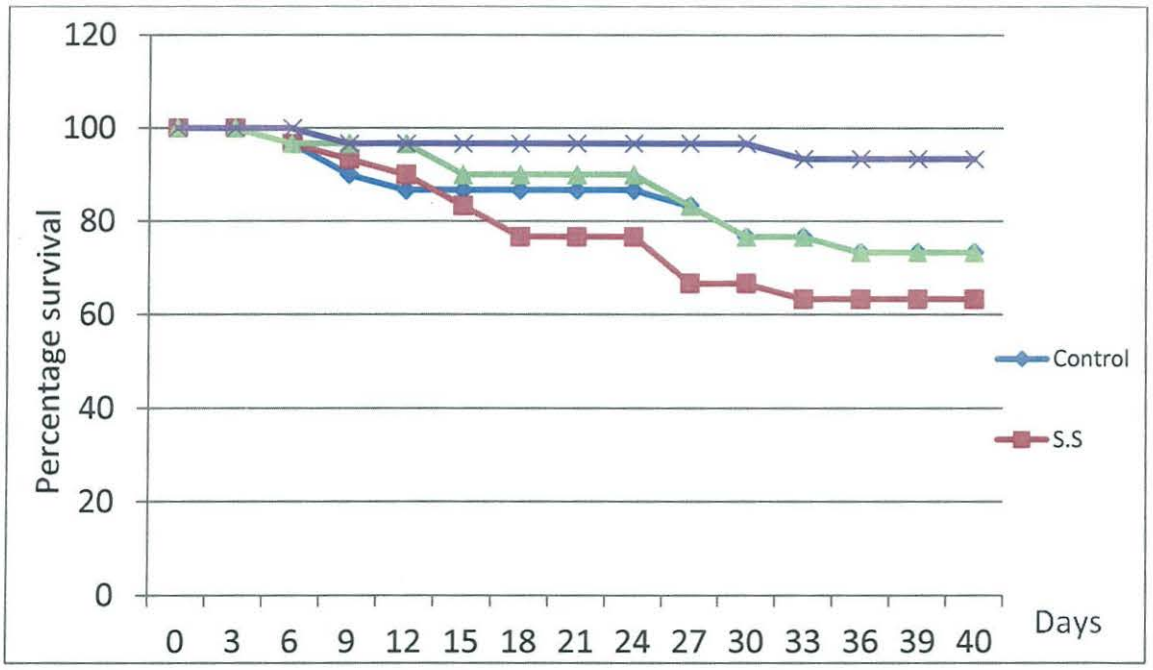

Figure 14: Percentage survival after 40 days of exposure to aqueous extract of *S. sesban (S.s)*, ARVs and ARVs plus *S. sesban* (ARVs+S.s).

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDETION

5.1 DISCUSSION

After compilation of healers' knowledge the results showed that remedies to treat herpes zoster (shingles) appear to be the commonest with 40% of the remedies recorded by diarrhea (20%; Table 1). An oral dosage is usually prepared and prescribed in 80% of cases, followed by topical formulations with 20%. The routine use of oral and topical formulations partly explained the lack of technical know-how by the traditional healers to produce parenterals and other advance formulations, also give an insight on their attempt to reduce production and product costs, since it is the only main source of heath care in rural settings (Orisatoki and Oguntibeju, 2010).

Percentage yield results (Table 2), showed a higher yield with aqueous extract of *S. sesban.* This could be due to hot percolation of 6hours (Pandhare *et al.* 2011) prior to maceration of 18hours (24hour of extraction). The results also shows slightly higher yield with ethanol extract compare to water extract of *W. ugandensis,* with loss of some crude extracts after partitioning aqueous extract with chloroform due to formation of three phased system (aqueous, chloroform and residues) after partitioning, middle layer being residue of clotting material.

Preliminary phytochemical screening (table 3), showed a high level of terpenoids and steroids in both aqueous and ethanolic extracts of *W. ugandensis*, which persist even after partitioning aqueous extract with chloroform. The presence of the two metabolites together is due to similar metabolic pathway of their synthesis from Acetyl CoA+Aceto acetylCoA to Squalene (C_{30}), although their quantities can differ (Trease and Evans 2002). Saponins were lost after partitioning aqueous extract with chloroform due to their inherent nature to form froth in mostly in water but not in chloroform and other organic solvents. Test for glycoside was positive with Keller killiani's test and negative with Lieberman's test probably due to the absence of the specific class of glycosides that react to Lieberman's test.

Acute toxicity is the adverse effects of a substance seen after an exposure or multiple exposures within a short term interval (IUPAC, 2006). Results of acute toxicity tests for both aqueous and ethanolic extracts of *W. ugandensis* (Figure 1 and 2) has showed to be non-toxic after one hour of exposure with standard deviation of 0 to 0.57.This hows that the local irritation caused by this plant does not really reflect same systemically. The results create a room form another question,

which is wether the extracts are toxic on chronic exposure or not. Which was answered by climbing assay and longevity study.

Climbing assay provides behavioral measure to determine motor activity, neurological damage and aging in *Drosophila* (Feany and Bender, 2000). Climbing assay showed a significant difference between control and flies treated with 100mg/ml (10%) of both aqueous and ethanolic extracts of *W. ugandensis* with $P<0.05$ and $P<0.01$ respectively (Figure 3, 4 and 5). The cause for significant decreased in negative geotaxis activity is not established in this study.

Copulation in *Drosophila* involves a series of activities that occur prior to copulation which occur in a stage known as copulation latency. These behaviors includes orientation, tapping, wing song licking and copulation attempt (Charles *et al.,* 2012). Results of copulation latency, copulation duration and copulation index of flies treated for 7 days (Figure 6, 7 and 8) respectively, showed strong inter-individual variability between the couples associated with increase in copulation duration in female flies treated with both ARVs and *S. sesban* with $P<0.05$ which is statistically not different from when both couples or only males received the same treatment.

Results for copulation latency, copulation duration and copulation index of flies treated for 14 days (figure 9, 10 and 11) respectively showed similarities in copulation behaviors that received the same treatment. The assay also showed significant decrease in copulation latency in flies treated with only *S. sesban* or ARVs plus *S. sesban* both having $P<0.001$, and significantly increased in copulation latency in flies treated with ARVs alone with $P<0.05$ calculated using T-test. Significant increase in copulation duration was also seen with flies treated with *S. sesban* ($P<0.05$) using. Finally copulation index was significantly high in flies treated with ARVs plus S. sesban ($P<0.001$) and significantly low in flies treated with ARVs alone ($P<0.05$) compared to the control, calculated using T-test.

Increase in copulation latency and decrease in copulation duration seen in flies treated with ARVs alone can partly be linked to the side effect of Zidovudine, as Zidovudine causes decreased in libido and gynecomastia in humans (BNF, 2014).

Longevity test result of aqueous extract of W. ugandensis (figure 12) is in agreement with that of acute toxicity, but ethanolic extract has shown to be significantly toxic at high dose. Combination of *S. sesban* plus ARVs has the lowest number of deaths

5.2 CONCLUSION AND RECOMMENDETIONS

These results shows how concomitant administration of ARVs plus *S. sesban* decrease copulation latency (increase libido) and increase copulation duration in *Drosophila melanogaster* especially after exposure for 14 days, at the same time results shows increase copulation latency (decrease libido) and decrease copulation durations ("short lasting") in flies treated with ARVs alone. And this combination has shown to be safe with both climbing assay and longevity study. My study provides some basic information on interaction between first line ARVs combination in Uganda and a commonly prescribed herb by Rukararwe traditional healers to treat herpes zoster and also give an insight into potential discovery of new drug or supplement for the improvement of sexual performance.

Further studies should be carried out to confirm the activity of *S. sesban* in human beings, as well as the mechanisms by which these agents affect copulation behaviors at molecular level. Studies should also be carried out to find the genes affected by water and ethanolic extracts of *W. ugandensis* that leads to decrease in negative geotaxis performance at high dose. Finally both *S. sesban* and *W. ugandensis* were found to be non-toxic at therapeutic doses on experimental model and they can continue to be used without much fear.

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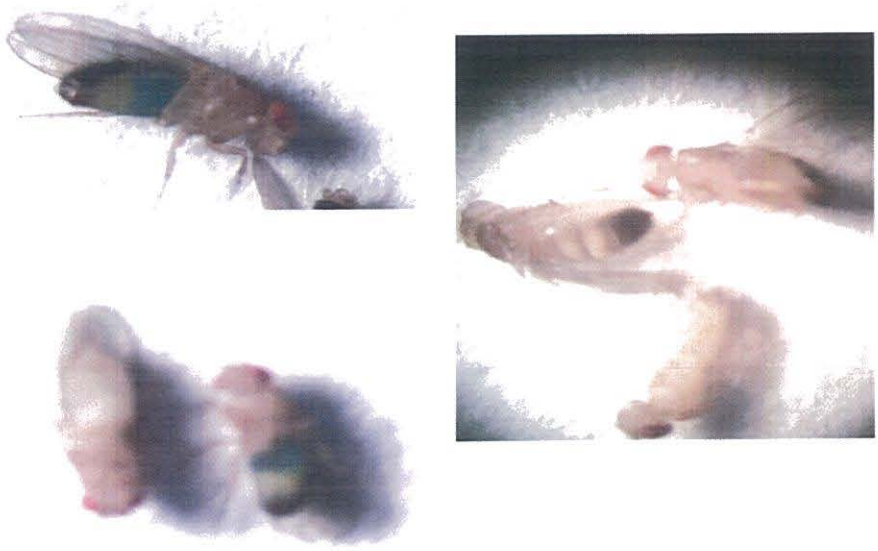
APPENDICES

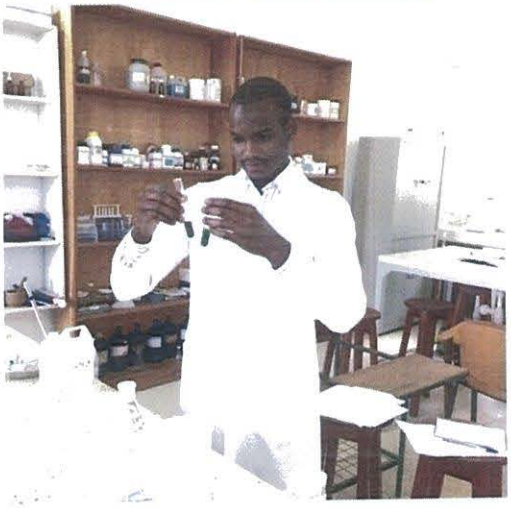
Appendix I: Time frame

ACTIVITY	MONTHS				
	February	March	April	May	June
Proposal development and presentation					
Data collection					
Data analysis					
Report writing and defense					
TOTAL	FIVE MONTHS				

Appendix II: Drosophila melanogaster before and after feeding with *W. ugandensis* extract containing food colorant during acute toxicity test



Appendix III: Pre-extraction and laboratory work during collection, particle size reduction, extraction and phytochemical screening exercise.



Appendix IV: Laboratory work during sex differentiation of virgins *D. melanogaster* and acute toxicity test.