DETERMINANTS OF USE AND ANTIVIRAL ACTIVITY OF MORINGA OLEIFERA EXTRACTS AMONG PEOPLE LIVING WITH HIV AND AIDS ATTENDING COMPREHENSIVE CARE CLINIC AT MIGORI COUNTY REFERRAL HOSPITAL, KENYA

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Determinants of Use and Antiviral Activity of *Moringa Oleifera* Extracts among People Living With HIV and Aids Attending Comprehensive Care Clinic at Migori County Referral Hospital, Kenya

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DECLARATION

This thesis is my original work and has not been presented to any other University or Institution for the award of a degree.

Signature…… Date……..……

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

To my husband Meshack Mwiti Maitima, our children Angela Nkatha Mwiti, Alan Arbiter Mwiti, my mum Silveria Nkirote and siblings for their love, patience, support and encouragement during this study. God bless you.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACV	Acyclovir
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ATCC	America type culture collection
AVMA	American veterinary medical association
CCC	Comprehensive Care Clinic
CO_2	Carbon dioxide
CTMDR	Center for Traditional Medicine and Drug Research
DMSO	Dimethylsulphoxide
EC_{50}	Effective concentration required to induce a 50% effect
FBS	Fetal bovine serum
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus type 1
IC_{50}	Inhibitory concentration required to inhibit 50%
JKUAT	Jomo Kenyatta University of Agriculture and Technologies
KEMRI	Kenya Medical Research Institute
MEM	Minimum essential media
MTT	2-(2,5-dimethyl-2-thiazoly)-3-,5-diphenyl-2h-tetrazolium Bromide
M.O	*Moringa oleifera*
OD	Optical density
PBS	Phosphate buffer solution
PFU	Plaque forming unit
PI	Primary investigator
PLWHA	People living with HIV/AIDS
PPE	Personal protective equipment

SERU	Scientific and Ethical Review Unit
$TCID_{50}$	Tissue culture infectious dose 50%
TI	Therapeutic index
VERO	Lineages of cell isolated from kidney epithelial cells of African Green monkey
WHO	World Health Organization

DEFINITION OF OPERATIONAL TERMS

Antiretroviral Therapy: Treatment with drugs that inhibit the ability of the HIV virus from multiplying in the body

Antiviral: An agent that kills or suppresses a virus and inhibits its ability to replicate and multiply

Cytotoxicity: This is an in vitro test which determines whether a process or substance will lead to the damage or death of a cell

In vitro: To perform a process outside a living organism such as in a test tube or culture dish

Opportunistic infection: **These** are infections that occur as a result of weakened immune system among PLWHA

Determinant: A factor which affects the outcome of use of M. Oleifera

ABSTRACT

Moringa oleifera (M. Oleifera) is widely used as source of nutrition and as traditional medicine for the treatment of various diseases. Herpes simplex infection is among the major opportunistic infections among people living with HIV/AIDS (PLWHA). To date, there is no vaccination against the Herpes simplex virus type 1(HSV-1) and the recommended antiviral drugs have only a modest effect against the virus. The aim of this study was to determine the determinants of use and antiviral activity of *Moringa oleifera* extracts among PLWHA attending Comprehensive Care Clinic (CCC) at Migori County Referral Hospital. The study was cross-sectional in which 278 PLWHA attending CCC at Migori County Referral Hospital were selected by systematic sampling while 9 herbalists were sampled through the snowballing technique. Quantitative data was collected using semi structured questionnaires and analyzed in Statistical Package for Social Sciences (SPSS V.22). Chi-square test was used to compare categorical variables with a level of significance at $p \leq 0.05$. *M. Oleifera* leaves were collected from the field and botanically identified at the University of Nairobi Herbarium. Aqueous and methanol extracts were tested for cytotoxicity by tetrazolium dye (MTT) and anti-herpes activity screened by cytopathic effect reduction and MTT assay using Vero cells and HSV-1. The study revealed that *M. Oleifera* is commonly (75.5%) used among PLWHA attending comprehensive care clinics (CCC) at Migori County Referral Hospital. The socio-demographic profile indicates that, age was the only significant factor associated with the use of *M. Oleifera* among PLWHA attending comprehensive care clinic. The commonly used part of the tree was the leaves (64.3%). The majority of the respondents (99%) had not experienced any side effects after using *M. Oleifera* together with HIV drugs. The findings indicate that distance to the health facility and the long awaiting time during health facility visits were the two significant health systems level factors associated with the use and non-use of *M. Oleifera.* Aqueous and methanol extracts of *M. Oleifera* are not toxic to vero cells and have antiviral activity against HSV-1. There is a need to formulate policy and legal framework to govern the herbal medicine practices among PLWHA attending CCC at Migori County Referral Hospital. Sensitization of people against stigma towards PLWHA is required. Specific phytochemicals responsible for antiviral activity against HSV-1 in aqueous and methanol extracts need to be isolated and further investigated for anti- HSV-1 activity *in vivo* and clinical trials.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Throughout the world conventional medical care systems continue to co-exist with traditional medical care systems. The use of traditional medicine continues to increase with people using it to treat various health problems. The World Health Organization estimates that 80% of the World's population use traditional and complementary medicine in some aspects of their health care (WHO, 2019a). The WHO Strategy on Traditional medicine showed that the ratio of traditional healers to the population in Africa is 1:200-400 while the ratio of biomedical experts to the population is 1:200000 (Asiimwe, 2012). Thus, in real sense traditional medicine is carrying the burden of clinical care in Africa.

The use of traditional medicine is particularly common in people with compromised immune systems such as those with HIV/AIDs. Globally, since the beginning of the HIV/AIDs epidemic, traditional and complementary medicine has been used by PLWHA due to various reasons including inaccessibility to effective treatment, management of side effects of Antiretroviral Therapy (ART), treatment of opportunistic infections, for relieving pain and stress, and as dietary supplements (Ekor, 2014). A study by (Gurmu, *et al,* 2017) showed that more than half of HIV/AIDS patients used traditional and complementary medicine is used along with ART medications. This include 67% in Chicago and Ohio, USA, 54.7% in Thailand, 53% in South Africa, 57.9% in Nigeria, 53% in Zimbabwe and 53.2% in Ghana. In Africa, between 36 percent and 68 percent of HIV patients use both complementary and alternative medicine (Gurmu, *et al,* 2017). This is attributed to various factors such as expensive or unavailable anti-retroviral drugs, claims of efficacy of herbal medicine and stigma for many of the HIV/AIDS patients hence they turn to traditional healers (Ekor, 2014; Asiimwe, 2012). People living with HIV/AIDs in African countries with strong

traditional systems of medicine often use herbal medicine both as primary and secondary treatment for HIV/AIDs (OHTN, 2013).

One of the widely used plant in herbal medicine because of its vast medicinal properties is *M. Oleifera*. Various parts and tissues from the tree such as seeds, roots, leaves, and immature pods have been used for a variety of purposes including herbal medicine, food, and fodder (Alegbeleye, 2018; Kumssa *et al.,* 2017; Razis *et al,* 2014). Several biochemical and ethno-botanical studies on *M. Oleifera* growing in other parts of the world have shown that different parts of the tree have varying health benefits, nutritional value, antioxidants compounds and antiviral properties (Singh, et al., 2019; Rajbhar, et al., 2018; Tshingani, et al., 2017; Gopalakrishnan, et al., 2016). For instance, the flowers of *M. Oleifera* grown in Oman are reported to contain various types of antioxidants compounds such as ascorbic acid, tannins, flavonoids, phenolic, and carotenoids in a study by (Alhakmani *et al*, 2013). Similar findings were reported by (Vongsak *et al.,* 2014) in a study on leaves of *M. Oleifera* grown in different regions of Thailand. On antiviral activities, various observations indicate that various preparations of *M. Oleifera* extract are used for their anti-inflammatory, antihypertensive, antimicrobial, antidiabetic, antihyperlipidemic, antipyretic, antiulcer, cardiprotectant, and hepatoprotectant activities (Razis *et al*., 2014; Mbikay, 2012). Despite its varied uses, the chemical composition of the different extracts of the *M. Oleifera* tree may vary depending on the source and cultivar (Kumssa *et al.,* 2017).

The reported benefits of *M. Oleifera* have led to an increasing recommendation for its use in people with compromised immune systems. *M. Oleifera* is recommended for use as a nutritional supplement and immune booster among Persons living with HIV and AIDS. The escalation of inflammatory reactions as a result of HIV infections increases basal metabolism and energy expenditure leading to a higher likelihood of PLWHA having malnutrition (Tsingani, et al, 2017). PLWHA experience nutritional deficiencies which results in decline in immunity, accelerated rate of HIV replication and

opportunistic infections such as HSV virus (Gopalakrishnan, et al., 2016). Due to the antimicrobial activities, there is increased research focusing on *M. Oleifera* as a safe alternative traditional medicine for the treatment of opportunistic infections such as HSV virus which has developed resistant pathogens to the available therapies (Jiang, et al, 2016; Hodge and Field, 2013).

Studies on the effects of *M. Oleifera* on PLWHA have shown positive outcomes. A study by (Ogbuagu, et al. 2016) in Nigeria established that *M. Oleifera* has the potential of improving the CD4 count of HIV positive patients on ART translating to better treatment outcome. The results of the study indicated a significant increase in the CD4 value of both male and female participants after using 20g of *M. Oleifera* daily for a period of two months. A study by (Tete-Benissani et al., 2013) showed that *M. Oleifera* powder is rich in proteins, micronutrients and induced BMI increase in HIV positive patients. Biochemical parameters determination showed an increase in triglycerides, LDL-cholesterol, atherogenicity index (AI) correlated with HDL-cholesterol decrease. Total cholesterol decreased more in HIV positive asymptomatic and increased in patients treated with ARV drugs. Glycaemia level also decreased in the patients who participate in the study (Tete-Benissani et al., 2013). *M. Oleifera* also has an impact on the body mass index and immune response of HIV patients on antiretroviral therapy. A study by (Tshingani, et al., 2017) established that patients who took *M. Oleifera* leaf powder daily for a period of six months exhibited a significantly greater increase in BMI and albumin levels.

1.2 Statement of the Problem

Over the years, great strides have been made to decrease the impact of HIV/AIDs on patients through the Antiretroviral Therapy (ART). Despite this, the use of herbal medicine including *M. Oleifera* among PLWHA continues to increase throughout the world. Several reasons have been given to explain this including the persistence of latent reservoirs of HIV-infected cells in the organs of patients treated with ART and

burdensome lifelong ART with side effects (Tshingani, et al., 2017). According to (Laila et al., 2019), antiretroviral drugs are associated with serious side effects like lipodystrophy causing peripheral fat loss and central fat accumulation leading to thin facial pads, thin legs and arms, buffalo humps and pot bellies which leave the patient stigmatized. Other reasons include various claims on the efficacy or effectiveness of *M. Oleifera*, belief that *M. Oleifera* is more potent compared to other pharmaceutical drugs and the readily availability of *M. Oleifera*.

Although several studies conducted have shown that *M. Oleifera* is used as medicine, there is limited evidence on its benefits as part of HIV/AIDs treatment. Additionally, how *M. Oleifera* used, which parts of the tree are consumed as meals and medicine, and the nutrient composition vary depending on the geographical region (Kumssa et al., 2017; Gopalakrishnan, et al, 2016). Thus the generalization of findings from studies on the different properties of *M. Oleifera* is inappropriate. This presents a challenge as herbal remedies such as *M. Oleifera* have the highest potential for contraindication as they can interfere with how antiretroviral drugs are metabolized, creating early inhibition and decreasing the length of drug exposure (OHTN, 2013). The PLWHA who continue to use herbal medicine particularly in areas where complementary medicine is not regulated are at great risk of adverse effects that undermine their quality of life.

The increasing HIV/AIDs infection and the subsequent rise in opportunistic infection such as HSV have become a public health concern in both developing and developed Countries. Despite the HSV-1 virus being a public health concern, there is no vaccination against it and the therapies with the recommended antiviral drugs having only a modest effect (WHO, 2016). The discovery of antiviral agents in herbal medicine including *M. Oleifera* is crucial for treatment of resistant pathogens to the available therapies. Studies have suggested that different crude extracts from different tissues of *M. Oleifera* show antibacterial activities against various opportunistic infections such as

HSV (Waiyaput, et al., 2012). Despite this, the antiviral activities of *M. Oleifera* in Kenya are also not well documented.

Herbal medicine including *M. Oleifera* is widely used by PLWHA in Kenya. However, the data on the prevalence and pattern on use, and the associated factors among HIV/AIDs patients while on antiretroviral therapy is scarce. A study by (Kumssa *et al*., 2017) established that there was little evidence about the nutritional and therapeutic values of Kenyan *M. Oleifera*. There is also a lack of database of medicinal plants which is one of the hindrances to herbal medicine studies (Kigen *et al.,* 2013). As such, many claims of the nutritional and medicinal value of *M. Oleifera* therefore remain unsubstantiated.

This study sought to establish the extent of *M. Oleifera* usage among the HIV/AIDS patients in Kenya and the factors associated with its use. The study also examined the antiviral activity of Kenyan *M. Oleifera* extracts on HSV -1. This was aimed at establishing whether *M. Oleifera* can be used as an anti-HSV remedial therapy.

1.3 Justification of the Study

Traditional and complementary medicine continues to be part of primary and secondary healthcare of people across the world. Despite the introduction of Antiretroviral Therapy (ART) which has resulted in significant reduction of mortality and improved quality of life for PLWHA, they continue to use herbal medicine including *M. Oleifera*. Although efficacies of a number of these medicines have been established through some studies and patient' self-reports, the nutritional, antiviral activities and prevalence of use in Kenya remain unknown. Additionally, the use of herbal medicine particularly among PLWHA is rarely or poorly monitored. This indicates that there is inadequate knowledge on the mode of action of herbal medicine, contraindications and potential adverse reactions with other medication including antiretroviral drugs. Thus, PLWHA are often

poorly informed about the decisions they make to use herbal medicine including *M. Oleifera*.

Previous reports have shown that there has been unregulated use of herbal remedies by Kenyan communities with risks of lack of intended benefits or adverse effects (Kigen *et al.,* 2013; Mamothena 2014). In Kenya herbalist are known to use *M. Oleifera* extracts to manage various illnesses and opportunistic infections due to the herb's nutritional and medicinal values. The studies done on the *M. Oleifera* have focused primarily on the plants ethnobotany (Kamau *et al.,* 2016) with limited information on its antiviral effects. Although studies have shown the potential efficacy of some medicinal plants, there are few studies that have looked at determinants of *M. Oleifera* that influence its use. Therefore, this warrants a scientific investigation to fill this gap and conclusively establish the veracity of the Kenyan *M. Oleifera* extracts.

The increase in opportunistic infections such as HSV and the rise in resistant strains to treatment drugs point out to a need to explore new and effective solutions including the use of herbal medicine. There is need for more evidence data for adoption of indigenous plants and herbs as a treatment regime. Moreover, knowledge on correct and effective dosage of herbal medicine such as *M. Oleifera* is needed so as to avoid contraindication with ART. The purpose of this study was, therefore to determine the determinants of use and antiviral activity of *M. Oleifera* extracts among PLWHA attending the Comprehensive Care Clinic at Migori County Referral Hospital.

1.4 Research Questions

1. What is the usage of *M. Oleifera* among PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital?
2. What are the patients level factors associated with the use of *M. Oleifera* use among PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital?

3. What are the health systems level factors health facility and treatment level factors associated with the use of *M. Oleifera* among PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital?
4. Is there *in vitro* cytotoxicity and antiviral activity of *M. Oleifera* extracts on herpes simplex virus type -1 (HSV-1)?

1.5 Study Objectives

1.5.1 General Objectives

To determine the determinants of its use and antiviral activity of *M. Oleifera* among people living with HIV/AIDS (PLWHA) attending a Comprehensive Care Clinic (CCC) at Migori County Referral Hospital.

1.5.2 Specific Objectives

1. To determine the usage of *M. Oleifera* among PLWHA attending comprehensive care clinics (CCC) at Migori County Referral Hospital.
2. To establish the patients level factors associated with the use of *M. Oleifera* among PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital.
3. To determine the health systems level factors associated with the use of *M. Oleifera* among PLWHA attending a comprehensive care clinics (CCC) at Migori County Referral Hospital
4. To determine *in vitro* cytotoxicity and antiviral activity of *M. Oleifera* extracts on herpes simplex virus type -1 (HSV-1).

CHAPTER TWO: LITERATURE REVIEW.

2.1 Overview of the HIV/AIDS situation

Human Immunodeficiency Virus (HIV) is a chronic infection that targets and weakens the immune system making it difficult to fight many infections. It causes the potentially life threatening chronic disease Acquired Immunodeficiency Syndrome (AIDs) (Laila et al., 2019). Globally, HIV/AIDS still remains a major public health burden as it is estimated that about 37.9 million persons are living with HIV worldwide having claimed nearly 36.3 million lives since the start of the pandemic (WHO, 2019b). Sub-Saharan Africa has a majority of the infected persons attributed to the significantly high populations, endemic malnutrition and low economic status. In Kenya, generally the prevalence of HSV is estimated at 26.6%. Adults are the highest affected populations estimated at 31.0% while adolescents at 10.7% (Akinyi *et al*., 2017). It is estimated that in 2017, 1.5 million people were living with HIV (NACC, 2018). Most of the persons living with HIV/AIDS are faced with limited access to adequate food thus they are in constant need for nutritional support. Since HIV/AIDs destroy the human immune system, PLWHA are at a significant risk of acquiring a variety of life-threatening opportunistic infections such as HSV. According to WHO report of 2015, among the PLWHA, those who were co-infected with HSV in different populations ranged between 60-90%. The WHO report also has shown that the risk of acquiring new HIV infection increased by approximately threefold amongst people with HSV infection, and in addition, people infected with both HSV and HIV were more likely to spread HIV to others (WHO, 2016).

Achieving accessible, quality healthcare for persons with HIV and AIDS is a critical need for patients living in Kenya and worldwide. Worldwide access to comprehensive health services is required to lessen significantly HIV related morbidity and mortality globally. To be able to manage the HIV/AIDS situation, Comprehensive Care Center (CCC) was established. These are outpatient medical facilities aimed at offering medical

care and providing support to PLWHA so as to improve their quality of life as well as increasing life expectancy.

2.2 Herpes Simplex Virus

Herpes Simplex Virus (HSV) is a member of the Herpesviridae family of viruses whose genomes are made up of a single large double-stranded DNA molecule. HSV is known to have two serological subtypes, namely, HSV-1 as well as HSV-2. The HSV- 1 is mainly spread through contact with oral secretions while HSV-2 is mainly spread through contact with genital secretions. Both HSV-1 and HSV-2 can infect genital surface and oral (Looker *et al.*, 2015). Following host cell entry, HSV must establish main infection at the site of entrance by attaching to cell-surface of receptors, fuse its envelope to the plasma membrane, and then allow the de-enveloped capsid to be moved to the nuclear pores as well as sensory ganglia for life long latency with periodic recurrence to cause recurring infections back to the entrance site (Du, Zhou & Roizman 2013).

2.2.1 Risk of HSV Infection

Herpes simplex viruses have a worldwide distribution and are found throughout the animal kingdom. They are among the common infections and worldwide rates of HSV-1 and HSV-2, range between 60% and 95% (Field and Hodge, 2013). HSV-1 is more common compared to HSV-2, with rates increasing as people age (Jiang et al., 2016). HSV-1 rates are between 70% and 80% among populations of low socioeconomic status and 40% to 60% in populations of developed socioeconomic status (Chayavichitsilp, Buckwilte, Krakowski, *et al.*, 2009). Prevalence of HSV-2 in those amongst the ages of 15 and 50 is approximately 535 million as of 2003 or 16% of the population, with utmost rates in sub-Saharan Africa and lowest in Western Europe, and with higher rates in those in the developing world and among women (Looker et al., 2015).

2.2.2 Transmission of HSV

Herpes simplex is the leading cause of genital ulcers throughout the world. Typically, HSV is acquired in childhood and HSV-1 causes primarily oral labial ulcers including the mouth, eye, face, throat, and central nervous system infections, while HSV-2 is sexually transmitted and causes ulcers and infections of the anal and genital areas. HSV-1 is mainly associated with oral and perioral infections and HSV-2 with genital infections both can infect at any site (Chayavichitsilp, Buckwalte, Krakowski, *et al.*, 2009). Infections with HSV-2 are acquired through sexual contact and, therefore, antibodies to this virus are rarely found before the age of onset of sexual activity. Even though most genital HSV infections are caused by HSV-2, an ever-increasing percentage is attributable to HSV-1.

2.2.3 Clinical Manifestation

Clinical presentations of HSV-1 and HSV-2 include, localized lesions, generally in orofacial and genital areas respectively (Field and Hodge, 2013). After the resolution of the primary infection, latent virus resides in sensory neurons. During a recurrence, the HSV in a nerve cell reactivates, and the virus travels down the axon to infect peripheral tissue to give localized lesions of the skin and mucus membranes. Recurrent lesions are common but the frequency of recurrences can vary widely. In recent decades, primary infection of the genital area by HSV-1 has become more common and can be severe, but the recurrence rate is usually much lower than with HSV-2 infections; similarly for HSV-2 infections of the orofacial area. Hence, the two viruses appear to retain their site preferences. Less common clinical manifestations include ocular lesions and bells palsy (Field and Hodge, 2013).

Diagnosis by detection of HSV antibodies by PCR and the new Immonodot glycoprotein G-specific (igG) test is more than 98% specific at differentiating HSV-1 from HSV-2. Management by antiviral medications can reduce the frequency, and topical anesthetic to

prevent itching and pain. Prevention is through the use of protective measures such as condom, and antiviral agents.

2.2.4 Management of HSV

There is no effective vaccine against HSV infections or its recurrences; management only focuses in reducing the virus number, transmission and severity. There are three categories of drugs that have been approved for treatment of HSV infections: Acyclic guanosine analogues, acyclic nucleotide analogues and pyrophosphate analogues (Jiang et al., 2016). Typical drugs from these classes include acyclovir, famciclovir, cidofovir and valacyclovir. These drugs target the Thymidine Kinase (TK) enzyme as well as or DNA polymerase of herpes viruses. The most common prescribed and clinically effective antiviral medication for the treatment for HSV infections which has been used ever since 1980s is acyclovir (9-{2- hydroxyethoxymethyl} guanine) which is a synthetic acyclic purine nucleoside analogue (Jiang et al., 2016).

Initial genital HSV infection can be treated with oral, intravenous or topical acyclovir. While a topical application of Acyclovir reduces the period of viral shedding and the length of period before a lesion become crusted: this treatment is less effective compared with oral or intravenous acyclovir. Intravenous acyclovir is the most effective treatment for the first occurrence of genital herpes and results in a significant reduction in the median duration of viral shedding, pain, and length of time to complete healing. Since intravenous acyclovir therapy usually requires hospitalization, it should be reserved for patients with severe local disease or systematic complications.

Recurrent genital herpes is less severe and resolves more quickly than primary infection; thus, there is less period to effectively introduce antiviral chemotherapy. Oral acyclovir therapy shortens the length of viral shedding and also the length of period to healing (6 days vs. 7 days) when initiated early (within 24 hours of onset), but the length of time and duration of symptoms to the recurrence are not affected. Famciclovir and

valacyclovir likely provide little added benefit. However, the long-term oral administration of acyclovir, valacyclovir, or famciclovir effectively suppresses genital herpes in patients who have frequent recurrence's (Wald et al., 2016).

Several drugs have been used in the management of HSV; however their extensive and long-term use has yielded repeated drug resistant strains due to mutation in viral thymidne kinase and or DNA polymerase by altering the substrate sensitivity (Jiang, et al, 2016; Du, Zhou & Roizman, 2013; Piret & Boivin, 2011). The resistance to treatment drugs such as acyclovir by HSV strains is more common in immune-compromised patients such as those with HIV/AIDS infection (Hodge and Field, 2013). Despite antiviral drugs being developed over the years, they have been ineffective in dealing with the new strains.

The challenge in establishing new and innovative antiherpetic molecules that are highly effective and exhibit low toxicity against drug resistant HSV has led to focus on therapies with antimicrobial activities such as *M. Oleifera*. Lipilum *et al.,* (2008) revealed that the ethanol extracts of exhibit anti-HSV-1 action at a dose of 750mg /kg per day. Treatment with *M. Oleifera* extract could delay skin lesion occurrence, prolong the mean survival times, and reduce the mortality of HSV-1 diseased mice (Lipilum *et al.*, 2008).

2.3 Traditional medicine including antiviral herbs usage

Throughout history, mankind has relied on nature for therapeutic by using poultices and infusion of local plants. Modern medication has progressively developed over the years by observational and scientific efforts from traditional treatments, and even today the ancient wisdom of ethno-medicines is an important source of drug development. There is an increased acceptance and interest in herbal therapies in both developed and developing countries. It is estimated that about four billion people living in developing countries rely on herbal medicine (Ekor, 2014).

However, there is a growing necessity for new and alternative compounds with antiviral action since the management of viral infections with the available antivirals is often inadequate with the problem of viral latency, resistance and conflicting efficacy in recurring infections (Jiang, et al, 2016). Interestingly, traditional medications, like *M. Oleifera* are believed to be a good source for potential medication development. A widespread diversity of active phytochemicals including the coumarins, lignans, alkaloids, polyphenolics, flavonoids, saponins, terpenoids, peptides etc. are stated to have therapeutic applications compared to genetically and functionally diverse viruses, due to their extensive variety of bioactivities. Many of these compounds have antioxidant, free radical scavenging, and antiviral activities by inhibiting viral entry, replication, gene synthesis, or assembly (Debayan *et al.*, 2016).

A thorough examination of the antiviral properties of such plants, used in local healthcare, may aid in the development of invaluable herbal lead. This study has selected Kenyan *M. Oleifera* an ethnomedicinal herbal used for diverse ailment by different Kenyan communities to evaluate its efficacy against *in vitro* HSV-1 infection. *M. Oleifera* has been reported to contain thiocarbanates, nitriles, glucosinolates and isothiocyanate (Welch and Tietje, 2017), which may have clinical significance for the treatment of viral infections including HSV-1.

2.4 Usage of *Moringa Oleifera*

M. Oleifera is a woody tree traditionally used as a medicinal plant and as a nutritional source. *M. Oleifera* is native to Northwestern India and is widely grown in the tropical and subtropical areas of Asia, the Middle East and Africa (Flora & Pachuri, 2011). It is the most widely grown species of the genius *Moringa*. The species was introduced to Kenya from India (exact location unknown) at the beginning of this century (Jahn 1991). *M. Oleifera* was introduced to Kenya over 100 years ago, but until recently, the species was considered of marginal value and details on the genetic diversity, these

introductions and relationships within and amongst the introduced people have not been presented.

M. Oleifera has been utilized for various dietary and medicinal purposes earning it the name miracle tree. *M. Oleifera* is a multipurpose plant used as food, as medicine, a spice, cosmetic oil and a source of cooking. All parts of *M. Oleifera*, the leaves, flower, bark, seeds, fruit and roots are all reported with a wide variety of nutritional, prophylactic and therapeutic virtues (Alegbeleye, 2018). A study conducted by Kumssa *et al.,* (2017) showed that palatable parts of *M. Oleifera* such as the fresh leaves, young shoots as well as fresh flowers were used as vegetables while leaf powder was mixed with other foods and also used in tea. In Kenya, *M. Oleifera* leaves have been used as an alternative vegetable for human consumption, they taste like spinach. The young tender and green pods are eaten as beans (Kumssa *et al*., 2017).

M. Oleifera is shown to be a rich source of bioactive compounds with diverse pharmacological activities. Many studies have shown that the bark, leaf, root, flower, and nearly all types of *M. Oleifera* extracts exhibit antimicrobial activity including anti-inflammatory, antibacterial, antiviral and ant parasitic activity, antioxidant, anti-cancer, anti-hyperlipidemic, antifungal and anti-hyperglycemic properties (Razis *et al*., 2014). As such, it has been widely used in treatment of bacterial, fungal, viral, parasitic diseases and ailments such as malaria, typhoid fever, arthritis, skin disease, hypertension and diabetes (Welch and Tietje, 2017). The study by Kumssa *et al.,* (2017) reported that therapeutic uses of *M. Oleifera* included treatment of high blood pressure, ulcers and stomach complications, food poisoning, joint and general body pain.

The prevalence of use of *M. Oleifera* among persons living with HIV/AIDS is high particularly in developing countries. PLWHA commonly look for alternative therapy that includes herbal and nutritional supplements parallel to their conventional therapy. According to (Lubinga *et al.* 2012), *M. Oleifera* has been reported to be used in up to 60% of HIV/AIDS patients in Africa. The reasons for the high number of patients

seeking herbal medicine include unsatisfactory results, high cost, unavailability and inaccessibility, and adverse side effects of the antiretroviral therapy (Gurmu, et al., 2017).

2.5 Determinants of use of *M. Oleifera*

Several patients and health systems level factors have been associated with the use of *M. Oleifera*.

2.5.1 Patient level factors

2.5.1.1 Social demographics and socioeconomic factors

The use of traditional and complementary medicine is dependent on various social demographic and socioeconomic factors. According to a study by (Gandji et al, 2018) people's socio-demographic attributes such as gender, age and education level; and socioeconomic factors such as occupation and professional activity often correlate with the use of plants. In the study women (86%) often used *M. Oleifera* compared to men while adults were more likely to use it compared to younger people. Similar findings were found in a study by (Monera and Maponga, 2012) which showed that females were more likely than males to use *M. Oleifera.*

Studies conducted in high income countries found out that use of complementary medicine among PLWHA was popular among high-income earners, women and those with high education (OHTN, 2013). A study by (Oyebode, et al., 2016) established that income quintile, education and geography were associated with the use of traditional medicine including *M. Oleifera* with poorer, less educated and rural participants more likely to report the use of traditional medicine. In Ghana older participants were more

likely to use traditional medicine while in China users of traditional medicine were younger.

2.5.1.2 HIV status

The health status of the patient is a factor that can influence their use of *M. Oleifera*. Experiencing improved health when using ARV drugs increases confidence in the medication for PLWHA and limits their use of herbal medicine. However patients who experience negative effects from their status such as loss of energy from depressed immune system can have reduced adherence to the treatment regime and be influenced to use *M. Oleifera*.

The patient's perception on the importance of supplementing modern medicine with herbal medicine in the treatment of chronic illness such as HIV/AIDs is a determinant in their use. PLWHA are more likely to use *M. Oleifera* if they believe that it is effective in alleviating some of the challenges they face as a result of their positive status.

2.5.1.3 Effectiveness of alternative medicine

M. Oleifera is reported to be a wonder plant with multiple uses including as a nutrition booster and a medicine for various ailments (Alegbeleye, 2018). These claims of efficacy of herbal medicine such as *M. Oleifera* have been shown to influence its uptake by many of the HIV/AIDS patients (Ekor, 2014).

The minimal reports of side effects associated with the use of *M.Oleifera* are also a determinant for it's widely acceptance. Some HIV patients stop treatment with ARV drugs and switch to medicinal plants which they believe is more effective after reporting negative effects on their health or resistant to ARV drugs (Noumi & Manga, 2011).

2.5.1.4 Source of information

The source of information on *M. Oleifera* also plays a critical role in influencing the PLWHA decisions to use the herb. A study in Ethiopia by (Gurmu, et al, 2017) found out that recommendations from family members (19.08%) was one of the key motivating reason why most patients used traditional medicines. According to (Monera and Maponga 2010), friends or relatives influenced the use of *M. Oleifera* among HIV patients on antiretroviral therapy as they were the most common source of recommendation for use.

2.5.1.5 Affordability and accessisbility of *M. Oleifera*

One of the reasons stated for the use of herbal medicine among PLWHA is the high cost of conventional medicine and the unavailability of anti-retroviral drugs (Ekor, 2014). This forces some of the PLWHA to turn to herbal medicine which they can easily access and that it's affordable.

2.5.2 Health systems level factors

2.5.2.1 Patient-health care personnel relationship

Health care workers can influnce patients' compliance to treatment and the use of herbal medicine. A confidential and good relationship with the health care personnel results in better adherence. The patients also are at ease to disclose their use of alternative treatment. Howevr, dissatisfaction with health care services and health care workers can be a determinant in non-adherence to ARV (Heestermans, et al., 2016). The increasing utilization of herbal medicine including *M. Oleifera* is also attributed to lack of trust for medical professional. Patients particularly those PLWHA being uncomfortable about discussing their medical problems and fear of lack of confidentiality may lead to non-disclosure on their use of herbal medicine.

2.5.2.2 Accessibility of health facilities

Health facility determinants are also associated with the use of herbal medicine and the non adherence to HIV/AIDS treatment. The distance to health facilities has an effect on the PLWHA ability to access essential services such as drugs and counselling. Patients who travel longer distances to access health facilities are highly likely to take *M. Oleifera* when compared to those who travel shorter distances (Heestermans, et al., 2016).

2.5.2.3 Side effects of treatment

The side effects of ARV treatment can be a determinant for the use of *M. Oleifera*. According to (Asiimwe 2012) side effects has a negative influence on the adherence to antiretroviral therapy. ARV has been reported to have side effects such as chronic diarrhea and body shaping effects. In a study by (Noumi & Manga, 2011), established that HIV positive patients sought alternative treatment since ARV drus provoked unbearable headache, vomiting, hunger, general body weakness, scabies, diarrhea and temporary memory loss.

2.5.2.4 Delay in accessing services

The waiting times and duration clinic hours is a determinant in the use of herbal medicine. According to (Heestermans, et al., 2016), long waiting times and limited clinical hours can lead to non-adherence to HIV treatment and push the PLWHA to turn to herbal medicine including *M. Oleifera*.

2.5.2.5 Stigma

Several studies have shown that stigma is a factor that can influence the ue of herbal medicine including *M. Oleifera*. Some of the HIV/AIDS patients experience stigma or have internalized stigma due to the nature of the disease and hence they turn to traditional healers for treatment. According to (Noumi & Manga, 2011) some patients

shy away from getting ARV drugs because of the stigma attached to the HIV/AIDS disease.

2.6 *In vitro* cytotoxicity and antiviral activity of *M. Oleifera*

As traditional plant, *M. Oleifera* has shown antiviral activities in several different studies. (Waiaput, *et al.,* 2012) suggested that 80% ethanol crude extracts of *M. Oleifera* fruit revealed anti-HBV activity by preventing HBV replication with mild cytotoxicity on HepG2 cells. An antiviral activity study by (Debayan *et al.,* 2016), using three different test systems like CPE reduction, MTT and PRA, showed that extracts from *M. Oleifera* has detectable antiviral activity compared to the drug acyclovir, as it effectively exhibited potent anti-HSV action in Vero cells without reducing cell viability. These extracts also inhibited the growth of the human isolate VU-09, isolated from a patient infected with HSV-1, indicating that *M. Oleifera* extracts need to be studied further with other viruses of herpes virus family (Debayan *et al.,* 2016).

2.7 Gaps identified in literature

A number of studies have been published by researchers from various parts of the world indicating numerous medical and functional benefits of *M. Oleifera*. These studies have focused on the nutritive, environmental, phytochemical and medicinal capabilities of the plant. Apart from the nutritional benefits of *M. Oleifera,* the studies have also claimed that it contains anti-inflammatory, antibacterial and antiviral properties and can be used in treatment of various illnesses. Due to the reported benefits of *M. Oleifera,* studies have also shown that the plant is being used by several patients with immune-compromised systems including PLWHA.

A deeper review however indicates that the studies only provide preliminary experimental evidence on the therapeutic potential of *M. Oleifera.* Standardization is also a critical problem as there is insufficient data on the properties and effectiveness of different extracts from the different parts of the plants which have been shown to have

unique therapeutic effects. Ascertaining which part of the tree is more beneficial and effective in the treatment of a specific disease is difficult. The evidence on specified preparation procedure, concentration or dosage of *M. Oleifera* that can be effective in providing the desired outcome is also scanty. Due to the differentials in the uses and chemical composition of *M. Oleifera* based on the geographical region in which it is planted; there is a challenge with generalization of the findings. As such it is important for *M. Oleifera* planted in the different regions be properly chemically characterized and standardized before utilization.

Despite the increasing use of *M. Oleifera* across the world populations including among the PLWHA, there still exist a gap regarding the potential pharmaceutical side effects, contraindications, allergies, and drug interaction with prescribed medicine. This is particularly true for PLWHA who use both *M. Oleifera* and ARV. There is also limited research on the factors that compel the PLWHA to use both herbal medicines such as *M. Oleifera* and ARV drugs.

2.8 Conceptual Framework

Figure 2.1 represents the conceptual framework for this study. It indicates the study variables and their relationship with each other. The independent variables for the research study were the determinants of *M. Oleifera* use among PLWHA attending Comprehensive Care Clinic (CCC) at Migori County Referral Hospital and the *invitro* cytotoxicity and antiviral activity of *M. Oleifera*. The determinants were subdivided into two categories the patient level factors and the health systems level factors. The depended variable was the usage of *M. Oleifera.*

Independent Variables **Dependent Variable**

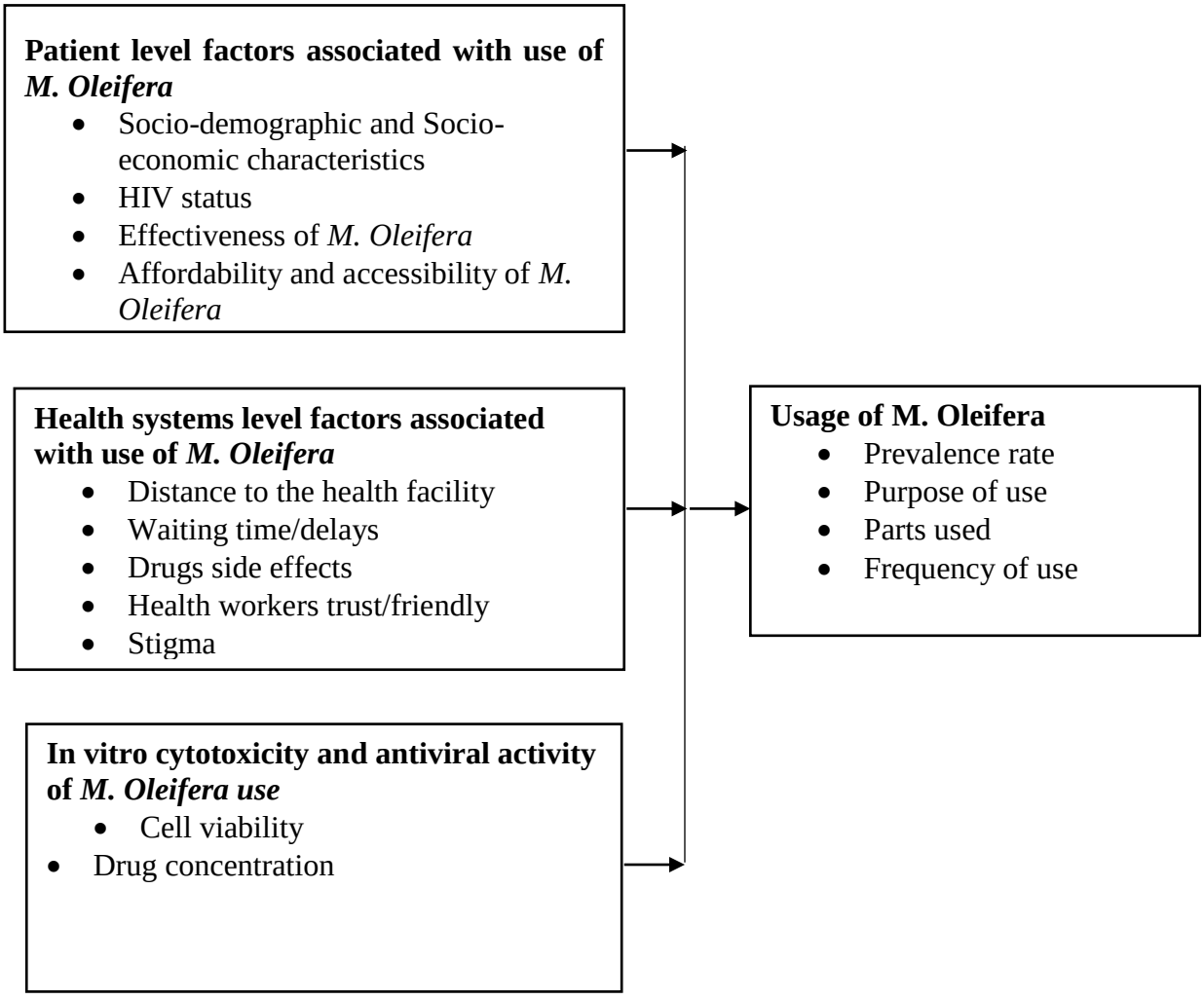


Figure 2.1: Conceptual Framework (Author, 2021)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out in Migori County located in the South Western region of Kenya. It borders Homa Bay County to the North, Republic of Tanzania to the South, Narok County to the East and Lake Victoria to the west. It covers an area of 2,596.5 km^2 including approximately 478 km2 of water surface. The County has ten (10) Sub Counties namely Suna East, Suna West, Uriri, Mabera, Awendo, Nyatike, Ntimaru, Rongo, Kuria East and Kuria West (The map of the County is shown in figure 3.1). The main economic activity in Migori County is agriculture. Agricultural activities occupy approximately 63% of the total land with 60% under food crop cultivation and the remaining 40% under cash crop cultivation. Other activities include tourism, industry and trade, and fishing (ROK, 2018).

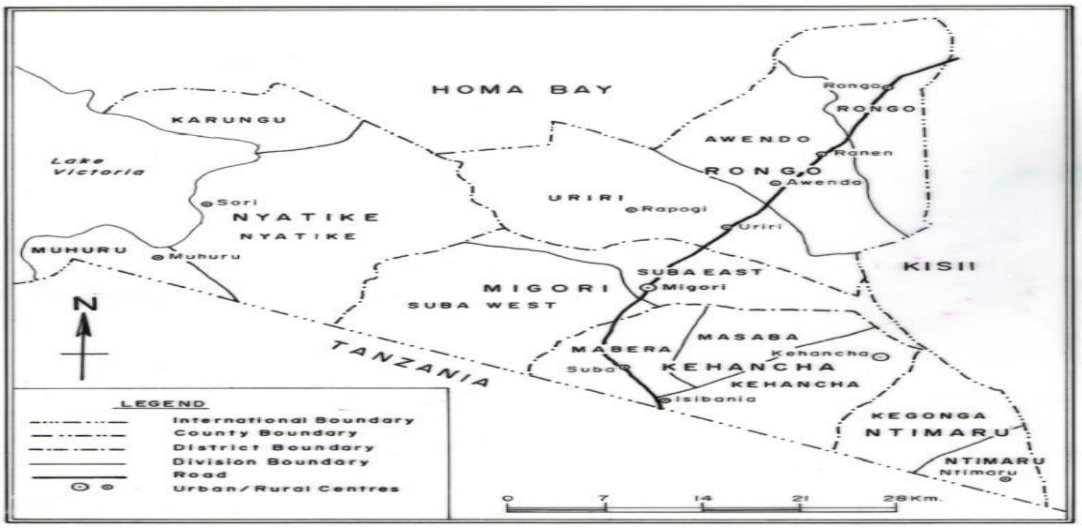


Figure 3.1: Map of Kenya showing the location of Migori County *(Google maps)*

The study survey was carried out at Migori County Referral Hospital in Migori County which serves patients from the wider Migori County and those in the nearby Counties.

The study was hospital based as it is not possible to identify the respondents who are patients due to the stigma associated with HIV/AIDS in the communities. Migori County was purposefully selected because of the high HIV prevalence. Migori County has a HIV prevalence of 14.3% which is nearly 2.5 times higher than the national prevalence (Kenya HIV Estimates, 2015). Migori County contributed to 5.5% of the entire number of persons living with HIV in Kenya, and is ranked the fifth highest contributor to the HIV incidences in the Country. By the end of 2015, the persons who were living with HIV were 83,603 with 22% being young persons at the age 15 - 24 years as well as children (under the age of 15 years) constituting 6% of those living with HIV in the County. In 2015, the County had about 5619, new HIV infections. Adult ART coverage stood at 76% and children ART coverage at 88%. Approximately 1,749 persons in the County died of AIDS related conditions in 2015 (Kenya HIV County Profiles, 2016). The County has one referral hospital, ten Sub-County hospitals, 25 health centers, 112 dispensaries, 8 FBO run health facilities, 10 private run hospitals, 9 nursing homes and 56 private clinics. The doctor-population ratio stands at 1:55,000 and the nurse-population ratio stands at 1:1,500 (CIDP, 2013). Migori County Referral Hospital was sampled as it offers CCC services to PLWHA across the entire County and offers an opportunity to get a diverse sample.

M. Oleifera leaves from the region was collected for testing in Laboratory based experiments which were performed at Kenya Medical Research Institute (KEMRI) Nairobi, Kenya at the Centre for Traditional Medicine and Drugs Research (CTMDR) laboratories.

3.2 Study Design

Cross-sectional study design was used for the assessment of determinants of *M. Oleifera* use among PLWHA attending CCC at Migori County Referral Hospital. In the laboratory, (Pre-clinical) experimental study design was used to determine the effect of

M. Oleifera extracts on Vero cells and HSV-1 as the indicators of cell cytotoxicity and antivirus effect respectively.

3.3 Study Population

The study population was PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital and are above the age of 18 years. Key informants were the herbalists who prescribe *M. Oleifera* to their patients also participated in the study.

3.3.1 Inclusion and Exclusion Criteria

3.3.1.1 Inclusion Criteria

PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital and are above the age of 18.

Herbalists, who prescribe *M. Oleifera* to their patients, are 18 years and above, willing to divulge information and maintained records were included in the study.

3.3.1.2 Exclusion Criteria

PLWHA and herbalists who refused to give consent.

3.4 Sampling and Sample Size Determination

3.4.1 Sample Size Determination

The sample size was determined using Cochran's formula: (Cochrans, *et al.*. 1977).

$$n_0 = \frac{z^2 pq}{e^2}$$

The study was conducted at 95% confidence, and at least ± 0.05% precision. A 95% confidence level gives us Z values of 1.96. Therefore: *e* the margin of error was 0.05, *p* the proportion of the population with the said characteristics was 0.5 while *q = 1 – p =* 0.5

$$n_0 = \frac{1.96^2 \times 0.5 \times 0.5}{0.05^2}$$

$$n_0 = 385$$

Since the population is less than 10,000, (there are approximately 1,000 PLWHA who attend CCC at the referral hospital in a month) the sample size was modified using the formula.

$$n = \frac{n_0}{1 + \frac{n_0}{N}}$$

$$n = \frac{385}{1 + \frac{385}{1000}}$$

$$n = \frac{385}{1.385}$$

$$n = 278$$

3.4.2 Sampling Methods

Systematic sampling was used to selects respondents to participate in the study where every kth person was interviewed.

$$k^{th} = \frac{Total\ number\ of\ population}{Sample} = \frac{1000}{278} = 3.6$$

Thus every 4th patient was enrolled as they exit the hospital at the pharmacy after receiving their medication.

Snowballing technique was used to select the herbalists (Kurant, *et al.,* 2010). This sought to collect in depth information and further understanding of individuals and health systems reasons for the use of *M. Oleifera.* The comprehensive care clinic (CCC) pharmacy staff together with the nurse at the clinic who were trained prior to the start of the study and facilitated in data collection, were requested to ask every 4th adult patient who met the inclusion criteria if they were interested in the study and therefore, redirected them to the researcher for study consent and their questionnaire interviewing which was conducted outside the CCC.

3.5 Data Collection Methods

Both quantitative and qualitative data was collected. The data was collected by principal investigator together with research assistants who understand the local language. They were recruited, trained and participated in pre-testing of the data collection tools in order to test and improve the validity of the results.

3.5.1 Questionnaires

An interviewer administered semi-structured Questionnaires (Appendix 3) was use to collect qualitative data from the respondents. The semi-structured questionnaires which contained close and open ended questions were used to obtain information on the usage of *M. Oleifera*, the patient level factors and health system level factors influencing the usage.

3.5.2 Key Informant Interview

In-depth semi-structured interviews with Key Informants who were herbalists were being carried out. A Key Informant Interview guide consisting of open ended questions divided according to themes in line with the research objectives was used to guide the conversation.

3.6 Validity and Reliability

3.6.1 Validity

Validity is important in ensuring that the methods used yield the desired results. Construct validity involves assessing whether the research instruments measure what the researcher intended to measure while content validity evaluates whether the tests capture all the aspects needed to be measured. To achieve construct and content validity the researcher sought opinion from lecturers and other professionals on the adequacy of the research instruments in producing valid results and achieving the objectives of the study.

3.6.2 Reliability

Reliability involves the consistency of the research instruments in producing similar results when the research is repeated under simila conditions. To achieve reliability there was pre-testing of the research instruments so as to monitor their effectiveness.

3.7 Laboratory Experiments

The experimental design involved the collection of leaf extracts of *M. Oleifera* from Migori County and conducting a Laboratory-based (pre-clinical) testing.

3.7.1 Chemical reagents, and assay kits

The chemical reagents and other experimental supplies were procured from the list of pre-qualified suppliers according to JKUAT/KEMRI procurement policies and stored in standard condition as stated on their labels within the duration of the study.

3.7.2 Collection of Plant Materials

Leaves of *M. Oleifera* were collected from Migori County and identified by a plant taxonomist at Nairobi University Herbarium of Kenya, Nairobi and given a voucher number (NNJ2019/001).

3.7.3 Preparation of Plant Extracts

3.7.4 Drying Process

The freshly collected Kenyan *M. Oleifera* plant material was dried in well-ventilated room at room temperature and not submitted to direct sunlight to avoid loss of active compounds. The dried *M. Oleifera* plant materials were grounded into powder using a laboratory mill (Christy and Norris Ltd., Chelmsford, England) at CTMDR, KEMRI and weighed with a Mettler® balance. The powdered sample was then packed in air tight polyethylene bags and kept away from direct sunlight until extraction was performed.

3.7.5 Extraction

3.7.5.1 Aqueous extraction

The extraction process was carried out based on a slight modification to the method showed by (Awoyinka *et al.,* 2007). One hundred grams (100g) of the powdered plant material was soaked in 400ml of distilled water and placed in a water immersion at 80^0 C for 1 hour. Then it was left to cool and decant and finally filtered through a Whatmanº

Cat (No 1001 185) filter paper. The filtered extract was freeze-dried using a Freeze Dryer (Edwards freeze dryer Modulyo). Finally, the powder was weighed, recorded, labeled and stored in an air tight 50ml centrifuge tubes at 4^0 C until use.

3.7.5.2 Methanol extraction

The extraction process was carried out based on a slight modification to the methods used by (Parekh *et al.*, 2005). One hundred grams (100g) of the dried powder plant material was soaked in 400ml methanol in a flat-bottomed conical flask at room temperature for 3 days in a dark room covered by cotton gauze. After 3 days it was filtered using Whatmanº Cat (No 1001 185) filter paper and concentrated using a rotary evaporator at 40-60^0 C. Finally, the extract was weighed, recorded, labeled and stored in a cap tight round bottom flask at 4^0 C until use.

3.7.6 Positive and Negative controls

Secondary pharmaceutical grade Acyclovir with purity of 94.8% obtained from Cosmos Limited was used as a positive control drug, whereas cell culture media was used as the negatve control.

3.7.7 Virus

The HSV-1 stock was obtained from Center for Traditional Medicine and Drug Research (CTMDR) laboratory and propagated in Vero cells.

3.7.8 *In vitro* assay (Cytotoxicity and Antiviral activity tests)

3.7.8.1 Preparation of Vero cells and HSV-1 virus culture

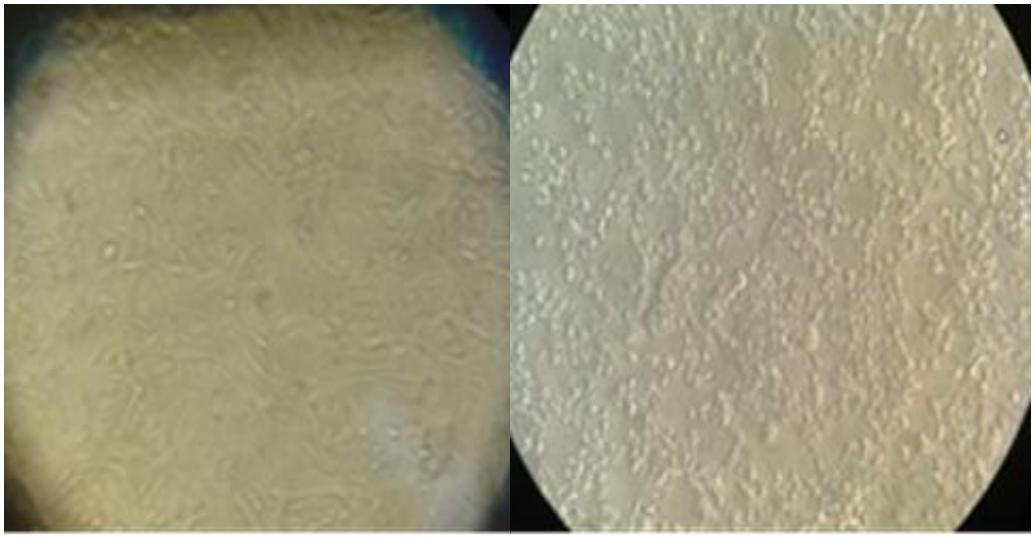
Herpes Simplex Virus type -1, (HSV-1) and the Vero cells used in this study were obtained from the Center for Traditional Medicine and Drug Research (CTMDR)

laboratory of the Kenya Medical Research Institute (KEMRI). A cryovial of Vero E6 cell line at Passage 27 previously cryopreserved in liquid nitrogen was revived as described by (Tolo *et al.*, 2006). The vial was placed in a water bath at 37^0 C to thaw before being suspended in a T -75cm^2 cell culture flask containing 20ml of Eagles minimum essential growth media (MEM) supplemented with 1% of 2mM L-glutamine, 10% (v/v) fetal bovine serum, 2.5% (v/v) of 7.5% (W/V) Sodium bicarbonate and 1% (v/v) of pen strep (10,000I.U/ml penicillin combined with 10,000µg/ml streptomycin). The seeded culture flask was incubated in a humidified environment at 37^0 C in a 5% CO_2 incubator until cell culture was about 90% confluent. The test medium used for antiviral assays and cytotoxic assays contained only 2% of fetal bovine serum (maintenance medium). One vial of cryopreserved virus stock was removed from liquid nitrogen storage and immediately thawed at 37^0 C in a water bath. The thawed virus was innoculated in monolayer of Vero cells, previously grown and maintained in T - 75cm^2 tissue culture flasks. The infected cells were incubated at 37^0 C in 5% CO_2 for 24 hours. The virus was harvested by 3 times repeated freezing of culture at -80^0 C and thawing at 37^0 C. The culture medium was centrifuged and the recovered virus in the supernatant kept in a freezer at -80^0 C until required for titration and bioassay.

3.7.8.2 Tissue Culture Infective Dose (TCID)

Virus titres were determined by virus-induced cytopathic effect technique in Vero cell culture and expressed as 50% tissue culture infective dose ($TCID_{50}$) per mL using Sperman - Karber's method. Briefly, Vero cells suspension ($2x10^4$cells/mL) were seeded into 96 well plates and incubated for 24 hours to form a monolayer. Tenfold serial dilutions of the virus stock were made and 100µL of each dilution was inoculated into the wells. A well that contained only the Vero cells without any virus served as the cell control. The 96 well plates were incubated at 37^0 C, 5% CO_2 and daily CPE scoring was done using x 10 power objective of an inverted microscope and recorded in a titration

sheet. The $TCID_{50}$ values were determined using Spearman-Karber's method, (WHO, 1997) and 100 $TCID_{50}$ was used for the assay.



A B

Plate 3.1: Vero cells with and without the cytopathic effects. A. Cells showing no cytopathic effect B. Cells showing the cytopathic effect

3.7.8.3 Determination of Cytotoxicity by MTT Assay

The MTT (3-)4, 5 –dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay, which is known to be a reliable measure of cell viability, was used to determine the methanol and aqueous cytotoxicity of *M. Oleifera* extracts. The principle of this assay involves the reduction of yellow tetrazolium dye by mitochondrial succinate dehydrogenase to an insoluble, coloured (dark purple) formazan product. The purple insoluble formazan crystals are then solubilized with an organic solvent dimethylsulphoxide (DMSO) to release the solubilized formazan dye which is measured by spectrophotometry. This assay was carried out with a slight modification to the methods indicated by (Mosmann, 1983). Briefly, Vero cells were grown in 96-well plates at concentration of 2×10^4 cells/well (rows 1, 2, 4, 5, 7, 8, 10, 11 filled with

maintenance media containing cells, while rows 3, 6, 9 were used as controls filled with only the media and no cells) in a 5% CO_2 incubator at 37°C for 24 hours. After 24 hours cell monolayers were treated with aqueous and methanol extracts at dilutions at a starting dose 1000µg/mL, followed by three-fold serial dilution in 6 steps. Briefly, row H of the plate contained the highest concentration 1000µg/mL and all the rows upward contained a serially 3-fold dilute concentration up to row B, while cell control contained only medium (row A). The cells were incubated for 48 hours at 37°C in a 5% CO_2 incubator. After 48 hours, 10µl of MTT solution (5mg/ml dissolved in PBS) was added to the plates in all the wells and incubated for 3-4 hours at 37°C until crystals of formazan were clearly visible. After the removal of MTT, 100µl DMSO was added to dissolve the formazan crystals. The optical densities (OD) were measured in a spectrophotometer at a range of 540nm to 720nm. The percentage cell viability was then calculated as [(A - B / C-B) x 100], where A, B, and C indicate the mean of 2 optical density readings of treated cells, blank and control respectively. The experiment was done in a triplicate for each treatment. The 50% cytotoxic concentration (CC_{50}) was determined from the graph of % cell viability against drug concentration. It is the extract concentration (µg/ml) that can reduce 50% cell viability to compare with cell control.

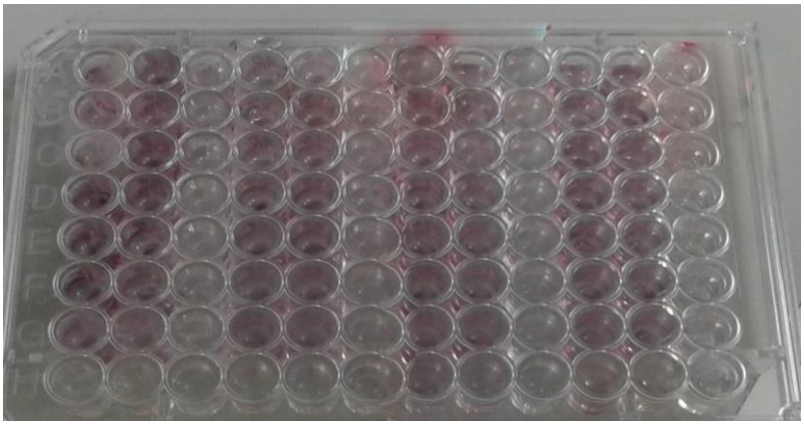


Plate 3.2: The setup of cytotoxicity effects of plant extracts on Vero cells. Clear wells were cell free while the purple colours were the purple formazan-containing cells.

3.7.8.4 Determination of Antiviral Activity of *M. Oleifera* Extracts against HSV-1 by Cytopathic Effect Reduction and MTT Method:

The antiviral activity of *M. Oleifera* extracts for both methanol and aqueous against HVS-1 was determined by a slight modification to the methods described by (Mohamed *et al*., 2015).

Briefly, Vero cells at a seeding density of 1×10^4 seeded in 96-well plates were incubated in humidified CO_2 incubator at 37^0 C for 24hours. Two different experiments in a quadriplicates wells were carried out for pre-treatment and post-treatment, and the antiviral assay was carried out in two different ways as follows:

3.7.8.4.1Treatment before virus infection (Pre – Treatment Assay)

96 well plates were seeded with cells at a density of 1 $x10^4$ for 24 hours. Four different concentrations (1000µg/mL, 500µg/mL, 250µg/mL and 125µg/mL) from each treatment

were added to the monolayer of previously incubated cells for cells drug interaction for 1h. The extract dilutions were removed and the cells incubated with 3.6232 x 10^2 PFU/mL $TCID_{50}$/0.1ml of virus suspensions in serum free MEM for 1 hour. After 1 hour incubation, then the cells were washed with PBS and incubated with fresh MEM. The cells were incubated under CO_2 humidified at 37^0 C for 48 hours.

3.7.8.4.2Treatment after virus infection (Post – Treatment Assay)

Confluent Vero cells previously seeded at $1x10^4$ monolayers were incubated with 3.6232 x 10^2 PFU/mL $TCID_{50}$ /0.1ml virus for 1 hour at 37° C in 5% CO_2 humidified incubator. The viral inoculum was aspirated off the wells and the drug extracts added in their respective concentration and incubated for 48 hours at 37° C in 5% CO_2 humidified incubator.

Cell control (only test medium with Vero cells) and viral control (virus suspension without extract) were included in all assays. The percentage cell protection was determined spectrophotometrically at 540 nm and 720 nm by MTT method as described above for evaluation of extract cytotoxicity and it was calculated as [(A-B)/(C-B) x100], where A, the mean absorbance of treated cells; B, the mean absorbance of virus control; C, the mean absorbance of cell control. The 50% inhibitory concentration (IC_{50}) was determined from the graph of % cell protection against drug concentration. The 50% inhibitory concentration (IC_{50}) was defined as the extract concentration of the plant that protects 50% of treated infected cells from HSV-1 induced destruction to compare with cell control.The selectivity index, (SI), defined as CC_{50} over IC_{50}, for each extract both methanol and aqueous were also determined. All experiments were carried out in quadriplicates.

3.8 Data Management and Analysis

Data collected from questionnaires and laboratory experiments was registered on notebook, stored in password protected computer and hard disks. Backups of the data collected were made by storing the information on flash disks.

Data was analyzed statistically using software SPSS (V.22) where Chi Square test was used to compare categorical variables. A $p \leq 0.05$ was considered statistically significant. Quantitative data was presented in frequency distribution tables and graphs while the qualitative data was analyzed through scrutiny of phrases and words mentioned by interviewees and categorized into themes and presented in narrative form to complement the quantitative data.

3.8.1 In vitro assay data Analysis

The experiments were conducted in quadriplicate. The 50% cytotoxic concentration (CC_{50}) and the 50% inhibitory concentration (IC_{50}) for both extracts were determined from concentration effect curves after linear regression analysis using Microsoft Excel. The therapeutic index (i.e, selectivity index) for methanol and aqueous extracts was also determined. Therapeutic index is a comparison of the amount of a test agent that causes the toxicity to the amount that causes the inhibitory effect expressed as the ratio of CC_{50}/IC_{50}.

3.9 Ethical Considerations

Approval was obtained from the Kenya Medical Research Institute (KEMRI) Scientific and Ethical Review Unit (SERU). Permission to carry out the study was further sought from the Sub County administration at Migori County and medical superintendent at Migori Referral Hospital. The research was conducted in accordance with KEMRI guidelines on the international accepted conduct of experimental research and the internationally accepted principles for laboratory research.

Before the interview, the participants were taken through the consent form (Appendix 2). The consent was written in English and Kiswahili languages. An explanation into the study including the purpose of the study, procedures, and benefits was explained to the participants. This was explained to make the participants familiarize themselves with the study before appending their signatures to respond to the questionnaire (Appendix 3). Voluntary participation in the study and right to withdraw at any point without any negative consequences was clearly explained to the participants. The confidentiality of the information from the study participants was maintained throughout the study. In addition, codes were used to maintain the anonymity of all participants and keep their information confidential.

Appointments with the herbalists were made on appropriate time and venues for the interviews. Participants were assured that all information obtained from them was treated with maximum confidentiality and that no names would be used in any report.

3.10 Hazardous Material Management

All the hazardous wastes generated were segregated, treated and appropriately disposed.

CHAPTER FOUR

RESULTS

4.1 Response Rate

The targeted number of participants for the study was two hundred and seventy eight (278) patients and nine (9) herbalists. The response rate for the study was 100%. The researcher was able to administer the questionnaire to two hundred and seventy eight respondents and conducted nine key informant interviews. This was achieved as the researcher administered the questionnaire in person.

4.2 Characteristics of respondents

Table 4.1 describes the participants' gender, age, marital status, education level and occupation. Female respondents were 164 (59%) of the respondents were females while 114(41%) were males. Of the respondents a majority 95 (34.2%) were aged between 36 and 45 years and 159 (57.2%) were married. Secondary level of education was the highest level of education attained by 38.8% of the respondents. Patients who were farmers were 31.9% while 36% were operating businesses.

Table 4.1: Characteristics of Respondents

Variable	Category	No. of Respondents (n=278)	Percentage (%)
Age	18- 25 years	44	15.8
	26 - 35 years	86	30.9
	36 - 45 years	95	34.2
	46 - 55 years	37	13.3
	Above 55 years	16	5.8
Sex	Male	114	41
	Female	164	59
Highest level of Education	No formal education	13	4.7
	Primary	99	35.6
	Secondary	108	38.8
	College	45	16.2
	University	13	4.7

Marital status	Single	52	18.7
	Married	159	57.2
	Divorced/Separated	35	12.6
	Widowed	32	11.5
Occupation	Farming	88	31.9
	Employed	82	29.5
	Business	100	36
	Unemployed	8	2.9

The number of respondents who had lived with the HIV for more than 5 years was 69(24.8%), 90(32.4%) had lived with the virus for between one and three years while 70 (25.2%) had lived with the HIV for less than one year.

Table 4.2: Number of years over which the respondents had lived with HIV

Years living with HIV	Frequency	Percentage (%)
Below 1 year	70	25.2
Between 1 - 3 years	90	32.4
Between 3 - 5 years	49	17.6
Above 5 years	69	24.8
Total	278	100

4.3 Usage and pattern of use of *M. Oleifera* among PLWHA attending CCC at Migori County Referral Hospital

4.3.1 Usage of *M. Oleifera*

The findings show a high usage of *M. Oleifera* among PLWHA attending CCC at Migori County Referral Hospital. Out of a total of 278 participants included in the study, 210 patients had used *M. Oleifera* while on antiretroviral therapy. This indicates a 75.5% prevalence of use of *M. Oleifera* among the respondents (Table 4.3).

Table 4.3: Usage of *M. oleifera* among PLWHA attending CCC at Migori County Referral Hospital

Do you use *M. Oleifera*	Frequency	Percentage (%)
Yes	210	75.5
No	68	24.5
Total	278	100.0

Table 4.4 shows the data on the distribution of *M. Oleifera* users and non-users by socio-demographic and socio economic characteristics. The number of females using *M. Oleifera*, among the respondents was 127(60.5%) while 83(39.5%) were males. Those aged between the age of 26 and 35 years were 67(31.9%) and 62(29.5%) were aged between 36 and 45 years. Of the respondents 117(55.7%) were married and 84(40%) had secondary level of education as the highest level of education. Those engaged in business were 76(36.1%), 71(33.8%) were farmers and only 6(2.9%) were unemployed.

Table 4.4: Distribution of *M. oleifera* users and non-users by socio-demographic and socio economic characteristics

Variable/ Characteristic	No. of respondents	*M. Oleifera* use	
		Users(n=210)	Non-users(n=68)
	Frequency (%)	Frequency (%)	Frequency (%)
Sex			
Male	114(41)	83(39.5)	31(45.6)
Female	164(59)	127(60.5)	37(54.4)
Age			
18 - 25 years	44(15.8)	35(16.7)	9(13.2)
26 - 35 years	86(30.9)	67(31.9)	19(27.9)
36 - 45 years	95(34.2)	62(29.5)	33(48.5)
46 - 55 years	37(13.3)	33(15.7)	4(5.9)
Above 55 years	16(5.8)	13(6.2)	3(4.4)
Marital Status			

Single	52(18.7)	40(19.0)	12(17.6)
Married	159(57.2)	117(55.7)	42(61.8)
Divorced/Separated	35(12.6)	24(11.4)	11(16.2)
Widowed	32(11.5)	29(13.8)	3(4.4)
Highest level of education			
No formal education	13(4.7)	9(4.2)	4(5.9)
Primary	99(35.6)	78(37.1)	21(30.9)
Secondary	108(38.8)	84(40)	24(35.3)
College	45(16.2)	33(15.7)	12(17.6)
University degree	13(4.7)	6(2.6)	7(10.3)
Main occupation			
Farming	88(31.9)	71(33.8)	17(25)
Employed	82(29.5)	57(27.2)	25(36.8)
Business	100(36)	76(36.1)	24(35.3)
Unemployed	8(2.9)	6(2.9)	2(2.9)

The herbalists who were the Key Informants provided in-depth details on the utilization of herbal medicine and particularly *M. Oleifera.* The years of experience for the herbalists ranged from seven years to forty years. The Health Act 2017 law makes it mandatory for practitioners of alternative medicine or herbalists to be registered by the Pharmacy and Poisons Board. This is geared towards ensuring appropriate, safe and effective use of traditional medicine. However, the study findings show that none of the herbalist had been registered. This means that none of them had a certificate. The patients however did not request to see any registration or affiliation to any professional body. The herbalists indicated that the patients just asked for treatment and nothing else.

The study showed quite a number of patients visited the herbalists. On average the number of patients attended to daily by the herbalists was five. A few of the herbalist attended to over ten patients per day. The period that the herbalists had used *M. Oleifera* was varied with a majority of them having used the plant for between four to eight years.

4.3.2 Purpose of *M. Oleifera* use

M. Oleifera is used by patients living with HIV/AIDs for various reasons. The findings show that most (49%) of the respondents used *M. Oleifera* as medicine followed by thirty two percent 32% consisting of those who used it as both food and medicine (Figure 4.1). 19% used *M. Oleifera* as food for nutritional supplement. The data shows the patients who used *M. Oleifera* as medicine, used it for the treatment of other diseases such as ulcers, stomach upset, and skin rashes while at the same time taking the HIV drugs for viral suppression.

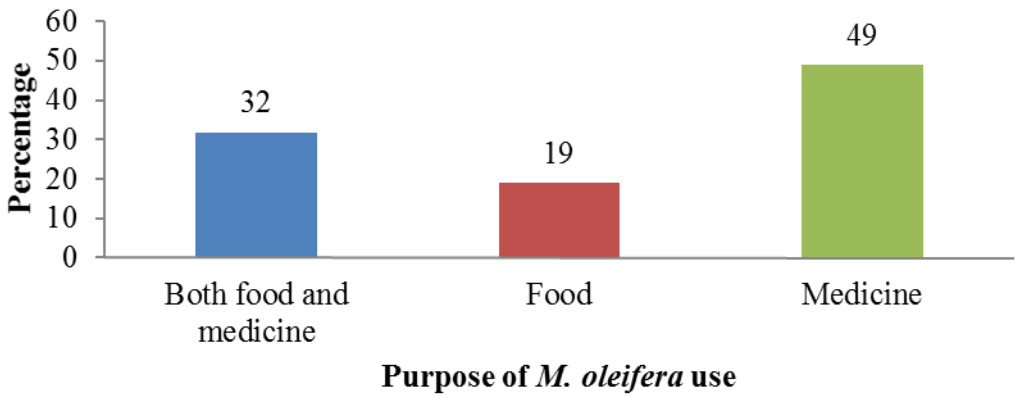


Figure 4.1: Uses of *M. Oleifera*

Information from the key informants showed that *M. Oleifera* is used to treat various diseases. The herbalists indicated that *M. Oleifera* had also been prescribed for use with patients who were HIV positive to deal with certain side effects of the drugs such as rashes and stomachaches. The findings indicate that other diseases often treated by *M. Oleifera* were headaches, skin infections, hypertension, diabetes, arthritis, high blood pressure and stomach aches.

4.3.3 Parts of *M. Oleifera* used by PLWHA attending CCC at Migori County Referral Hospital

Table 4.5 indicates the parts of *M. Oleifera* used by PLWHA attending CCC at Migori County Referral Hospital. The data shows that the part of *M. Oleifera* used by all the patients was the leaves. The percentage of respondents who used the leaves alone was 64.3%. Others used the leaves together with other parts including seeds (20.5%), dry pods (9%), bark (3.3%) and drypods and seeds (1.9%). The finding from the respondents is congruent with that given by the herbalists who indicated that the part of *M. Oleifera* that was prescribed for use to the patients was the leaves.

Table 4.5: Parts of *M. oleifera* used by PLWHA attending CCC at Migori County Referral Hospital

Part of *M. Oleifera* used	Frequency	Percentage %
Leaves	135	64.3
Leaves and seeds	43	20.5
Leaves and dry pods	19	9.0
Leaves and bark	7	3.3
Leaves, dry pods & seeds	4	1.9
Whole plant	2	1.0
Total	210	100.0

4.3.4 Frequency of use of *M. Oleifera* by PLWHA attending CCC at Migori County Referral Hospital

The study also sought to establish the frequency of use of *M. Oleifera* by PLWHA attending CCC at Migori County Referral Hospital. The findings indicate that more than a third (37.6%) of the respondents used *M. Oleifera* on a weekly basis. Some of the respondents 11% took *M. Oleifera* once in a while. The length of usage of *M. Oleifera* varied with 28.1% of the participants indicating that they had used *M. Oleifera* for more than five years.

4.3.5 Sources of and form in which *M. Oleifera* was used by PLWHA attending CCC at Migori County Referral Hospital

Figure 4.2 shows the sources of *M. Oleifera* used by PLWHA attending CCC at Migori County Referral Hospital. A combination of the market and friends/relatives was the main source of *M. Oleifera* according to 31% of the respondents. A similar percentage of respondents reported to have obtained *M. Oleifera* exclusively from their relatives and friends. Very few patients (14%) had grown *M. Oleifera* in their farms. This finding is however different from that by the herbalists who reported that *M. Oleifera* they used was mostly sourced from their own farms with only a few sourcing it from the market.

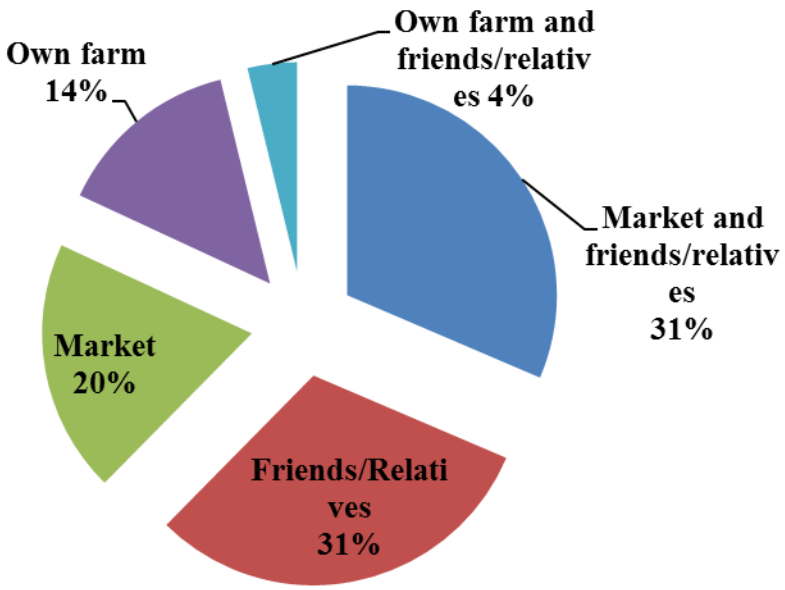


Figure 4.2: Sources of *M. Oleifera*

The study further sought to establish the form that *M. Oleifera* was used by PLWHA attending CCC at Migori County Referral Hospital. The findings indicate that *M. Oleifera* was commonly used in crushed form (small pieces) (38.1%), while 36.24% of the participants used *M. Oleifera* in grounded powder form. *M. Oleifera* is mainly taken

orally as food or drunk in teas by 89% of the respondents, a small percentage (1.0%) however indicated they smeared it on their bodies.

According to the herbalists *M. Oleifera* is taken in powder form. The powder is usually mixed with water at the ratio of 1 teaspoonful of *M. Oleifera* powder to 1 litre of water. The decision on the dosage of *M. Oleifera* however depends on several factors including the nature of the disease and age, with children and the elderly given smaller doses than the adult patients. The length for the dosage varies from a few days to a few months depending on the diseases. Other herbalists however indicated that they advise their patients to take *M. Oleifera* until the disease is cured and not based on dates. *M. Oleifera* is usually taken alone according to majority of the herbalists since it is more effective than when it is mixed. One herbalist however indicated that *M. Oleifera* is 80% effective when used alone and 20% effective when combined with other herbs.

4.4 Patient-level factors associated with the use of *M. Oleifera* used among PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital

4.4.1 Socio demographic and socio-economic factors

Socio demographic and socio-economic factors associated with the use of *M. Oleifera* used among PLWHA attending comprehensive care clinic (CCC) at Migori County Referral Hospital The findings indicate that age was the only significant variable associated with the use of *M. Oleifera* with a p-value of 0.039 (Table 4.6).

Table 4.6: Socio Demographic and Socio-economic factors associated with use of *M. oleifera*

Characteristic	Variable	*M. Oleifera* use		Chi square	p-value
		Users(n=210)	Non-users(n=68)		
		Frequency (%)	Frequency (%)		
Sex	Male	83(39.5)	31(45.6)	0.781	0.377
	Female	127(60.5)	37(54.4)		
	Total	210(100)	68(100)		
Age	18 - 25 years	35(16.7)	9(13.2)	10.086	0.039
	26 - 35 years	67(31.9)	19(27.9)		
	36 - 45 years	62(29.5)	33(48.5)		
	46 - 55 years	33(15.7)	4(5.9)		
	Above 55 years	13(6.2)	3(4.4)		
	Total	210(100)	68(100)		
Marital status	Single	40(19.0)	12(17.6)	5.244	0.155
	Married	117(55.7)	42(61.8)		
	Divorced/Separated	24(11.4)	11(16.2)		
	Widowed	29(13.8)	3(4.4)		
	Total	210(100)	68(100)		
Highest level of education	No formal education	9(4.2)	4(5.9)	7.332	0.119
	Primary	78(37.1)	21(30.9)		
	Secondary	84(40)	24(35.3)		
	College	33(15.7)	12(17.6)		
	University degree	6(2.6)	7(10.3)		
	Total	210(100)	68(100)		
Main occupation	Farming	71(33.8)	17(25)	7.176	0.305
	Employed	57(27.2)	25(36.8)		
	Business	76(36.1)	24(35.3)		
	Unemployed	6(2.9)	2(2.9)		
	Total	210(100)	68(100)		

To establish whether the use of *M. Oleifera* can be predicted based on gender, binomial logistic regression was used. The logistic regression model was not statistically

significant, $\chi^2 = 0.776$, $p > 0.05$. The model explained (0.4%) of the variance in the use of *M. Oleifera* and correctly classified 75.5% of the cases. Males were 12.83% less likely to use *M. Oleifera* compared to females.

Table 4.7: Logistic Regression Output

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	308.511[a]	.003	.004

a. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

Classification Table[a]

Observed			Predicted		
			Patients_using_Moringa		Percentage Correct
			Yes	No	
Step 1	Patients_using_Moringa	Yes	210	0	100.0
		No	68	0	.0
	Overall Percentage				75.5

a. The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Gender (1)	.248	.281	.779	1	.377	1.282	.738	2.226
	Constant	-1.233	.187	43.579	1	.000	.291		

a. Variable(s) entered on step 1: Gender.

4.4.2 HIV status as a determinant of *M. Oleifera* use

The disease status of a patient can determine their use of herbs such as *M. Oleifera*. The study sought to establish whether HIV status was a determinant of *M. Oleifera* use among PLWHA attending CCC at Migori County Referral Hospital. The findings indicate that 64.3% of those patients who used *M. Oleifera* started using it before they knew their HIV status. However, 35.7% started using it after knowing their status.

Figure 4.5 shows the findings of the study on whether *M. Oleifera* can be used to cure HIV. The data indicates that a majority of the respondents (85%) believed that it *M. Oleifera* cannot cure HIV. The percentage of those who had the opinion that *M. Oleifera* can cure HIV was 10% while 5% didn't know. (Figure 4.5)

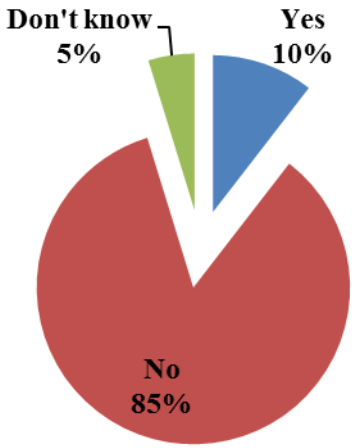


Figure 4.3: *M. Oleifera* as a cure for HIV

On appropriateness of supplementing modern treatment with herbal medicine, the study established that 88.6% of the respondents thought that it is appropriate to supplement modern treatment with herbal medicine since when this is done some of the diseases that cannot be treated by modern medicine can be treated by traditional medicine. However, some of the respondents (11.4%) thought it was not appropriate as traditional medicine has not yet been tested and may cause serious health problems when used. The findings are shown in Figure 4.3.

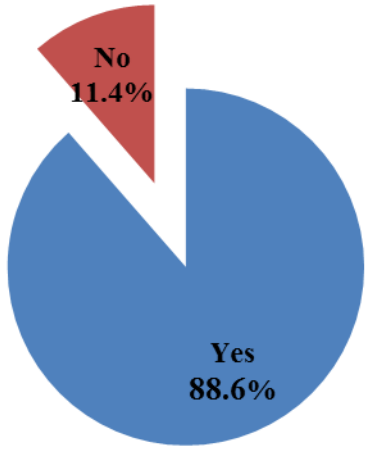


Figure 4.4: Supplementing Modern Medicine with Traditional Medicine

4.4.3 Effectiveness of *M. Oleifera*

The study sought to assess the level of knowledge of the patients on whether a problem would arise when using *M. Oleifera* and HIV drugs at same time. As shown in table 4.8, the findings indicate that 125(59.5%) thought that a problem would not arise when one takes both *M. Oleifera* and HIV drugs together while 8(3.8%) indicated that a problem would arise. However, 77(36.7%) stated that they did not know whether a problem would arise. This point out to lack of awareness among the patients on the possible contraindication and interaction between *M. Oleifera* and HIV drugs.

Table 4.8: Consequence when using *M. oleifera* and ARVS at the same time

A problem will arise when *Moringa* and ARVs are used at same time	Frequency	Percentage (%)
Yes	8	3.8
No	125	59.5
Don't know	77	36.7
Total	210	100.0

The study further sought to find out whether there were side effects arising from the use of *M. Oleifera.* Nearly all (99%) of the respondents indicated that they had never experienced any side effects after using *M. Oleifera* with only 1% indicating that they had some side effects that included rashes and stomach aches. The findings correspond to those of the key informants who stated that there were no reported side effects of the herb and thus it is safe. In rare cases those who use *M. Oleifera* may experience some itchiness.

4.4.4 Sources of information on the uses and benefits *M. Oleifera*

Figure 4.4 shows the main source of information on the uses and benefits of *M. Oleifera*. The percentage of the respondents who reported to have gotten the information from family members was 25.2% while 21.4% indicated that had obtained it from friends. The findings on the herbalists' source of knowledge on *M. Oleifera* indicated that majority had acquired it from their family members especially grandparents and fathers. Only one of the herbalists had nurtured the practice through reading books. This implies that most of the purported benefits of *M. Oleifera* are from folk sources, with minimal involvement of healthcare personnel.

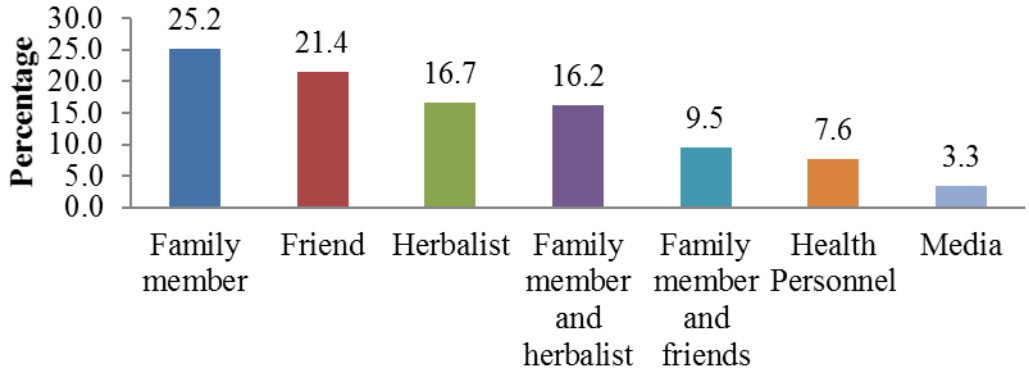


Figure 1.5: Sources of information on *M. Oleifera* use

4.4.5 Affordability and accessibility of *M. Oleifera*

The availability and cost of herbal medicine is can influence its use among patients. This study sought to determine how affordable and accessible *M. Oleifera* was among PLWHA attending CCC at Migori County Referral Hospital. The findings indicate that *M. Oleifera* was affordable according to 82.4% of the respondents. The rating of the accessibility of *M. Oleifera* was high with 80.2% of the respondents indicating that *M. Oleifera* was accessible. However 17.6% of the respondents indicated that *M. Oleifera* was not affordable while 19.8% stated that it was not accessible.

4.5 Health Systems level factors associated with the use of *M. Oleifera* among PLWHA attending CCC at Migori County Referral Hospital

Several factors are associated with the use of *M. Oleifera.* The study sought to establish health systems level factors associated with the use of *M. Oleifera.*

4.5.1 Patients – health care personnel relationship

Patient's trust of the health care providers is paramount for the healthcare services to be effective. The study sought to establish the extent to which the respondents trusted their medical providers to offer them high quality medical care. The findings indicate that

189(90.0%) of those taking *M. Oleifera* completely trusted the medical providers, 12(5.7%) somewhat trusted them while 9(4.3%) fairly trusted the health personnel. Among the non-users of *M. Oleifera*, 89.7%, 8.8% and 1.5% completely, somewhat and fairly trusted the providers respectively. In terms of respect, 204(97.1%) of the respondents had not experienced any hostility or lack of respect from the health care personnel while 6(2.9%) reported some disrespect. For the non users *M. Oleifera* 65(95.6%) stated the health care personnel were respectful while 3(4.4%) sttsed to the contrary Further analysis however indicated that there was no significant difference in terms of trust for medical providers (p = 0.387) between the users and non-users of *M. Oleifera*..A similar finding was found on the patients experience of disrespect (p = 0.529) between the users and non-users of *M. Oleifera.*

Table 4.9: Patients – Health care personnel relationship

		M. Oleifera use		Chi square value	p-value
		Users(n=210)	Non-users(n=68)		
		Frequency (%)	Frequency (%)		
Trust for medical providers	Completely	189 (90.0)	61(89.7)	1.899	0.387
	Somewhat	12(5.7)	6(8.8)		
	Fairly	9(4.3)	1(1.5)		
Total		210(100.0)	68(100.0)		
Disrespect for patients	Yes	6(2.9)	3(4.4)	0.396	0.529
	No	204(97.1)	65(95.6)		
Total		210(100.0)	68(100.0)		

The relationship between the patients and the healthcare personel is also critical for the disclosure of use of herbal medicine and HIV drugs. Informing the health care practitioners on the use of alternative forms of treatment one is taking is important as it informs on the possibility of drug interaction and the fact that mixing certain drugs can be dangerous. The study thus sought to establish whether the patients using *M. Oleifera*

had informed their doctors. The findings indicate that 90% had not disclosed to their health care providers that they were taking *M. Oleifera.*

4.5.2 Distance to the health facility

The study sought to establish whether the distance the patients had to travel to access health care services influenced their use and non-use of *M. Oleifera*. From the findings, distance to the health facility was a significant factor ($p = 0.033$) associated with the use and non-use of *M. Oleifera* (Table 4.10).

Table 4.10: Distance as a factor associated with use and non-use of *M. oleifera*

		M. Oleifera use		Chi-square value	p-value
		Users(n=210)	Non-users(n=68)		
Distance		Frequency (%)	Frequency (%)		
Those who faced distance as a challenge	(Yes)	54(25.7)	9(13.2)	4.564	0.033
Those who didn't face distance as a challenge	(No)	156(74.3)	59(86.8)		
Total		210(100.0)	68(100.0)		

4.5.3 Side effects experienced by users and non users of *M. Oleifera* among PLWHA attending CCC at Migori County Referral Hospital

The study sought to establish whether users and non users of *M. Oleifera* among PLWHA attending CCC at Migori County Referral Hospital had experienced any side effects from ARV treatment. From the findings, only 15(7.1%) of users of *M. Oleifera* affirmed that they had experienced some side effects including stomachaches while 195(92.9%) of the patients who were using *M. Oleifera* had no side effects. For those who were not using *M. Oleifera* 65(95.6%) had not experienced any side effects from the treatment they were receiving while 3(4.4%) stated they had. The findings however indicated that side effects was not a significant factor ($p = 0.426$) associated with the use and non-use of *M. Oleifera.*

Table 4.11: Side effects as a factor associated with use and non-use of *M. oleifera*

		M. Oleifera use Users(n=210)	Non-users(n=68)	Chi-square value	p-value
		Yes	No		
		Frequency (%)	Frequency (%)		
Side effects	Yes	15(7.1)	3(4.4)	0.633	0.426
	No	195(92.9)	65(95.6)		
Total		210(100.0)	68(100.0)		

4.5.4 Delay in accessing services

Patients are always in need of timely access of health care services. The study sought to find out whether delays in offering services at the health facility and long waiting times was associated with the use and non-use of *M. Oleifera*. Findings show that delay in getting services was a significant factor (p = 0.000) associated with the utilization of *M. Oleifera* (Table 4.12).

Table 4.12: Delay as a factor associated with use and non-use of *M. oleifera*

		M. Oleifera use Users(n=210)	Non-users(n=68)	Chi-square value	p-value
		Frequency (%)	Frequency (%)		
Delays in getting services	Yes	17(8.1)	19(27.9)	17.947	0.000
	No	193(91.9)	49(72.1)		
Total		210(100.0)	68(100.0)		

4.5.5 Stigma associated with HIV and AIDS positive status

Table 4.10 shows the finding on stigma among *M. Oleifera* by PLWHA attending CCC at Migori County Referral Hospital. Of the respondents 4(1.9%) of those using *M. Oleifera* had experienced stigma while 206(98.1%) had not experienced any stigma. For the non users of *M. Oleifera* only 1(1.5%) had experienced stigma due to their status while 98.5% had not experienced stigma. The analysis however indicates that stigma

was not a significant factor (p = 0.815) associated with the utilization of *M. Oleifera* between users and non users.

Table 4.13: Stigma as a factor associated with use and non-use of *M. oleifera*

		M. Oleifera use		Chi-square value	p-value
		Users(n=210)	Non-users(n=68)		
		Frequency (%)	Frequency (%)		
Stigma	Yes	4(1.9)	1(1.5)	0.055	0.815
	No	206(98.1)	67(98.5)		
Total		210(100.0)	68(100.0)		

4.5 *In vitro* cytotoxicity and antiviral activity of *M. Oleifera* Extracts against Herpes simplex Type 1 Virus

4.5.1 Tissue culture infective dose (TCID)

The determination of the virus titre ($TCID_{50}$) for Herpes Simplex Virus type -1, virus was determined by use of Sperman-Karber's method as described in section 3.7.8.2 and gave a value of 3.6232×10^2 PFU/mL. This value was achieved at a dilution of $x10^{-13}$.

4.5.2 Cytotoxicity

Cytotoxicity experiment was carried out as described in section 3.7.8.3 to determine concentration range within which Vero cells tolerate cytotoxic effect of extracts. The cytotoxicity of *M. Oleifera* for both methanol and aqueous extracts showed a curve with CC_{50} of more than a 1000µg/mL respectively as shown in Figure 4.6. Aqueous extracts of *M. Oleifera* exhibited a (CC_{50}) of 1965.23 ± 10.26µg/mL as compared to the methanol extract with CC_{50} of 1622.10±11.98µg /mL. It was obtained from the mean of 4 separate experiments.

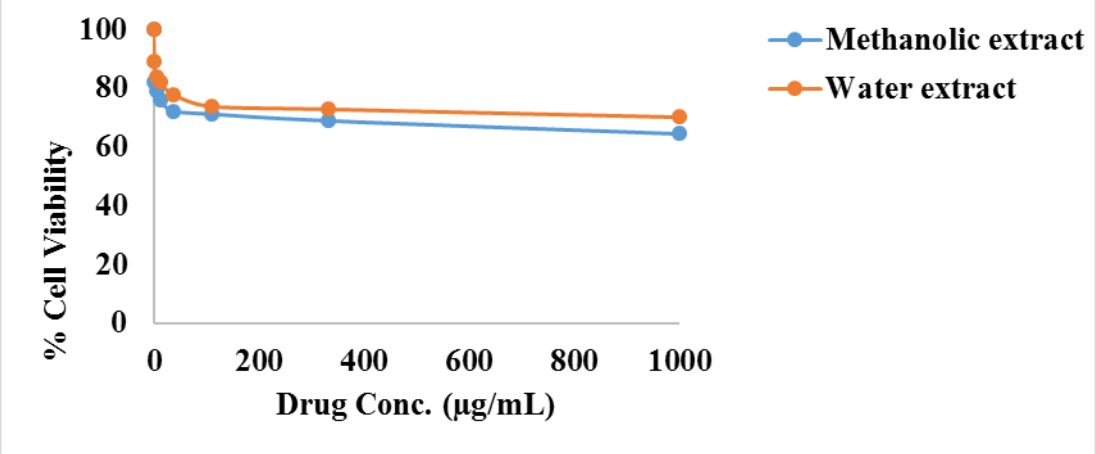


Figure 4.6: Interaction line plots of % cell viability against extract concentration in µg/ml of the methanol and aqueous extract from *M. Oleifera.* It indicates decline in cell viability as concentration of the extract increases.

4.5.3 *In vitro* antiviral activity of *M. Oleifera* Extracts against Herpes simplex Type 1 Virus

This was carried out as described in section 3.7.8.4 of the methodology. Anti HSV-1 activity of both methanol and aqueous extracts were evaluated using two different approaches. The selectivity index of the extracts was calculated by dividing obtained results of CC_{50} with IC_{50}. The aqueous extracts of *M. Oleifera* provided the highest cell protection during post and pretreatment as compared to methanol extract.

Both aqueous and methanol extracts tested, have shown antiviral activity against HSV-1 at different concentrations (1000µg/mL, 500µg/mL, 250µg/mL and at 125µg/mL) to the Vero cells both prior and after virus infection. The IC_{50} for the aqueous extract was 627.29± 0.33 with TI = 3.13 when incubated with the cells after the virus infection, and IC_{50} of 695.10±0.28 with TI = 2.83 when incubated prior to the virus infection while methanol had IC_{50} of 1350.61 ±0.24 and 2427.83 ± 0.23 respectively. Positive control by

acyclovir at 5µg/mL and 10µg/mL gave a moderate IC_{50} compared to aqueous and methanol extracts at 1000, 500, 250 and 125µg/mL.

Table 4.1: *In vitro* antiviral activity against HSV-1 evaluation of methanol and aqueous extracts of *M. oleifera*

	Pre-Treatment			**Post-Treatment**	
	CC_{50}	IC_{50} [A]	TI[B]	IC_{50} [A]	TI[B]
Aqueous	1965.23±10.26	695.10± 0.28	2.83	627.29 ±0.33	3.13
Methanol	1622.10±11.98	2427.83± 0.23	0.67	1350.61± 0.24	1.20

A: Concentration of extract in µg/ml that inhibits virus activity by 50%. B: Therapeutic index = CC_{50}/IC_{50}. Both CC_{50} and IC_{50} are the mean values of 4 different experiments.

4.5.4 Dose response test for cell protection of *M. Oleifera* aqueous and methanol extracts against HSV-1

Figure 4.7 and 4.8 respectively represents the percentage cell protection graphs against different extracts concentrations µg/mL during post and pretreatment.

This was carried out as described in section3.7.8.4 of the methodology. The aqueous extract provided the highest cell protection at both 500µg/mL and 1000 µg/mL during pre and post treatment as compared to methanol extract during pre and post treatment.

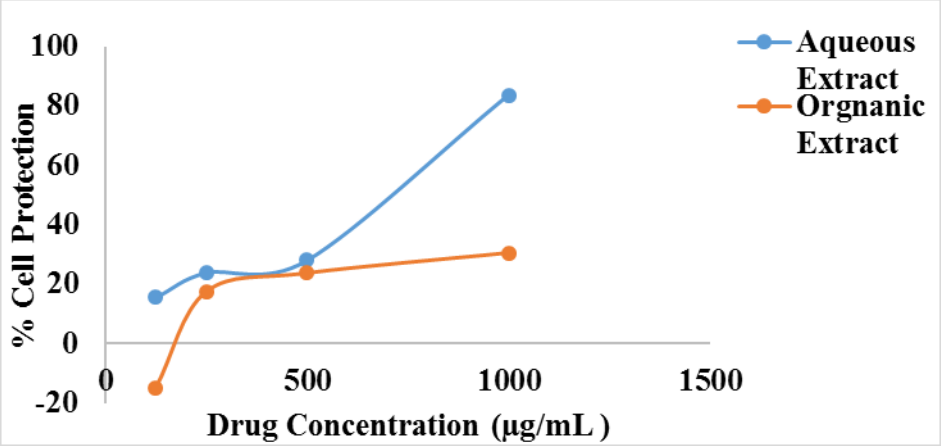


Figure 4.7: Percentage cell protection against HSV-1 at different extract concentration in µg/mL (Post treatment)

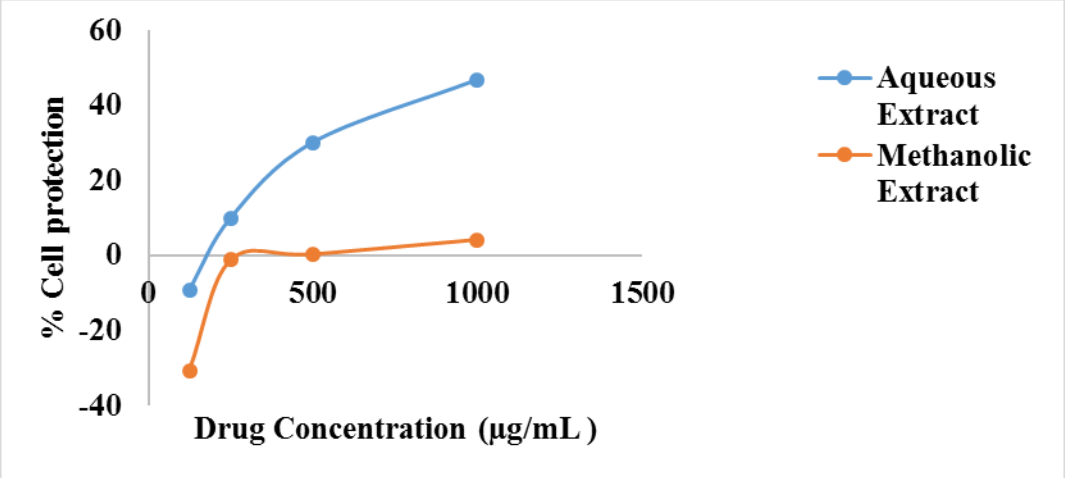


Figure 4.8: Percentage cell protection against HSV-1 at different extract concentration in µg/mL (Pretreatment)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The study findings indicate that there was a high usage of *M. Oleifera* (75.5%) among the people living with HIV (PLWHA) attending the CCC at Migori County Referral Hospital. This finding is similar to that of other studies that found out that herbal medicine (including *M. Oleifera*) use in HIV clinics and among PLWHA is high. A study by (Maponga and Monera 2012) conducted in Zimbabwe showed a high percentage of participants who stated to have used *M. Oleifera.*

The source of information can influence the use of herbal medicine by People living with HIV as a result of self-reporting. PLWHA self-report that traditional medicine helps in improving their quality of life as it has fewer risks and helps alleviate the symptoms related to HIV and side effects of its treatment (OHTN 2013). These claims lead to an erroneous belief that herbal medicines are superior to pharmaceutical products which are ineffective. The knowledge on the importance of *M. Oleifera* was mainly obtained from family members (25.2%) and friends (21.4%). Only a small percentage of the participants (7.6%) had obtained the information from health care personnel. Similar results were obtained in a study by (Monera and Maponga 2010) which showed that friends or relatives were the most common source of a recommendation for use of *M. Oleifera.* These findings are also consistent with several studies which showed that the main sources of information for herbal medicine were personal knowledge, media, friend, and family and not the health care provider (Shedlin 2013, Hassan, 2010).

M. Oleifera is a multipurpose plant that has been used as human food and an alternative for modern medicine for several years. It is believed to have a range of benefits including protecting and nourishing skin and hair, treating edema, protecting the liver,

preventing and treating cancer, fighting against bacterial diseases, making bones healthier, treating diseases such as stomach complaints, diabetes, asthma among others (Alegbeleye, 2018). The study sought to establish the reason as to why *M. Oleifera* was being used by PLWHA attending CCC. Although the reasons for using *M. Oleifera* varied, the majority of the respondents did not indicate that *M. Oleifera* was used to treat HIV. The most common reason for using *M. Oleifera* was that it was used as food due to its high nutritive value and as medicine due to its ability to boost immune system. Other respondents, however, stated that they used *M. Oleifera* as medicine for other diseases such as ulcers, stomach upset, skin infections, etc. as well as due to its affordability and accessibility.

The different benefits associated with the use of *M. Oleifera,* had made it a darling herb to many of the herbalists. The herbalists indicated that they used *M. Oleifera* to treat various diseases including skin infections, diabetes, high blood pressure, hypertension, and arthritis. Similar reasons for use of *M. Oleifera* were given in the study by (Monera and Maponga 2012) which found the most common reason for using *M. Oleifera* being as an immune booster (80% of respondents). Other indications were nutritional disorders, diabetes, digestive disorders, hypertension, and arthritis.

The commonly used part of the *M. Oleifera* tree was the leaves. It was either used alone or in combination with the seed and dry pods. *M. Oleifera* was supplied by relatives and friends with an equal number of respondents obtaining their supplies from the market, and friends/relatives. This is attributed to the fact that most of the farmers in Migori County have planted the *M. Oleifera* tree. The *M. Oleifera* was commonly used in crushed and powdered form by nearly majority of the participants. It was brewed in teas or added in foods as a spice. Other usage was cooking of the leaves as vegetables.

Several studies have indicated that there is a potential for interactions between the use of herbal medicine and modern medicine when used together. Leaf extracts of *M. Oleifera* inhibit CYP3A4 enzymes which can metabolize a large proportion of drugs, including

antiretroviral drugs (Monera, *et al.* 2008). According to (Monera-Penduka *et al*, 2017), the associated use of traditional herbs with modern drugs may result in herb-drug interactions through various pharmacokinetic and pharmacodynamic mechanisms. Therefore, self-directed herbal medicine use such as the use of *M. Oleifera* without directions from health care personnel puts HIV patients at a high risk of developing herb-drug interactions. The findings of the study indicated that the majority of the respondents had not experienced any side effects after using *M. Oleifera* together with HIV drugs. Those who had some side effects stated that they had some mild rashes and stomach aches after using it. The reported side effects of *M. Oleifera* in this study are in concurrence with the findings of a study by (Bepe et al 2012). In the study abdominal pain and rashes had significant association with using herbal medicine during antiretroviral therapy. Although *M. Oleifera* is stated as being safe with minimal side effects, this does not indicate that the whole plant is safe for use. The only parts used by the patients and herbalists were leaves, seeds and barks. Other parts such as roots may be having harmful substances that may have serious side effects to their users.

A significant socio-demographic factor that was associated with the use of *M. Oleifera* among PLWHA attending CCC at Migori County Referral Hospital was age. A binomial logistic regression model established that males were 12.83% less likely to use *M. Oleifera* compared to females. These findings are similar to those found by (Gandji et al, 2018) and (Monera and Maponga, 2012) who established that women were more likely to use *M. Oleifera* compared to men.

The use of herbal supplements can have a significant impact on the health and wellness of patients. However, combining herbs with conventional medicine is not necessarily safe. The majority of the patients stated that it is appropriate to supplement modern treatment with herbal medicine since when this is done some of the diseases that cannot be treated by modern medicine can be cured with traditional remedies. Others, however,

thought it was not appropriate as traditional medicine has not yet been tested and may cause serious health problems when used.

A critical issue of concern in the study was the high rate of nondisclosure of *M. Oleifera* use to the health care providers. This rate of not disclosing is consistent with findings from other studies which indicate that a majority of PLWHA are usually very hesitant to discuss, let alone inform their physicians on their use of herbal medicine. A study by (Furler *et al,* 2003) in Ontario, Canada found out that 53% of the HIV-infected outpatients did not disclose their usage of complementary and alternative medicine. Another study in Malaysia found a nondisclosure of 68% among patients with HIV/AIDS. Several reasons could explain this including the physicians not enquiring about the use of *M. Oleifera* or the fear among patients of disproval of the use by the physicians or punished by being denied treatment.

The patient is expected to reveal their relevant medical history, expose their bodies for examination and act on the instructions given by their healthcare provider upon giving out consent. The patient must trust their health care givers with these, for their own good. According to (Goold, 2002), "trust, in the healer is essential to healing itself. Trust, at least to some minimal extent, is undoubtedly a prerequisite to seeking care at all". The study findings indicate that majority of the respondents, of those taking *M. Oleifera* and of those not taking *M. Oleifera*, completely trusted the providers. In terms of respect, majority of the respondents of those taking *M. Oleifera* and those not taking it had not experienced hostility or lack of respect from the health care personnel. Further analysis however indicated that there was no significant difference in terms of trust for medical providers and patients experience of disrespect between the users and non users of *M. Oleifera*.

The distance that patients had to travel to access health care can influences their utilization of the services. A study by (Akullian *et al,* 2016) found out that PLWHA are likely to travel longer distances to access free services.From the findings, both the users

non users indicated that distance was a challenge as they lived far and had to use “boda bodas or matatus”. Additionally, the findings indicate that distance to the health facility was a significant factor associated with the use and non-use of *M. Oleifera*.

From the findings, a majority of the patients using *M. Oleifera* had not experienced side effects from the treatment they were receiving. The findings also indicate that side effect was not a significant factor associated with the use and non-use of *M. Oleifera.*

Patients are always in need of timely access of health care services. The study findings shows that delay in getting health services was a significant factor associated with the utilization of *M. Oleifera.* This indicates that the long awaiting time during health facility visits was a reason as to why some of the patients turned to using *M. Oleifera.*

Generally, stigma has been associated with poor utilization of HIV services where fear of gossip and being called names results in PLWHA avoiding HIV services near their residential places and therefore walk far distances to seek the same same services as well as disclosing (Underwood *et al* 2014). Although stigma was not a significant factor associated with the utilization of *M. Oleifera*, the findings of this study shows that stigma exists in the society with users and non-users of *M. Oleifera* having experienced stigma due to their status. Interestingly, (Underwood, *et al* 2014) cited a study where stigma was a motivator of adherence as PLWHA took their medicines to avoid a sickly appearance, which might draw community attention. Nevertheless, stigma and discrimination should be discouraged regardless of this finding. This will enable PLWHA to visit their nearest facilities whenever they need HIV services and might indirectly improve adherence to treatment.

However, differences exist in the plant based on the geographical location where it is grown. *M. Oleifera* differs in nutrient composition at different locations (Gopalakrishnan, et al, 2016). The *M. Oleifera* grown in India has slightly different nutritional components than *M. Oleifera* grown in Nigeria. (Asante *et al.* 2014), studied

the nutritional differences in the leaves from two ecological locations semi-deciduous and Savannah regions. It showed that the latter was less nutritious than the former and attributed this to high temperatures at the Savannah regions. At higher temperatures, protein and enzymes get denatured and this could be the cause for the difference in nutrient content. And therefore, it was inappropriate to generalize the findings to the species of *M. Oleifera* grown in Kenya.

This study was designed to evaluate cytotoxic and antiviral activities of *M. Oleifera* aqueous and methanol leaves extracts against HSV-1 by the use of MTT assay. Both methanol and aqueous extracts showed a curve with CC_{50} of more than a 1000µg/mL respectively as shown in figure 4.8. Methanol extracts of *M. Oleifera* exhibited a (CC_{50}) of 1622.10±0.68 µg/mL as compared to the aqueous extract with CC_{50} of 1965.23±0.31µg/mL.

The antiviral activity of *M. Oleifera* extracts for both methanol and aqueous against HVS-1 was determined by a slight modification to the methods described by (Mohamed *et al.,* 2015). Both extracts had detectable antiviral activity as they reduced HSV-1 replication in the infected Vero cells. The aqueous extract exhibited a higher inhibitory effect when incubated with the cells after the virus infection, and it exhibited a moderate inhibitory effect than the methanol extract when incubated prior to the virus infection as compared to acyclovir. The inhibitory concentration required to suppress 50% of the virus was significantly less in the post-treatment for both aqueous and methanol extracts. The extract may have affected the metabolic functions of the cell with some cell tolerance to structural effects. The aqueous extract exhibited a therapeutic index ranging from 2.83 to 3.13 while the methanol extract exhibited therapeutic index ranging from 0.67 to 1.20. It was obtained from the mean of 4 separate experiments.

The results are in accordance with other different studies previously conducted on *M. Oleifera*. For example, studies done by (Goswami *et al.,* 2016) showed that methanol extracts of *M. Oleifera* exhibited detectable antiviral activity against HSV-1 with IC_{50} of

74.8µg/ml. (Waiaput, *et al.,* 2012) suggested that 80% ethanol crude extracts of *M. Oleifera* fruit revealed anti-HBV activity by inhibiting HBV reproduction with mild cytotoxicity on HepG2 cells. An antiviral activity study by (Debayan *et al.,* 2016), using three different test systems like CPE reduction, MTT, and PRA, showed that extracts from *M. Oleifera* have detectable antiviral activity compared to the drug acyclovir, as it effectively exhibited potent anti-HSV action in Vero cells without reducing cell viability. These extracts also inhibited the growth of the human isolate VU-09, isolated from a patient infected with HSV-1, indicating that *M. Oleifera* extracts need to be studied further with other viruses of herpes virus family (Debayan *et al.,* 2016).

5.2 Conclusions

1. The usage of *M. Oleifera* among PLWHA attending comprehensive care clinics (CCC) at Migori County Referral Hospital is high.
2. The patient level factor associated with the use of *M. Oleifera* among PLWHA were the age of the patients and the time used to access healthcare services at the health facility.
3. Distance to the health facility and delays in getting the health services were significant health systems level factors that were associated with the use of *M. Oleifera* among PLWHA attending CCC.
4. Aqueous and methanol extracts of *M. Oleifera* are not toxic to vero cells and have antiviral activity against HSV-1.

5.3 Recommendations

1. There is a need to formulate policy and legal framework to govern the use of herbal medicine practices among PLWHA.
2. Sensitization of PLWHA on the possible contraindication and interaction between *M. Oleifera* and ARV drugs should be carried out.
3. Sensitization of people against stigma towards PLWHA is required.

4. Even though the *in vitro* safety evaluation of crude extracts did not show any significant toxicity, further safety evaluation should be done on pure isolates that could be having the potential of antiviral activity.
5. Specific phytochemicals responsible for antiviral activity against HSV-1 in aqueous and methanol extracts need to be isolated and further investigated for anti- HSV-1 activity *in vivo* and clinical trials.

5.4 Areas for further research

1. Studies to indicate the potential interactions between *M. Oleifera* and antiretroviral drugs.
2. Further clinical trials with patients to identify the antiviral activity of *M. Oleifera*.

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APPENDICES

Appendix I: Information sheet for participants

(KMRI/SERU/CTMDR/071/3792)

Invitation

How are you? I am Nkirote Judith, from Jomo Kenyatta University of Agriculture and Technology/KEMRI.

You are being invited to be engaged in this research study; before you consent to take part, it is vital for you to understand why the study is being carried out and what your participation will entail. Kindly take time to read through the following information carefully and you may discuss it with other people if you wish. You are allowed to contact the Principal Investigator if anything is unclear or in case you would like to inquire more information about the study. Take your time to decide whether, or not you would like to participate.

What is the purpose of this study?

The aim of this study is to determine the antiviral activity of extracts from *M. Oleifera* and determinants of its use, among HIV positive patients attending comprehensive care clinic at Migori County Referral Hospital.

Why have I been chosen?

You are randomly selected because you are among the patients attending Comprehensive Care Clinic at Migori County Referral Hospital.

What will participation involve?

You were asked a number of questions concerning individual, patterns and health system level factors associated with the use of *M. Oleifera* as well as the products services you draw from this species. Your responses to the questions were written down on a field note book.

What if I decide that I don't want to take part?

You are at liberty to decide that you don't want to take part in the study and can:

1. Object to answer any questions that you feel uncomfortable with
2. Decide to stop the interview at any time
3. Remove your consent for the data collected to be used

Will I be paid for my time?

There is no payment for taking part in this study. However there are numerous benefits such as the research acts as a tool to expand the knowledge on *M. Oleifera* benefits and its uses.

Will I be anonymous, and who will know my identity?

With your consent to take part in the interview, a Participant Number was created for you and that's the only thing that was used to identify you. Your identity will only be known by the interviewer, and will not be found in any record. Hard copy and electronic data was stored in a lockable drawer accessed on by the PI: this was deleted after 3 years, or if you withdraw your consent (whichever sooner).

What do I do next?

If you would like to be involved in this study we will interview you as you consult or collect your medication at Migori County Referral Hospital. At the interview you were issued with one of these information sheets, and were asked to sign a consent form.

Who shall I contact with any question?

To be part of this study, or ask any question, then please get in contact with the Principal Investigator (Nkirote Judith) through telephone number: 0723857263 or email judy.nthiori@gmail.com

If you have any questions or concerns regarding this study and would like to talk to someone other than the researcher, kindly you are encouraged to contact the following:

The Director,
Institute of tropical Medicine and Infectious Diseases (ITROMID) KEMRI,
Jomo Kenyatta University of Agriculture and Technology (JKUAT)
P.O BOX 62000 - 00200 Nairobi
Telephone No: 067- 52711
Email: itromid@nairobi.mimcom.net
OR
Head SERU,
Scientific and Ethics Review Unit,
S.L.P. 54840 00200, Nairobi
Telephone No: 0717719477,

Email:seru@kemri.org

Appendix II: Consent form provided to the respondents prior to interview.

You have been invited to participate in a research project; it is necessary for you to give your consent before you proceed with your participation. By completing this form you are consenting to take part in this research project; you can withdraw your consent at any point. To withdraw your consent, please either mention that to the interviewer during the interview or contact Mrs. Judith Nkirote by email, phone or any other means before (date). Kindly read the following statements and indicate that you agree with them by ticking next to them before appending your signature for consent

	Initial here
I have been issued with a Participant Information Sheet	
I have been informed what the purpose of this research is, and the nature of the study	
I have been informed how the data that are collected within the research was handled and stored	
I have been informed that I can remove my consent at any time either during or after the interview, and that withdrawal of consent will not harm me in any way	
I have been informed that the interview was written down on a field note book	
I have been informed that my anonymized quotes may be used as part of reports of this research finding	
I agree to take part in this study	

Consent signed by ……Date…….

Consent received by……..Date……..

Appendix III: Kiswahili version of the Information sheet for participants

Mwaliko

Habari yako. Jina langu ni Judith Nkirote kutoka chuo kikuu cha JKUAT/ITROMID (KEMRI).

Ningependa kukualika kushiriki katika utafiti kuhusiana na maswala kujua vile *Moringa oleifera* inaweza kutumiwa kwa baadhi ya wagonjwa wa UKIMWI ilikudhibitisha faida zake kwa wagonjwa wanaotembelea vituo vya matibabu katika hospitali ya Migori County Referrral.Utafiti huu unaongozwa na Judith Nkirote ambaye ni mwanafunzi katika chuo kikuu cha JKUAT. Ni vyema kufahamu sababu ya kufanya utafiti huu na ni vipi utakavyo shiriki.

Tafadhali chukua muda wako kusoma na kuelewa taarifa hii, umekubalishwa pia kujadiliana na wenzako kama ungependa ama kuuliza swali lolote kwa mtafiti ili kutoa uamuzi wa kushiriki ama kutoshiriki kwa hiari yako.

Kusudi la Utafiti huu

Utafiti huu utasaidia katika kujua na kuamua usalama na makali ya virusi kutoka kwenye Moringa oleifera na matumizi yake, kati ya wagonjwa wanaoishi na virusi vya ukimwi wanaotembelea kliniki ya kina hospitali ya rufaa Migori Kaunti kupata huduma.

Kwa nini nimechaguliwa?

Unaomba kushirikishwa katika utafiti huu kwa sababu wewe ni kati ya wagonjwa wanaohudhuria Huduma ya kina katika hospitali ya rufaa Migori Kaunti.

Utaratibu wa kufanya Utafiti huu

Utafiti huu utahusisha mahojiano ya moja kwa moja, utaulizwa maswali kadhaa ya kibinafsi,hali ya afya yako kwa kipindi ambacho umetumia *Moringa oleifera* na bidhaa ama faida ambazo umeweza kuzipata kutokana na matumizi ya *Moringa oleifera*.

Tutaomba kuyaandika majibu na maoni yako katika karatasi ama kijitabu kidogo.Majibu yako kwa maswali itaandikwa chini ya kitabu kidogo.

Haki ya kukataa na kujiondoa kwenye utafiti

Sio jambo la lazima wewe kushiriki katika utafiti huu.

Unaweza kuamua kutojihusisha na utafiti huu wakati wowote utafiti unapoendelea kwa:

a) Kuamua kutojibu maswali yoyote ambayo inakutia wasiwasi.

b) Kuamua kuwacha mahojiano wakati wowote

c) Kuondoa Idhini majibu yako isitumike katika kujumlisha majibu ya utafiti huu.

Uamuzi wa kutoshiriki hautadhuru matibabu yako katika kliniki hii kwa njia yoyote.

Malipo

Hakuna malipo yoyote utakayo pata kwa kushiriki katika utafiti huu.Hata hivyo majibu yako yatatusaidia kukuza kiwango cha elimu na utafiti kuhusiana na matumizi na faida za *Moringa oleifera* haswa katika matibabu ya wagonjwa wa Ukimwi.

Kudumisha siri

Yale tutakayozungumza na majibu utakayo tupatia yatawekwa siri na hakua mtu atakaye weza kukutambua.Kwa idhini yako ya kushiriki katika utafiti huu, majibu yako kwa

maswali utakayo ulizwa yatatambuliwa kwa nambari tutakayo kupa wala sio kwa jina lako.

Vile vile jina lako halitakuweko kwa matokeo ya utafiti huu wala halitakuwa kwenye rekodi zozote. Takwimu kwenye makaratasi na kwenye tarakilishi zitawekwa kwenye kabati ambayo itakuwa inafungwa na kutumiwa na mtafiti mkuu pekee yake.takwimu hizi zitaharibiwa baada ya miaka mitatu ama ukitoa idhini kuwa majibu yako yaondolewe.

Ushiriki katika utafiti

Kama ungependa kushiriki katika utafiti huu, utahojiwa wakati utakapokuja kupata huduma ya matibabu ama kuchukuwa dawa katika hospitali ya Rufaa Migori counti

Katika mahojiano utapatiwa nakala ya habari kuhusu utafiti na utaulizwa kupatiana idhini ya kushiriki utafiti kwa hiari yako.

Je, unaweza kuwasiliana na nani ikiwa una maswali?

Kama Ungalitaka kushiriki ama kuachana na utafiti huu, ama pengine unaswali, unaweza kuwasiliana na mchunguzi mkuu Nkirote Judith kupitia nambari ya simu 0723857263 ama barua pepe judy.nthiori@gmail.com

Kama ungalitaka kuuliza maswali kuhusu utafiti huu kwa mtu mwingine ambaye sio mchunguzi huyu wasiliana na:

Mkurugenzi, (ITROMID) KEMRI,
Jomo Kenyatta University of Agriculture and Technology (JKUAT)
S.L.P 62000-00200 Nairobi
Nambari ya Simu: 067- 52711
Barua Pepe: itromid@nairobi.mimcom.net

Ikiwa una maswala yoyote kuhusu haki zako kama mtu anayeshiriki katika utafiti huu unaweza kuwasiliana na katibu wa kuchunguza maswala ya tabia zinazofaa utafiti wa sayansi huko KEMRI

Mwenyekiti SERU

Kenya Medical Research Institute,

S.L.P 54840- 00200, Nairobi

Nambari ya Simu: 0717719477

Barua Pepe: seru@kemri.org

Je una swali lolote kwa sasa?

Appendix IV: Kiswahili version of consent form provided to the respondents prior to interview.

Unaalikwa kuhusika katika utafiti huu ambao utasaidia katika kujua na kuamua usalama na makali ya virusi kutoka kwenye *Moringa oleifera* na matumizi na faida yake haswa kwa wagonjwa wa virusi vya Ukimwi.Kabla ya kushiriki kwenye utafiti ni vyema upeane idhini ya kushiriki kwa hiari yako mwenyewe na ueleweya kwamba unaweza amua kutojihusisha na utafiti huu wakati wowote ule.

Kwa kujaza fomu hii ni idhini tosha kwamba umekubali kuwa mshirika kwa hiari yako. Kuondoa idhini ya kushiriki tafadhali mjulishe mhojaji wako ama uwasiliane na mtafiti mkuu Mrs. Judith Nkirote kwa njia ya simu nambari 0723857263 ama barua pepe judy.nthiori@gmail.com

Tafadhali soma taarifa ama maneno yafataayo kwa utaratibu na uashirie kukubali kwa kuweka alama ya tick (√) kando ya taarifa kabla ya kutia sahihi ya kutoa idhini ya kushiriki.

Taarifa	**Alama hapa**
Mimi nimepewa karatasi iliyo na taarifa na maelezo ya kushiriki kwenye utafiti huu.	
Nimeelezwa kusudi na utaratibu wa utafiti huu	
Nimeelezwa vile takwimu na majibu yangu yatakavyo chukuliwa, kutumiwa na kuhifadhiwa vizuri wakati wa utafiti.	
Nimeelezwa sio jambo la lazima kushiriki utafti huu na naweza kubadili kauli yangu ya kushiriki wakati wowote bila kudhuru matibabu yangu	
Nimeelezwa kwamba majibu yangu yataweza kurekodiwa kwa kuandikwa kwenye kitabu.	
Nimepewa taarifa kwamba matamshi na majibu yangu bila utambulisho itaweza kutumika kama sehemu ya utafiti huu	
Nimekubali kushiriki katika utafiti huu	

Sahihi ya Mshiriki ……Tarehe……

Jina/Sahihi ya Mhojaji……Tarehe…….

Appendix V: Questionnaire

Questionnaire for patients

Background

- County……

- Sub County……
- Location……..
- Sub Location……..

GPS Location

- Latitude (X.Y0)……..
- Longitude (X.Y0)……
- Altitude (m)……
- Accuracy (m)……..

Date of interview:

……

Name of interviewer:

……..…….

Name of interviewee/patient:

……..…….

Participant number:

……...……

SECTION A: Social Demographic Characteristics

Please answer the questions below by ticking (√) in the boxes.

1. Gender:

   Male [ ]

   Female [ ]

2. Age:

   Below 25 yrs. [ ]

   Between 26 - 35 yrs. [ ]

   Between 36 - 45 yrs. [ ]

   Between 46 - 55 yrs. [ ]

   Above 55 yrs. [ ]

3. Marital status:

   Single [ ]

   Married [ ]

   Divorced/Separated [ ]

   Widowed [ ]

4. Highest level of Education attained:

   Illiterate [ ]

Primary Certificate [ ]

Secondary Certificate [ ]

College Certificate [ ]

University Degree [ ]

5. What is your main occupation?

Farming [ ]

Employed [ ]

Business [ ]

Other (specify) …….

6. For how long have you been living with HIV?

Below 1year. [ ]

Between 1 - 3 yrs. [ ]

Between 3 - 5 yrs. [ ]

Above 5 yrs. [ ]

7. What treatment are you on? And do you have another alternative of the treatment you are using?……

8. For how long have you been on treatment?

Below 1year. [ ]

Between 1 - 3 yrs. [ ]

Between 3 - 5 yrs. [ ]

Above 5 yrs. [ ]

SECTION B: PREVALENCE AND PARTS OF *MORINGA* USE

1. Do you use *Moringa*?

Yes [ ]

No [ ]

If No, explain
reasons……

(If the answer is No kindly skip the questions below and answer section C)

2. What do you use *Moringa* for?

Food [ ]

Medicine [ ]

Both Food and Medicine [ ]

3. How often do you take *Moringa*?

Daily [ ]

Weekly [ ]

Monthly [ ]

Other (specify) …….

4. For how long have you used *M. Oleifera* (years)?

Below 1year. [ ]

Between 1 - 3 yrs. [ ]

Between 3 - 5 yrs. [ ]

Above 5 yrs. [ ]

5. How did you know of the uses and benefits of *M. Oleifera* ? Through

Family member [ ]

Friend [ ]

Herbalist [ ]

Media [ ]

Health personnel [ ]

Others …….

6. Which part of the *Moringa* do you use as food or medicine or both? Please tick.

	Description of use	
Part	**Food**	**Medicine**
Leaves		
Bark		
Roots/tubers		
Green pods		
Stems		
Dry pods		
Branches		
Seeds		
Flowers		
Whole plant		

7. Where do you source/get your *Moringa* from?

Market [ ]

Own farm [ ]

Friends/Relatives [ ]

Other (specify) …….

8. In which form do you use *Moringa*?

Raw [ ]

Crushed [ ]

Grounded powder [ ]

Other (specify) …….

9. How do you use *Moringa*?

Orally [ ]

Smearing [ ]

Bathing [ ]

Other (specify) …….

10. After using the *Moringa* parts or its products, have you experienced any side effects?

Yes [ ]

No [ ]

If Yes (specify) ……

11. In your opinion, do you think it is appropriate to supplement modern treatment with herbal medicine like *Moringa*?

Yes [ ]

No [ ]

SECTION C: HEALTH SYSTEM LEVEL FACTORS ASSOCIATED WITH THE USE OF *MORINGA OLEIFERA*

1. When did you start using *Moringa?* Was it before or after knowing your HIV status?

   Before [ ]

After [ ]

2. If the answer is after (Q1), then did your status of HIV inform the use of *Moringa*?

Yes [ ]

No [ ]

3. Why do you use both ARVS and *M. Oleifera* at the same time?
   ……
   …….

4. Did you inform your health care provider that you are taking *Moringa*?

Yes [ ]

No [ ]

5. How much do you trust your medical providers to offer you high quality medical care?

   Completely [ ]

   Somewhat [ ]

   Fairly [ ]

   Not at all [ ]

6. Has anybody in the health care system ever exhibited hostility or lack of respect towards you?

   Yes [ ]

   No [ ]

7. Do you experience any challenges getting medical treatment?

   Yes [ ]

   No [ ]

8. If yes, (7) what problems do you face?

   Distance (….Km) [ ]

   Cost of treatment high [ ]

   No drugs in the hospital/HC [ ]

   Drug side effects [ ]

   Health workers unfriendly [ ]

   Long waiting time/delays [ ]

   Others (specify) …….

9. Explain your answer in Q8 above…..…….

……

10. Do you think a problem may arise if you take *Moringa* and HIV drugs at the same time?

Yes [ ]

No [ ]

Don't Know [ ]

11. How would you rate the affordability of *Moringa*?

Easily affordable [ ]

Affordable [ ]

Not affordable [ ]

12. How would you rate the accessibility of *Moringa*?

Easily accessible [ ]

Accessible [ ]

Not accessible [ ]

13. Do you think *Moringa* can be used to cure HIV?
Yes [ ]
No [ ]
14. Do you have any questions or comments about *Moringa* in general?

……

15. Would you like to receive or know more information about *Moringa*?
Yes [ ]
No [ ]

Thanks for participating in this study and for answering the questions.

Appendix VI: Kiswahili version of Questionnaires

Hojaji kwa wagonjwa

Maelezo ya sehemu Unayoishi

Kaunti…….....

Kaunti Ndogo…….…….

Eneo…….

Eneo Ndogo…….....

GPS Location

- Latitude (X.Y0)……..
- Longitude (X.Y0)……
- Altitude (m)……
- Accuracy (m)……..

Tarehe ya mahojiano.....

Jina la mhojaji……...

Jina la mhojiwa/mgonjwa.....

Nambari ya mshiriki

……

SEHEMU A: Demografia ya kijamii

Tafadhali jibu maswali hapo chini kwa kuweka alama ya tick (√) katika sanduku.

1. Jinsia

   Mwanamke [ ]

   Mume [ ]

2. Umri:

   Chini ya miaka 25 [ ]

   Kati ya miaka 26-35 [ ]

   Kati ya miaka 36-45 [ ]

   Kati ya miaka 46 – 55 [ ]

   Zaidi ya miaka 55 [ ]

3. Hali ya ndoa

   Nimeoa/Nimeolewa [ ]

   Sijaoa/olewa [ ]

   Nimetaliki [ ]

Mjane [ ]

4. Kiwango cha Elimu

   Sijasoma [ ]

   Shule ya Msingi [ ]

   Sekondari [ ]

   Kitengo cha mafunzo [ ]

   Chuo kikuu [ ]

5. Kazi yako kuu ni ipi?

   Kilimo [ ]

   Umeajiriwa [ ]

   Biashara [ ]

   Nyingine
   (taja)……

6. Umeishi na virusi vya ukimwi kwa muda gani?

   Chini ya mwaka 1. [ ]

   Kati ya miaka 1 - 3 [ ]

   Kati ya miaka 3 - 5 [ ]

Zaidi ya miaka 5 [ ]

7. Unatumia tiba gani? Na je, una mbadala ya matibabu unayotumia.....

……
……

8. Umekuwa kwenye matibabu kwa muda gani?

Chini ya mwaka 1. [ ]

Kati ya miaka 1 - 3 [ ]

Kati ya miak 3 – 5 [ ]

Zaidi ya miaka 5 [ ]

SEHEMU B: KIWANGO CHA MAAMBUKIZI NA MUUNDO WA MATUMIZI YA MZUNZE (*MORINGA OLEIFERA)*

1. Je, unatumia Mzunze/*Moringa*?

Ndio [ ]

La [ ]

Kama jibu lako ni la, eleza sababu zako…….……

……
……..

(Kama jibu lako ni la kwa ruka maswali mpaka sehemu ya C)

2. Unatumia Mzunze/Moringa kama?

   Chakula [ ]

   Dawa [ ]

   Chakula na Dawa [ ]

3. Ni mara ngapi wewe hutumia Mzunze/Moringa?

   Kila siku [ ]

   Kila wiki [ ]

   Kila mwezi [ ]

   Nyingine
   (taja…….

4. Umetumia Mzunze/moringa kwa muda gani (miaka)?

   Chini ya mwaka 1. [ ]

   Kati ya miaka 1 - 3 [ ]

   Kati ya mika 3 - 5 [ ]

   Zaidi ya miaka 5 [ ]

5. Je, ulijua aje kuhusu matumizi na faida ya Mzunze/Moringa?

Familia [ ]

Rafiki [ ]

Waganga wa kiasili [ ]

Chombo cha habari [ ]

Mfanyakazi wa Afya [ ]

Wengine……
…

6. Je,unatumia sehemu gani ya Mzunze/Moringa kama chakula au dawa ama vyote? Tafadhali weka alama ya tick(√) katika sehemu ifuatayo.

	Maelezo ya matumizi	
Sehemu ya mzunze/Moringa	Chakula	Dawa
Majani		
Maganda		
Mizizi		
Mbegu kijani		
Mashina		
Ganda kavu		
Matawi		
Mbegu		
Maua		
Mti kwa ujumla		

7. Je, unatoa wapi Mzunze/Moringa wako?

Sokoni [ ]

Shamba lako mwenyewe [ ]

Kwa rafiki / Jamaa [ ]

Nyingine
(taja)……..

8. Unatumia Mzunze ukiwa hali gani?

Mbichi [ ]

Umebondwa [ ]

Umesagwa unga [ ]

Nyingine
(taja)……

9. Je unatumia mzunze/Moringa kwa njia gani?

Kwa mdomo [ ]

Kupaka [ ]

Kuogea [ ]

Nyingine
(taja)……..

10. Baada ya kutumia sehemu za Mzunze au bidhaa zake, je umepata madhara yoyote?

    Ndio [ ]

    La [ ]

    Kama Ndiyo
    (taja)…….

11. Kwa maoni yako, je unafikiri ni vyema kuongeza matibabu ya kisasa na dawa za asili kama Mzunze?

    Ndio [ ]

    La [ ]

SEHEMU C: MFUMO WA AFYA UNAOHUSIANA NA MATUMIZI YA MZUNZE/MORINGA

1. Je, ulitumia Mzunze kabla au baada ya kujua hali yako ya kuwa na virusi vya ukimwi?

   Kabla [ ]

   Baada [ ]

2. Kama jibu lako ni baada ya (Swali1), kuna uwezekano kuwa hali yako ya kuwa na virusi vya ukmwi ilichangia matumizi ya Mzunze?

   Ndio [ ]

La [ ]

3. kwa nini unatumia mzunze na dawa za kisasa za ukimwi kwa wakati mmoja?

   …….....
   .....

4. Je, umemfahamisha muhudumu wako wa afya kuwa wewe unatumia Mzunze?

   Ndio [ ]

   La [ ]

5. Uko na imani kiasi gani kwamba muhudumu wako wa afya anakupa hudumu ya hali ya juu ya matibabu?

   Kabisa [ ]

   Kiasi [ ]

   Katikati [ ]

   Sina Imani hata kidogo [ ]

6. Je, kuna yeyote katika mfumo wa huduma za afya na siha bora amewahi kukuonyesha uadui ama madharau?

   Ndio [ ]

   La [ ]

7. Je, umepata matatizo yoyote kupata matibabu?

   Ndio [ ]

   La [ ]

8. Kama jibu lako ni ndio, (swali 8) ni tatizo gani?

   Umbali (....Kilomita) [ ]

   Gharama ya juu ya matibabu [ ]

   Ukosefu wa dawa katika hospitali [ ]

   Kupata madhara baada ya kutumia dawa [ ]

   Wafanyakazi wa afya wenye madharau [ ]

   Kusubiri muda mrefu / ucheleweshaji [ ]

   Matatizo mengine
   (taja)……..

9. Eleza jibu lako katika (swali 9)
   juu…….

   ……
   …….

10. Je, unafikiri kuna tatizo linaweza tokea kwako kwa kutumia Mzunze na dawa za ukimwi kwa wakati mmoja?

Ndio [ ]

La [ ]

Sijui [ ]

11. Unakadiria aje uwezo wa kununua Mzunze?

    Unanunulika kwa urahisi [ ]

    Unanunulika [ ]

    Bei ghali/haununuliki [ ]

12. Unakadiria aje upatikanaji wa Mzunze?

    Unapatikana kwa urahisi/uko kila sehemu [ ]

    Unapatikana [ ]

    Si rahisi Kupatikana [ ]

13. Je, unafikiri Mzunze unaweza kutumika kutibu virusi vya ukimwi?

    Ndio [ ]

    La [ ]

14. Je, una swali lolote au maoni kuhusu Mzunze/Moringa? Maoni yako kwa ujumla ni yapi?

……
……..

15. Je, ungependa kupokea au kujua habari zaidi juu ya Mzunze?

Ndio [ ]

La [ ]

Asante kwa kushiriki katika utafiti huu na kwa kutumia muda wako kujibu maswali haya.

Appendix VII: Key Informant Guide (Herbalists)

Background

Name of herbalist……..

Contacts address……..

Telephone number……..

- County……
- Sub County……
- Location……..
- Sub Location……..

1. How long have you been an herbalist?

……
…….

2. Where did you get knowledge on traditional medicine?

……
……

3. Are you registered or belong to any professional body?

……
…….

If yes which body?

……..

4. Do your patient request to see any registration or affiliation to any professional body?

……
……

5. How many patients do you see daily on average?

……
…….

6. For how long have you used *Moringa* as a medicine?

……
……

7. Where do you source/get your *Moringa* from?

……
……

8. Which part of *Moringa* do you use as medicine?

……
……

9. What types of ailments/diseases do you treat with *Moringa*?

……
……

10. Have you treated patients who are HIV positive with *Moringa*?

Yes [ ]

No [ ]

If the answer is Yes (10) above what ailments did you treat.....

……
……

11. In what form is *Moringa* taken?

……
……

12. How do you decide on the dosage and how long *Moringa* should be taken?

……
……

13. Is *Moringa* taken alone or is it mixed with other medicines?

……
……..

14. How effective has *Moringa* been in treating the ailments/diseases?

When used alone

……

……

…….

When mixed with other

medicines…..……

……

15. What side effects have patients using *Moringa* reported?

……

16. What can you comment about *Moringa*?

……

Thanks for participating in this study.

APPENDIX: VII CTMDR CSC Approval Letter

14th November, 2018

Judith Nkirote,
c/o Dr. Festus Tolo,
CTMDR, KEMRI.

Dear Madam,

RE: CSCP 071: SAFETY AND ANTIVIRAL ACTIVITY OF EXTRACTS FROM *MORINGA OLEIFERA* AND DETERMINANTS OF ITS USE, AMONG HIV POSITIVE PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC AT MIGORI COUNTY REFERRAL HOSPITAL, KENYA.

The CTMDR CSC acknowledges the resubmission of your proposal for review and also notes your revision of the proposal as required in the 141st meeting on 20th September, 2018. The CSC approves it for forwarding to SERU for further review. Please note that this approval does not allow you to start implementing the proposal- this is subject to the final approval by SERU.

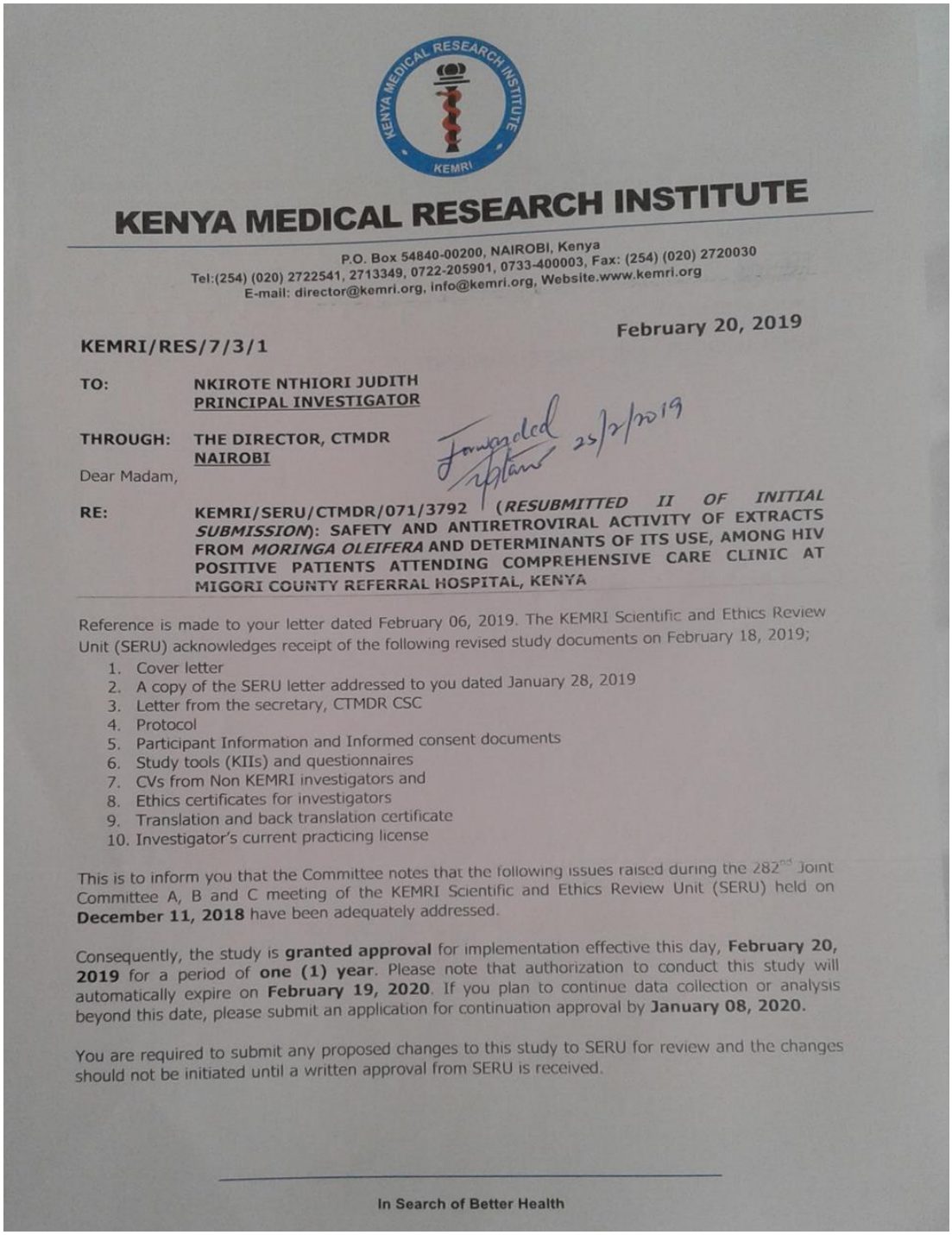
I wish you the best in your work.

Yours sincerely,

Dr James Kuria

CTMDR CSC Secretary

APPENDIX:IX KEMRI Scientific and Ethics Review Unit (SERU) Approval Letter



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel:(254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website.www.kemri.org

KEMRI/RES/7/3/1

February 20, 2019

TO: NKIROTE NTHIORI JUDITH
PRINCIPAL INVESTIGATOR

THROUGH: THE DIRECTOR, CTMDR
NAIROBI

Forwarded 25/2/2019

Dear Madam,

RE: KEMRI/SERU/CTMDR/071/3792 (*RESUBMITTED II OF INITIAL SUBMISSION*): SAFETY AND ANTIRETROVIRAL ACTIVITY OF EXTRACTS FROM *MORINGA OLEIFERA* AND DETERMINANTS OF ITS USE, AMONG HIV POSITIVE PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC AT MIGORI COUNTY REFERRAL HOSPITAL, KENYA

Reference is made to your letter dated February 06, 2019. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the following revised study documents on February 18, 2019;

1. Cover letter
2. A copy of the SERU letter addressed to you dated January 28, 2019
3. Letter from the secretary, CTMDR CSC
4. Protocol
5. Participant Information and Informed consent documents
6. Study tools (KIIs) and questionnaires
7. CVs from Non KEMRI investigators and
8. Ethics certificates for investigators
9. Translation and back translation certificate
10. Investigator's current practicing license

This is to inform you that the Committee notes that the following issues raised during the 282nd Joint Committee A, B and C meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **December 11, 2018** have been adequately addressed.

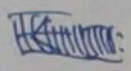
Consequently, the study is **granted approval** for implementation effective this day, **February 20, 2019** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **February 19, 2020**. If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval by **January 08, 2020.**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until a written approval from SERU is received.

In Search of Better Health

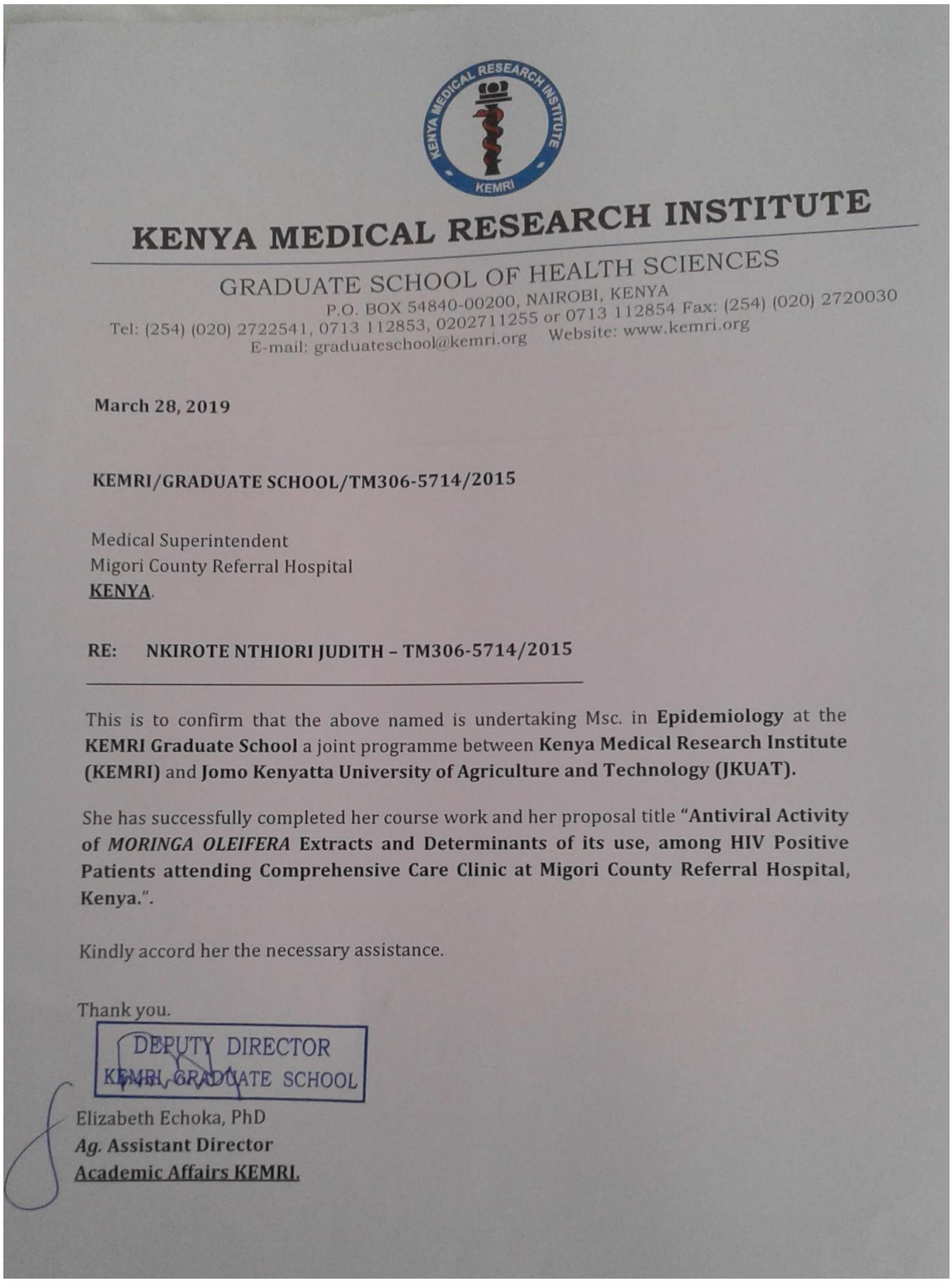
Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advice SERU when the study is completed or discontinued.

Yours faithfully,



ENOCK KEBENEI
THE ACTING HEAD
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

APPENDIX: X KEMRI Graduate School of Health Sciences Permission Letter



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E-mail: graduateschool@kemri.org Website: www.kemri.org

March 28, 2019

KEMRI/GRADUATE SCHOOL/TM306-5714/2015

Medical Superintendent
Migori County Referral Hospital
KENYA.

RE: NKIROTE NTHIORI JUDITH – TM306-5714/2015

This is to confirm that the above named is undertaking Msc. in **Epidemiology** at the **KEMRI Graduate School** a joint programme between **Kenya Medical Research Institute (KEMRI)** and **Jomo Kenyatta University of Agriculture and Technology (JKUAT).**

She has successfully completed her course work and her proposal title **"Antiviral Activity of *MORINGA OLEIFERA* Extracts and Determinants of its use, among HIV Positive Patients attending Comprehensive Care Clinic at Migori County Referral Hospital, Kenya.".**

Kindly accord her the necessary assistance.

Thank you.

DEPUTY DIRECTOR
KEMRI, GRADUATE SCHOOL

Elizabeth Echoka, PhD
***Ag.* Assistant Director**
Academic Affairs KEMRI.

Appendix XI: Freshly collected *M. Oleifera* leaves from Migori County

Appendix XII: Aqueous extract of *M. Oleifera* in a laboratory freeze drier

Appendix 13: Publication

Journal of
Health, Medicine and Nursing
(JHMN)

DETERMINANTS OF *MORINGA OLEIFERA* UTILIZATION AMONG HIV POSITIVE PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC AT MIGORI COUNTY REFERRAL HOSPITAL, KENYA

Nkirote N. Judith, Francis K. Njonge, Gideon Mutie Kikuvi and Festus M. Tolo

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