ANTI-HERPES SIMPLEX AND ANTIOXIDANT VALUE OF SELECTED MEDICINAL PLANTS CITED FOR MANAGEMENT OF HIV CONDITIONS IN WESTERN KENYA

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Anti-herpes simplex and antioxidant value of selected medicinal plants cited for management of HIV conditions in Western Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Benard Radol, to my wife Immaculate Omondi, and to my children Julie Radol, Petronila Adhiambo and Paschal Baylon

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ABBREVIATIONS AND ACRONYMS

AIDS: Acquired Immune deficiency syndrome

ART: Antiretroviral therapy

ARVs: Anti-retrovirals

CC_{50}: extract concentration killing 50% of cells

CHEWs: Community Health extension workers

CHU: Community Health Unit

CHWs: Community Health Workers

CPE: Cytopathic effect

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl

EBV: Epstein - Barr virus

EPTT: End point titration technique

GOK: Government of Kenya

HCMV: Human cytomegalovirus

HIV: Human Immune deficiency Virus

***HSV*-1**: Herpes Simplex type 1

IBC: International Bioethics Committee

IC_{50}: extract concentration inhibiting 50% of virus activity

Kgbwt: Kilogram body weight

MCPC: Maximum cell protection concentration

MEM: Minimum Essential Media

MNC: Maximum nontoxic extract concentration

MTT: Methyl thiazole tetrazolium base for 3 – (4, 5-Dimethylthiazole -2-y) -2, 5-diphenyltetra-zolium bromide salt. A substrate for mitochondria enzyme

NACC: National AIDS Control Council

***OD*:** Optical density

PLWHIV: People living with HIV

***RF*:** Titer reduction factor

Rsa_{50}: Concentration of sample reducing the absorbance of DPPH control by *50%.*

$TCID_{50}$: Tissue Culture infection doze that kills 50% of cell replicates

TI: Therapeutic index

UNAIDS: United Nations on AIDS

UNESCO: United Nations Educational, Scientific and Cultural Organization

VZV: Varicella zoster Virus

WHO: World Health Organization

DEFINITION OF TERMS

Cell control (cc): Wells in the 96 well plate containing only Vero cells and growth or maintenance media.

Cytotoxicity control (CTC): Wells in the 96 well plate designed to detect cytotoxicity. The wells contain Vero cells and experimental extract at concentrations prepared for antiviral experiments.

DPPH control: Mixture of DPPH and methanol.

Drug control (DC): Wells in 96 well plate containing acyclovir.

ODccb (Optical density of cell control blank): Optical density of wells in a 96 well plate that contained growth or maintenance media but no cells.

ODtb (Optical density of test blank): Optical density of wells in 96 well plate that contained test extract but no cells.

ODvcb (Optical density of virus control blank): Optical density of wells in a 96 well plate that contained VC but no cells.

Percentage cell protection: The ratio of MTT metabolism by cells exposed to virus and extract (ODt - ODtb) to metabolism of MTT by cells not exposed to virus and extract (ODcc - ODccb) multiplied by 100. ([ODt – ODtb] – [ODvc – ODvcb]/ [ODcc – ODccb] – [ODvc –ODvcb] x 100).

Percentage Cell viability: The ratio of MTT metabolism by cells exposed to extract (ODt – ODtb) to metabolism of MTT by cell not exposed

to extract (ODcc – ODccb) multiplied by 100. ([ODt – ODtb]/ [ODcc – ODccb] x 100)

Reduction factor (RF): Ratio of VC titer to the titer obtained in the presence of plant extract in the EPTT experiments.

Time of addition assays: Time in relation to presence or absence of extracts during adsorption events. Thus whether the extract is present only during adsorption (Adsorption assay), only after adsorption (Post adsorption assay) or before and after adsorption (Throughout assay).

Virus control (VC): Aliquots of virus stock whose titer was determined to be 10^7 by EPTT

Virus control (VC): Quantity of the virus obtained used in the experiments, determined by $TCID_{50}$

ABSTRACT

Medicinal plant products are common medication therapy in traditional healing practices. However, the product plant species and their health value are often not scientifically authenticated. Given multiple challenges of unmet needs in care and management of HIV conditions, People living with HIV (PLWHIV) are prone to utilization of the plants with consequent risk of lack of expected benefits and at worst, fatality. The aim of this study was to identify plants used by PLWHIV in Hamuyundi and Mukhwa communities in Western Kenya and to determine cytotoxicity, anti-Herpes activity, antioxidant value and to phytochemical groups of selected plant species. The identification of plants was carried out by qualitative ethnobotanical survey, using Community Health Workers as key informants. Plant species were collected from the field and botanically identified at the University of Nairobi herbarium. By literature search, secondary metabolites and pharmacological activity reported in previous studies on the plants were identified. Water extract of eight selected medicinal plants were tested for cytotoxicity and anti-herpes activity using Vero cell and Herpes simplex type 1 (HSV-1). The effect of extract on cell metabolism of tetrazolium dye (MTT) was measured to determine cytotoxicity. The anti-herpes activity was determined by measuring metabolism of MTT by cells exposed to HSV-1 in presence of extracts. The antioxidant value was determined by measuring reduction of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) by extracts of selected plant species. The extracts were then tested for the presence of flavonoids, terpenoids, alkaloids, saponins and phenols qualitative procedures. At Hamuyundi community, a total of 36 plant species from 26 families were identified while in Mukhwa, 29 species distributed in 17 families were identified. From literature, it was established that a third of plant species identified as medicinal in both Hamuyundi and Mukhwa have some of their pharmaceutical activity and or phytochemical contents identified. Except for *Garcinia buchananii* (Baker) (Bark) and *Croton macrostachys* (Hoechst) (Bark) that gave maximum nontoxic concentration (MNC) of 40 µg/mL each, all the other species; *Tithonia diversifolia* (Hemsl.) Gray (Roots), *Schkuria piñata* (Lam) O.Ktze (Leaves), *Entada abyssinica* (A. Rich) (bark), *Vernonia adoensis* Walp (roots), *Plumeria alba* L. (leaves), *Caesalpinia decapetala* (Roth. I. Alston) (root) gave MNC of 20 µg/mL and below. The extract concentration that was cytotoxic to 50% of the cells (CC_{50}) of *A. abyssinica*, *G. buchananii* and *C. macrostachys* were above 500µg/mL while CC_{50} of *C. decapetala* (roots), *V. adoensis* (roots) *T. diversifolia* (roots), *P. alba* (leaves) and *S. pinata* (leaves) were 500, 470, 460, 120 and 90 µg/mL respectively. The best anti-herpes activity was obtained from *G. buchanii* (stem bark), giving an extract concentration inhibiting 50% of virus activity (IC_{50}) at 20µg/mL) and *C. decapetala* (whole root) giving IC_{50} at 80µg/mL. Therapeutic index (TI) of *G. buchanii* was > 25 and that of *C. decapetala* was > 6. *Garcinia buchananii* extract was active against HSV-1 infection in mice at 500µg/mL, when compared with negative control by independent t-test, significantly

delaying onset of symptoms ($p = 0.006$), progression of symptoms, ($p= 0.005$) and day of death ($p =0.007$). The most potent antioxidant activity was given by *E. abyssinica*, *G. buchananii* and *C. decapetala*, giving sample concentration reducing DPPH by 50% (Rsa_{50}) of 20, 10 and 50µg/mL respectively. Major phytochemical groups detected in the selected plants were Alkaloids in *S. pinata*, terpenoids in *E. abyssinica*, flavonoids and phenols in *G. buchananii*. Results show that there is scientific basis for use of many plants in Hamuyundi and Mukhwa communities in Western Kenya. Further investigation is required to isolate and characterize compounds responsible for activity found in *G. buchananii* and *C. decapetala*

CHAPTER ONE

INTRODUCTION

1.1 Background information

Ethno-medical practice is a dynamic cultural adaptation associated with health problems that emerge from time to time (Eworinde and Erinoso, 2012). Ineffective remedies, the emerging and re-emerging diseases, the high cost of care and gaps in health care systems are some of the current issues in the health sector that threaten community confidence in conventional care. Viral infections are a major source of such health problems, since many of the diseases associated with the infections lacks effective cure. The control or eradication of viral diseases such as polio, chicken pox and rubella have mainly relied on vaccination, with very little success in curative products. The success of vaccination relies on host immunity and although host immunity frequently resolve many of the viral infections without exogenous intervention, diseases that compromise immunity itself or those that emerge because of lowered immunity such as Herpes Simplex Virus (HSV) can hardly be effectively resolved by vaccination or host immunity alone. Herpes Simplex Virus (HSV) poses major risk to People living with Human Immune Deficiency Virus (PLWHIV) due to extensive spread and involvement of vital organs such as liver and brain (Richman et al., 1987). There two types of HSV, the HSV type 1 and 2, which differ on genetic variation but have overlapping epidemiology and clinical manifestation. The HSV type 1 is mainly transmitted through contact with mucosal oral secretions while HSV type 2 is mainly transmitted through contact with genital secretions. Both type 1 and 2 can infect oral and genital surface (Looker et al., 2015). According to WHO report of 2015, among the PLWHIV, those who were co-infected with HSV in different populations ranged between 60 -90%. The WHO report also indicated that the risk of acquiring new HIV infection increased by approximately threefold among people with HSV infection, and in addition, people infected with both

HIV and HSV were more likely to spread HIV to others (WHO, 2016). Neonatal herpes is the acquired infection of HSV by newborns from a mother who experience active secretion of the virus at around the time of birth. It's a devastating illness with high mortality and morbidity and can be caused by either type 1 or type 2 (Katherine 2015). The none-HIV immune depressing conditions such as patients receiving chemo therapy suffer same serious HSV infection as HIV patients (Richman et al., 1987). However, due to the magnitude of those who experience it due to HIV, it remains mainly a problem of PLWHIV. Compounds based on the mode of action of nucleosides such acyclovir and valacyclovir are available for treatment but are facing resistance mainly from HSV-1 ((Richman et al., 1987). One of the viable approaches to the search of alternatives is the medicinal plants known to patients, patient care takers or traditional healers. The latter are specialist in ethno-medical practice but as suggested by Eworinde and Erinoso, ethno – medical cures are generally known by members of individual community as a matter of evolutionary response to health challenges (Eworinde and Erinoso, 2012). Traditional medicine men represent supernatural healing powers rather than sole custodian of knowledge of the curative products. The study used Community Health Workers (CHW) as key informants in the identification of medicinal plants used by PLWHIV. The CHW are the first formal contact person with PLWHIV at household level as described in the Ministry of Health (MOH) document for Community Health Strategy (GOK-MOH, 2012)

The use of medicinal plants by People living with HIV (PLWHIV) has been reported by previous studies (Hughes et al., 2012; Chisembu and Hedimbi, 2010a.). The studies revealed that PLWHIV used herbal medicine to overcome opportunistic infections attributed to Human Immune Deficiency Virus (HIV) and side effects of Anti-retroviral drugs (ARVs). Other issues that have been reported to account for the use of herbal medicine are; resistant HIV strains, inhibited access to treatment and lack of ARV adherence (Chisembu and Hedimbi 2010a; Manish et al., 2010, DiazGranados, 2010).

However, as noted by the International Bioethics Committee (IBC) of United Nations Educational, Scientific and Cultural Organization (UNESCO), the issues of effectiveness and quality of traditional medicines remain key concerns (UNESCO, 2013).

1.2 Statement of the problem

From the WHO (2016) statistics on HSV, it's evident that HSV infection is a major problem of PLWHIV. The condition is considered as a 4th stage defining feature of HIV disease and therefore a determinant of quality of life of PLWHIV. The current anti-HSV drugs are facing resistance from the virus (Chien – Yu et al., 2001*)*. On the other aspect, a relationship between HIV disease and antioxidants has previously been established by previous studies (Baum et al., 1995; Semba et al., 1994; Greenberg et al., 1997; Nduati et al., 1995; John et al., 1997; Mostad et al., 1997; Tang et al., 1997). All the authors concurred that individuals who were at an advanced HIV disease were also antioxidant deficient. The relationship between investigation of plant cure for HSV and antioxidant value is considered on the basis of plants being a major source of compounds associated with antioxidant activity and known to complement immune functions by protecting cells against pro-oxidant damage (Rui, 2004; Gosvenor and Smoli, 2002). Any relief of disease symptoms resulting from use of medicinal plants would therefore require an investigation into the role of antioxidant content.

1.3 Justification

The key ideological rationale informing this study is the view expressed by Eworinde and Erinoso, about the dynamism of ethno-medical practices being influenced by health challenges interacting with cultural and environment factors, leading to unique lessons learnt (Eworinde and Erinoso 2012). In terms of plant species used as medicine, it is envisaged that the lessons learnt by the communities can be translated to scientific information that can benefit the wider global community.

The medicinal plants that portend harmful effect to the health of consumers are identified in this study and will be useful for educational intervention. Previous reports have indicated that there has been unregulated utilization of herbal medicines by Kenyan communities with risks of lack of intended benefits or adverse effects (Kigen et al., 2013; Geissler et al., 2000; Mamothena 2014).

This study provides a baseline data for herbal medicines in the broad scope of conditions associated with HIV and can stimulate further researches that are specific to each condition. One of the obstacles to herbal medicine studies is the lack of database of medicinal plants (Kigen et al., 2013). The findings on plant medicines against HSV-1 as a specific HIV condition provides hope for innovation of anti-herpes compound with different mode of activity, away from the current ones, such as acyclovir which are facing resistance

The purpose of the study was therefore to identify the species of medicinal plants used in the management of HIV conditions, investigate the phytochemical content and pharmacological activity and to select and evaluate them for anti-HSV and antioxidant value.

1.4 RESEARCH QUESTIONS

1. What species of medicinal plants are used in the management of HIV conditions in Western Kenya?
2. What has been reported on medicinal plants cited for management of HIV conditions in Western Kenya?
3. Are there prospects of finding anti-herpes remedy among the plants identified?
4. What is the antioxidant activity of the plants selected for anti-herpes determination?

5. Are the phytochemical groups known for antimicrobial and or antioxidant activity present in plants used for treatment of herpes conditions?

1.5 Objectives

1.5.1 General Objective

To identify medicinal plants used for HIV conditions in Western Kenya and investigate selected medicinal plants for activity against Herpes simplex virus (HSV) and antioxidant value.

1.5.2. Specific objectives

1. To identify medicinal plants used in the care and management of HIV disease in Kakamega and Vihiga counties in Kenya using Community Health Workers as key informants.
2. To identify what has been reported in plant species cited as medicine in Kakamega and Vihiga counties.
3. To determine cytotoxicity and anti HSV activity of plants used for treatment of herpes infections through experiments.
4. To determine antioxidant activity of plants used for anti- herpes treatment through chemical measurements
5. To screen selected plants for common antimicrobial phytochemical groups by chemical detections

CHAPTER TWO

LITERATURE REVIEW

2.1 The Practice of Traditional Medicine

The world Health Organization estimates that about 80% of population in some developing countries use traditional medicine (TM) for primary health care, and that herbal medicine in particular is a major source of revenue for those involved in production and retail (WHO, 2002). The integration of TM in mainstream health care has been successful in many Asian nations and in the US, the department of food and drug administration has put in place a mechanism of regulating quality of the medicines (Calixto, 2000). Kenya is still stuck on the historical colonial regime about TM, when the Medical Practitioners and Dentist board under the colonial government granted some tolerance to TM under cultural practice, rather than a health practice (Harrington 2016). The evangelical churches considered the practice evil, as was the stand of missionaries who opposed the practice as evil (Harrington 2016). This historical view of TM has largely influenced the philosophy of medical training and practice, where the role of TM is hardly recognized. The attitude of health professionals against TM appear to have softened since the WHO's Alma-Ata declaration of 1978 on TM's role in primary health care (WHO, 2002). However, against expectations, not much has changed despite the Kenya's 2010 constitution provided a basis for more intensive and wide ranging engagement with practitioners of TM (Harrington 2016). Enabling parliamentary legislation and policies are yet to be formulated and implemented to provide the public with benefits of TM and address issues regarding ineffective and adverse effects of herbal medicines.

The WHO document on standardization of methodologies for assessing TM, differentiates herbal medicine from other forms of TM and recognize herbal medicines as having characteristics similar to biomedical pharmaceutics (WHO 2000). A review of

herbal medicine practice by Andrew *et al* drew similar observations on herbal medicines, noting that both conventional and herbal medicines interacts with organism cells to modify their functions (Andrew et al., 2001). The implication of these observations is that the standards of monitoring and evaluation of both medicines ought to be similar.

A common view among herbal medicine users is that the medicines have been used since time immemorial, is natural and safe. The WHO delegates at the 1978 Almata conference noted the holistic characteristic of TM care and recommended its integration into biomedical for effective delivery of primary health care (WHO, 2002). Andrew *et al* described the diagnostic practices of TM practitioners as involving physical examination and wide scope of inquiry about patient's biological and social functions such as elimination, sleeping, eating and concerns about peace of mind (Andrew *et a*l., 2001). A decision about how to restore the functions may include calming down the patient psychologically and dispensing a cocktail of herbal medication. The strength of the practice is manifest in emotional involvement with the patient, rigorous follow-up and persistent partnership in wellbeing of the client. It can be deduced that this kind of bio-social approach to health care would well be suited for chronic conditions such as HIV and cancer.

Herbalists have explained that the rationale behind the use of crude plant extracts or cocktail of extracts is the working together of ingredients in synergy and that the effect of the whole herb or mixtures is better than the sum total of individual constituents. Further it's claimed that toxicity is reduced when whole herb is used instead of purified constituents, a phenomenon that is analogous to buffering effect (Andrew et al., 2001). Such claims may be true but cannot be assumed for all the herbal medicines.

2.2 Challenges in the practice of traditional medicine

The UNESCO report on ethical issues in TM indicated violation of autonomy and responsibility by practitioners and exercising the same on the part of the patient

(UNESCO, 2013). This was emphasized on the aspect of spiritual healing where the patient ability to make informed consent was limited. Unhygienic handling of herbal medicine materials and uncontrolled entry of individuals claiming skills in herbal medicine practice were pointed out in the report. The report observed that assessment of efficacy and safety was made difficult by complex mixtures of medicines and varied naming conventions of the medicinal plants. In the report, some traditional medicine practitioners were cited as involved in persuading their clients not to seek conventional treatment even when situation was acute and required urgent modern medicine intervention. As a remedy, the report suggested involvement of community in determining genuine practitioners, researches that focuses on safety and efficacy, improved system of regulation, registration of practitioners, quality assurance programmes, controlled harvesting of medicinal materials, and improved coexistence between TM and health professionals. From this report, it is clear that value validation and safety consideration are key aspects of assessing the worth of any TM material.

There has been reports of rapid progressive interstial renal fibrosis on women who took Chinese herbs, an association of gastrointestinal disturbances with *Prunus africana* treatment, acute renal failure with *Securidaca longependunculata*, convulsions and incoordination movement with *Catharanthus roseus* (Benard and Clove, 2014; Andrew et al., 2001). Laboratory investigations have established that plant compounds comprising alkaloids, cardiac glycoside and flavonoids are responsible for toxicity in cells and in animal models (Peter, 1995). Yet the same phytochemical groups are associated with antiviral activities (Hudson, 1990). These reports show that herbal medicine cannot be generalized as safe. The magnitude of adverse response to administration of herbal medicines may be high but difficult to report, considering the secretive nature of TM practitioners' engagement with clients. When concomitantly used with conventional drugs, some herbal medicines have been reported to produce adverse drug-herb interaction and some have potential to modify the action of effective conventional drug. Examples of adverse-herb interactions with conventional medicine

include; garlic interaction with Warfarin Sodium, producing altered bleeding time, *Echinacea* reaction with anabolic steroids, producing hepatoxicity, *ginseng* reaction with phenelzine sulphate producing headache and maniac episodes and St. John's Wort compromising effect of ARVs (Andrew et al, 2001). These examples are cited in developed countries with effective monitoring and reporting of adverse drug reactions. With scarce health resource in many development countries, the health sector may hardly gather data for basic ADRs of conventional medicine.

2.3 Use of complementary and alternative medicines in HIV Care

The use of complementary and alternative medicine in HIV care has been associated with lack of or sub optimal adherence to anti-retrovirals (ARVs), adverse drug-herb interactions, late or lack of access to evidence based conventional treatments and adverse health outcomes (Hsiao et al., 2003; Owen and Lweith, 2004; Dhalaa et al., 2006). Some of the predisposing factors to the use of herbal medicines are reported to be; psychological distress, adverse effect of conventional medicine, unsatisfactory emotional support, cost of traveling to health facility, belief that herbal medicine provides better cure, high cost of conventional care, non-availability of conventional care, heightened awareness regarding availability of herbal therapies, uncontrolled disease symptoms, chronic conditions and conditions whose cure is not available (Dhalaa et al., 2006; Joseph et al., 2007; Metcalfe et al., 2010). Research indicates that many patients who use complementary and alternative treatments do not inform conventional health service providers (Hsiao et al., 2003; Hassan, 2010). The implication of none-disclosure of the use of herbal medicines to health workers is the risk of undetectable adverse effects of the herb itself or compromising effectiveness of conventional medicine

2.4 The role Traditional medicine in HIV management and research

Collaboration with traditional healers has proved effective in increasing access to HIV testing, prevention and care (UNAID, 2000). The UNAID review report cites useful experiences based on 8 projects in sub-Saharan African countries;

In Botswana the Ministry of Health/ National AIDS programme for traditional healers implemented the Botswana Dingaka AIDS Awareness and Training Project. The project objective was to train traditional healers as trainers who would pass AIDS information on to other traditional healers in selected pilot areas and promote cooperation and collaboration between traditional and biomedical health services. An independent assessment of the Botswana Dingaka AIDS awareness and Training project found that 72% of the traditional healers reported to have changed something in their practice in relation to the new information on AIDS and 80% reported to have recommended condom to their patients, while 31 out of 32 had referred patients to clinics or to hospitals. Out of 19 nurses interviewed, 17 had referred patients to traditional healers but only 7% of medical doctors reported to have done the same. The second assessment that took place one year later showed lower findings of participation with only 3 out of 12 healers reporting to have disseminated information learnt to the community. These findings indicate that there are opportunities for involving traditional healers in HIV programmes but the strategies must be sustainable and be followed up. The findings also show that some advocacy might be required to bring on board all health workers to support such collaboration. Thus attitude problem of health workers may occasionally be an issue to overcome in such endeavors.

In a bid to increase traditional healers' capacity to deliver preventive messages, provide support to PLHIV, and modify their own risk behavior, the Central African Republic conducted 36 hours of STD/AIDS information training on community education to 103 traditional healers. The assessment results showed that traditional healers' knowledge had significantly improved regarding: the role of STD in increasing risk of HIV, condom

protection against HIV, the causes of genital discharge and ulcers, STD complications and the mode of HIV transmission and prevention. However, the objective of supporting persons living with HIV/AIDS and changing healers' practices were not measured in detail although 76% reported to have integrated partner referral into their STD treatments.

Although the initiative by Guinea's Ministry of Public health and its department of traditional medicine did not proceed to conclusion due to lack of funding, it made useful suggestions about collaboration with traditional healers. It was suggested that educational messages should be specifically designed to reinforce, and not contradict the traditional concept of disease and illness among the healers (e.g., using the same names of disease, which helps to gain interest and trust of traditional healers). In one district of the country, it was reported by AIDS office that traditional healers working in collaboration with AID programme were able to: carry out health education, promotion and distribution of condom, treatment of opportunistic infections, early referral, and participation in research. The district did significant research and documentation of plants used in STD and AIDS treatment by traditional healers.

In Malawi a baseline survey was conducted among 89 healers regarding their knowledge, attitude, belief and practices surrounding AIDS. The results were used to design a curriculum for one day workshop that was conducted in 14 sites focusing on community education and condom distribution. Healers were selected through recommendation from community leaders to participate in training sessions. An evaluation conducted six months later on 61 randomly selected healers showed that 89% had distributed condoms. Those who reported to have combined patient education and condom distribution managed to attend to more clients than those who issued condoms on demand, implying that knowledge of client and enthusiasm of the health provider made a positive difference in project objective. It was not however clear what determined the enthusiasm on some traditional healers and not others. When the

condoms ran out, the healers did not seek to replenish the stocks from the health center implying that sustenance strategy was not put in place.

In the South African case participation of traditional healers was desired to tame the rising incidence of HIV. The approach to recruitment and training was quite remarkable in terms of the level of involvement of the traditional healers. The first generation of healers were selected by the project team and trained but the second group was selected and trained by the first generation of trained healers. The cycle was repeated with each generation of trained healers selecting and training the subsequent group under the supervision of the project team until the 27,000 target was reached. On evaluation, it was found that healers had retained correct information that they used to educate their clients. The generation trained by peer healer appeared to be more motivated and effective than the first generation trained by biomedical trainers. This experience provides important lessons about how genuine healers can be accessed and motivated to participate in a similar health intervention in the absence of a formal criteria of identifying genuine healers. The report was however not clear on the parameters used to measure effectiveness or motivation.

The report on Uganda's programme of traditional and modern health practitioners together against AIDS (THETA) provide lessons on how collaboration with traditional healers can be used to enhance opportunity for research and learning for health workers. Apart from the traditional healers' participation in community education and counseling, they volunteered their treatment materials for evaluation and helped set up community resource center. The resource center is reported to contain a library of traditional medicines and produces health information that are important in the care and management of HIV.

Through collaboration with traditional healers, the Ministry of Health of the Republic of Tanzania managed to test 1,600 clients for HIV, being referred by traditional healers.

The healers made 237 home visits to PLWHIV and 5,400 clients received counseling services from the Tanga Working group (TAWG), a collaborating agency

The Zambian report is similar with the other African countries with regards to client education objectives and health promotion achievements. There was increased knowledge and better practices by traditional healers. Many healers discussed HIV and STD prevention, HIV testing, condom use and care of PLWHIV with their clients.

These case series of traditional medicine collaboration show that, if well trained, the traditional healers can be useful change agents in disease prevention education messages, treatment literacy and care of PLHIV. With a well-designed healers education emphasizing the value of their client's health over profit, coupled with little incentives if possible, TMP can be used to increase access to ART, adherence and be involved in drug-herb risk management.

2.5 Use of herbal medicine by Kenyan population

Conventional medicine provision was reported to cover only 30% of Kenyans and that two thirds of Kenyans depended on TM for primary health care, especially in rural areas (NCAPD, 2008). The challenges responsible for this scenario was reported to include; lack of drugs, shortage of health professionals, holistic value attached to TM, cultural appropriateness of TM and less expense of TM compared to conventional medicine (NCAPD, 2008). The popularity of TM has also been associated with availability of TM among communities living in areas where indigenous plants are still available such Kakamega forest (NCAPD, 2008).

In an interview of PLWHIV at Mfangano Island, Nagata *et al* established that 70.1% of PLWHIV had used herbal medicines after HIV diagnosis (Nagata et al., 2011). The medicines had been used for symptoms related to opportunistic infections in HIV. The study recommended a collaboration between herbalists and biomedical scientists, noting the importance of understanding pharmacological, toxicological and the constituent

ingredients. The plant medicines identified were; *Azadiracta indica* A. Juss, *Carissa edulis* (Forssk.) Vahl and *Ximenia americana* L (Nagata et al, 2011). Kenyans are reported to prefer hospitals when the cost of health care is affordable and seek care at clinics when hospitals are too far (Lambert *et al*, 2011). Traditional herbal medicine is also sought, but when inadequate care is not too dangerous (Lambert et al, 2011). The assertion by Lambert *et al* suggests that Kenyans are aware of the risks of lack of effectiveness and safety assurance of using herbal medicine.

Although the use of herbal medicine is associated with long distance to a health facility and a rural setting, findings by Mamothena among pregnant women in Nairobi, living with a radius of 5 Kilometers to a health facility suggests other needs in herbal medicine (Mamothena, 2014). The study reported that 12% had used herbal medicine, only 12.5% of the users disclosed the use to health care professional and of the users, 20% combined herbal medicine with conventional medicine. The risk of using herbal medicine by Kenyans is not limited to lack of disclosure to health professionals, an equally dangerous finding was lack of adherence to Tuberculosis (TB) treatment due to use of herbal medicine (Muture ***et al***, 2011).

2.6 Community Health Workers' as informants on community use of traditional medicine

Internationally, CHW are the key link persons between a particular community and the health workers on education and treatment for variety of diseases and public health issues (US department of health, http://www.diversityrx.org/HTML/RCPROJ_D.htm.) According to a report by Utah Lehman and David Sanders for WHO there are broad trends on the selection of CHW, but they are comprised of men, women, old, young who may be literate or illiterate but whose role is to respond to local societal and cultural norms and customs to ensure acceptance and ownership of health programmes (WHO, 2007). The effectiveness of CHW in the management of HIV/AIDS in the community and specifically among PLWHIV was first noted in Uganda at the initial phase of

epidemic (Green et al, 2002). In Kenya, the role of CHWs and the concept of home based care (HBC) for PLWHIV was originally adopted in Western Kenya where CHWs would provide basic health care to individual persons with HIV, and households with PLWHIV. The activities of HBC include visiting clients in their homes, attending Ministry of health sponsored trainings, initiating and supervising income generating activities for supporting HBC work such as cultivating and processing of medicinal plants. To become a CHW, one is nominated by a community- a process that takes place at a chief's meeting (Becky and Sunil, 2004). Given the close relationship between the CHWs and the PLWHIV, including their households, and being members of the particular community themselves, it is reasonable to expect them to be aware of ethno-medical practices of the PLWHIV and the community.

2.7 Limitations of conventional HIV management

Several reports on HIV services acknowledge problems in management and care of HIV diseases. The identifies PLWHIV who are above 50 years, comprising about 4.2 million as being affected by lack of access to care, and suggesting that this could be due to long distance covered to care centers (UNAID, 2014). The report further indicates that 3 out 5 people who require ART do not access it (Chisembu and Hedimbi, 2010a). The side effects of ART treatments is now causing new form of stigma associated with lipodystrophy (Chisembu and Hedimbi, 2010a). Lipodystrophy is characterized by loss of fat from one region of the body and accumulation in another region of the body. This has been reported to be the cause of thin facial pads, thin arms and legs, pot-bellies, or 'buffalo humps (Chisembu and Hedimbi, 2010a). Other than effects of ARTs, their short half-lives require frequent dosages, which becomes a burden to PLWHIV. The virus evades the antiviral compounds by residing in drug inaccessible sites, leading to requirement of lifetime compliance. In its 2013 report, The United Nations on AIDS (UNAID) reported dwindling donor support for the cost of ART, indication threat to sustainability of care (UNAID, 2014). This scenario in developing countries had been

previously reported by Chou and Huffman, 2005 and Hawkins (2006) and D'Arminiomonforte (2000).

2.8 Health Conditions associated with HIV

The WHO case definition of HIV for sero surveillance and revised clinical staging and immunological classification of HIV-related diseases in adults and children provides a list of diseases and health problems associated with HIV (WHO, 2007). According to the WHO document, the clinical events which must be confirmed by an HIV test are categorized in 4 stages as shown in the table 2.0 for adults and adolescents and table 2.1 for children

Table 2.1: WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection

Clinical stage 1
Asymptomatic persistent generalized lymphadenopathy
Clinical stage 2
Un explained persistent hepatosplenomegaly
Papular pruritic eruptions
Fungal nail infection
Angular chelitis
Lineal gingival erythema
Excessive molluscum contagiosum
Unexplained persistent parotid enlargement
Herpes zoster
Recurrent or chronic upper respiratory tract infections (Otitis media, otorrhoea, sinusitis or tonsillitis
Clinical stage 3
Un explained severe weight loss (> 10% of presumed or measured body weight)
Unexplained chronic diarrhea for longer than one month
Unexplained persistent fever 9 above 37.6^{o}C intermittent or constant for longer than one month)
Persistent oral candidiasis
Oral leukoplakia
Pulmonary tuberculosis (Current)
Severe bacterial infection (such as pneumonia, emphysema, pyomiosistis, bone or joint infection, meningitis or bacteremia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Unexplained anaemia (< 8 g/dl), neutropenia (< 0.5 x 10^9per liter)
Or chronic thrombocytopenia (<50 x 10^9per liter)
Clinical stage 4
HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (oralabial, genital or anorectal of more than one month duration or visceral at any site
Oesophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)
Extra pulmonary tuberculosis
Kaposi's sarcoma
Cytomegalovirus infection (retinitis, or infection of the organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extra pulmonary cryptococcosis including meningitis
Disseminated non-tuberculosis mycobacterial infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis 9 with diarrhea)
Chronic isosporiasis
Disseminated mycosis (coccidiomycosis or histoplasmosis)
Recurrent non-typhoid Salmonella bacteremia
Lymphoma (Cerebral or B-cell non Hodgkin) or other solid HIV associated tumor
Invasive cervical carcinomab
Atypical disseminated leishmaniosis
Symptomatic HIV –associated nephropathy or symptomatic HIV- related cardiomyopathy
Table 2.2: WHO clinical staging of HIV/AIDS for children with confirmed HIV infection
Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy
Clinical stage 2
Unexplained persistent hepatosplenomegaly
Popular pruritic eruptions
Fungal nail infection
Angular chelitis
Lineal gingival erythema
Extensive wart virus infection
Extensive molluscum contagiosum
Recurrent oral ulcerations
Unexplained persistent parotid enlargement
Herpes zoster
Recurrent or chronic upper respiratory tract infections 9 otitis media, otorrhoea, or

tonsillitis)
Clinical stage 3 Unexplained moderate malnutrition or wasting not adequately responding to standard therapy Unexplained persistent diarrhea (14 days or more) Unexplained persistent fever (above 37.5^{o}C intermittent or constant for longer than one month) Persistent oral candidiasis (after 6 – 8 weeks of life) Oral hairy leukoplakia Acute necrotizing ulcerative gingivitis or periodontitis Lymph node tuberculosis Pulmonary tuberculosis Severe recurrent bacterial pneumonia Symptomatic lymphoid interstitial pneumonitis Chronic HIV-associated lung disease including bronchiectasis Unexplained anaemia (<8g/dl), neutropenia (<0.5 x 10^9 per liter) and or chronic thrombocytopenia (<50 x 10^9 per liter)
Clinical stage 4 Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy Pneumocystis pneumonia Recurrent severe bacterial infection (such as empyema, pyomyositis, bone or joint infection or meningitis but excluding pneumonia) Chronic herpes simplex infection (oralobial or cutaneous of more than one month' duration or visceral at any site) Oesophageal candidiasis (candidiasis of trachea, bronchi or lungs) Extra pulmonary tuberculosis Kaposi's sarcoma Cytomegalovirus infection : retinitis or cytomegalovirus infection affecting another organ with onset at age older than one month Central nervous system toxoplasmosis (after one month of life) Extra pulmonary cryptococcosis (including meningitis) HIV encephalopathy Disseminated non-tuberculosis mycobacterial infection Chronic cryptosporidiosis (with diarrhea) Chronic isosporosis Cerebral or B-cell non-Hodgkin lymphoma Progressive multifocal leukoencephalopathy Symptomatic HIV- associated nephropathy or HIV- related cardiomyopathy

2.9 Herpes Viruses

The group of viruses referred to as Herpes belong to the family, *Herpesviridae.* They are morphologically identical and biologically closely similar. Their replication in the nucleus results in inflammatory response and destruction of host cells (Richman *et al*., 1987). The agents are host specific and establish lifelong latent infection, with occasional reactivation. Infection sometimes results in malignant forms of host cells (Metselear and Simpson, 1982).Viruses within the family include Varicella zoster Virus (VZV), Epstein - Barr virus (EBV), Human cytomegalovirus (HCMV), HSV and Human herpes viruses (Reinke et al., 1999). Herpes Simplex Viruses are classified in the sub family *Alphaherpesvirinae* together with VZV. The subfamily characteristics include relative rapid replication cycle *in vitro*, ability to establish lifelong infection in the sensory ganglia of the host and variable host range. Herpes Simplex virus type 1 and 2 vary in their clinical manifestation, but in both cases, symptomatic infections are characterized by painful vesicular or ulcerative lesions at various mucocutaneous sites (Lenette *et al.,* 1995). Immunocompromised patients and infected neonates suffer severe complications due to extensive local disease, visceral involvement, general dissemination and or encephalitis (Richman et al., 1987). Most immunocompetent individuals experience sub-clinical infections with either HSV-1 or HSV-2, mainly following reactivation of latent virus and subsequent mucocutaneous replication (Lenette *et al.,* 1995). Infections may sometimes occur without symptoms and this usually lead to asymptomatic virus shedding, which is significant for oral HSV-I transmission among children, sexually transmitted HSV in adults and mother to child transmission during birth (Kinghorn, 1993). Acyclovir resistant strains are much less virulent in immune competent individuals but cause serious and persistent symptoms in the immunocompromised (Lenette et al., 1995). Pharmaceutical studies have confirmed existence of active principles against several viruses in plants (Hudson, 1990). However,

it is expected that other researchers will discover more compounds since majority of plants have not been fully evaluated.

2.9.1 Herpes infections and HIV

Genital herpes is the most common cause of genital ulceration worldwide and its association with HIV was noted as early as 1981 (Siegal et al., 1981). The ulcer becomes a potential portal of entry of HIV due to its' infiltration with CD4 lymphocytes, the target cells for the HIV virus (Carrasco *et al*., 2002).This is in addition to the fact that the ulcers lack the protection of surface epithelium that becomes portal of entry of other infectious agents. Herpes simplex virus cause recurrent symptoms in HIV infection and is a common cause of morbidity and complication amongst HIV infected persons (Armstrong et al., 1985). Based on the on the observation it was qualified as one of the case definitions of HIV since 1987 by Center for Disease Control (CDC, 1987; NASCOP, 2009).

2.9.2 Anti-herpes drugs

The most common antiviral agents used against herpes infections are nucleoside analogs such as acyclovir, valacyclovir, penciclovir and brivudin. They are targeted at the virus encoded DNA polymerase (De Clercq, 2001). Acyclovir is widely used due to its safety profile and cost but resistant virus strains are known to have emerged several years back (Chien – Yu et al., 2001*)*. The individuals that are mostly by the resistant strains are those who are immuno-suppressed individuals (Stamm et al., 1998). The activity of acyclovir is dependent on virus encoded thymidine kinase activity, which converts the nucleoside analog to monophosphate form. It is further phosphorylated by cellular kinase(s) to triphosphate form, which is the active compound, that interact with viral DNA polymerase as a competitive inhibitor (De Clerq, 2001). Because of resistance owing to the emergence of thymidine kinase deficient strains, the search for new compounds still continue. Two more classes of nucleoside analog, the D-and L-enantiomers of cyclohexanenylguanine and bicyclic furopyrimidine are the latest compounds reported to offer marked potential (De Clerq, 2001). Combination therapy

with agents such as inosinate dehydrogenase inhibitors was considered but did not make a significant difference (De Clerq, 2001). The challenge of the pathogen resistance is a dynamic phenomenon and continuous effort is required to discover new therapeutic compounds. Plants are known to possess antiviral principles that need to be carefully selected on the basis of safety and efficacy of what seems to work.

2.10 Antioxidants and Plant medicines

The increase of pro-oxidants such as free radicals and reactive oxygen species accompany many chronic conditions such as arthritis, cancer and HIV. By the nature of their reduction potential, antioxidants protect against such diseases by being free radical acceptors and ability to convert reactive oxygen to neutral molecular form (Halliwel and Gutterrridge, 1992). Other diseases associated with increase of free radicals includes; atherosclerosis, arthritis, ischemia, cancer and other immune deficiency syndrome (Pourmorad *et al,* 2006). The pro-oxidant compounds attack the unsaturated fatty acids in the cell bio membranes, resulting in leakage of cytoplasmic contents (Dean and David, 1993). The cell DNA is another site of damage to the cell caused by excess pro-oxidant molecules, resulting in carcinogenic behavior of cells (Riu, 2004). The body's normal use of oxygen for respiration and some cell-mediated functions are key physiological sources of oxidant molecules such as reactive oxygen O_2^-, hydrogen peroxide (H_2O_2), hydroxyl radical (OH) (Chia et al, 2012). According to a report by Riu, consumption of plant products are protective against chronic conditions due to their antioxidant content (Riu, 2004). The author reported that conditions such as cancer and degenerative disorders was low on individuals who consume fruits, whole grains and vegetables and that whole plant product was more effective in disease prevention than isolated phytochemicals. The strong antioxidant value of plants are associated with vitamin C, A and E, with polyphenols being the most potent for antioxidant activity (Lamien-Menda et al, 2008). From the observed association between plant medicines, antioxidants and the health effect, it is considered that an evaluation of antioxidant value is an important consideration when investigating antimicrobial property of medicinal

plants. The role of antioxidants in restoring adverse immune response and its role in reducing pro-oxidants may be responsible for empirical health value.

2.10.1 Antioxidants and HIV infection

Epidemiological and clinical evidence suggest that low plasma or serum Vitamin A levels are associated with accelerated HIV progression (Baum et al., 1995), increased mortality (Semba et al., 1993), higher vertical transmission of HIV (Semba *et al*., 1994; Greenberg *et al*., 1997), child growth failure (Semba et al., 1997) and increased HIV load in breast milk and the birth canal (Nduati et al., 1995; John et al., 1997; Mostad *et al*., 1997). Studies have shown that HIV infected individuals with high serum vitamin E levels have a 30% lower risk of progression to AIDS (Tang et al., 1997). In Toronto, a reduction in oxidative stress and decrease in viral load were noted in clinical trials of vitamin E and C in HIV infected adults (Allard et al., 1998).

Apart from their immunological roles, it has been established that Vitamin E, C and beta carotenes prevent HIV generated oxidative stress and associated factors that activate the virus replication (Semba and Tang, 1999). Pro-oxidant compounds and free radicals are involved in release of Factor NF-$_K$B that is involved in transcription of HIV-1 (Semba and Tang, 1999). The trace elements such as zinc and selenium comprise elements that are necessary constituent enzymes involved in defenses against oxidative damage of body cells and tissues (Gosvenor and Smoli, 2002) that are associated with HIV disease (Baruchel and Wainberg, 1992).

The importance of micronutrient constituent of plant materials in the maintenance of health has been established to be in their superior bioavailability. Studies show that natural food complex nutrients are more bioavailable than isolated vitamins or inorganic mineral salts or mineral chelates (Schuan, 1997). Abundant source of antioxidant compounds and essential minerals and trace elements have previously been established in the crude extracts of fruits, herbs, vegetables, cereals and other plant materials (Idowu et al., 2006; Singelton and Ross, 1965). This study seeks to establish the antioxidant

content of selected medicinal plants as a basis of recommending their use as an alternative to food supplements given to PLWHIV to slow the progress of the disease.

2.11 Conceptual Frame Work

Substitute of effective conventional medicine with ineffective herbal medicine or reliance on the latter as a primary form of treatement has a negative impact on the outcome of HIV management. Further, herbal medicines may be effective but might compromise effectiveness of conventional drugs, it may have unregonized toxicity or delay the effectiveness of conventional drugs and result in HIV morbidity and mortality. Figure 2.3 shows Herpes Simplex Virus and other opportunistic infection in HIV interacting with variables of herbal medicine use resulting in HIV morbidity and mortality.

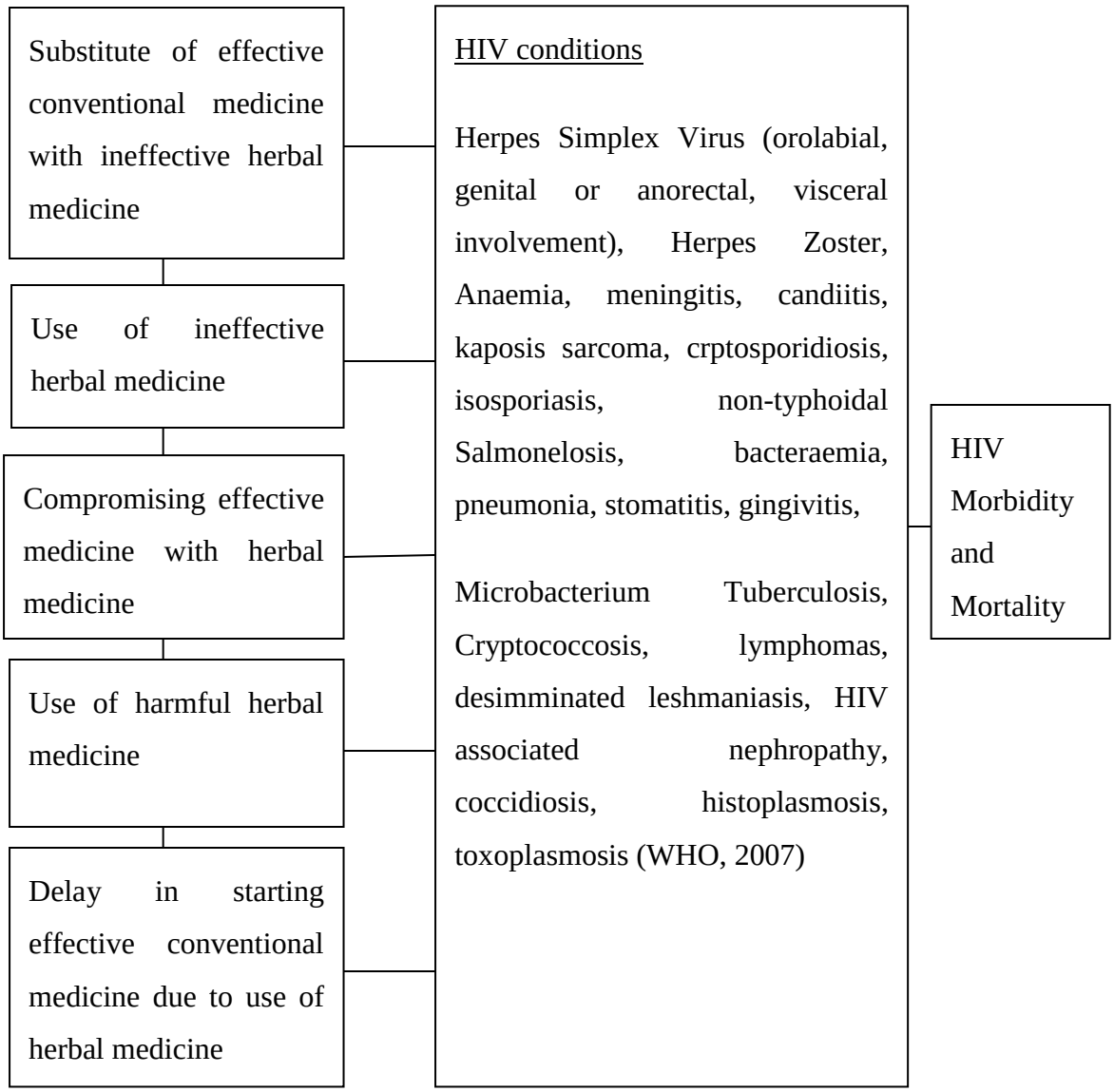

Figure 2.3: Conceptual framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The study design for identification of plants used as medicinie for HIV conditions was qualitative ethnobotanical survey. The survey was followed by collection and scientific identification of the plant species, and literature search for pharmacological and phytochemical finding on the identified plants species. In the laboratory, experimental study design was used to determine the effect of selected plant extracts on Vero cells and HSV-1 as the indicators of cell cytotoxicity and antivirus effect respectively. The effect of plant extract on 2,2-Diphenyl-1-picrylhydrazyl was used as an indicator of antioxidant activity.

3.2 Target population and Study units for identification of herbal medicines used by PLWHIV

Based on the fact that HIV is still a stigmatizing condition in the study sites, and therefore inhibiting direct interview of PLWHIV, Community Health Workers as the community care givers were chosen as the target population. The CHWs are the key link persons between the health department and the community they represent. The other criteria considered for choosing CHWs' was the fact that they are knowledgeable about local societal cultural norms and customs, the same criteria being a qualification for community nomination to ensure acceptance and ownership of health programmes (GOK-MOH, 2012).

3.3 Sampling of Study sites and study units

The study was carried out at Hamuyundi sub-location in Sabatia sub-county, Vihiga County (Figure 3.1) and Mukhwa sub-location in Mumias sub-county, Kakamega County (Figure 3. 2). The two sub-locations were identified for the study because of

having high numbers of long term serving CHWs and high number of cumulative number of HIV clients registered under community care as reflected on HIV programme reports from the year 2008 (PHMT- Western province, 2012). The Maragoli predominantly inhabits Hamuyundi while the Wanga sub-tribes of the Luhya community predominantly inhabit the Mukhwa sub-location in Kakamega County.

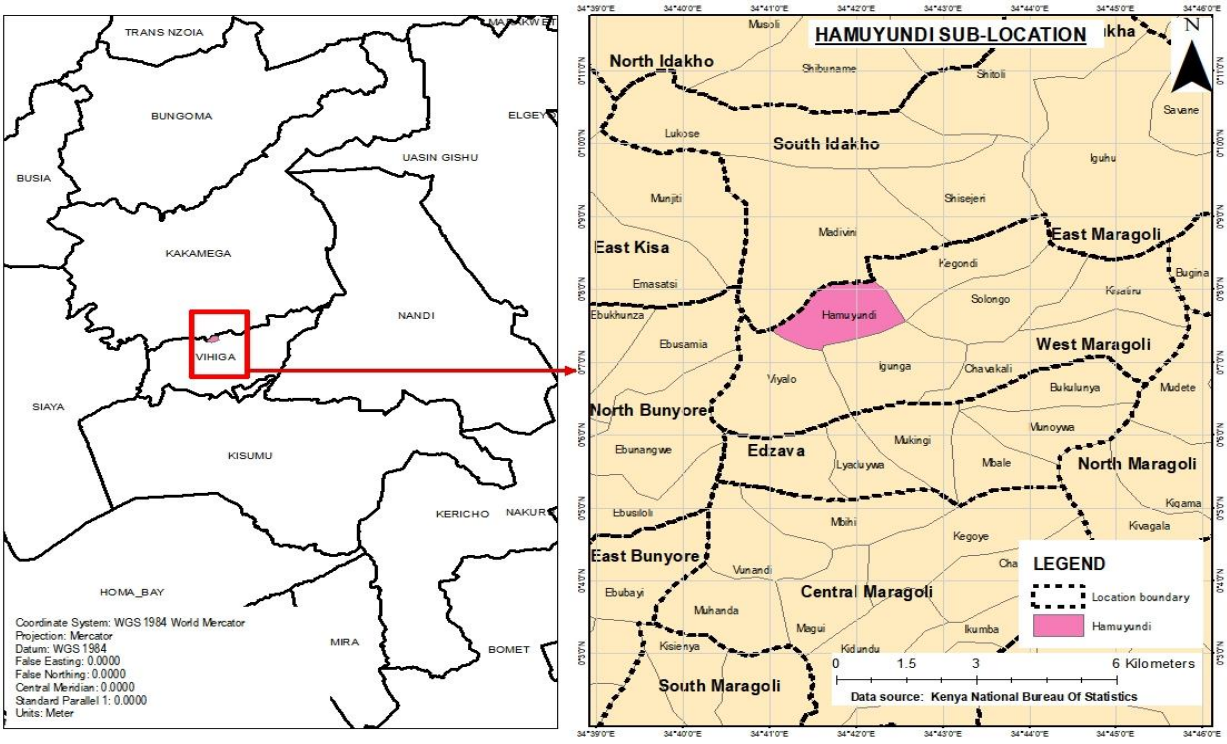

Figure 3.1: Hamuyundi Sub-location in Vihiga County

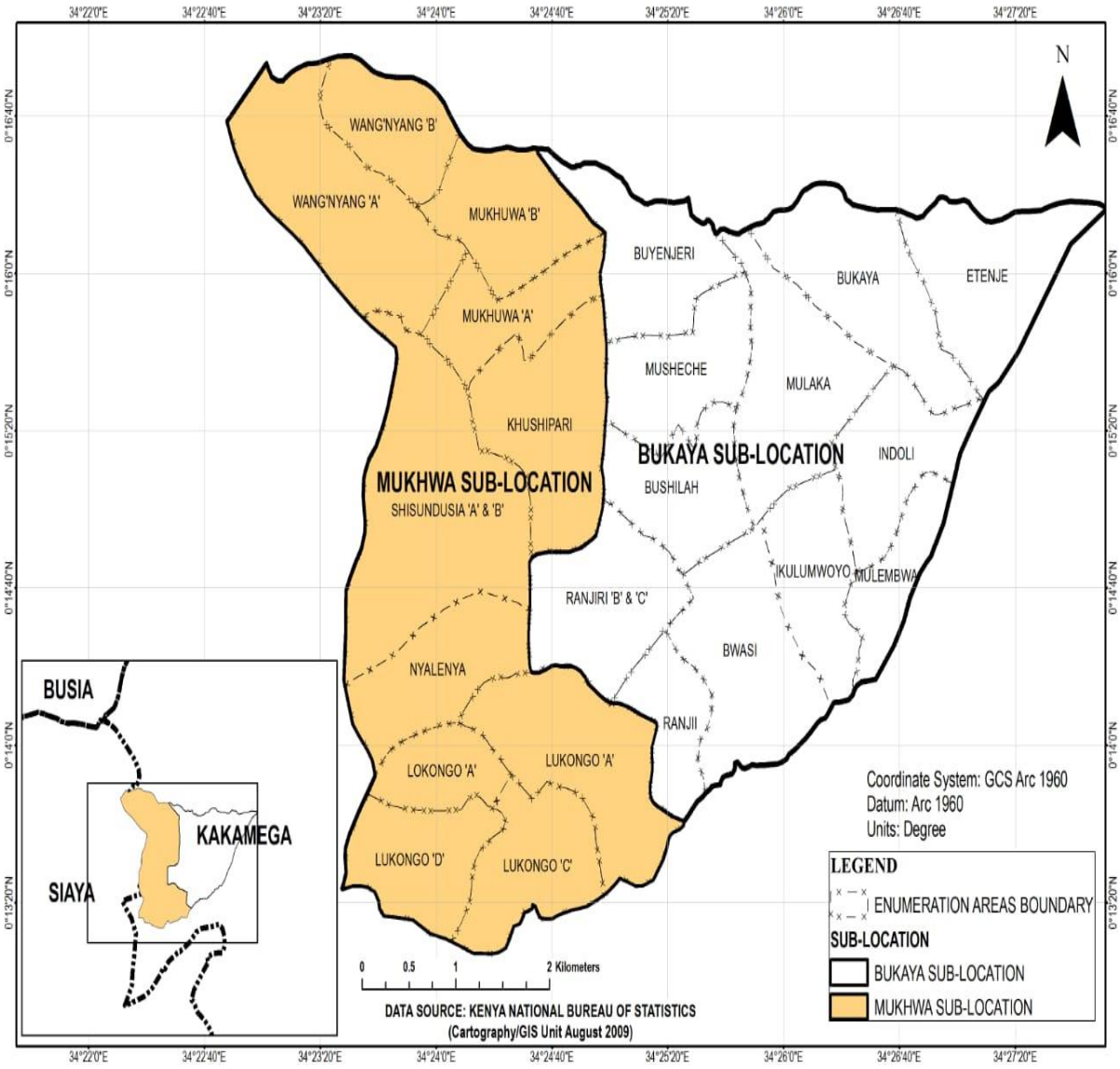

Figure .3.2: Mukhwa Sub-location in Kakamega County

Data was obtained from all the 12 CHWs in Mukhwa and 11 CHWs in Hamuyundi who were serving under the jurisdiction of Mukhwa and Hamuyundi Community Health Units (CHU) respectively. Each CHU is equivalent to a sub-location and are the smallest health administration unit as per the MOH structure for community health strategy. They comprised 7 females and 5 males in Mukhwa, and 8 females and 3 males in Hamuyundi. At the time of interview, the registered PLWHIV were 62 in Mukhwa and 51 in Hamuyundi.

3.4 Sampling of plant species for HSV and antioxidant determination

Plant species for HSV and antioxidant determination were selected on the basis of being cited for anti-Herpes zoster Virus infection (HZV), HSV or description of conditions closely resembling HZV or HSV. The consideration for antioxidant determination of the same plant species was based on the association of plant products and antioxidants, as explained in section 2.10.

3.5 Data collection

3.5.1 Key informant interviews

Key informant interview and sample collection was carried out in September 2014 in Mukhwa and December 2014 in Hamuyundi. A key informant interview guide (Appendix 4) was used to collect the data. Information was obtained on knowledge of conditions associated with HIV infection and the status of herbal medicine use by PLWHIV in their respective communities. Further inquiry was about the herbal medicines used by their clients for care and management of HIV conditions, plant parts used, the method of preparation and administration, and the local names. The study finding on plant part used and the method of preparation was used to design methods of plant preparation for laboratory experiments.

3.5.2 Collection of medicinal plants

The CHW were used as guides during field trips to collect samples for laboratory analysis and voucher specimens. Plant collection and handling was done according to the method used by Tolo et al, (2006). For scientific identification, the specimens comprising leaves, flowers and or seeds were taken and pressed between old newspapers and transported to the University of Nairobi herbarium within 36 hours. In the herbarium, the plants were identified in accordance with taxonomic practice. The same method of collection and handling was applied for laboratory samples. At least one kilograms of relevant parts of the plants was wrapped in old newspapers and parked in cartons. The samples were delivered to the laboratory drying room within 36 hours of collection. Further treatment before extraction was done according the method of Tolo et al, 2006. The medicinal part of the plant, as specified by the informant was dried at room temperature for 2 weeks before being ground to form powdered for hot water extraction. The extraction procedure was carried out as described under preparation of extract in section 3.5.4.1.

3.5.3 Literature search for Secondary metabolites and ethno pharmaceutical use of plants mentioned by CHW

Secondary metabolites and ethno pharmaceutical use of cited medicinal plants were identified by electronic literature search engines, using google scholar and PubMed. The name of plant species was used as search phrase and refined by adding ‘ phytochemistry’ , ‘secondary metabolites’ , ‘pharmaceutical activity’ and or ethno-pharmaceutical use.

3.5.4 Cytotoxicity and *in vitro* antiviral activity tests

3.6.4.1 Plant selection for extract preparation and anti-herpes

Plants for anti-herpes investigation were selected on the basis of being cited for use by CHW for either HSV disease or Herpes Zoster (HZ) disease. Based on the criteria, species collection codes; KK01, KK02, KK03, KK04, KK05 , KK07, KK08 and KK09,

corresponding to *Tithonia diversifolia* (whole root) *Schuhria pinata* (Leaves) , *Entada abyssinica* (Bark), *Garcinia buchananii* (Stem Bark), *Croton macrostachys*(Stem Bark), *Vernonia adoensis* (Whole root), *Plumeria alba* (Leaves) and *Caesalpinia decepetala* (Whole root) were applied. Prior to anti-herpes investigation selected plants were investigated for cytotoxicity to determine their effects on the cells used for both virus cultivation and anti-herpes experiments. Extract preparation was carried out according to method previously used by Tolo et al, (2006). Plant materials were dried at room temperature for 2 weeks. The dried material was ground to form powder using an electric grinding machine. Fifty grams of powdered material was soaked in 500ml of distilled water and heated to 80°C for one hour (Hot water extraction was the popular method of plant preparation as reported by the community). The extract was cooled to room temperature and filtered using cotton wool. The filtrate was then frozen using dry ice in acetone, freeze dried and kept at $^{-}$20°C until required for use. For experiments, the freeze dried material was dissolved in phosphate buffered saline to make a stock concentration of 1000µg/ml. Working concentrations were made from the stock. The stock concentration was prepared by obtaining 5mg of freeze dried material and dissolving in 5 mL of phosphate buffered saline.

3.5.4.2 Preparation of Cell and virus culture

A cryovial of Vero cell line, ATCC CCL 81 was obtained from viral hemorrhagic fever (VHF) research laboratory, of the Kenya Medical Research Institute (KEMRI). The preparation of cell and virus culture was done as described by Tolo et al. (2006). The vial was placed in a water bath at 37°C to thaw. The content was transferred into a 75cm^2 cell culture flask containing 25ml of Eagles minimum essential growth media (MEM) supplemented with 1% of 2mM L-glutamine, 10% (v/v) fetal calf serum, 2.5% (v/v) of 7.5% (W/V) Sodium bicarbonate and 1% (v/v) of pen strep (10,000 I.U/ml penicillin combined with 10,000µg/ml streptomycin). The seeded culture flask was incubated in a humidified environment at 37°C in a 5% CO_2 incubator until cell culture was about 90% confluent. The 75cm^2 cell culture flask was used to prepare virus stock

of herpes simplex type 1 (ATCC® VR -1789™) purchased from American Type culture collection (ATCC) as explained below;

The flask was emptied of growth media and 1ml vial of herpes simplex virus type 1, HSV 1 ATCC® VR -1789™ in Eagles maintenance medium (EMEM) (ATCC®30-2003®) at multiplicity of infection (MOI 0.1), was emptied into the flask. Virus inoculum was adsorbed on to the cell monolayer for 1 hour in 5% CO_2 incubator at 37°C. The inoculum was removed and 25 ml of maintenance media made of the same chemically defined constituents as for growth media except fetal calf serum, used at 2.5%. The virus culture was incubated at 37°C in 5% CO_2 incubator and the virus stock harvested after 48 hours when about 90% cytopathic effect (CPE) was observed on the monolayer.

The virus was harvested by 3 times freezing in -80°C freezer and thawing in 37°C water bath. The maintenance media containing the virus was then centrifuged at 3,000 rpm for 10 minutes and the supernatant aliquoted in 1ml cryovials and kept at -80°C. The stock titer was determined by endpoint titration technique (EPTT) as previously described by Bentacur *et al,* (2002).

One cryovial of the stock virus was removed from the freezer and immediately thawed in a water bath at 37°C. The EPTT was carried out in a 24 hour growth culture of Vero cells in a 96- well plate, previously seeded with 100µl of 2 x 10^4 cells /ml. The growth media was removed from the wells and the cells infected in quadruplicates with 100µl of each tenfold dilution of the virus suspension from 10^{-1}- 10^{-12}. The plate was incubated for 48 hours in 5% CO_2 at 37°C and examined for proportion of cells showing CPE in each dilution replicate using x 10 and x 40 inverted microscope objective. The cells showing CPE and those without CPE is as shown in plate 3.3. The virus titer was calculated using spearman-karba formula (Bentacur *et al*, 2002), based on the proportion of replicate virus dilutions showing above 75% cytopathic effect on the cells. The

dilution of virus required to cause lysis of 50% of replicate cultures ($TCID_{50\ [0.1mL]}$) was found to be 10^{-7}. Thus the virus titer was 10^{7} and was labelled as Virus control (vc).

Cells showing no cytopathic effect Cells showing cytopathic effect

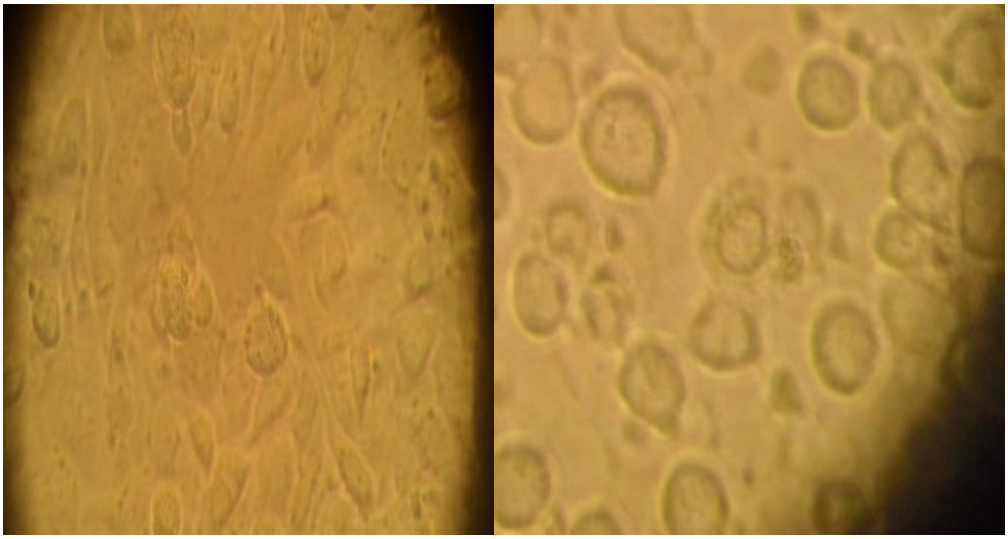

Plate 3.1: Vero cells with and without CPE

3.5.4.3 Cytotoxicity test

Cytotoxicity was tested by determining viability of Vero cells after exposure to graded concentration of extracts as previously used by Bentancur *et al* and Jaime et al, (Bentancur et al., 2002; Jaime *et al*.,2013). The method is based on the metabolism of 3 – (4, 5-Dimethylthiazole -2-y) -2, 5-diphenyltetra-zolium bromide (MTT), converting the yellow solution of the dye to purple colour, hence indicating cell viability. The intensity of the purple colour is directly proportional to the viability of the cells and is determined using a spectrophotometer.

Three different MTT experiments in triplicate wells of 96 well plates were carried out using Vero cells (ATCC CCL 81). Each 96 well plate was designed for 3 extracts. Vero cells were seeded at 2 x 10^{4} cells per well in columns 1,2,4,5,7,8,10 and 11 in EMEM

supplemented with 10% fetal calf serum, 1% penicillin/ Streptomycin, 1% glutamine and 2.5% of 7.5% (W/V) sodium bicarbonate solution. Wells in row 3, 6, 9 and 12 received only growth media to serve as test extract blank (tb) and cell control blank (ccb).

The cells were grown in 5% Carbon dioxide at 37°C for 24 hours. The growth media was removed and replaced with 100µl of maintenance media containing all the ingredients similar to growth media except fetal calf serum at 2.5%. To make initial 500 µg/mL for serial doubling dilution, a 100µl of 1000 µg/ml of Plant extract was added to row H of 96 well plate and serial doubling dilutions carried out up to row C, representing 500, 250, 125, 62.5, 31.25, 15.625, leaving row B and A to serve as cell control (CC). Cells were incubated at 37°C for 48 hours. The wells were emptied of the media and 10µl of MTT prepared at 5mg/ml in phosphate buffered saline added to all the wells, and incubated for 4 hours. The MTT was removed and 100µl of dimethyl Sulphoxide (DMSO) added. The setup of cytotoxicity was as shown in Plate 3.1. Clear wells were cell free or wells where cells were destroyed by cytotoxic effect of extracts.

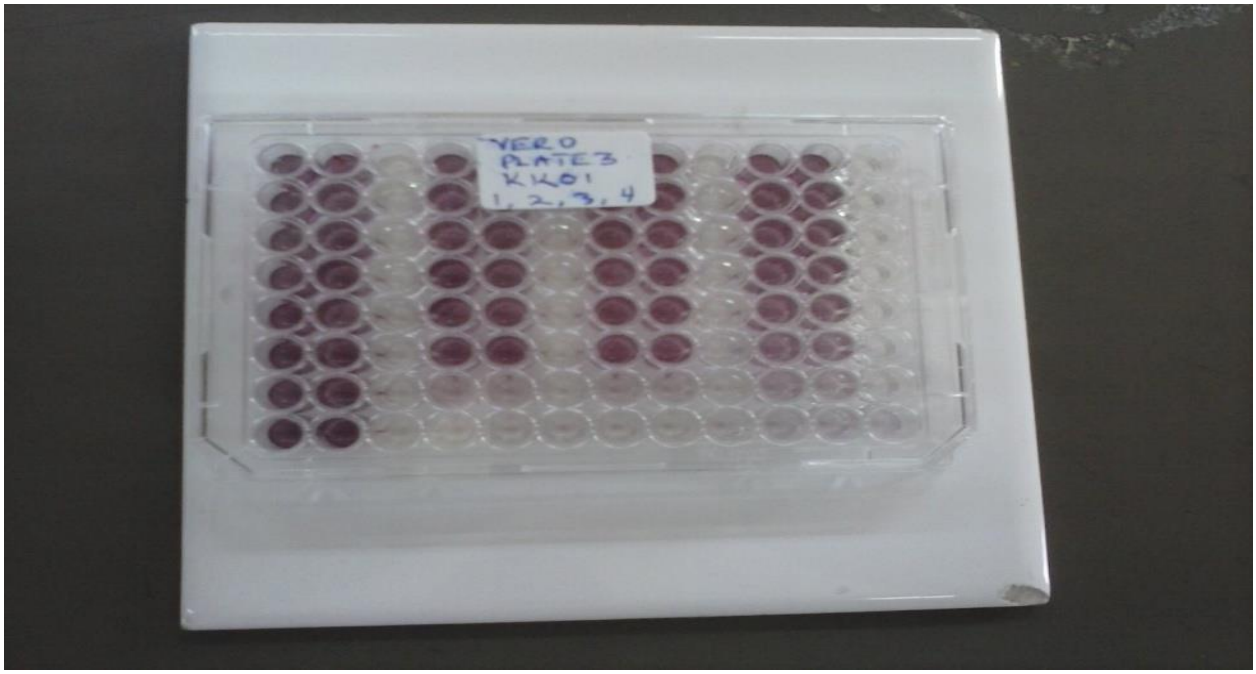

Plate 3.2: Cytotoxicity effects of plant extracts on Vero cells

The Optical density of the wells were read at 562 nm and the cell toxicity expressed as percentage cell viability at each extract concentration calculated as follows;

Percentage cell viability = [ODt – ODtb]/ [ODcc – ODccb] x 100 (Bentacur et al., 2002)

Where ODt = Optical density of test extract

OD tb = Optical density of test extract blank

OD cc= Optical density of cells control

OD ccb = Optical density cell control blank

The concentration of extracts reducing viability of cells by 50% (CC_{50}) was obtained by plotting a graph relating mean percentage cell viability of 3 different experiments to respective extract concentration.

3.5.4.4 Anti-herpes screening

End point titration technique (EPTT) as described by Betancur et al (2002) was applied to detect anti- Herpes activity in the extracts at 100µg/ml. The 96 well plate was seeded with the same Vero cell strain (ATCC CCL 81) at same density as for cytotoxicity test and incubated in the same chemically defined growth media and condition for 24 hours.

A portion of 100µl of 100µg/ml of extract in maintenance media was added to quadruplicate cell wells 1 hour before a tenfold dilutions of previously titrated virus was added at 100µl per well. The 96 well plate virus culture was incubated for 48 hours in a humidified atmosphere at 37°C in a 5% CO_2. Virus control (VC) consisting of maintenance media added 1hour in quadruplicate wells before a tenfold dilution of

previously titrated virus was added at100µl per well. Drug control (DC) was set up in the same way as extracts except 100µl of acyclovir tablet prepared at 5µg/ml in phosphate buffered saline was used instead of the extract solution. Cell control (CC) and extract/acyclovir cytotoxicity controls (CTC) were included in respective experiments, consisting of non-infected cells without extract/acyclovir and cells with extract/acyclovir respectively. The virus titer was calculated using spearman-karba formula as for the determination of stock virus titer.

3.5.4.5 Dose response test of extract concentration against HSV

Determination of dose response of HSV to extract concentration was carried out using cell metabolism of tetrazolium dye (MTT) as described by Jaime et al (2013). The selected extracts were initially tested at two concentrations of 100 and 25µg/ml using control virus ($TCID_{50\ (0.1ml)} = 1\ x10^{7}$). The 96- well plate was seeded as for EPTT assay and cells grown for 24 hours. The growth media was removed and cells pre-incubated in triplicates with 100µl of extract at 100µg/ml and 25µg/ml for 1 hour. The extracts were replaced with 90µl of control virus followed by 10µl of extract concentration at 1000 and 250µg/ml to make 100µg/ml and 25µg/ml respectively. Each test concentration had its own cytotoxicity control (CTC) containing test extract without the virus. Virus control (VC) and cell control (CC) was included in the experiments. The VC contained the virus without test extract while CC contained maintenance media without the virus. Acyclovir at 5µg/mL was used as reference drug and treated the same way as test extract. For each of the wells containing cells; test extract at 25µg/ml, test extract at 100µg/ml, CC and VC, a blank well containing respective solutions without cells were included. The wells were treated for spectrophotometric analysis by MTT treatment as explained for the cytotoxicity test. The experimental set up was as shown in plate 3.2. Clear wells were cell free or wells where the virus destroyed the cells.

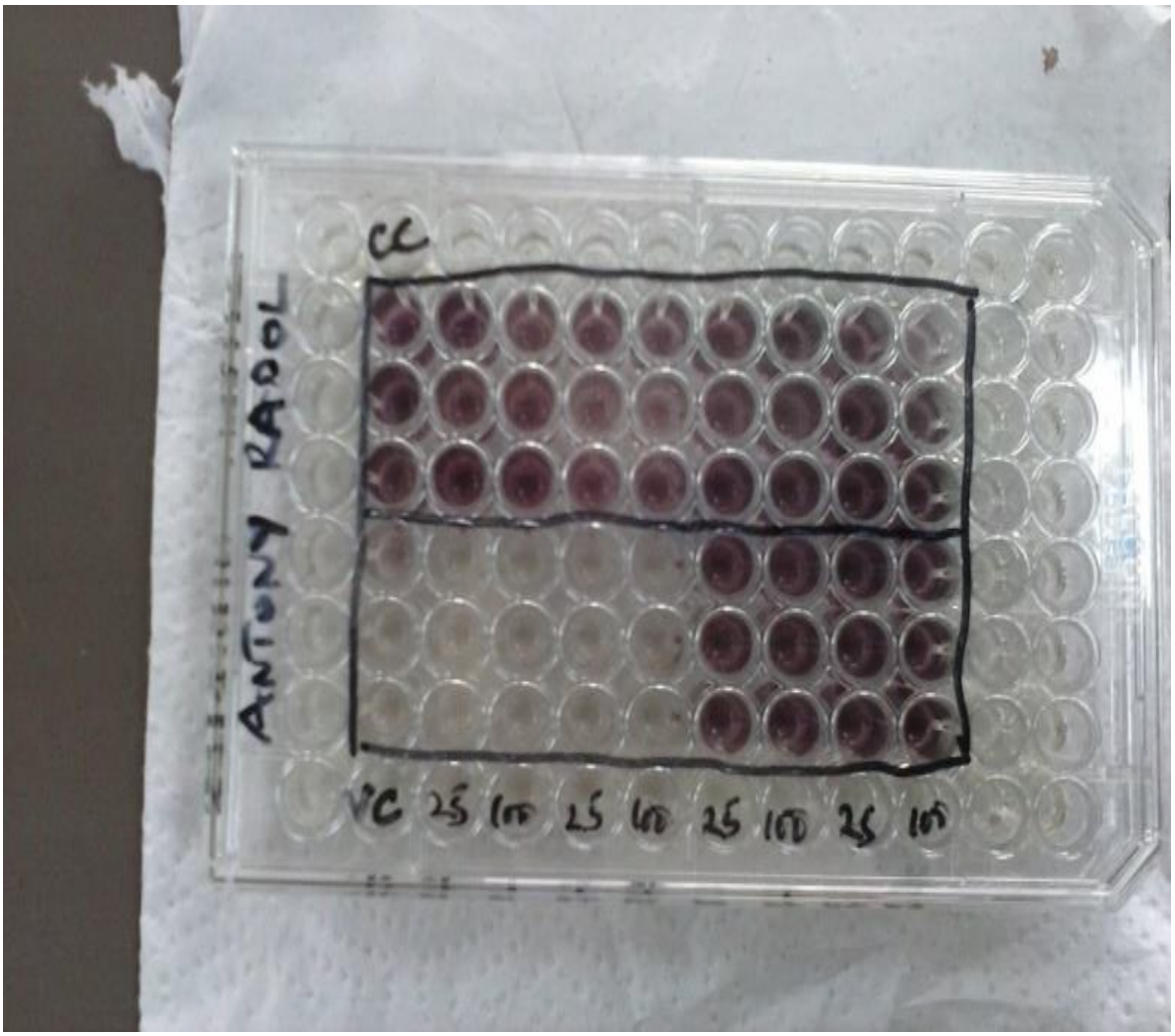

Plate 3.3: *In vitro* anti-herpes screening of plant extracts at 25µg/ml and at 100µg/ml on Vero cells.

The antiviral activity was expressed in terms of percentage cell protection and calculated using the formula below

% Cell protection = (ODt – ODtb) – (ODvc – Odvcb)/ (ODcc –ODccb)-(ODvc –Odvcb) x 100

ODt = Optical density of test extract, ODtb = Optical density of test blank, ODvc = Optical density of virus control, ODvcb = Optical density of virus control blank, ODcc = Optical density of cell control and ODccb= Optical density of cell control blank (Bentacur *et al*, 2002)

3.5.4.6 Determination of inhibitory concentration 50 (IC_{50}) and therapeutic index

The effectiveness of a compound in killing a pathogen is measured by the concentration of the compound required to reduce infectivity of a pathogen by 50% (IC_{50}). Therapeutic index (TI) measures selective killing of the pathogen while sparing the cell. The method of Bentancur ***et al*** (2002) was used to determine IC_{50} of extract giving more than 50 % cell protection at both 25 and 100µg/ml. Growth media was removed from a 24 hour monolayer of Vero cells and replaced with extracts in duplicates at a final concentration of 500, 250, 125, 62.5, 31.25, 15.625µg/ml with their respective CTC, blanks, CC and VC included and treated as explained above. The respective % cell protection was calculated and the IC_{50} obtained by plotting a graph relating mean percentage protection of 3 different experiments and extract concentration. Therapeutic index (i.e. selective index) was expressed as the ratio of CC_{50}/IC_{50}.

3.5.4.7 Characterization of *In vitro* antiviral activity

Characterization experiments were carried out for extracts of two plants giving pronounced antiviral activity, using the method of Jaime et al with some modification. Three different experiments in triplicate wells were carried out for pre-treatment, virucidal and time of addition assays.

Pre- treatment assay: Growth media was removed from a 24 hour Vero cells in a 96-well plate and replaced with 100µl of extracts at 25 and 100µg/mL. The cells were incubated with the extract in a humidified 5% CO2 atmosphere at 37°C for 7 hours. The extract was then removed and cells washed with phosphate buffered saline (PBS) before addition of 100µL of control virus. Experiment controls consisting of CC and VC were

included. For the CC, the growth media in a 24 hour Vero cells in a 96 well plate was replaced with 100µL of maintenance and incubated for 7 hours in a humidified 5% CO2atmosphere at 37°C. Fresh maintenance media was added after emptying the wells of the previous media and the cells incubated further for 48 hours in a humidified 5% Co2 atmosphere at 37°C. The VC was made up of cells pre – incubated with maintenance media which was replaced by 100µl of control virus and further incubated for 48 hours in the same conditions as the test. The blanks for tests and controls were included in the experiments. After 48 hours, the cells were processed for optical density reading and calculation of percentage cell protection.

Virucidal assay: A 90µL volume of control virus was mixed with10µl of extract at 250 and 1000µg/mL to make a virus suspension in 25 and 100µg/mL of extract in triplicate wells, in cell free 96-well plate. The virus was suspended in the extract at 37°C for 30 minutes. Simultaneously, CC and VC were set up. The CC was set up by incubating 100µl of maintenance media at the same temperature and duration. The VC wells contained 90µl of control virus and 10µl of maintenance media and was incubated at same temperature and duration. The blanks were set up for the test, CC and VC wells. After incubation, the content of the wells were transferred to respective wells containing Vero cells and further incubated for 48 hours in a humidified 5% CO_2 atmosphere at 37°C. The cells were processed for optical density reading and calculation of percentage cell protection as previously described.

Time of addition Assay: Time of addition assays was carried out to study the effect of the extracts in the adsorption and post adsorption events. Three different treatment of cells against the control virus with extracts at 25 and 100µg/mL was carried out. The extracts were present (i) only during the adsorption period for adsorption assay (AA), (ii) after adsorption and until the end of experiment for Post- adsorption assay (PAA) and (iii) during and after adsorption for throughout assay (TA). A 24 hour Vero cell culture in a 96-well plate were precooled for 1 hour at 4°C. A 90µl of control virus was added to AA and TA wells followed by10µL of a 250µg/mL or 1000µg/mL of extract. For PA wells,

90µl of virus was added followed by 10µl of maintenance media. In order to allow only adsorption step to take place, the cells were further incubated at 4°C for 1 hour. Cells were then washed with PBS, and then 100µl of maintenance media with extract for TA or without extracts for AA and PA was added. The CC and VC controls were included in the experiment. For CC wells, the precooled wells received 100µl of maintenance media before and after washing with PBS. The VC received 90µl of control virus followed by 10µl of maintenance media and 100µl of maintenance media after PBS washing. The respective blanks for AA, PA, TA, CC and VC were included in the assay. After 48 hours of incubation in a 5% CO_2 humid atmosphere at 37°C, the cells were processed for optical density and calculation of percentage cell protection as described for MTT antiviral assay.

3.5.4.8 *In vivo* experiments

The two extracts showing strong ***in vitro*** antiviral activity were evaluated for ***in vivo*** activity, using Swiss female albino mice. An HSV type 1 strain 7401H previously used by Kurokawa for ***in vivo*** antiviral experiments was used (Kurokawa ***et al.,*** 2001). The strain is known to inflict progressive symptoms in 7-week-old mice resulting in death in about 7 days if no effective anti-herpes medicine is given. Prior to antiviral experiments, acute toxicity test was performed on 7 weeks old Swiss albino mice weighing between 18 – 20grams. A limit dose test (OECD, 2001)), using a1000mg/kilogram body weight (mg/Kgbwt) of extract doze was chosen. The aim was to detect or rule out any toxicity associated with administration of the extract, twice above the intended antiviral test doze of 500, 250 and 125mg/kgbwt.

3.5.4.9. Preparation of extracts

With the objective of preparing extracts for acute toxicity experiments that is equivalent to administering 1000mg/kgbwt in 0.2 ml volume to mice weighing 18 – 20grams, a 100 mg of extracts was dissolved in 1ml of normal saline. For antiviral experiments, 50mg was dissolved in normal saline with the goal of preparing a dose equivalent to giving

500mg/kgbwt in 0.2ml oral volume. Lower doses of 250 and 125mg/kgbwt were prepared from the 500mg/kgbwt.

3.5.4.10 Preparation of the virus

One vial of HSV- 1 7401H was removed from -80°C cold storage and immediately thawed in 37°C the water bath. The virus was cultivated and titrated in same Vero cells and using same technique as for in vitro experiments. A titer of 10^4 $TCID_{50\ (0.1ml)}$ was obtained and used for *in vivo* experiments.

3.5.4.11 Acute oral toxicity tests

Based on ***in vitro*** results, two plant extract were selected for oral toxicity test prior to ***in vivo*** antiviral experiments. A limit test dose of 1000 mg/kgbwt of albino Swiss mice was carried out accordin to international environmental chemical toxological assessment (ENV/JM/MONO, 2001). Six 7 weeks old female albino Swiss mice weighing between 18 – 20grams each were used for each extract. The animals were taken to the experimental room 5 days prior to commencement of experiment. They were starved of food except water for 3 hours prior to determination of weight and oral administration of 0.2ml of extract solution and 0.2ml normal saline for positive control. The mice were observed for signs of toxicity (change in skin colour and fur, eyes and mucous membrane, change in movement, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma) at 30minutes and 1 hourly interval for the next 3 hours with feeding withheld except water over the period. Feeding was resumed after 3 hours and observations made at 6 hours, 12 hours, 24 hours. Based on outcome of observation made over 24 hours, further observations were made daily for 14 days and weight of mice taken at 7 and 14 days to determine the difference in weight between experimental and positive control groups.

3.5.4.12 In *vivo* antiviral experiments

In vivo antiviral tests were carried out as described by Kurokawa et al, (2001). For antiviral experiment, 6 mice of same species, sex (female), and age and weight range of 18 – 20g, as described for toxicity test were used. The mice were selected from the breeding room and randomly allocated to each experimental and control group cages. The cage size measurements were 15 x 21 x 29 cm and made of transparent plastic material. The cages were bedded with wood chips and equipped with continuous-flow nipple watering devices. The cages were labelled according to test extract group, positive control (acyclovir at 5mg/kgbwt) negative control (Normal saline). Additionally, toxicity control cage comprising six mice for the highest dose of test extract treatment was set up. Two days before onset of experiment, the animals were shaved on the right mid flank using an electric shaver machine and Veet hair remover applied and washed off after 3 minutes. At the onset of experiment, the shaved area measuring about 2x2 cm was scratched with a 27gauge needle. The experimental group, positive and negative control groups were infected on the abraded skin with 10µl of Herpes Virus type 1, strain 7401 H (HSV-1 7401H) at 10^4 $TCID_{50\ (0.1ml)}$). The strain previously caused symptoms and death to mice in the absence of effective antiviral treatment (Kurokawa et al., 2001). Treatment began 4 hours after infection. A 0.2ml volume equivalent to desired mg/kgbwt (Milligram per Kilogram body weight) was orally administered and the same volume equivalent to 5mg/kgbwt acyclovir orally administered to positive control three times a day. Treatment was given for 7 days and observation recorded daily for 14 days. The specific observation made were lesion symptoms on the skin scored as 0 (no symptom), 2 (vesicle), 4 (ulcer), 6 (mild zosteriform), 8 (moderate zosteriform), 10 (serious zosteriform) and 12 (death).

3.6 Determination of Antioxidant activity

The aqueous extracts of plants screened for antiviral activity were also tested for antioxidant value. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) reduction assay as described by Igbinosa (2013) was used with slight modification (wave length used was

518nm instead of 517nm). The DPPH reagent was prepared at a concentration of 0.116mM in methanol. The samples were plant extracts prepared in duplicate concentrations ranging between 3.9 - 500µg/mL in methanol, and reference standard of ascorbic acid prepared in duplicate concentrations ranging between 8 – 0.25µg/ml in methanol. The experiment was carried out by mixing 1ml of the sample with 1ml of DPPH reagent. A DPPH control was set up by mixing 1ml of DPPH reagent and 1ml of methanol. The mixtures were vortexed thoroughly and incubated in the dark for 30 minutes at room temperature. Extracts were first tested for antioxidant activity at 500µg/mL, as a screening assessment and only extract giving more that 50% DPPH reduction were further evaluated for the concentration of the sample reducing DPPH by 50% (RSa_{50})

The absorbance of the samples and the control were read spectrophotometrically at 518nm and using the mean absorbance of each duplicate, the reduction power was obtained using the formula below;

Reduction power (%) = [(Absorbance $_{Control}$- Absorbance $_{Sample}$)]/ [(Absorbance $_{Control}$)] x 100. Where

Absorbance $_{control}$ = Absorbance of control,

Absorbance $_{sample}$= Absorbance of reference standard or extract

3.7 Screening of extracts for phytochemical groups

The extracts were tested for presence of flavonoids, terpenoids, alkaloids, saponins and phenols. For each fraction, a 1mg/ml concentration was prepared in methanol and used for chemical tests as explained below.

3.7.1 Flavonoids

The method of Edeoga et al (2005) was applied. To 1ml of extract solution in methanol, 5mL of dilute ammonia was added; the solution was observed for development of yellow colour upon addition of 1mL of sulphuric acid. Further, the yellow colour was observed for disappearance on standing.

3.7.2 Terpenoids

The method of Pavithra and Vadivukkarasi (2012) was applied. To 5ml of extract, 2ml of chloroform and 3ml of concentrated, H_2SO_4 was added and observed for development of a layer of reddish brown coloration at the interface of the two solutions as an indication of the presence of terpenoids.

3.7.3 Alkaloids

The method of Houghton and Raman (1998) was applied .To 5ml of the extract, few drops of Mayer's reagent were added and the solution observed for the formation of white precipitate as an indication of the presences of alkaloids.

3.7.4 Saponins

The method of Pavithra and Vadivukkarasi (2012) was applied. The extract was diluted with 10ml of distilled water and shaken for 15 minutes. The solution was observed for formation of stable foam that indicates presence of saponins.

3.7.5 Phenols

The method of Mace (1993) was applied. To 5mL of extract solution, drops of 10% Ferric chloride was added and observed for the formation of intense blue colour indicating presence of phenols

3.8 Data management and analysis

Respondents to key informant interview were represented by codes and their responses to questions on diseases caused by HIV and status of herbal medicine use captured verbatim. The plant species name was recorded in the language used by the respondent and details on the part used, conditions treated, method of preparation and administration recorded in a table attached to the interview schedule. The recorded narrative and table contents were entered into MS word computer programme. The table was later expanded to include the scientific name of the plant and family group name, obtained upon the plant specimen identification. The plant specimen for identification and sample for laboratory analysis were given the same code. The plants cited as medicine were analysed for concensus among the informants by frequency of mention by different informants for a particular condition and intercommunity concurrence on citation as medicine.

Results of literature search on plants mentioned by CHW were recorded against scientific name of the plant in a table created in MS word. Plants mentioned but with no information about secondary metabolites and or pharmaceutical use were not included in the data.

Cytotoxicity test data was recorded as optical density (OD), representing photometric measure of MTT metabolism by cells remaining viable after exposure to extracts. Results were expressed as percentage cell viability representing the ratio of OD of cells exposed to extracts to OD of cells not exposed to extracts, multiplied by 100. Concentration of extracts reducing the viability of cells by 50% was obtained by plotting a graph relating mean percentage of cell viability of 3 different experiments to respective extract concentration.

Data for anti-herpes screening experiments were recorded as $\log_{10}TCID_{50\ [0.1ml]}$, indicating strength of the virus. Results were recorded as Reduction factor (RF)

indicating the difference between the strength of the virus cultivated in presence of the extract and strength of the virus cultivated in extract free media.

For antiviral determination, data were recorded as OD, representing photometric measure of MTT metabolism by cells protected from virus destruction by extracts. The results were expressed as percentage cell protection, obtained by the ratio of optical density of infected cells in the presence of extracts to optical density of infection free cells multiplied by 100. The IC_{50} was obtained by plotting a series of extract concentration against corresponding percentage protection and identifying the extract concentration where the percentage protection is 50%. Therapeutic index was obtained by the ratio of CC_{50} to IC_{50}.

To determine the mode of anti- herpes activity, the percentage cell protection of selected extracts under different conditions of cell treatments were compared by post hoc analysis of variance (ANOVA).

For acute toxicity experiments, the weights of mice in the study were recorded at onset of the experiment and at day 7 and 14. The difference between the weights of mice at onset of experiment and at day 7 and 14 was determined and the mean of experimental group compared with the positive control.

The *in vivo* antiviral experiments data were recorded in terms of days it took for specific symptoms to be initially observed or death occurrence. The mean day at which symptoms were observed or occurrence of death was compared between experimental groups and negative control. Data were entered in excel spread sheet windows 2010 and analyzed in SPSS version 17. A p value was calculated using independent t-test and value equal to or less than .05 was considered significant for antiviral activity.

Antioxidant data were recorded as the difference in OD between DPPH control and mixture of DPPH and extract. The ratio of the OD difference to the OD of DPPH control was multiplied by 100 and termed percentage reduction power. A graph relating

percentage mean reduction power and concentration of sample was used to estimate the concentration of sample reducing the absorbance of DPPH control by 50% (Rsa_{50}).

The presence or absence of phytochemicals was indicted by use of signs; + for presence, trace (for very little indication), and – for absence, based on the interpretation of the colour of the product obtained between the reaction of reagent and extract.

3.9 Ethical considerations

Approval for the study was sought and obtained from Ethics Review Committee of the Kenya Medical Research Institute (ERC), for SSC Protocol number 2285 (Appendix 5). Further approval was sought and obtained from the Ministry of health, granting access to community health structures in Kakamega and Vihiga counties.

Before interview, the informant were taken through the consent form and requested to sign if in agreement. The consent was written in English, Kiswahili and Luyha languages (Appendix 1, 2 and 3)

Animals dying or sacrificed as a result of experimentation were put in biohazard container bag and either put in a freezer to await incineration or taken directly for incineration, depending on incineration schedule. The ones alive at the end of experiment were euthanized using compressed carbon dioxide gas from CO_2 gas cylinder. The cage housing the animals was placed in the CO_2 chamber and the gas turned on to flow at the rate of 2 liters per minute. The flow of the gas was maintained for 1 minute after apparent death of mice. Death of mice was confirmed by palpation to ascertain the absence of heart beat before being put into bio-hazard container and a tag attached showing investigator's name, date and protocol number. The container was then placed in a freezer in case incineration could not be done immediately.

3.10 Study limitations

The limitation of the study is as per the disadvateges of qualitative surveys (Harrie 2010). Thus, the information provided by key informants for plant species used as medicine may not accurately represent the varied species of plants used as medicine, the methods of preparation or conditions for which the plants are applied as medicine.

CHAPTER FOUR

RESULTS

4.1 Plants used for treatment of HIV conditions

The plants mentioned for treatment of HIV conditions were grouped according to community at the source and plant families (Appendix 7: Plants used for treatment of HIV conditions). At Hamuyundi community, a total of 38 plant species from 26 families were identified while in Mukhwa, 29 species distributed in 17 families were identified. Specific parts of the plant used and methods of preparation is included in the table of plants used for treatment of HIV conditions (Appendix 7). Appendix 8 and 9 shows collection numbers adopted as voucher numbers. All the field specimens were found to match the herbarium collections and were therefore not preserved. Appendix 10 and 11 shows frequency of plant mention as medicine and the corresponding HIV conditions. The HIV conditions were grouped according to pathophysiologic criteria. At Hamiyundi *Justicia betonica* (L.) and *Cassia occidentalis* (L.*)* were the most frequently mentioned plants for gastrointestinal and malaria/fever respectively (Appendix 10). Appendix 11 shows that at Mukhwa, *P. alba* L. for Herpes zoster was most frequently cited. Table 4.1 show that six plant species were cited at both Hamiyundi and Mukhwa. Table 4.1 also shows that ***Ajuga intergrifolia*** Buch-Ham**,** ***Psydium guajava*** L. and *Microglossa pyrifolia* (Lam.) Kuntze were cited for same condition in both communities

Table 4.1: Intercommunity citation of plant species as medicine

Plant species	Condition cited in Hamiyundi	Condition cited in Mukhwa
Ajuga intergrifolia. Buch-Ham	Gastrointestinal	Gastrointestinal
Croton macrostachys	Respiratory	Malaria/Fever and skin / Mucocutaneous
Microglossa pyrifolia (Lam)O.Kutze	Skin	Mucocutaneous
Tylosema fassoglensis (Kotschy)Torre & Hill C.	Malaria/Fever	Gastrointestinal ,
Aloe spp	Respiratory	Syphilis
Psydium guajava L.	Gastrointestinal	Gastrointestinal

4.1.1 Knowledge of diseases associated with HIV and comments on herbal medicine use in the community

The responses shown in Appendix 6 (Table of responses to knowledge of diseases caused by HIV and comments on the use of herbal medicine) show that CHW were knowledgeable on diseases associated with HIV. The use of herbal medicine was regarded positively by most of them as the following responses show. 'The conditions treated by the medicinal plants include Herpes zoster, TB, and diarrhea'. A community health worker from Hamuyundi.

'I have observed some herbal medicine that are very effective against Herpes simplex, and coughs'. A community health worker from Mukhwa.

'Conditions that are not easily treated in modern hospitals such as TB, Oral thrush, and Herpes are well managed by herbal medicine' another community health worker from Mukhwa.

On the question about status of herbal medicine use by PLWHIV in the community, most CHWs indicated that they were widely used. 'I know of some PLWHIV who have been using it for the last five years'. A CHW from Hamuyundi.

'Many people use herbal medicines frequently for problems like fever, headache and stomach aches'. Another CHW from Hamuyundi.

'Those who have used the herbal medicines and experienced their benefits use them a lot'. A CHW from Mukhwa.

Not all the CHW had positive regard for the herbal medicines as seen from the following responses. 'They are not effective for HIV management at all'. A CHW from Mukhwa.

'It is not effective and cause more harm on the patients' health because it doesn't have dosage'. Another CHW from Mukhwa.

'I discourage herbal drugs to be used by HIV patients but instead use hospital drugs which are effective'. A CHW from Hamuyundi.

4.2 Phytochemistry and pharmacology reports on cited medicinal plants

Of the 67 plant species identified as medicine by CHW, 40 were cited in literature as having medicinal application and or pharmaceutical activity (Appendix 12: Phytochemical and Ethno-pharmacological reports of the cited medicinal plants). Out of the 40 plant species, 23 have been investigated for secondary metabolites (Table 4.2a – 4.2c), and 7 of the medicinal plants mentioned by CHWs were reported as having anti-malarial application (Table 4.2a), 14 for anti-microbial application (Table 4.2b), and 16 as physiology modulators (4.2c). Additionally, *C. macrostachys* Hoechst (Tariku *et al.*, 2010), *Tetradenia urticifolia* (Bak.) Phlipson, and *C. macrostachus* Hoechst. (Nyunja *et al.*, 2009) were cited for use against leishamnia, helminthes and measles, respectively.

Table 4.2a: Plants cited for anti-malarial use

Plant species	*Secondary metabolites*
Tetradenia urticifolia (Bak.) Phlipson(Satish and Ranjana, 2013)	Polyphenol compounds (Aprotosoae et al., 2013)
Ajuga integrifolia (Buch-Ham) (Satish and Ranjana, 2013) (Cocquyt *et al*, 2011)	Ergosterol-5,8-endoperoxide (6) ajugarin-(1), 8-O-acentylharpagide(5) (Cocquyt et al., 2011)
Ocimum gratissimum (L.) (Tchoumbougnang et al., 2005),	Gamma-terpene, beta-phellandrene, limonene and thymol (Tchoumbougnang *et al.,* 2005), Tannins, steroids, terpenoids, Flavonoids and cardiac glycosides (Akinmoladun et al., 2007) and Phenols ((Igbinosa et al., 2013), eugenol and methyl eugenol (Josh, 2013)
Fuerstia africana T.C.E Fr (Lilian et al., 2013; Ester et al., 2012; Kingondu et al., 2011)	Sterols, terpenoids, Alkaloids, Saponins, Glycosides, Flavonoids and Tannins (Okach et al., 2013)
Croton macrostachys Hoechst. (Leychilu *et al*, 2014)	Benzyl benzoate, Linalool, gama-muurolene, alpha-farnesene, delta-cadinene and alpha curcumene (Tariku et al., 2010)
Anthocleista vogelii Planch. (Gboeloh, Okon, & Udoh ., 2014)	Saponins, cardiac glycosides, flavonoids, terpenes , alkaloids, and steroids (Gboeloh, Okon, & 2014)
Plumeria alba L. (Boampong et al., 2013)	Leaves; Terpenoids, Flavonoids, Alkaloids, Glycosides and Phytosteroids (Radha et al., 2008), Flowers; Steroids, Flavonoids and Alkaloids (Zaheer et al., 2010)

Table 4.2b: Plants cited for anti-microbial activity

Kedrostis foetidissima (Jacq.) Cogn, ((Nirmala and Pandian, 2015)	7, 10- hexadecadienoic acid. 2- hexadecen-1-ol, 3,7,11,15-tetramethyl-[R-[RR-(E)]] and 1H-1, 2, 4-triazole-3, 5-dicarbaldehyde and docosanoic acid (Pavithra and Vadivukkarasi, 2012).
Cucumis aculeatus Cogn (Ogutu et al., 2012)	Terpenoids, phenolic (Ogutu *et al.*, 2012)
Ajuga integrifolia (Buch-Ham)	Ergosterol-5,8-endoperoxide (6)ajugarin-(1), 8-O-acentylharpagide(5) (Cocquyt et al., 2011)
Fuerstia africana T.C.E Fr (Lilian *et al*., 2013; Ester et al., 2012; Kingondu *et al.*, 2011)	Sterols, terpenoids, alkaloids, Saponins, glycosides, Flavonoids and Tannins (Okach *et al*., 2013)
Ocimum gratissimum (L.) (Adebolu and Oladimeji, 2005; Matasyoh *et al*., 2008; Emeka and Eze, 2009; Josh, 2013)	Gamma-terpene, beta-phellandrene, limonene and thymol (Tchoumbougnang *et al.,* 2005), Tannins, steroids, terpenoids, Flavonoids and cardiac glycosides (Akinmoladun et al., 2007) and Phenols ((Igbinosa *et al.*, 2013), eugenol and methyl eugenol (Josh, 2013)
Thuribergia alata Sims(Jeniffer et al., 2004)	Glucosides:Thunaloside and alatoside, iridoid glycosides, stilbericosides, 6-epi-stilbericosides and thunbergioside (Damltoft et al., 1994)
Microglossa pyrifolia (Lam.) Kuntze(Dicson et al, 2006)	Dihydrobenzofurans; (methyl 2-(5-acetyle-2,3-ihydrobenzo[beta]furan-2yl) propenoate]., 3(methyl2-(6-acetyl-5-hydroxy-2,3dihydrobenzofuran-2-yl)propenoate] and 7(6-acetyl-5 hydroxy-2-(1-hydroxy-2-(1-hydroxy-2-propenyl)-3-methoxy-2,3-dihydrobenzofuran., Triterpenes; 3beta-acetoxy-25-hydroxydammara-20,23-diene(9)., 3beta-acetoxy-24-oxo-dammara-220,25-diene(11)., 17beta-hydroxy-3,16-dioxo-28-

	norolean-12-ene(12) and 17beta-hydroxy-3,11,16-trioxo-28-norolean-12-ene (Schmidtt, *et al*., 2003)
Croton macrostachys Hoechst.	Benzylbenzoate, Linalool, gama-muurolene, alpha-farnesene, delta-cadinene and alpha curcumene (Tariku *et al.,* 2010)
Zanthoxylum giletti (De wild) P.G.Waterman (Wagate et al., 2010; Belay et al., 2011) (Tariku et al., 2010)	Alkaloids:Peroxysimulenoline, Sanguinarine, Faragarine 1, Norchelerythrine, Dihydronitidine (Gaya et al., 2013)
Cassia occidentalis L (Saganuwan and Gulumbe, 2006) (Vedpriya et al., 2010)	Alkaloids, tannins, saponin, glycoside and flavonoids (Saganuwan and Gulumbe, 2006)Achrosin,, Aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol and chrysoeriol (Yadav et al.,2010)
Entada abyssinica A. Rich (Teke et al., 2011; Tchana et al., 2014)	Alkaloids, flavonoids, Tannins, Saponins and cardiac glycosides; (5S,6R,8AR) -5-(carboxymethyl) -3,4,4a,5,6,7,8,8a –octahydro – 5,6,8a – trimethylnaphalene-carboxylic acid ; methyl 3,4,5 – trihydroxybenzoate(methyl galate) ; benzene – 1,2,3 –triol (Pyrogallol); and 2,3 – dihydroxypropyltriacontanoate. Lipids; Hexadecanoic acid, 9-Octadecenoic acid and Octadecanoic acid
Plumeria alba L. (Kumari et al., 2012) (Syakira and Brenda, 2010)	Leaves; Terpenoids, Flavonoids, Alkaloids, Glycosides and Phytosteroids (Radha *et al.,* 2008), Flowers; Steroids, Flavonoids and Alkaloids (Zaheer et al., 2010)
Caesalpinia decepetala (Roth. I. Alston) (Erasmus et al., 2012)	6’-hydroxy-epoxy-propane)-2’,3’-(1”β-hydroxy-2carbonyl-cyclobutane)-1,1’-diphenyl; Octacosyl 3,5-dihydroxycinannamate; 2’4,4’-trihydroxychalocone; bonducellin; 7,3’5’-trihydroxyflavanone, daucosterin and β-sitosterol (Zhang et al., 2008), Cassane diterpenoid (spathulenol; 4,5-epoxy-8(14)-caryophyllene,; squalene; lupeol; trans-resveratrol; quercetin; astragalin and stigmasterol (Kiem et al., 2005), lupeol acetate; lupeol; oleanoic acid; pentacosanoic acid 2,3-dihydroxypropylester; 1-(26-hydroxyhexacosanoyl)-glycerol;

Microglossa pyrifolia (Dickson et al., 2006)	stigmasterol; beta-sitosterol (Li *et al*., 2002) Dihydrobenzofurans (methyl 2-(5-acetyl -2, 3-dihydrobenzo [beta] furan-2-yl) propenoate; methyl 2-(6-acetyl-5-hydroxy-2, 3-dihydrobenzofuran-2-yl) propenoate; 6-acetyl-5-hydroxy-2-(1-hydroxy-2-propenyl)-3-methoxy-2, 3-dihydrobenzofuran. Triterpenes 3 beta-acetoxy-25-hydroxydammara-20,23-diene; 3beta-acetoxy-24-oxo-dammara-20,25-dien; 17beta-hydroxy-3,16-dioxo-28-norolean-12-ene; 17beta-hydroxy-3,11,16-trioxo-28-norolean-12-ene (Schmidt *et al*., 2003)

Table 4.2c: Plants cited as Physiology modulators

Ocimum gratissimum (L.) (Akinmoladun et al., 2007; Igbinosa et al., 2013; Mahapatra *et al.*,2009; Josh, 2013) (Chiu *et al*., 2012) (Okon et al.,2015) (Ajayi et al., 2014) (Ofem and Eno, 2012) (Okoli et al., 2010)	Gamma-terpene, beta-phellandrene, limonene and thymol (Tchoumbougnang *et al.*, 2005), Tannins, steroids, terpenoids, Flavonoids and cardiac glycosides (Akinmoladun *et al*., 2007) and Phenols ((Igbinosa et al., 2013), eugenol and methyl eugenol (Josh, 2013)
Justicia betonica (L.)	10H-Indolo[3,2-b] quinolone (Andy et al., 2007)
Psidium guajava L. (Ju –Wen et al., 2009)	Gallic, Catechin and quercetin (Ju –Wen et al., 2009)
Markhamia lutea (Benth.) K. Shum(Lacroix et al., 2009)	Cycloartane triterpenoids (Lacroix et al., 2009)
Aloe sp (Lisa et al., 2008)	Diverse species specific Contents including Alcohols, aldehydes, Ketones, Pyrimidines, Indole, alkaloids, Sterols, Fatty acids, Dicarboxylic acid (Lisa et al., 2008)
Croton macrostachys Hoechst. (Nyunja et al., 2009) (Kamanyi et al., 2009)	Benzylbenzoate, Linalool, gama-muurolene, alpha-farnesene, delta-cadinene and alpha curcumene (Tariku et al., 2010)
Zanthoxylum giletti (De wild) P.G.Waterman(Kokwaro, 1993)	Alkaloids: Peroxysimulenoline, Sanguinarine, Faragarine 1, Norchelerythrine, Dihydronitidine (Gaya et al., 2013)

Cassia occidentalis L. (Laximi et al., 2010) (Vipin et al., 2007)	Alkaloids, tannins, saponin, glycoside and flavonoids (Saganuwan and Gulumbe, 2006)
Tylosema fassoglensis Brenan, J.P(Jafri et al.,1999; Sadique et al., 1987)	High seed content of Linoleic acid, Oleic and Palmitic acid. Proteins are characterized by high levels of lysine, proline and tyrosine (Dubois *et al.*, 1995)
Melia azedarach L. (Gayatri and Rajani, 2010)	(20S) -5,24(280-ergostadiene-3β,7α, 16 β,20-tetrol, (20S)- 5-ergostene - 3 β, 7α, 16 β,20-tetrol 2α, 3β-dihydro-5-pregnen-16-one (Shi-Bao *et al.*, 2009)
Anthocleista vogelii Planch. (Abuh et al., 1990)	Saponins, cardiac glycosides, flavonoids, terpenes , alkaloids, and steroids (Gboeloh, Okon & Udoh 2014)
Entada abyssinica A. Rich(Teke et al., 2011; Tchana et al., 2014)	Alkaloids, flavonoids, Tannins, Saponins and cardiac glycosides; (5S,6R,8AR) -5-(carboxymethyl) -3,4,4a,5,6,7,8,8a –octahydro – 5,6,8a – trimethylnaphalene-carboxylic acid ; methyl 3,4,5 – trihydroxybenzoate(methyl galate) ; benzene – 1,2,3 –triol (Pyrogallol); and 2,3 – dihydroxypropyltriacontanoate. Lipids; Hexadecanoic acid, 9-Octadecenoic acid and Octadecanoic acid (Chibikwa, 2015)
Plumeria alba L. (Radha and Sivakumar, 2009)	Leaves; Terpenoids, Flavonoids, Alkaloids, Glycosides and Phytosteroids (Radha *et al.*, 2008), Flowers; Steroids, Flavonoids and Alkaloids (Zaheer et al 2010)
Caesalpinia decepetala (Roth. I. Alston) (Parveen et al., 2014) (Hussain et al., 2014) (Gallego et al., 2015; Pawar and Surana, 2010) (Modh, 2012)	6'-hydroxy-epoxy-propane)-2',3'-(1"β-hydroxy-2carbonyl-cyclobutane)-1,1'-diphenyl; Octacosyl 3,5-dihydroxycinannamate; 2'4,4'-trihydroxychalocone; bonducellin; 7,3'5'-trihydroxyflavanone, daucosterin and β-sitosterol (Zhang *et al.*, 2008), Cassane diterpenoid (spathulenol; 4,5-epoxy-8(14)-caryophyllene,; squalene; lupeol; trans-resveratrol; quercetin; astragalin and stigmasterol (Kiem *et al.*, 2005), lupeol acetate; lupeol; oleanoic acid; pentacosanoic acid 2,3-dihydroxypropylester; 1-(26-hydroxyhexacosanoyl)-

	glycerol; stigmasterol; beta-sitosterol (Li et al., 2002)
Ageratim conyzoides (Bayala *et al.*,2014) (Choudhury et al ., 2015) (Diallo et al., 2014)	Precocene (Bayala et al.,2014)
Microglossa pyrifolia (Lam)O.Kutze(Bum et al., 2011)	Dihydrobenzofurans (methyl 2-(5-acetyl -2, 3-dihydrobenzo [beta] furan-2-yl) propenoate; methyl 2-(6-acetyl-5-hydroxy-2, 3-dihydrobenzofuran-2-yl) propenoate; 6-acetyl-5-hydroxy-2-(1-hydroxy-2-propenyl)-3-methoxy-2, 3-dihydrobenzofuran. Triterpenes 3 beta-acetoxy-25-hydroxydammara-20,23-diene; 3beta-acetoxy-24-oxo-dammara-20,25-dien; 17beta-hydroxy-3,16-dioxo-28-norolean-12-ene; 17beta-hydroxy-3,11,16-trioxo-28-norolean-12-ene (Schmidt *et al*., 2003)

4.3 Cytotoxicity and Antiviral activity of selected medicinal plants

4.3.1 Plants selected for cytotoxicity and anti-herpes experiments

Using criteria explained in section 3.5.4., eight medicinal plants shown in table 4.3, cited for Herpes simplex or Herpes Zoster were selected for cytotoxicity and anti-herpes determination. All the 8 medicinal plants were cited and collected in various villages within Mukhwa sub-location (Geographical detail of the villages are as shown in Figure. 3.2).

Table 4.3: Plants selected for cytotoxicity and antiviral experiments

Plant name	HIV condition cited for use	Village	Part used	Method of preparation	Method of application
Tithonia diversifolia	Herpes zoster	Wang Nyang 'A'	Whole root	Boiling	Drinking
Schuhria pinata	Mouth ulcers, cold sores	Mukhwa 'B'	Leaves	Boiling	Topical
Entada abyssinica	Skin ulcers, Herpes simplex lesions	Mukhwa 'A'	Stem bark	Boiling	Drinking
Garcinia buchananii	Herpes zoster	Mukhwa 'A'	Stem bark	Boiling	Drinking
Croton macrostachys	Fever and skin conditions, Herpes simplex	Mukhwa 'A'	Stem bark	Boiling	Drinking
Vernonia adoensis	Herpes simplex Genital ulcers, Herpes zoster	Lukongo 'C'	Whole root	Boiling	Drinking
Plumeria alba	Herpes zoster	Mukhwa 'B'	Leaves	Pounding (Crushing) Cold infusion or boiling	Topical
Caesalpinia decepetala	Genital ulcers (1), Herpes simplex Poor appetite, Mouth sores	Wang nyang 'A'	Whole root	Boiling	Drinking

4.3.2 Cytotoxicity of selected medicinal plants

Cytotoxicity experiment as described in section 3.5.4.3 was carried out to determine concentration range within which Vero cells tolerate cytotoxic effect of extracts. Table 4.4 shows that Vero cells retained above 50% viability at extract concentrations of 125µg/mL and below, except for *S. pinata* which showed values of 17.4% at 125µg/mL, but showed good viability below the concentration.

Table 4.4: Cell Viability response to extracts of selected plant species

Extract concentration µg/ml	% Mean Vero Cell Viability response to plant species ± SD							
	Tithonia diversifolia (Whole root)	*Schuhria piñata (Leaves)*	*Entada abyssinica (Stem bark)*	*Garcinia buchananii* (Stem bark)	*Croton macrostachys* (Stem bark)	*Vernonia adoensis* Walp (Whole root)	*Plumeria alba* L.(Leaves)	*Caesalpinia decepetala* (Whole root)
500	44.5± .95	6.0± .92	58.8± 3.02	57.0± 2.00	40.4± 2.08	46.5± 1.30	2.6±.62	67.7 ±1.53
250	82.8± 1.01	14.2± .56	72.5± 2.78	68.0± 2.00	78.0± 1.73	81.2± 1.85	13.4±.87	76.50± 1.34
125	85.7± .92	17.4±1.40	80.0± 3.46	92.6± 1.51	86.0± 1.73	83.6± 1.32	51.0±2.65	78.5± 2.72
62.5	86.2± 1.11	82.0± 4.36	94.0± 2.65	98.0± .44	87.0± 1.00	88.5± .82	62.0±3	92.6± 1.71
31.25	96.0± 1.40	95.3± .70	97.0± 1.20	98.4± .53	88.0± 3.21	93.3± .66	68.0± 2.64	91.0± 2.6
15.625	100.0± .00	97.0± 1.11	97.8± .53	98.6± .26	92.0± 1.73	98.2± .60	70.0± 5.29	98.1± 1.20
0	100.0± .00	100.0± .00	100.0± .00	100.0± .00	100.0± .00	100.0± .00	100.0± .00	100.0± .00

4.3.3 Extract concentration reducing Cell viability by 50% (CC_{50})

As described in section 3.5.4.3, selected medicinal plants were further investigated for CC_{50}. The CC_{50} for *T. diversifolia* (KK01) *S.pinata* (KK02), *E. abyssinica* (KK03) , *G. buchananii* (KK04), *C. macrostachys* (KK05), *V.adoensis* (KK07) *P. alba* (KK08) and *C. decapetala* (KK09) were; 460, 90 and >500 , >500, 440 and 470, 120 and >500 µg/ml, respectively (Figure 4.1).

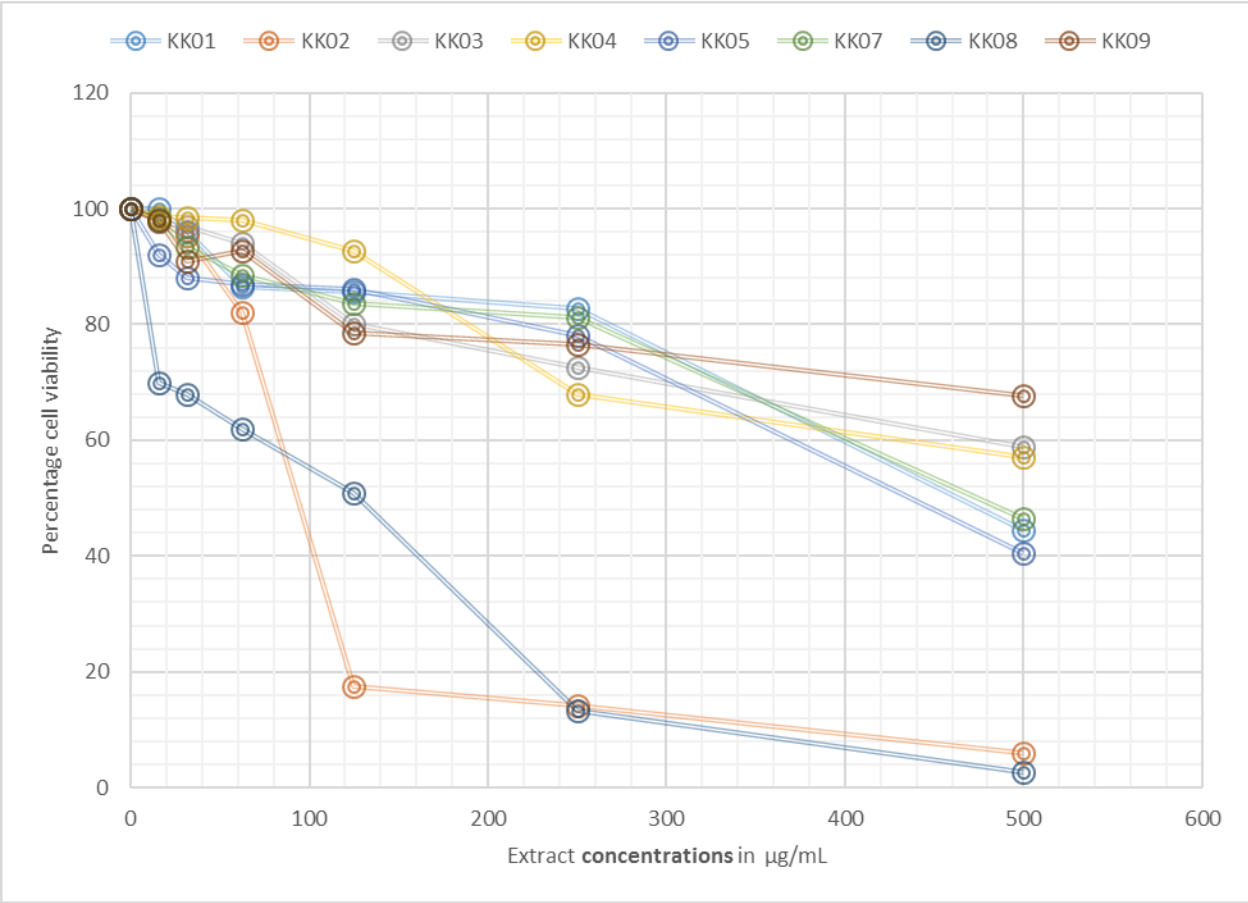

Figure 4.1: Cell viability at different concentrations of extract.

The legends in the figure represent plant species as follows; KK01 (*T. diversifolia*), KK02 (*S.pinata*), KK03 (E. *abyssinica*), KK04 (*G. buchananii*), KK05 *(C. macrostachys*). KK07 (*V.adoensis*), KK08 (*P. alba*), KK09 (*C. decapetala)*

4.3.4 Anti-herpes screening test

Section 3.5.4.4 of the methodology describes the screening method for anti-herpes activity at 100 µg/mL by EPTT. The extracts of *S. pinata, E. abyssinica, P. alba* and *C. decepetala* provided at least 10 fold titer reduction of HSV-1 compared to negative control (Table 4.5).

Table 4.5: Titer reduction factor (RF) of plant extracts

Plant extract	Part used	RF
T. diversifolia	Root	0.4
S. pinata	Leaves	1.0
E. abyssinica	Stem bark	1.2
G. buchananii	Stem bark	0.7
C. macrostachys	Stem bark	0.2
V. adoensis	Root	0.9
P. alba	Leaves	1.2
C. decepetala	Root	1.3

4.3.5 Dose response test for cell protection of plant extracts against HSV- 1

This was carried out as described in section 3.5.4.5 of the methodology. The extracts of *G. buchananii* and *C. decepetala* provided the highest cell protection at both 25 and 100µg/mL by metabolism of MTT (Table 4.6). Positive control by acyclovir at 5 and 10µg/mL gave lower percentage protection compared to *G. buchananii* at 25 and 100µg/mL.

Table 4.6: Cell protection of plant extracts against HSV - 1

Experimental Plant extract/Acyclovir (Positive control	Part used	Percentage cell protection		Acyclovir Tablet	
		25µg/ml	100µg/ml	5µg/ml	10 µg/ml
T. diversifolia	Whole root	0.02%	4.9%		
S. pinata	Leaves	1.85%	4.85%	N/A	N/A
E. abyssinica	Stem bark	13.7%	21%	N/A	N/A
G. buchananii	Stem bark	91.4%	100%		
C. macrostachys	Stem bark	0%	0%		
V. adoensis	Whole root	0%	0%	N/A	N/A
P. alba	Leaves	5%	6.9%	N/A	N/A
C. decepetala	Whole root	17.1%	68.5%	N/A	N/A
Acyclovir	N/A	N/A	N/A	64%	84%

N/A: not applicable because plant part does not apply to acyclovir (Control drug) or the concentration at which acyclovir was tested does not apply to extracts.

4.3.6 Inhibitory concentration 50 (IC_{50}), therapeutic index (TI), maximum cell protection concentration (MCPC) and maximum non-toxic concentration (MNC)

Based on dose response test results at 25µg/mL and 100µg/mL, against HSV- 1, *G. buchananii* and *C. decapetala* were selected for inhibitory concentration 50 (IC_{50}), therapeutic index (TI) maximum cell protection (MCPC) and maximum non-toxic concentration (MNC) tests as described in section 3.5.4.6.

4.3.6.1 The IC_{50}, TI, MCPC) and MNC of *G. buchananii*

The IC_{50} of *G. buchananii* was 20µg/mL, MCPC 60µg/mL, MNC 40µg/mL (Figure 4.2).The therapeutic index was obtained from the ratio of CC_{50} to IC_{50} (CC_{50} >500µg/ml)/IC_{50}~20µg/ml) > 25.

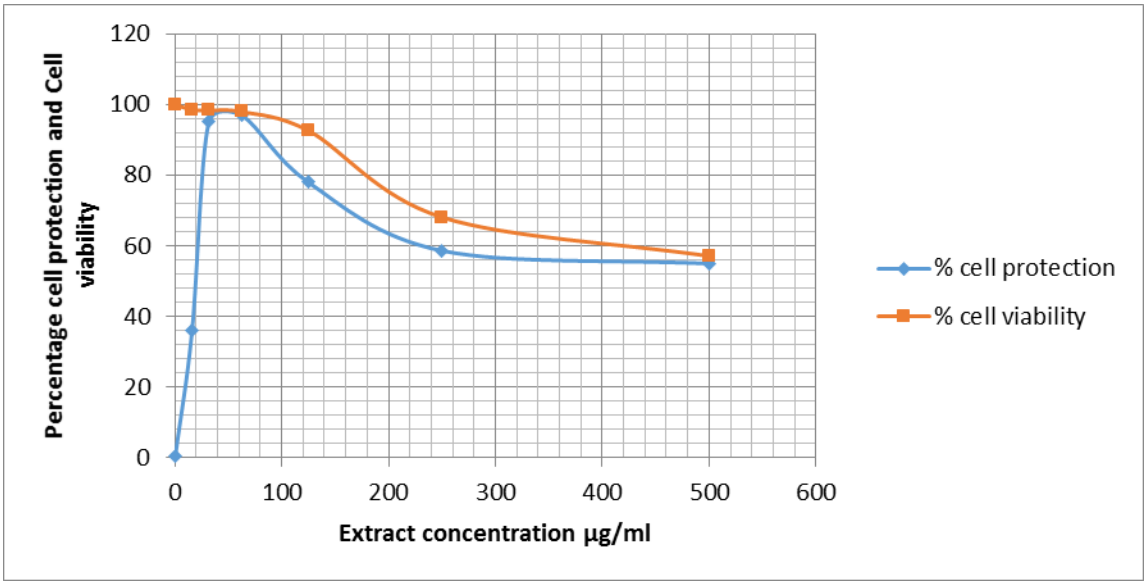

Figure 4.2: Cell protection against HSV and cell viability at different extract concentrations of *G. buchananii*.

4.3.6.2 The IC_{50}, TI, MCPC) and MNC of C. *decapetala*

The IC_{50} for *C. decapetala* was approximately 80µg/ml, MCPC was approximately 120µg/ml and MNC was approximately 20µg/mL (Figure 4.3). Therapeutic index: (CC_{50} >500µg/ml)/IC_{50} 80µg/ml) > 6

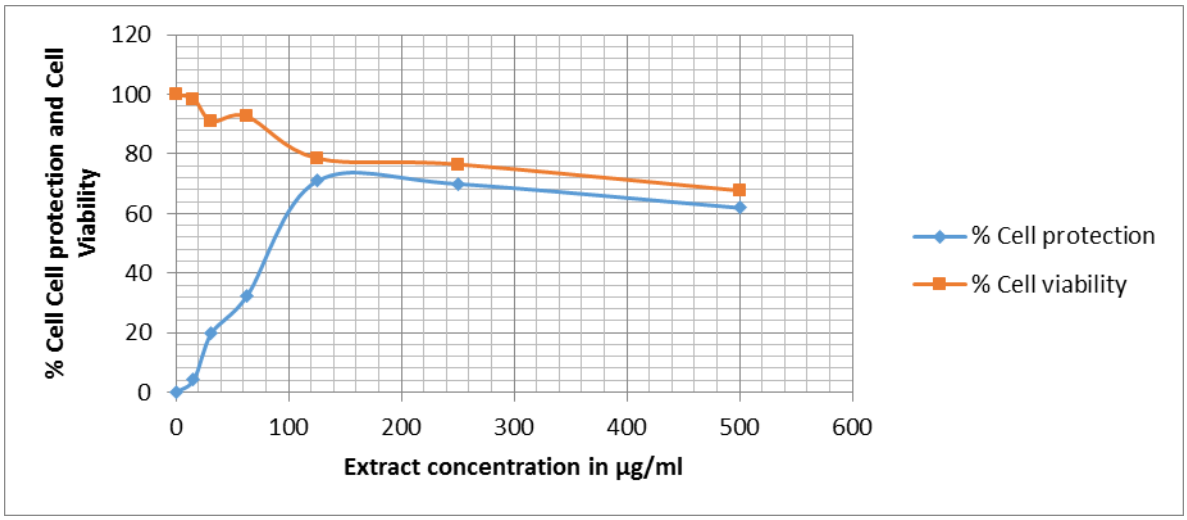

Figure 4.3: Cell protection against HSV and cell viability at different extract concentrations of *C. decapetala.*

4.3.7 Characterization of antiviral activity of *G. buchananii* and *C. decapetala* at 25µg/mL and 100µg/mL

This was carried out as described in section 3.5.4.7. Cell protection by the presence of extract throughout and post adsorption treatment was significantly higher than cell protection with pre-treatment, Virucidal and during adsorption treatment at all concentrations $p \leq .05$ by ANOVA (Multiple comparisons Bonferroni post Hoc test) (Table 4.7)

Table 4.7: Characterization of antiviral activity of *G. buchananii* and *C. decapetala*; Mean % Cell protection at 25µg/mL and100µg/mL ±SD

Cell treatment	***G. buchananii***		***C. decapetala***	
	25µg/mL	100µg/mL	25µg/mL	100µg/mL
Pre-treatment	.5±.12	.5±.06	1 ±.21	.7±.06
Throughout treatment	92.3±1.53	98.1± .94	18.5 ±1.29	68.4±1.69
Post adsorption treatment	92.7±1.53	97.0±1.0	18.9±1.79	68.3± 3.51
Virucidal treatment	8.5±.56	9.1±1.10	8 ±.35	5.5±.61
Adsorption treatment	7.5± .61	8.5±.64	7.8 ±.32	5.8±.35

4.4 In *vivo* Assay

4.4.1 Acute oral toxicity of extracts

Toxicity of *G. buchananii* and *C. decapetala* extracts was determined by mortality and change in mice weight at day 7 and day 14 after they were given a dosage of 1000 mg/kgbwt of the extract as described in section 3.5.4.8. No death was observed in mice receiving extract and normal saline. There was also no significant difference in mean change of weight between the control mice and those receiving the extracts (Table 4.8)

Table 4.8: Acute oral toxicity of extracts at 1000mg/kgbwt compared to normal saline as a control.

Treatment	**Mean change in weight (g)±SD**		Mortality ratio
	(Day 1-7)	**(Day 1-14)**	
Control (normal saline)	0.6 ±0.10	1.2±0.12	0/6
G. buchananii	0.5 ±0.12	1.0±0.10	0/6
C. decapetala	0.6±0.05	1.1±0.10	0/6

4.4.2 *In vivo* oral antiviral response

Section 3.5.4.8 describes the procedure for *In vivo* experiments. The *In vivo* carried out for *G. buchananii* and *C. decapetala* at 125, 250 and 500 µg/mL on mice infected with HSV-1 7401H. The response was compared with that of a positive control (Acyclovir) and negative control (normal saline). Acyclovir delayed symptom onset, further development and prevented death of the mice. The treatment with *G. buchananii* at 500 mg/kgbwt gave significant different response compared to negative control for mean day of symptom onset (p = .006), progression to mild zosteriform (p = .005) and day of death (p = .007). Treatment with below 250mg/kgbwt of *G. buchananii* and the extracts of *C. decapetala* at 500mg/kgbwt did not give significant difference with negative control (Table 4.9).

Table 4.9: *In vivo* oral antiviral response

Treatment	Mean time (days) of initial symptom scores ±SD		Mean time (days) of death ±SD	Mortality ratio
	Score 2	**Score 6**		
Positive control (Acyclovir 5mg/kgbdwt)	5.0±.00	**NC**	**NC**	0/6
Negative control (Normal saline)	3.5±0.55	4.8±0.45	6.7±0.52	6/6
G. buchananii 500 µg/mL	4.8*±*0*.50	6.3*±0.58	7.7*±0.52	6/6
G. buchananii 250 µg/mL	3.5±0.55	4.7±0.58	6.7±0.52	6/6
G. buchananii 125 µg/mL	3.5±0.55	5.0±0.82	6.5±0.55	6/6
C. decapetala 500 µg/mL	4.0±0.71	5.3±0.50	6.8±0.5	4/6
C. decapetala 250 µg/mL	3.3±0.52	5.0±0.82	6.3±0.52	6/6
C. decapetala 125 µg/mL	3.2±0.41	4.8±0.45	6.0±0.63	6/6

NC; Not calculated because the symptom was not observed.
** p< .05 compared to negative control.*

4.5 Antioxidant activity

4.5.1 Screening of extracts for antioxidant activity at 500µg/mL

Determination of antioxidant activity was carried out for the eight medicinal plants selected for anti-Herpes activity, based on relationship between microbial infections and antioxidants as explained in section 2.6 of literature review. The experiment was carried out as explained in section 3.6. At 500µg/mL, *S. pinata* (KK02) gave the highest DPPH reduction. The extract of *P. alba* (KK08) did not reduce DPPH (Table 4.10). Extracts giving more than 50% DPPH reduction were further investigated for the sample concentration giving 50% DPPH reduction (RSa_{50}) and compared with ascorbic acid. The extracts for *C. macrostachys* and *P. alba* were not considered because each gave a screening reduction of less than 50%.

Table 4.10: Screening of extract for antioxidant activity at 500µg/mL

Plant species	*Part used*	*Percentage DPPH reduction*
T. diversifolia	Whole root	86.9
S. *pinata*	Leaves	97.3
E. abyssinica	Stem bark	96.1
G.buchananii	Stem bark	89.1
C. macrostachys	Stem bark	28.2
V. adoensis	Whole root	58.7
P. alba	Leaves	0
C. decapetala	Whole root	92.3

4.5.2 Sample concentration reducing DPPH by 50% (RSa_{50})

For the samples that showed over 50% DPPH reduction at 500µg/mL, sample concentration reducing DPPH by 50% (RSa_{50}), was determined. The RSa_{50} of extracts were; 110, 150, 20, 10, 240, 50 µg/mL for *T. diversifolia* (KK01), *S. piñata* (KK02), E. *abyssinica* (KK03), *G. buchananii* (KK04) , *V. adoensis* (KK07) , and *C. decapetala* (KK09) respectively (Figure 4.4). The RSa_{50} of the extracts was equivalent to 0.8µg/mL of ascorbic acid (Figure 4.5).

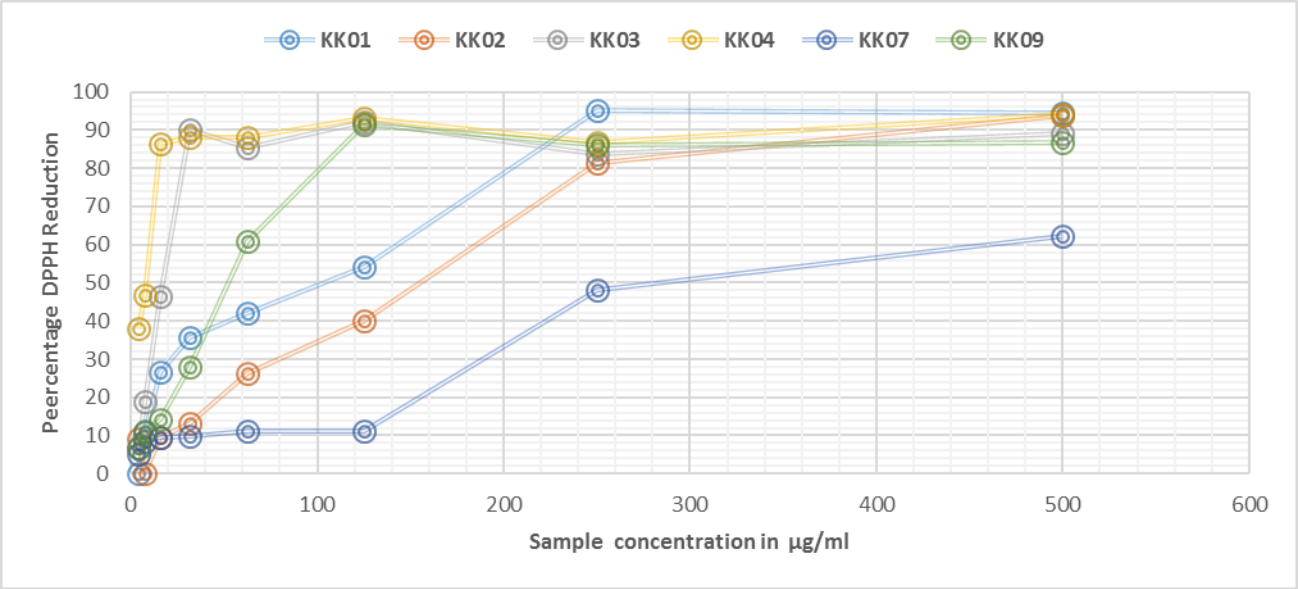

Figure 4.4: Sample concentration and percentage reduction of DPPH.

The legends in the figure represent plant species as follows; KK01 (T. diversifolia), KK02 (*S.pinata*), KK03 (*E. Abyssinica*), KK04 (*G. buchananii*), KK07 (*V. adoensis*), KK09 (*C. decapetala*)

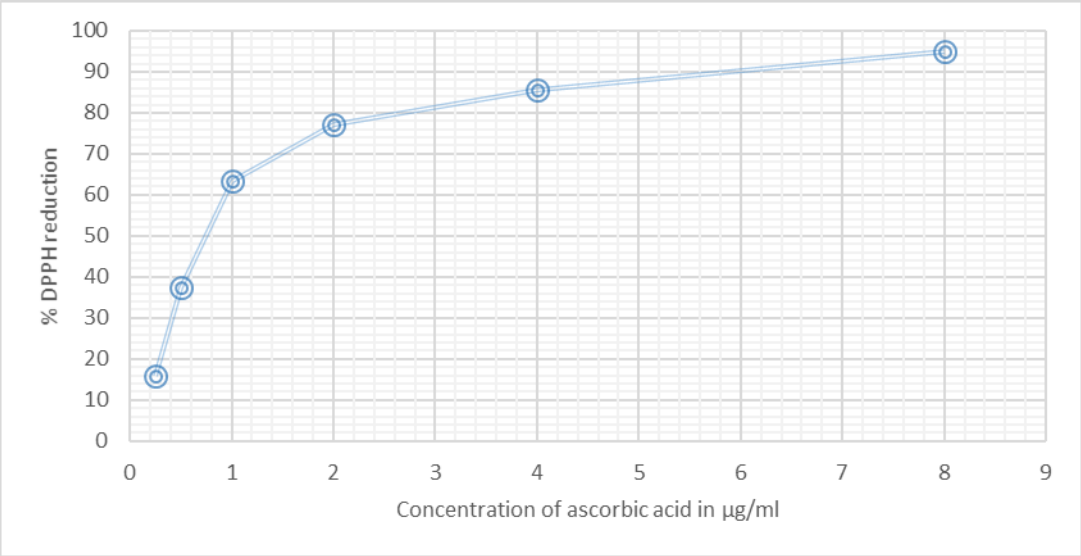

Figure 4.5: Ascorbic acid concentration and percentage reduction of DPPH

4.6 Phytochemical screening of extracts

The 5 chemical groups selected for detection comprised majority of reported antiviral compounds (Hudson, 1990). Major phytochemical groups detected in the plant extracts were; alkaloids in *S. pinata*, terpenoids in *E. abyssinica*, flavonoids and phenols in *G. buchananii* (Table 4.11).

Table 4.11: Phytochemical screening of extracts for common pharmacologically active principles against Microbial agents

Sample	Plant part used	Alkaloids	Terpenoids	Saponins	Flavonoids	Phenols
T. diversifolia	Whole root	Trace	Trace	-	Trace	Trace
S. pinata	Leaves	+	Trace	-	Trace	-
E. abyssinica	Stem bark	Trace	+	-	Trace	Trace
G. buchananii	Stem bark	Trace	Trace	-	+	+
C. macrostachys	Stem bark	Trace	-	-	Trace	-
V. adoensis	Stem bark	Trace	Trace	-	Trace	-
P. alba	Stem bark	Trace	-	-	Trace	-
C. decapetala	Stem bark	Trace	Trace	-	Trace	-

+ Chemical group present

Trace: Chemical present in trace amounts

- Chemical group absent

CHAPTER FIVE

DISCUSSION, SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Medicinal plants used in the care and management of HIV disease in Vihiga and Kakamega Counties in Western province of Kenya

Prior to inquiry on plants used for management and care of HIV conditions, the study sought to assess the knowledge of CHW on HIV conditions and the status of herbal medicine use in Hamiyundi and Mukhwa. The aim was to determine awareness of the relationship between HIV diseases and application of alternative medicine, existence of herbal medicine use in the community, the attitude about herbal medicine use, and observations on people using them.

Except for a few none-HIV related conditions mentioned such as syphilis, trachoma and gonorrhea, all other conditions are consistent with the WHO symptomatic staging of HIV disease (WHO, 2006). The knowledge of HIV conditions by CHW was not surprising, given that they are often the target of training programmes in HIV as part of community health strategy for primary health care (CHS) (GOK- MOH, 2012a; GOK-MOH, 2007).

The use of CHWs as respondents to inquiry of health behavior of PLWHIV was premised on the concept of representation in qualitative survey. Qualitative survey is capable of determining diversity of cognition or behavior of a population, represented by a small group if the small group is well integrated with the target population (Harrie (2010). According to community health strategy, each CHW take charge of primary health care of at least 20 households, and may be expected to act as an intermediary between a sick member of the community, including PLWHIV and formal health care service provider (GOK-MOH, 2012b).

The attributes of herbal medicine as perceived by the community was deduced from verbatim quotes of respondents. The majority regarded plant medicines positively, with some implying that herbal medicine have withstood test of time and possess benefits lacking in modern medicine. However, some comments reflected health sector policy forbidding the use of herbal medicine for patients on ARVs (GOK-MOH, 2007). Despite the two contradictory stance and given the attitude implied in the opinion of the respondents, unmet needs in the care and management of HIV still persits as indicated in the Kenyan demographic HIV survey report (GOK-MOH, 2014). The latter appear to be a significant factor that will sustains the seeking and use of alternative medicine,

A total of 65 different plants from 43 families were cited as used in the care and management of HIV conditions in Vichuga and Kakamega counties. By considering species in each family, the most used plant families in Hamuyundi were *Lamiaceae, Solanaceae* and *Rubiaceae*, with 5 species in *Lamiaceae* and 3 species in *Solanaceae* and *Rubiaceae. Lamiaceae* family is known for abundance of polyphenols (Aprotosoaie et al, 2013). The family is recognized for antimicrobial and anti-oxidant value (Liu, 2004). Other uses reported for plants in this family is aromatherapy, a property that provides soothing effect to users (Raja, 2012). Other medicinal benefits reported by Raja are sedative effect, diuretics, tonics and antispasmodic properties (Raja, 2012). The physiological effects reported by Raja are requirements needed for relief of side effects of ARVs as reported by Manish et al (Manish et al., 2014). Manish et al reported several adverse drug reactions experienced by PLWHIV taking ARV, including disturbances in nervous system, gastrointestinal musculoskeletal and dermatological functions.

Plants in *Rubiaceae* family are known for important metabolites and medicinal applications. Kala reported medicinal applications for antihypertension, antimicrobial, antidiabetic, antioxidants and anti-inflammatory uses (Kala, 2015). Use of family species is supported by reported presence of bioactive metabolites including alkaloids, indole, alkaloids, terpenoids and anthraquinones (Kala, 2015). The symptoms for application such as heart problems, coughs, chest pain and oral thrush reported at

Hamiyundi are consistent with applications cited in the literature for many species in the family (Kala, 2015).

The Solanaceae family contains many species of plants known for nutritional and medicinal value. This includes the *Capsicum* species that have shown antimicrobial activity (Cichewicz et al., 1996). Capsaicin and dihydrocapsaicin are the two ingredients that have been suggested to account for antimicrobial activity against *Bacillus cereus, Bacillus subtillis, Clostridium sporogenes, Clostridium tetanii* and *Streptococcus pyogenes* (Cichewicz et al., 1996). In Israel, *Lycium europium* L., *Solanum nigram* L., *Hyoscyamus aureus* L. and *Hyoscymus albus* L. have been reported to be useful for external applications, suggesting that they are active against skin pathogens (Dafni et al., 1994). The *Lycopersicon esculentum* (L.) H. Karst (tomato) is known for its nutrition value (Bohs, 1988) and has also shown activity against Herpes simplex (Thorne et al., 1985).

In Mukhwa, most of the species identified as medicine belonged to *Fabaceae* family. The family is known for plant species of nutritional and medicinal value. The nutritionally valued species include; *Glycine max (L.) Merr*. (Soya bean), *Phaseolus vulgaris L* (beans), *Pisum sativum L* (pea), *Cicer arietinum L* (Chicken pea), *Medicago sativa Lam.* (Alfalfa) *Arachis hypogaea L* (Peanut), *Ceratonia silique L* (Carob), and *Glycyrrhiza glabra* L (Liquorice) (Rahman and Parvin, 2014). Ethno-medical uses of *Abrus precatorius L.*, for respiratory and *Albizia* genus for skin problems in Mukhwa is supported by findings by Rhamand and Parvin in Bangladesh (Rahman and Parvin, 2014).

In both Hamiyundi and Mukhwa, it was observed that one condition could be managed by different plant species, as in the case of *Cassia occideantalis, Tyloseama fassoglensis* and *Melia azedarach* for malaria/fever in Hamiyundi and *Ximemia Americana, albizia coreana* and *Ananas comosa* for gastrointestinal problems. In some cases, one plant species was indicated for more than one conditions as in *Fuerstia* Africa for respiratory

and skin conditions, and *Croton macrostachys* for malaria/fever and skin conditions. Similar findings have been reported by Chisembu and Hedimbi, and Tabuti *et al* (Chisembu and Hedimbi, 2010b; Tabuti *et al* 2009). Both studies attributed the phenomena to dynamism in the ethno-medical practice and the fact that plants contain several and diverse chemical groups that could be remedial to many health problems. In the case of incurable conditions like HIV and the associated opportunistic infection such as HSV, the communities are likely to try several plant species and in different combination to try and treat or manage problematic symptoms.

The plant species with highest frequency of mention in Hamiyundi were; *J. betonica* (4 out of 11 informants) and *C. occidentalis* (4 out of 11 informants). *J. betonica* has been cited in literature for ethno-pharmacological use and potential benefits (Jeruto *et al*., 2008; Sasikamar *et al*., 2007; Bbosa *et al*., 2013). In Nandi County, bordering the County of Vihiga, the community uses leaves of *J. betonica* for coughs, anti-diarrhea and orchitis (Jeruto *et al*., 2008). The plant was also reported to possess broad antibacterial activity when tested against both gram positive and gram-negative bacteria (Sasikumar *et al*., 2007). A study by Bbosa *et al* indicated anti-malaria activity from *J. betonica*, confirming its wide ethno-pharmaceutical application (Bbosa *et al*., 2013)

Saganuwan *et al* evaluated *C. occidentalis* for antibacterial and phytochemical properties. The results showed that the extracts have activity against both gram positive and gram-negative bacteria. Chemical screening revealed the presence of alkaloids, saponins and glycosides (Saganuwan *et al*., 2006). The plant species has also showed anti-diabetic activity when tested on rat experimental model (Verma, 2010). However, consumption of plant seeds of *C. occidentalis* was associated with hepato-myo-encephalopathy among young children in India (Vedpriya *et al*., 2010). A study by Jafri *et al* showed that the leaf extract of *C. occidentalis* was hepato-protective and anti-inflammatory when given orally to experimental rats (Jafri *et al*., 1999). The different results reported for seed and leaves of *C.occidentalis* show that the distribution of

phytochemical ingredients in seeds and leaves might be different, hence different pharmacological effects.

At Mukhwa, *P. alba* (Cited by 8 out of 12 informants) was the most frequently cited plant for medical application. Leaves of *P. alba* have been reported to contain terpenoids, flavonoids, alkaloids, glycosides and phytosteroids (Radha *et al.,* 2008). Flowers of *P. alba* have been reported to contain steroids, Flavonoids and Alkaloids (Zaheer *et al*2010). The aqueous leave extract was shown to possess *in vivo* antimalarial activity (Boampong *et al* 2013). The *in vitro* antifungal activity against *Candinda albicans*, *Aspergillus nige*r and *Penicillum chrysogenum* was reported in the aqueous extract of flowers (Kumari *et al.,* 2012). The *In vitro* antibacterial activity of methanolic flower extracts against *Escherichia coli*, *Staphylococcus saprophyticus, Proteus vulgaris* and *Serretia marcescens* has been reported (Syakira and Brenda, 2010). *In vitro* and *In vivo* anti- cancer activities have also been established in methanolic leave extracts (Radha and Sivakumar, 2009). From these reports, it is evident that *P.alba* has wide range of bioactivity and its pharmacological potential may increase with further investigation.

For majority of plants, consensus was low on use for particular remedies within each community. However, intercommunity consensus on use plants as medicine is worth noting. *A. intergrifolia. C. macrostachys, M. pyrifolia, T. fassoglensis, Aloe spp,* and *Psydium guajava* represent intercommunity consensus for plants of medicinal value. The recognition of such plants by the wider population as having health benefits is a significant lead to drug discovery and therefore required further investigation

Results indicating gastro-intestinal (GI) and Skin problems for which herbal medicines were used points to the needy areas for special care focus. These findings concur with previous studies which have indicated GI and skin problems as the major reasons for use of plant medicines (Maroy, 2014; Chisembu and Hedimbi, 2010; Riberio *et al*., 2010;

Mustapha, 2014). The two conditions are among the symptoms of immune suppression in HIV associated diseases as that defines HIV clinical staging (WHO, 2007).

Findings in previous studies of herbal medicine utilization by PLWHIV is confirmed by results in this study. The most frequently mentioned plant species and the intercommunity concurrence on plants as medicine indicate their potential as medicinal and therefore require further investigation

5.1.2 Pharmaceutical activity and secondary metabolites reported in plant species cited as medicine

With the aim of identifying possible ingredients accounting for their pharmacological use, plant species identified as medicine by CHWs, and whose metabolites and use are cited in literature were classified according to pharmacological activity or application. The cited metabolites could be prime targets for isolation and testing for relevant activity. Additionally, the literature citation of plants identified by CHWs is an indicator of the potential of such plants, and therefore need for further study of the metabolite.

It was noted that *Ocimum gratissimum, Fuerstia Africana*, *Croton macrostachys,* and *Plumeria alba* were cited in literature as both antimalarial and antimicrobial (antibacterial and antifungal). Although none of the four plants was mentioned by CHWs for cure of malaria, their application for skin symptoms as mentioned by CHWs indicate possibility of being active against microbial infection of the skin. Combination of at least two of the five classes of compounds; terpenoids, alkaloids, sterols, phenols and flavonoids, known to comprise antimicrobials, (Igbinosa *et al*., and 2013) were reported in each of the species. The combinations indicate that synergistic or complementary mechanism was responsible for activity against the infectious agents.

Apart from comprising compounds against bacteria and fungi, flavonoids have been associated with anti-HIV activity (Singh *et al.,* 2005). Terpenoids are recognized for enhancement of immunity (Wagner and Emadfa, 2003). The plants mentioned by CHWs

and reported to comprise detectable amounts of flavonoids were; *C. occidentalis, E. abyssinica* and *A. vogelii*, and the detectable amounts of terpenoids were; *C. aculeatus, O. gratissimum, F. Africana, A. integrifolia* and *P. alba*. The cited plant species require investigations to determine their potential against bacteria and fungi.

In *O. gratissimum*, the two phytochemicals; *Eugenol* and *methyl eugenol* are structurally related to *Eugenin*, which is reported to be active against HSV-1 (Kurokawa et al, 2001). The symptoms for application as cited by CHWs and in literature show that O. *gratissimum* could posess ingridients with broad spectrum antimicrobial activity.

Kedrostis foetidissima was reported by CHW as a remedy for skin rashes and administered as an antiseptic. Similarly in literature, the plant is reported to possess wide spectrum antibacterial activity (Nirmala and Pandian, 2015). None of the compounds established in the plant species has been isolated and tested individually for the gram negative and gram positive. However, the fatty acids, noted as one of the contents are known to inhibit bacterial growth (Agoramoorthy, *et al*., 2007).

The presence of unsaturated compounds in *Melia azedarach* could possibly accounts for the high antioxidant reported (Gayatri and Rajani, 2010). The use by PLWHIV for fever and or malaria could be justified by the fact that fevers is associated with generation of pro-oxidants (Igbinosa et al..., 2013).

5.1.3 Anti HSV activity of selected plants

All the eight plants selected for anti HSV determination possessed compounds known to be pharmaceutically active (Dowole and Oni, 2013; Anywar et al..., 2016;Balemba et al..., 2010; Kareru et al..., 2007; Luseba et al..., 2007; Muhindi et al..., 2016;Teke et al..., 2011; Tchana et al..., 2014;Tariku et al..., 2010; Wagate et al..., 2010; Belay*et al.*,2011; Nyunja et al..., 2009; Kamanyi et al..., 2009; Radha et al..., 2008; Zaheer et al..2010; Boampong et al..., 2013; Kumari et al..., 2012;Syakira and Brenda, 2010; Radha and Sivakumar, 2009; Zhang et al..., 2008;Kiem et al..., 2005; Li et al..., 2002; Parveen et

al..., 2014; Hussain et al..., 2014;Gallego et al..., 2015; Pawar and Surana, 2010; Modh, 2012; Erasmus et al..., 2012;). However, none of the plants have previously been investigated for activity against Herpes simplex type 1. This is the first time the finding on anti-herpes activity is being reported. Before the antiviral experiments, cell viability was carried out with the aim of detecting limits beyond which the materials could cause harm to people using them and to detect the concentration that would allow the study of antiviral effect while minimizing toxicity.

On toxicity findings, it was observed that *S. piñata* and *P. alba* were the most toxic. This finding supports their external use as administerd by the community. The high antiviral response by *S. piñata* and *P. alba* by EPPT and lower protection by MTT was attributed to antiviral and anti-ellular effect of the extracts. The extracts affected the metabolic functions of the cell with some cell tolerance to structural effects. The protection of cells by *G. buchananii* by MTT and lower antiviral effect was attributed to antiviral effect of the extract with low cell tolerance to structural effect. Since it is possible that the antiviral ingredients might be different from causing toxicity, future studies should focus on isolation of individual compounds and testing each separately.

Based on high percentage protection shown by *G. buchananii* and *C. decapetala* extracts, further determination was carried out at 7 different values. For *G. buchananii*, maximum cell protection concentration (MCPC), was at approximately 60µg/mL and maximum non-toxic concentration (MNC) was approximately 40µg/mL. The results show that MCPC is higher than MNC, but the toxicity of extract do not severely affect the cell compared to effect on the virus. The relationship between the two values is evident in the favourable therapeutic index (TI) indicating good selectivity against the virus at > 25. The TI compares well with the TI established for acyclovir (Witink and Janknegt, 1991). The findings indicates that the plants could provide useful compounds agaist HSV.

Phytochemical studies have previously reported the presence of; *Isomanni flavanone, an ent-eriodictyyol – (3a →6)* - *dihydroquecetin* linked *bio flavanone; 1, 5-dimenthoxyyajacareubin; depsidonegarcinisidone – G; (2"R, 3R)-Preussiannon; euxanthone; Jacareubin; isogarcinol and garcinol* in ethanolic-aqueous extract of *G. buchananii* (Timo et al..., 2015). None of these compounds have been investigated for antiviral activity but it is worth noting that phenolic groups comprising a significant proportion of ethanolic extracts, such as caffeic acid, chrysoplenois, leuteolin have been associated with anti - HSV activity (Hudson, 1990). The strong antioxidant activity of the phytochemicals isolated from *G. buchananii* by Timo et al.. is a factor of cell health and therefore could possibly enhance viability and antiviral performance.

The MCPC for *C. decapetala* was approximately 120µg/mL and MNC was 20µg/mL. The TI was >6, and like *G. buchananii,* there was good selectivity of extract activity against the virus and favourable comparison with TI of acyclovir (Witink and Janknegt, 1991). Previous studies have shown that the main constituent of *C. decapetala* belongs to terpenoids and flavonoids groups (Kiem et al..., 2005). The terpenoid compound previously associated with anti-HSV activity was tetrahydro-cannabinol (Hudson, 1990). As with *G.buchananii*, high antioxidant activity has been reported in *C. decapetala* extracts (Gallego et al..., 2015; Pawar and Surana, 2010). Strong antioxidant property of the plants may also explain the maintence of cell viability and tolerance against virus destruction.

To understand the mechanism of antiviral activity of *G. buchananii* and *C. decapetala,* cells were exposed to extracts at different treatment conditions to test for inhibition of the virus at different stages of replication. During replication, Herpes viruses are known to undergo the stages of attachment, uncoating, gene expression and replication, assembly and release of the virion (Richman et al..., 1987). The less than 1% protection observed at for pre-treatment means that the extract does not activate any antiviral state on the host cell, while the less than 10% for virucidal protection means that there was no direct virion inactivation. The presence of extract during adsorption also gave less than

10% cell protection. The latter observation shows that the presence of extract did not interfere with attachment. The significantly high protection of Vero cell by extract at post adsorption and throughout experiment treatment indicates that compounds in the extract inhibits some steps of viral replication, would need to be identified in future studies.

Even though *G.buchananii* did not prevent death of mice, there was significant delay of symptom onset indicating *in vivo* antiviral effect, thus supporting the *in vitro* findings. The results show that *G. buchananii* has therapeutic value but is only effective at high dose, given that lower doses did not prevent death or delay symptom development. Isolation and experimentation with pure active component of the extract could provide a clearer range of effectiveness and characterization of mode of activity. Results of *C.decapetala* show that the plant extract has no *in vivo* antiviral activity at the tested doses. However, like *G.buchananii*, a purified compound at *in vitro* level need to be isolated for better understanding of antiviral activity.

5.1.4 Antioxidant activity of selected plants

The relationship between progression of HIV to AIDS and oxidative stress has been documented by Tang Graham, Semba and Saah (1997) and Dhalwal, Deshpande & Purohit (2007). Individuals who have high level of free oxidative radicals tend to experience faster progression to AIDS (Tang et al..., 1997). Dhalwal et al.. explained that microbial infections cause release of highly oxidative molecules from cells due to enhanced metabolism of oxygen leading to extensive damage to cells and tissues (Dhalwal et al..., 2007). The cycle of increase in pro-oxidant molecules due to infection and progression of HIV due to increase of pro-oxidants indicate the central role of antioxidant in care and management of HIV. A part from the relationship between HIV and antioxidants, wide range of chronic diseases are known to be associated with oxidative stress (Ames and Gold, 1991). Liu cited plants as important source of antioxidant and cure of many chronic ailments including those associated with

endogenous viruses (Liu (2003). From the preceding observations, it is clear that the antioxidant factor in plants must be considered together with using plant products for cure of infections. Such consideration is more relevant when viral infection is being evaluated, given that viability of a cell is central in resistance to infection. The value of plants as antioxidant source for PLWHIV and whether the observed cell protection against HSV-1 could partly be attributed to antioxidant property justifies the need for determination in selected plants. The results show that *E. abyssinica*, *G. buchananii* and *C.decapetala* were the potent source of antioxidant. These findings support results by previous studies on antioxidant values of the plant species (Teke et al..., 2011; Tchana et al..., 2014; Gallego et al.., 2015; Pawar and Surana, 2010; Timo *et al*., 2015). There medicinal use can be attributed to the antioxidant content. The findings on to *G. buchananii* and *C. decapetala* which emerged as the most antiviral can be attributed to synergistic contribution of antioxidant property supporting the antiviral content of the plant materials.

5.1.5 Phytochemical groups in selected plants

Phenolic acid and flavonoids have been identified as providing protection against pathogens and predators to plant species that synthesize (Liu, 2003). Large number of alkaloids, terpenoids and flavonoids have been cited as being antiviral (Hudson (1990). The same phytochemical groups including saponins were cited as constituting majority of metabolites exhibiting antimicrobial against human pathogens (Nirmala and Pandian 2015). The antioxidant properties in plants that are associated with general good health are closely related to the concentration of flavonoids and phenolic acids. The aim of pharmaceutical screening was to examine the possible ingredients accounting for benefits claimed by the population of study. The findings would also explain results obtained on anti-herpes and antioxidant determinations. The chemical groups selected for screenings were those identified as comprising the majority of antiviral compounds from plants (Hudson, 1990). The chemical groups not detected by the methods employed, means that there was complete absence or very little presence of the groups,

while detectable amounts means presence in significant to large amounts. From the results it is noted that *G. buchananii* contained large amounts of flavonoids and Phenols and apparently accounting for its relatively high scores in *in vitro* and *in vivo* antiviral activity, as well as its antioxidant value. The anti HSV-1 activity has been specifically associated with quercetin flavonoids (Hudson, 1990). Fergusion et al.. observed that compounds that are rich in phenols are most effective in antioxidant activity (Fergusion et al..., 2006). The β- carbolines, furanoquinolines and camptothecin alkaloids are reported to be antiviral against DNA viruses (Hudson, 1990). The possible mode of action against the viral DNA could as well affect the cell, and this might explain both the antiviral and cytotoxic activity of *S. piñata* and *P. alba*

5.2 Summary

- A total of 62 plant species distributed in 46 families were used in the treatment and management of HIV conditions in Hamuyundi and in Vihiga and Kakamega counties, respectively.
- Secondary metabolites and pharmaceutical activity of 23 plant species identified at the population of study was documented in literature.

- The best anti-herpes activity was obtained from *G. buchananii* (Stem bark) and *C. decapetala* (Whole root).

- The most potent plant species for antioxidant activity were; *E. abyssinica, G. buchananii* and *C.decapetala.*

- Major phytochemical groups present in selected plants were; alkaloids in *S. pinata,* Terpenoids in *E. abyssinica, Flavonoids and phenols* in *G. buchananii*.

5.3 Conclusions

- Herbal medicine is part of remedies used for HIV conditions in Vihiga and Kakamega counties in Western Kenya
- About a third of plant species have been studied for their phytochemical content and pharmaceutical activities
- Two plant species; *G. buchananii*, and *C. decapetala* have a promising potential as alternative to existing anti-herpes remedies and can be developed for management therapies for anri-herpes infections.
- Three plant species; *E. abyssinica*, *G. buchananii* and *C.decapetala* have a promising potential as alternative to existing antioxidant supplements.
- Phytochemicals known for antiviral activities are present in *S. pinata, E. abyssinica, and G. buchananii.*

5.4 Recommendations

- Community in Vihiga and Kakamega should be made aware of health implications of using herbal medicines
- Plant species cited by the community but with no previous literature reports and not covered within the scope of current study should be investigated against claimed pharmaceutical activity and secondary metabolites
- Specific phytochemicals responsible for antiviral activity against HSV-1 in *G. buchananii* and *C.decapetala* need to be isolated and further investigated for anti- HSV-1 activity
- Specific phytochemicals accounting for antioxidant activity in *E. abyssinica, G. buchananii* and *C.decapetala* need to be isolated and further investigated for antioxidant activity

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APPENDICES

Appendix 1: Consent form in English

I (Name of respondent)……..

ID No……freely volunteer information on my ethno botanical knowledge of medicines used in HIV conditions. I understand that:

(i)This information will be used for research purposes and that I will not be acknowledged by name in the publication of the findings in order to protect my privacy and confidentiality but will be entitled to the report upon request directed to the principal investigator

(ii) I will be entitled to economic benefits arising from medicinal

Products developed from this research according laws of Kenya on

Industrial and property rights and Policy on natural product

Industry

Signature/ Thump print

……

Respondent, ethno botanical Survey

Date:……..

Postal Adress

Signature

……

Principal Investigator or Representative, Ethno botanical Survey, Date;

Postal Address;

Appendix 2: Consent form in Kiswahili

Mimi (Jina) ……...

Nambari ya kitambulisho……napatiana habari hii kwa hiari kuhusu ujusi yangu kwa tiba ya miti shamba kwa magonjwa ina letwa na ukimwi. Nina elewa ya kwamba:

i) Maelezo nita patiana itatumika kwa utafiti na kwamba juhudi hii haitatambuliwa kwa maelezo ya matokeo ya utafiti ili maelezo kuhusu mimi iwe siri lakini ninaweza kupata matokeo ya utafiti kwa ku tuma maombi kwa mtafiti mkuu.

ii) Ikiwa kutakuwa na dawa itatengenezwa kutokana na juhudi hii ya utafiti, nita nufaika ki uchumi kulingana na sheri ya Kenya.

Sahihi/Alama ya Kidole

……..

Mtalamu ya dawa ya kiasili

Tarehe……

Sanduku ya posta

Sahihi

……

Mtafiti mkuu/Mwakilishi ya Matafiti mkuu

Tarehe

Sanduku ya Posta

Appendix 3: Consent formin Luhyia language

Esie (Elira lio omufuchiriri)…….

Inamba yie shipande……ndiyamanga okhuhana ekemanyire okhulondana nende emisala/amalesi kakhurumishili kitandamu khulworkhusilikha obulwale bwo bukimwi. Emanyire mbu:

i) Amakhuba kano kali khulwokharurumikha khulio obutafiri bwonyene.

ii) Shilesi nafaidike okhurulana nende emisala/amalesi ketsa okhunyolekhana okhurulana nende utafiti buno; akhulonndana nende amalako ke shibala shiefwe Kenya

Isaini/Eshitere

……..

Elira……..

Omwesi……

Esanduku ya posta

Isaini

……..

Omutafiti/Omwakilishi wo mutafiti

Omwesi…….

Esanduku ya posta

Appendix 4: Interview Schedule

INTERVIEW SCHEDULE FOR COMMUNITY HEALTH WORKERS ON KNOWLEDGE OF MEDICINAL PLANTS USED BY HIV CLIENTS

Serial No. ---------- Date of interview. ----------

-

Socio-Demographic Data

1. Name of Community Health Worker (Optional). ----------
   ----
2. Sex: Male (------) Female (------)
3. Age (Years): 20 – 30 (----) 31 – 40 (-----) 41 – 50 (----)
   51 – 60 (----) 61 – 70 (----) ≥71 (----)
4. Marital Status: Married (------) Single (------)
5. Educational level. ----------
   -
6. Other professional activity? ----------

   --
7. Place of information gathering. (a)sub-Location/Community Unit ----------
   -
   (b) District/Sub-County ----------
-

8. Place of Practice (Village). ----------

9. Duration of practice. ----------

Knowledge of HIV diseases and Use of medicinal plants by HIV clients

10. Name at least five diseases that are caused by HIV (1). ---------- (2). ---------- (3). ----------

    (4). ---------- (5). ----------

11. How many HIV clients are you caring for----------?

12. Do you know of clients who used or are using medicinal plants for HIV Conditions? Yes. ----------. No ----------

    If yes, do you know the medicinal plants? Yes---------- No----------

    If yes, proceed to page 2, on plants used by HIV clients to treat HIV conditions

Give the name of plants and place where the plant can be obtained. (Use the Table below)

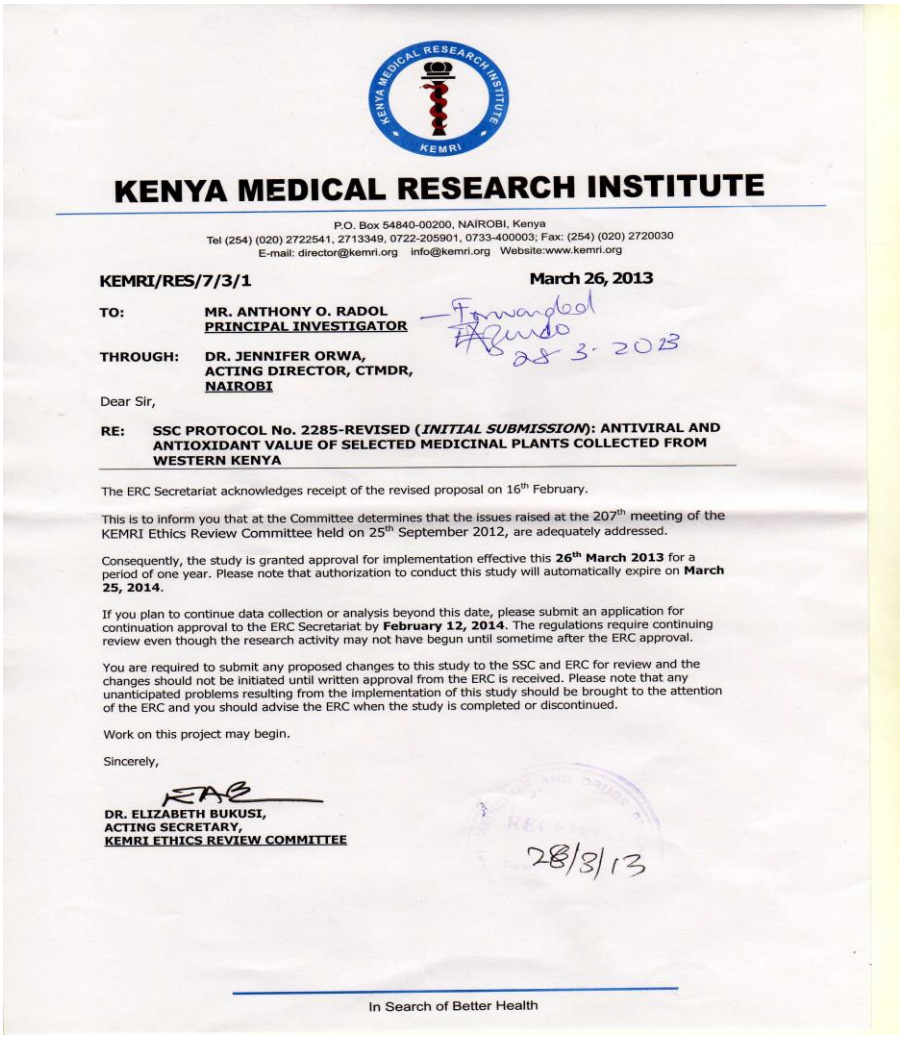
Plant Local Name	**Place found**	**HIV Condition treated**	**Parts Used**	**Preparation Method**	**Method of** *Administration*

Name of interviewer----------

Signature---------- Date----------

THANK YOU

Appendix 5: Ethics Review Committee approval letter



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KEMRI/RES/7/3/1 **March 26, 2013**

TO: **MR. ANTHONY O. RADOL**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. JENNIFER ORWA,**
ACTING DIRECTOR, CTMDR,
NAIROBI

Dear Sir,

RE: **SSC PROTOCOL No. 2285-REVISED (*INITIAL SUBMISSION*): ANTIVIRAL AND ANTIOXIDANT VALUE OF SELECTED MEDICINAL PLANTS COLLECTED FROM WESTERN KENYA**

The ERC Secretariat acknowledges receipt of the revised proposal on 16th February.

This is to inform you that at the Committee determines that the issues raised at the 207th meeting of the KEMRI Ethics Review Committee held on 25th September 2012, are adequately addressed.

Consequently, the study is granted approval for implementation effective this **26th March 2013** for a period of one year. Please note that authorization to conduct this study will automatically expire on **March 25, 2014**.

If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to the ERC Secretariat by **February 12, 2014**. The regulations require continuing review even though the research activity may not have begun until sometime after the ERC approval.

You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

Work on this project may begin.

Sincerely,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

28/3/13

In Search of Better Health

Appendix 6: Response on Knowledge of diseases caused by HIV and comments on the use of herbal medicine

Community Unit	Informant code	Response on Knowledge of HIV diseases	Response on state of herbal medicine use in the community	
Hamuyundi	VGA/HAM/01;	Herpes zoster, TB, Diarrhea	'I know of some who have been using it for the last five years'	
	VGA/HAM/02;	Gonorrhea, Syphilis	'I used herbal medicine, had developed herpes and had general body weakness'	
	VGA/HAM/03;	Herpes simplex, coughs	'It has healing effect on the disease'	
	VGA/HAM/04;	TB, Oral thrush, Herpes	'It is good for support of patients with side effect and	

			herpes zoster’	
	VGA/HAM/05;	TB, Diarrhea, Gonorrhea, Trachoma	‘Many people use them for problems like fever, headache and stomach aches’	
	VGA/HAM/06;	Diarrhea, Oral thrush, TB, Rashes, Herpes, Fever	‘Herbal medicine can be the best compared to ARVs because most of the herbs are for drinking only’	
	VGA/HAM/07;	Boils, Mouth sores, Skin rashes, Vomiting, Headache	‘The people living with HIV are not supposed to use herbal medicine for effectiveness of ARVs’	
	VGA/HAM/08;	TB, Headache, Swelling of feet, Rashes, Mouth rash, weight loss	‘It is not good to use both HIV medicine with herbal medicine, need advice from health facility’	

	VGA/HAM/09;	Skin rashes, Diarrhoea, Body wearing	'There is no need to use herbal medicine since hospital drugs are refined before being brought to the facility'	
	VGA/HAM/10;	TB, Typhoid, Anaemia, Diarrhoea, Breathing difficulties	'Can affect one's health, can cause side effects when combined with ARVs'	
	VGA/HAM/11;	Malaria, Dysentery, Typhoid, skin rashes, frequent, coughing, stomach ulcers	'People use herbal medicine but fear to volunteer the information to health workers'	
Mukhwa	KK/BKA/01	Diarrhea, skin problems, mouth infections	'The herbal medicines are useful to HIV patients'	
	KK/BKA/02	Stomach problems, Fever, poor	'Those that work are used a lot'	

		appetite, rashes		
	KK/BKA/03	Herpes zoster, Headache, Syphilis, coughs	'Some people use them and get well, they can be given a trial'	
	KK/BKA/04	TB, Weight loss, rashes, diarrhea	'They are not effective for HIV management'	
	KK/BKA/05	Fever, coughs, skin problems, diarrhea	'It is not effective and cause more harm on the patients' health just because it doesn't have dosage	
	KK/BKA/06	Tiredness, pneumonia, TB, rashes	'I discourage herbal drugs to be used by HIV patients but instead use hospital drugs which are effective	

	KK/BKA/07	Wounds, stomachache, Malaria, mouth sores	'A lot of people use them'	
	KK/BKA/08	Anaemia, vomiting, TB, coughs, fever, diarrhea	'Some people combine ARVs and herbal medicine'	
	KK/BKA/09	Diarrhea, skin problems, genital ulcers, eye problems	'Herbal medicine can be a good alternative because may be you can take for some time and stop. Not like ARVs where you take for the rest of your life'	

	KK/BKA/10	Pneumonia, skin rashes, mouth ulcers, diarrhea	'They are useful for stomach problems'	
	KK/BKA/11	Skin problems, headache, stomach ache, fever	'I know of HIV patients who have been using herbal medicine for the last 5 years'	
	KK/BKA/12	Herpes zoster, mouth sores, diarrhea, fever	' People like them very much'	

Appendix 7: Plants used for treatment of HIV conditions

Community of source	Collection number	Family	Scientific name	Local name	Frequency of plant mention as medicine	Part used	HIV condition treated and frequency of plant mention for the condition	Method of preparation	Mode of application
Hamuyundi	VGA/16 VGA/65	Curbiteraceae	*Kedrostis foetidissima* (Jacq.) Cogn *Cucumis aculeatus*Cogn.	Livunyabukundu Kaseveve	2 2	Leaves Leaves	Skin Rashes (2) Mouth sores(2)	Cold infusion Ashing	Drinking and bathing Licking ash

	VGA/17 VGA/25 VGA/18	Lamiaceae	*Tetradenia urticifolia* (Bak.)Phlipson *Ajuga integrifolia* (Buch-Ham) *Fuerstia africana* T.C.E Fr.	Okemba Imbusi yo Mtakha Muvunyanyingu	1 1 2	Leaves Leaves Leaves	Coughs (1) Diarrhea and Malaria (1) Mouth sores, STI (2)	Cold infusion Cold infusion Cold infusion	Drinking Drinking Topical Drinking
	VGA/19 VGA/34	Acanthaceae	*Justicia betonica* (L.) *Thuribergia alata* Sims	Indulisia SSanda	4 1	Leaves Leaves	Diarrhea and Stomachache (4) Swollen breast	Cold infusion Ashing, Emulsifying in	Drinking Rubbing

								fats	
	VGA/20	Myrtaceae	*Psidium guajava* L.	Mapera	2	Young leaves	Stomachache (2)	Pound or chew	Swallow juice
	VGA/21	Bignoniaceae	*Markhamia lutea* (Benth.) K. Shum	Olusiola	2	Young leaves	Stomachache (1), Eye spots and (1) Typhoid (1)	Chewing Ashing, cold infusion Add honey for typhoid	Swallow juice Drop in the eye Drinking
	VGA/22	Solanaceae	*Solanium inacanum* (L.)	Kitatula/Sirandalwa Litsutsa	1	Roots	Stomach pains (1)	Chewing	Swallow juice

	VGA/31 VGA/47		*Solanum nigrum* (L.) *Nicotiana rabacum* (L.)	Tobacco	1 1	Leaves Leaves	Chicken pox (1) Typhoid (1)	Decoction Cold infusion	Drinking decoction and eating leaves Drinking
	VGA/23	Umbelliferae	*Centella asciatica* (L.) Urb	Liru lala	1	Leaves	Abscess(1)	Chewing and spiting juice on the boil	Topical
	VGA/24	Papilionaceae	*Indigofera arrecta* (A. Rich)	Unknown by local name	1	Roots	Breathing difficulty (1)	Chewing	Swallow juice

	VGA/26	Asteraceae	*Microglossa pyrifolia* (Lam.) Kuntze	Ingwe	1	Leaves	Skin infection (1)	Cold infusion	Drinking
	VGA/28 VGA/29	Xanthorrhoeae/Aloaceae Combined with Euphorbiaceae	*Aloe sp* *Croton macrostachus* Hochst.	Ligakha Mutswitswi	1	Leaves Leaves	Coughs (1)	Ashing	Licking
	VGA/30	Sapindaceae	*Cardiospermum halicacabum* L.	Obulili	1	Seeds	Eye problems (1)	Taken intact	Swallow seeds
	VGA/33	Verbenaceae	*Clerodendrum myricoides* (Hochst.) Vatke	Esuchi	2	Leaves Stem Bark	Pneumonia (1) Stomachache(1)	Decoction Decoction	Drinking Drinking
	VGA/35 VGA/3	Rubiaceae	*Spermacoce princeae* (K. Schum) Verdc. *Psydrax Shimperiana*	Lisienjili Esikhuli	1 1	Leaves Leav	Diarrhea (1) Stomachache (1)	Cold infusion Cold infusion	Drinking Drinkin

	8		(A. Rich) Bridson			es			g
	VGA/39 VGA/34	Rubiaceae Combined with Acanthaceae	*Keetia gueinzili* (Sond.) Bridson *Thuribergia alata* Sims	Lisengele Essanda	1	Leaves	Chest pain (1)	Pound leaves and mix with fat	Rub on the chest
	VGA/68	Rutaceae	*Zanthoxylum giletti* (De wild) P.G.Waterman	Kikuma	2	Stem bark	Oral thrush (2) Heart diseases and Coughing (1)	Decoction	Drinking
	VGA/36	Pteridaceae	*Pellea adiatoides* (Willd.) J.SM	Fern	1	Leaves	Aching feet (1)	Cold infusion	Immersing feet in the infusion
	VGA/37 VGA/7	Lamiaceae	*Salvia coccinea*L. *Leucus calostachys*	Mfunyanyungu Kiguduri	2 1	Leaves Leav	Oral thrush (2) STI (1)	Chewing Decocti	Swallow juice Drinkin

	5		Oliv.			es		on	g
	VGA/40	Oxalideceae	*Oxalis corniculata* L.	Nakhabulu	1	Leaves	Poor appetite (1)	Chewing	Swallow juice
	VGA/41	Mimoceceae	*Albizia grandibrateata* Taub	Omusenjeli	1	Stem bark	Indigestion (1)	Decoction	Drinking
	VGA/42 VGA/56	Caelspineceae	*Cassia occidentalis* L. T*ylosema fassogleuse.* Brenan, J.P	Imindi Local name unknown	4 1	Roots Roots	Malaria/Fever (4) Malaria/ fever (1)	Decoction Decoction	Drinking Drinking
	VGA/46	Melaceae	*Melia azedarach* L.	Mwarubaine	1	Roots and leaves	Malaria (1)	Cold infusion	Drinking
	VGA/51 VGA/55	Polygoneceae	*Rumex abyssinicus* (Jascq) *Rumex stendelii* A.Rich	Likachi sacha Mnyerangokho/ Alukava	1 2	Leaves Leaves	Stomachache (1) Ulcers (1), HIV (2), Stomachache (1), Malaria	Cold infusion Cold infusion	Drinking Drinking

	VGA/52	Gentianceae	*Anthocleista vogelii* Planch.	Sikhuma	2	Leaves and stem bark	Typhoid (2)	Decoction	Drinking
	VGA/61	Convolvulaceae	*Ipomuea kiliensis* L.	Libilibizi	1	Roots	Constipation (1)	Cold infusion	Drinking
Mukhwa	KK01	Compositae	*Tithonia diversifolia (Hemsl.) Gray*	Amabinzo	1	Roots	Herpes zoster (1)	Decoction	Drinking
	KK02	Compositae	Schuhria pinnata (Lam) O.Ktze	Olwayi	1	Leaves	Mouth ulcers, cold sores, Herpes simplex (1)	Cold infusion	Topical
	KK07		Vernonia adoensis Walp	Khumululusia kumuseja	1	Roots	Genital ulcers, Herpes zoster (1)	Decoction	Drinking
	KK015	Compositae	Erlangea tomentosa (Oliv. & Hiern). S.Moore	Ikhole	1	Roots	Skin and mouth sores (1)	Decoction	Drinking
	KKO3	Mimosaceae	*Entada abyssinica(A. Rich*	Musembe	2	Stem Bark	Skin ulcers, Herpes	Decoction	Drinking

						Leaves	simplex lesions (2)		
	KKO4	Guttiferae	*Garcinia buchanii*	Khumukhomeli	1	Bark	Herpes zoster (1)	Decoction	Drinking
	KK05	Euphorbiacea	*Croton macrostachys*	Omutswitswi	1	Bark	Fever and skin conditions, Herpes simplex (1)	Decoction	Drinking
	KK08	Apocynaceae	*Plumeria alba* L.	Frangipani	8	Leaves or sap	Herpes zoster (8)	Crush leaves or apply sap	Topical
	KK09	Caesalpinaceae	*Caesalpinia decepetala (Roth.I. Alston)*	Lunani	1	Roots	Genital ulcers (1), Cold sores	Decoction	Drinking
	KK 010		*Ageratim coryzoider*	Olunywele/ Liliveri	1	Leaves	Poor appetite, mouth conditions (1)	Chew	Swallowing juice, topical application of juice
	KK011	Caesalpinaceae	*Chamaecrista mimosoides (L.)*	Unknown by local name	1	Leaves	Mouth sores (1)	Chew	Apply juice to sores

	KK012	Lamiaceae	*Ocimum gratissimum (L.)*	Mtashe	1	Roots	Skin infections and mouth sores (1)	Decoction	Drinking
	KK013	Asteraceae	*Microglossa pyrifolia (Lam)O.Kutze*	Ingwe	1	Roots	Mouth sores (1)	Decoction	Drinking
	KK014	Liliflorae	*Gloriesa superba (L.)*	Unknown by local name	1	Aria l part	Skin and mouth sores (1)	Decoction	Drinking
	KK084	Lamiaceae	*Ajuga intergrifolia. Buch-Ham*	Imbusi ya mutashi	2	Leaves	Stomachache (2)	Decoction	Drinking
	KK085	Fabaceae	*Tylosema fassoglensis (Kotschy)Torre & Hill C.*	Imbasa	1	Roots	Stomachache (1)	Decoction	Drinking
	KK086	Olacaceae	*Ximemia Americana L.*	Olemo	1	Stem bark	Abscess (1)	Decoction	Drinking
	KK087	Fabaceae	*Albizia coreana Welw.*	Ober	1	Stem bark	Absces (1)	Decoction	Drinking

	KK088	Fabaceae	*Entada abyssinica A.Rich*	Musembe	1	Stem bark	Absces (1)	Decoction	Drinking
	KK089	Olacaceae	*Ximemia Americana L.*	Olemo	1	Stem bark	Diarrhoea (1)	Decoction	Drinking
	KK090	Fabaceae	*Albizia coreana Welw.*	Ober	1	Stem bark	Diarrhoea (1)	Dicoction	Drinking
	KK092	Bromeliaceae	*Ananas comosus (L.) Merr*	Pineapple	1	Fruit bark	Diarrhoea (1)	Decoction	Drinking
	KK093	Fabaceae	*Acasia lahal Steud and Hochst*	Alaktar	1	Stem bark	Wound (1)	Decoction	Drinking
	KK094	Xanthonhoeeaceae	*Aloe spp*	Ogaka	1	Roots	Syphilis (1)	Decoction	Drinking
	KK095	Myrtoideae	*Psydium guajava L.*	Mapera mayom	1	Young leaves	Stomachache (1)	Chew	Swallow
	KK096	Rutaceae	*Toddolia asciatica (L) Lam*	Nyalwet kwach	1	Stem bark	Sores in the mouth (1)	Chew	Swallow

	KK097	Polygonaceae	*Oxygonum sinuatum Hoest and Steud*	Nyalwet gweno	1	Roots	Stomachache (1)	Decoction	Drinking
	KK098	Fabaceae	*Abrus precatorius L.*	Okita	1	Leaves	Coughs (1)	Ashing	Licking
	KK099	Lamiaceae	*Ocimum kilimanddscharicum L*	Ombulu	1	Leaves	Coughs (1)	Ashing	Licking

Appendix 8: Plants and Reported HIV Condition for use in Hamuyundi

Voucher number	Family	Scientific name	Local name	Medicinal use
VGA/16 VGA/65	Curbiteraceae	*Kedrostis foetidissima* **(Jacq.) Cogn** ***Cucumis aculeatus* Cogn.**	Livunyabukundu Kaseveve	Skin rashes Mouth sores
VGA/17 VGA/25 VGA/18 VGA/37 VGA/75	Lamiaceae	*Tetradenia urticifolia* **(Bak.)Phlipson** *Ajuga integrifolia* (Buch-Ham) *Fuerstia africana* **T.C.E Fr**. *Salvia coccinea* L. *Leucus calostachys* **Oliv**.	Okemba Imbusi yo Mtakha Muvunyanyingu Mfunyanyungu Kiguduri	Coughs Diarrhea and malaria Mouth sores and STI Oral thrush STI
VGA/19 VGA/	Acanthaceae	*Justicia betonica* **(L.)** *Thuribergia alata* **Sims**	Indulisia SSanda	Diarrhea and Stomachache Swollen breasts Chest pain
VGA/20	Myrtaceae	*Psidium guajava* **L**.	Mapera	Stomachache

VGA/21	Bignoniaceae	*Markhamia lutea* (Benth.) **K.** Shum	Olusiola	Stomachache, Eye spots and Typhoid
VGA/22 VGA/31 VGA/47	Solanaceae	*Solanium inacanum* **(L.)** *Solanum nigrum* **(L.)** *Nicotiana rabacum* **(L.)**	Kitatula/Sirandaa Litsutsa Tobacco	Stomach pains Chicken pox Typhoid
VGA/23	Umbelliferae	*Centella asciatica* **(L.) Urb**	Liru lala	Abscess
VGA/24	Papilionaceae	*Indigofera arrecta* **(A. Rich)**	Unknown by local name	Breathing difficulty
VGA/26	Asteraceae	*Microglossa pyrifolia* **(Lam.) Kuntze**	Ingwe	Skin infection
VGA/28	Xanthorrhoeae/ Aloaceae	*Aloe sp*	Ligakha	Coughs
VGA/29	Euphorbiaceae	*Croton macrostachys* **Hochst.**	Mutswitswi	Coughs
VGA/30	Sapindaceae	*Cardiospermum halicacabum* **L.**	Obulili	Eye problems
VGA/33	Verbenaceae	*Clerodendrum myricoides* **(Hochst.) Vatke**	Esuchi	Pneumonia

VGA/35	Rubiaceae	*Spermacoce princeae* (K. Schum) Verdc.	Lisienjili	Diarrhea
VGA/38		*Psydrax Shimperiana* **(A. Rich) Bridson**	Esikhuli	Stomachache
VGA/39		*Keetia gueinzili* **(Sond.) Bridson**	Lisengele	Chest pain
VGA/68		*Zanthoxylum giletti* **(De wild) P.G.Waterman**	Kikuma	Oral thrush Heart diseases and Coughing
VGA/36	Pteridaceae	*Pellea adiatoides* **(Willd.) J.SM**	Fern	Aching feet
VGA/40	Oxalideceae	*Oxalis corniculata* **L.**	Nakhabulu	Poor appetite
VGA/41	Mimoceceae	*Albizia grandibrateata* **Taub**	Omusenjeli	Indigestion
VGA/42	Caelspineceae	*Cassia occidentalis* **L**.	Imindi	Malaria/Fever
VGA/56		T*ylosema fassogleuse.* **Brenan, J.P**	Local name unknown	Malaria/ fever
VGA/46	Melaceae	*Melia azedarach* L.	Mwarubaine	Malaria

VGA/51 VGA/55	Polygoneceae	*Rumex abyssinicus* (Jascq) *Rumex stendelii* **A.Rich**	Likachi sacha Mnyerangokho/Alukava	Stomachache (1) Ulcers (1), HIV Stomachache (1), Malaria
VGA/52	Gentianceae	*Anthocleista vogelii* **Planch.**	Sikhuma	Typhoid
VGA/61	Convolvulaceae	*Ipomuea kiliensis* **L.**	Libilibizi	Constipation

Appendix 9: Plants and Reported HIV conditions for use in Mukhwa

Voucher number	Family	Scientific name	Local name	Medicinal use
KK01 KK02 KK07 KK015	*Asteraceae*	*Tithonia diversifolia* ***(Hemsl.) Gray*** Schuhria pinata **(Lam) O.Ktze** Vernonia adoensis **Walp** Erlangea tomentosa **(Oliv. & Hiern). S.Moore**	Amabinzo Olwayi Khumululusia kumuseja Ikhole	Herpes zoster Mouth ulcers, cold sores, Herpes simplex Genital ulcers, Herpes zoster Skin and mouth sores
KK03	*Mimosaceae*	*Entada abyssinica* ***(A. Rich)***	Musembe	Skin ulcers, Herpes simplex lesions
KKO4	*Guttiferae*	*Garcinia buchananii* **(Baker)**	Khumukhomeli	Herpes zoster
KK05	*Euphorbiacea*	*Croton macrostachys* **(Hochst)**	Omutswitswi	Fever and skin conditions, Herpes simplex
KK08	*Apocynaceae*	*Plumeria alba* **L**.	Frangipani	Herpes zoster

KK09	*Caesalpinaceae*	Caesalpinia decepetala **(Roth.I. Alston)**	Lunani	Genital ulcers , Herpes simplex
KK011		Chamaecrista mimosoides **(L.)**	Local name unknown	Poor appetite, Mouth sores
KK 010	*Asteraceae*	*Ageratium conyzoides***(L)**	Olunywele/ Liliveri	Mouth conditions
KK013		*Microglossa pyrifolia* **(Lam) O.Kutze**	Ingwe	Mouth sores
KK012	*Lamiaceae*	*Ocimum gratissimum* **(L.)**	Mtashe	Skin infections and mouth sores
KK084		*Ajuga intergrifolia*. **Buch-Ham**	Imbusi ya mutashi	Stomachache
KK099		*Ocimum kilimanddscharicum* **L**	Ombulu	Coughs
KK014	*Liliflorae*	*Gloriesa superba* **(L.)**	Unknown by local name	Skin and mouth sores
KK085	*Fabaceae*	*Tylosema fassoglensis* **(Kotschy) Torre & Hill C.**	Imbasa	Stomachache
KK087		*Albizia coreana Welw.*	Ober	Abscess
KK088		*Entada abyssinica* **A.Rich**	Musembe	Abscess
KK090		*Albizia coreana* **Welw.**	Ober	Diarrhea
KK093		*Acasia lahal* **Steud and Hochst**	Alaktar	Wound
KK098		*Abrus precatorius* **L.**	Okita	Coughs
KK086	*Olacaceae*	*Ximemia Americana* **L.**	Olemo	Abscess
KK089	*Olacaceae*	*Ximemia Americana* **L.**	Olemo	Diarrhea

KK092	*Bromeliaceae*	*Ananas comosus* **(L.) Merr**	Pineapple	Diarrhea
KK094	*Xanthonhoeeaceae*	*Aloe spp.*	Ogaka	Syphilis
KK095	*Myrtoideae*	*Psydium guajava* **L.**	Mapera mayom	Stomachache
KK096	*Rutaceae*	*Toddolia asciatica* **(L) Lam**	Nyalwet kwach	Sores in the mouth
KK097	*Polygonaceae*	*Oxygonum sinuatum* **Hoest and Steud**	Nyalwet gweno	Stomachache

Appendix 10: Plants and frequency of mention as medicine in Hamuyundi

Voucher number	Plant species	Condition	Frequency
VGA/19	*Justicia betonica* **(L.)**	Gastrointestinal	4
VGA/42	*Cassia occidentalis* L.	Malaria/Fever	4
VGA/16	*Kedrostis foetidissima* **(Jacq.) Cogn**	Skin	2
VGA/65	*Cucumis aculeatus* Cogn	Skin	2
VGA/18	*Fuerstia africana* **T.C.E Fr**	Skin	2
VGA/20	*Psidium guajava* **L**	Gastrointestinal	2
VGA/21	*Markhamia lutea* **(Benth.) K. Shum**	Gastrointestinal	2
VGA/68	*Zanthoxylum giletti* **(De wild) P.G.Waterman**	Skin	2
VGA/37	*Salvia cocernia* L	Skin	2
VGA/55	*Rumex stendelii* **A.Rich**	Gastrointestinal	2
VGA/52	*Anthocleista vogelii* **Planch**	Gastrointestinal	2
VGA/17	*Tetradenia urticifolia* **(Bak.)Phlipson**	Respiratory	1
VGA/25	*Ajuga integrifolia* ***(Buch-Ham)***	Gastrointestinal	1
VGA/34	*Thuribergia alata* Sims	Neoplastic	1
VGA/22	*Solanium inacanum* **(L.)**	Gastrointestinal	1
VGA/31	*Solanum nigrum* **(L.)**	Skin	1

VGA/47	*Nicotiana rabacum* **(L.)**	Gastrointestinal	1
VGA/23	*Centella asciatica* **(L.) Urb**	Skin	1
VGA/24	*Indigofera arrecta* **(A. Rich)**	Respiratory	1
VGA/26	*Microglossa pyrifolia* **(Lam.) Kuntze**	Skin	1
VGA/28	*Aloe sp*	Respiratory	1
VGA/29	*Croton macrostachys* **Hochst**	Respiratory	1
VGA/30	*Cardiospermum halicacabum* **L**.	Eye	1
VGA/33	*Clerodendrum myricoides* **(Hochst.) Vatke**	Gastrointestinal	1
VGA/35	*Spermacoce princeae* ***(K. Schum) Verdc***	Gastrointestinal	1
VGA/38	*Psydrax shimperiana* (A. Rich) Bridson	Gastrointestinal	1
VGA/39	*Keetia gueinzili* **(Sond.) Bridson**	Respiratory	1
VGA/34	*Thuribergia alata* **Sims**	Respiratory	1
VGA/36	*Pellea adiatoides* **(Willd.) J.SM**	Musculoskeletal	1
VGA/18	*Fuerstia africana* **T.C.Fr**	Respiratory	1
VGA/75	*Leucus calostachys* **Oliv**	Respiratory	1
VGA/40	*Oxalis corniculata* **L**	Nutrition	1
VGA/41	*Albizia grandibrateata* **Taub**	Gastrointestinal	1
VGA/56	T*ylosema fassogleuse.*	Malaria/Fever	1

	Brenan, J.P		
VGA/46	*Melia azedarach* **L.**	Malaria/Fever	1
VGA/51	*Rumex abyssinicus* **(Jascq)**	Gastrointestinal	1
VGA/61	*Ipomuea kiliensis* **L**	Gastrointestinal	1

Appendix 11: Plants and frequency of mention as medicine in Mukhwa

Voucher number	Plant species	Conditions	Frequency
KK08	*Plumeria alba* **L.**	Herpes zoster	8
KKO3	*Entada abyssinica* ***(A. Rich)***	Skin/Mucocutaneous	2
KK084	*Ajuga intergrifolia.* ***Buch-Ham***	Gastrointestinal	2
KK01	*Tithonia diversifolia* ***(Hemsl.) Gray***	Herpes zoster	1
KK02	*Schuhria pinata* **(Lam) O.Ktze**	Skin/ Mucocutaneous	1
KK07	*Vernonia adoensis* **Walp**	Herpes zoster/ Mucocutaneous	1
KK015	*Erlangea tomentosa* **(Oliv. & Hiern)S.Moore**	Skin and Mucocutaneous	1
KKO4	*Garcinia buchanii* **(Baker)**	Skin	1
KK05	*Croton macrostachys* **Hochst.**	Malaria/Fever and skin / Mucocutaneous	1
KK09	*Caesalpinia decepetala* **(Roth.I. Alston)**	Skin/ Mucocutaneous	1

KK 010	*Ageratim coryzoides* **(L)**	Gastrointestinal	1
KK011	*Chamaecrista mimosoides* **(L.)**	Mucocutaneous	1
	Ocimum gratissimum (L.)	Skin/Mucocutainous	1
KK013	*Microglossa pyrifolia* **(Lam)O.Kutze**	Mucocutaneous	1
KK014	*Gloriesa superba (L.)*	Skin/ Mucocutaneous	1
KK085	*Tylosema fassoglensis* **(Kotschy)Torre & Hill C**.	Gastrointestinal	1
KK086	*Ximemia Americana L.*	Skin	1
KK087	*Albizia coreana* **Welw.**	Skin	1
KKO3	*Entada abyssinica* **A.Rich**	Skin	1
KK089	*Ximemia Americana* **L**.	Gastrointestinal	1
KK090	*Albizia coreana* **Welw.**	Gastrointestinal	1
KK092	*Ananas comosus* **(L.) Merr**	Gastrointestinal	1

KK093	*Acasia lahal* **Steud and Hochst**	Skin/ Mucocutaneous	1
KK094	*Aloe spp*	Syphilis	1
KK095	*Psydium guajava* **L.**	Gastrointestinal	1
	Toddolia asciatica **(L) Lam**	Mucocutaneous	1
KK097	*Oxygonum sinuatum* **Hoest and Steud**	Mucocutaneous	1
KK098	*Abrus precatorius* **L**.	Respiratory	1
KK099	*Ocimum kilimanddscharicum* **L**	Respiratory	1

Appendix 12: Phytochemical and Ethno-pharmacological reports of the cited medicinal plants

Family	Scientific name	Phytochemical compounds	Ethno-Pharmacological use /activity
Curbiteraceae	*Kedrostis foetidissima* (Jacq.) Cogn	7, 10- hexadecadienoic acid., 2- hexadecen-1-ol, 3,7,11,15-tetramethyl-[R-[RR-(E)]] and 1H-1, 2, 4-triazole-3, 5-dicarbaldehyde and docosanoic acid (Pavithra and Vadivukkarasi, 2012).	Antibacterial activity against gram negative and gram positive bacteria (Nirmala and Pandian, 2015).
	Cucumis aculeatus Cogn.	Terpenoids, phenolics (Ogutu et al..., 2012)	Activity against Pseudomonas aeruginosa (Ogutu et al..., 2012)
Lamiaceae	*Tetradenia urticifolia* (Bak.)Phlipson	Polyphenol compounds is abundant in the two species of the family (Aprotosoaie*et al* , 2013)	Ethno-medical use for antimalarial (Satish and Ranjana, 2013)
	Ajuga integrifolia (Buch-Ham)	Ergosterol-5,8-endoperoxide (6)ajugarin-(1), 8-O-acentylharpagide(5) (Cocquyt et al.., 2011)	Ethno-medical use for antimalaria and invitro antimycobacterium and antiplasmodial activity(Cocquyt et al.., 2011)
	Fuerstia africana T.C.E Fr	Sterols, terpenoids, alkaloids, Saponins, glycoids, Flavanoids and Tannins (Okach et al..., 2013)	Invitro antimicrobial activity against Gram negative and gram positive bacteria and anti plasmodial activity (Lilian et al..., 2013; Ester et al..., 2012; Kingondu et al..., 2011)
	Salvia cocernia L.		Ethno-medical use for inflammatory diseases. Causes release of allergic mediators (Osadebe and Okoye, 2003). Causes side effects of water and salt retention and cancer (Wang et

			al.., 2008) and gastro-intestinal disturbances (Gooch et al.., 2007)
	Leucus calostachys Oliv.		In vitro Anti-plasmodial activity (Nyambati et al.., 2013)
	Ocimum gratissimum (L.)	Gamma-terpene, beta-phellandrene, limonene and thymol (Tchoumbougnang et al..., 2005), Tannins, steroids, terpenoids, Flavonoids and cardiac glycosides (Akinmoladun et al..., 2007) and Phenols ((Igbinosa et al..., 2013), eugenol and methyl eugenol (Josh, 2013)	*In vivo* Anti –Plasmodium activity (Tchoumbougnang et al.., 2005), High antioxidant activity,(Akinmoladun et al.., 2007; Igbinosa et al.., 2013; Mahapatra et al..,2009; Josh, 2013), *In vitro* antimicrobial activity against Staphylococcus aureus, *Escherichia coli, Salmonella typhimurium and Salmonella typhi , Bacillus spp, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Proteus mirabilis, Candida albicans,* Serretia marcescens (Adebolu and Oladimeji, 2005; Matasyoh et al.., 2008; Emeka and Eze, 2009; Josh, 2013), High antioxidant, *In vivo* hepatoprotective activity (Chiu et al.., 2012),*In vivo* restoration of digestion and absorption abnormalities derived from diabetes mellitus (Okon et al..,2015), *In vivo* anti-inflammatory and anti-nociceptive effect (Ajayi et al.., 2014), *In vivo* increased hematological

	Ajuga intergrifolia. Buch-Ham		parameters (Ofem and Eno, 2012), anticonvulsant and anxiolytic activity (Okoli et al.., 2010) Ethnobotanical use for Taenia capitis infection(Teklay *et al..*, 2013)
Acanthaceae	*Justicia betonica* (L.) *Thuribergia alata* Sims	10H-Indolo[3,2-b] quinolone (Andy et al.., 2007) Glucosides:Thunaloside and alatoside, iridoid glycosides, stilbericosides, 6-epi-stilbericosides and thunbergioside (Damltoft et al.., 1994)	Ethno-medical use for coughs, anti-diarrhea and Orchitis (Andy et al.., 2007; Jeruto et al.., 2008) Antibacterial activity (Sasikumar et al.., 2007) Anti-plasmodial activity (Bbosa et al., 2013) Ethno-medical use for cough and backache (Jeruto *et al..*, 2008) In vitro antibacterial activity against Salmonella typhi (Jeniffer et al.., 2004)
Myrtaceae	*Psidium guajava* L.	Gallic, Catechin and quercetin (Ju –Wen et al.., 2009)	Antiglycation activity (Ju –Wen et al.., 2009)
Bignoniaceae	*Markhamia lutea* (Benth.) K. Shum	Cycloartane triterpenoids (Lacroix et al.., 2009)	Toxic to MRC5 and KB cell lines (Lacroix et al.., 2009)

Solanaceae	*Solanium inacanum* (L.) *Solanum nigrum* (L.) *Nicotiana rabacum* (L.)		In-vitro antifungal activity (Al-Fatima et al.., 2007) Strong antioxidant activity on DPPH ([Al-Fatima et al.., 2007) Ethno-medical use for stomach ulcer. Mode of action is by inhibition of H^+K^+ ATPase activity (Malika and Chennam, 2006)
Umbelliferae	*Centella asciatica* (L.) Urb		Strong antioxidant activity on Ferric thiocyanate and Thiobarbituric acid (Zainol et al.., 2003)
Papilionaceae	*Indigofera arrecta* (A. Rich)		Anti-hyperglycemic activity (Marian and Alex 2006)
Asteraceae	*Microglossa pyrifolia* (Lam.) Kuntze	Dihydrobenzofurans; (methyl 2-(5-acetyle-2,3-ihydrobenzo[beta]furan-2yl)propenoate]., 3(methyl2-(6-acetyl-5-hydroxy-2,3dihydrobenzofuran-2-yl)propenoate] and 7(6-acetyl-5 hydroxy-2-(1-hydroxy-2-(1-hydroxy-2-propenyl)-3-methoxy-2,3-dihydrobenzofuran., Triterpenes; 3beta-acetoxy-25-hydroxydammara-20,23-diene(9)., 3beta-acetoxy-24-oxo-dammara-220,25-diene(11)., 17beta-hydroxy-3,16-dioxo-28-norolean-12-ene(12) and 17beta-hydroxy-3,11,16-trioxo-28-norolean-12-ene (Schmidtt, et al.., 2003)	In vitro antimicrobial activity of ethanol extract (Dicson et al., 2006)

Xanthorrhoeae/ Aloaceae	*Aloe sp*	Diverse species specific Contents including Alcohols, aldehydes, Ketones, Pyrimidines, Indole, alkaloids, Sterols, Fatty acids, Dicarboxylic acid (Lisa et al.., 2008)	Species specific indications including Arthritis, skin cancer, burns, eczema, psoriasis, digestive disorder, hypertension and diabetes (Lisa et al.., 2008)
Euphorbiaceae	*Croton macrostachus* Hochst.	Benzylbenzoate, Linalool, gama-muurolene, alpha-farnesene, delta-cadinene and alpha curcumene (Tariku et al.., 2010)	Invivo anti-plamodial activity (Leychilu et al., 2014) *In vitro* antibacterial activity against wide range of gram positive and gram negative bacteria (Wagate et al.., 2010; Belay*et al.,* 2011) Antileishmanial activity, moderate toxicity to human monocytic leukemia celline and erythrocytes from sheep (Tariku et al.., 2010), Ethnobotanical use for sore throat, TB, Uvula, heamostatics, stomachache, measles, STD, dysentery, diarrhea and malaria (Nyunja et al.., 2009). *In vivo*Anti-Nociceptive and Anti-Inflammatory effect (Kamanyi et al.., 2009)
Sapindaceae	*Cardiospermum halicacabum* L.		Antioxidant and anti-inflammatory activity (Ming –Hsing *et al..*,2011)
Verbenaceae	*Clerodendrum myricoides* (Hochst.) Vatke		In vivo anti-malarial activity (Tekalign *et al..*, 2010)
Rubiaceae	*Spermacoce princeae* (K. Schum) Verdc. *Psydrax Shimperiana* (A. Rich) Bridson		Ethno-medical use for bacterial infections. Causes constipation, cardiovascular problems, hepatoxicity and nephrotoxicity at high doses

	Keetia gueinzili (Sond.) Bridson		(Ntemafack et al.., 2015) Ethno-medical indication for cancer (Ochwangi*et al.,* 2014) Ethno-medical use for malaria (Njoroge and Rainer, 2006)
Rutaceae	*Zanthoxylum giletti* (De wild) P.G.Waterman	Alkaloids:Peroxysimulenoline, Sanguinarine, Faragarine 1, Norchelerythrine, Dihydronitidine (Gaya *et al..*, 2013)	Ethno-medical use as liniment for back pain and treatment for urogenital infections, stomach ulcers (Kokwaro, 1993)
Oxalideceae	*Oxalis corniculata* L.		High in vivo antioxidant activity in rats (Muhammad, 2013). In vitro antibacterial activity (Satish et al.., 2008; Shahedur et al.., 2010). Anti-seizure activity in rats (Senthil and Rajkapoor 2010). Antiimplantation and abortifacient activity in rats (Sharangoud et al.., 2007)
Fabaceae	*Cassia occidentalis* L. *Chamaecrista mimosoides*	Alkaloids, tannins, saponin, glycoside and flavonoids (Saganuwan and Gulumbe, 2006) Achrosin,, Aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol and chrysoeriol (Yadav et al..,2010)	In vitro antibacterial activity (Saganuwan and Gulumbe, 2006). In vitro antibacterial and antifungal activity (Vedpriya *et al..*, 2010). In vitro Anti-hyperglycemic activity in rats (Laximi et al.., 2010). Causes acute hepato-myo-encephalopathy (Vipin et al.., 2007) *In vitro* acetylcholinesterase inhibitory

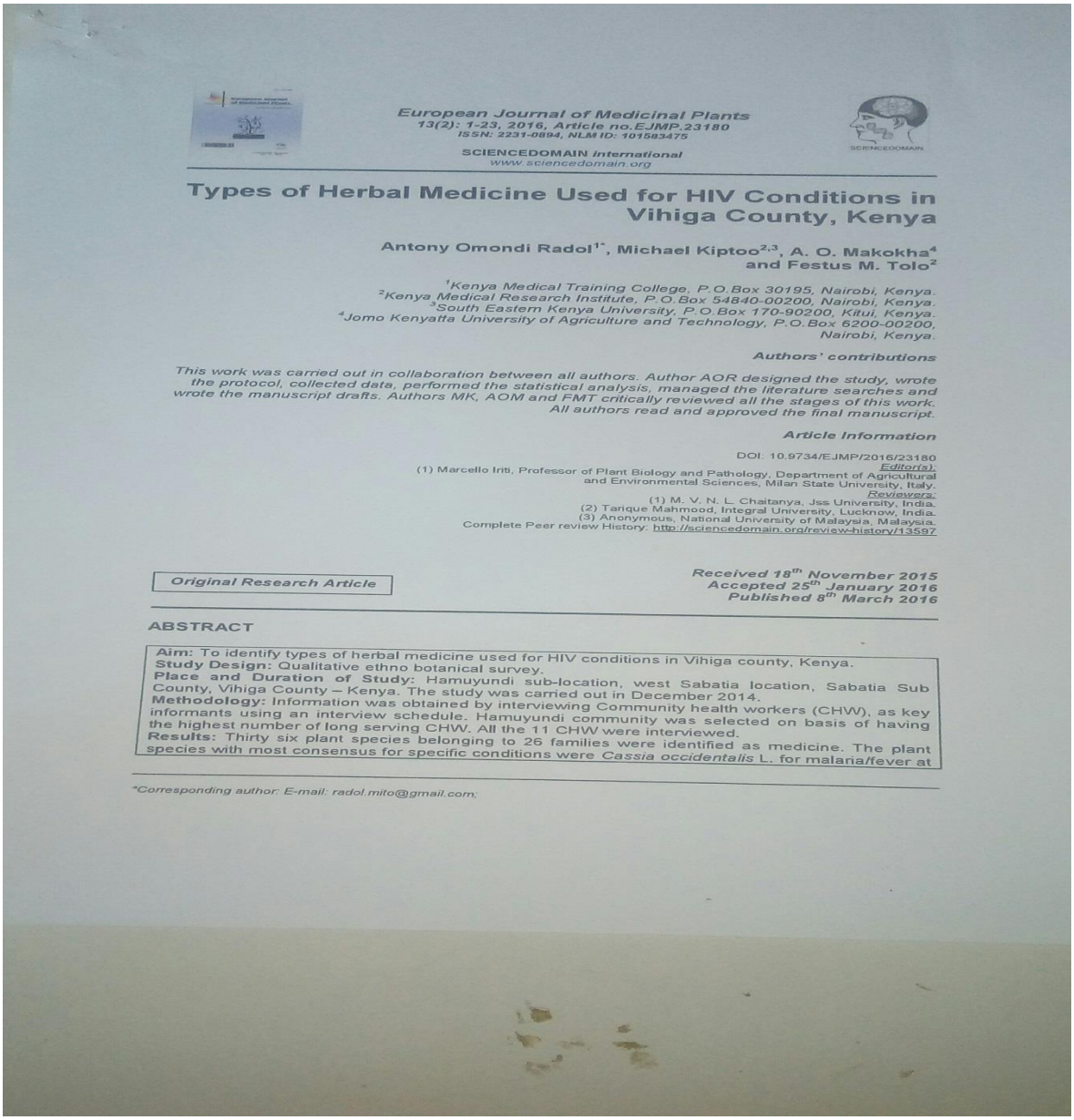
	(L.) T*ylosema fassoglensis*Brenan, J.P	High seed content of Linoleic acid, Oleic and Palmitic acid. Proteins are characterized by high levels of lysine, proline and tyrosine (Dubois et al.., 1995)	activity (Adewusi et al.., 2011). Hepatoprotective and anti-inflammatory (Jafri et al..,1999; Sadique et al.., 1987)
Melaceae	*Melia azedarach* L.	(20S) -5,24(280-ergostadiene-3β,7α, 16 β,20-tetrol, (20S)- 5-ergostene - 3 β, 7α, 16 β,20-tetrol 2α, 3β-dihydro-5-pregnen-16-one (Shi-Bao et al.., 2009)	High Antioxidant activity on DPPH (Gayatri and Rajani, 2010)
Polygoneceae	*Rumex abyssinicus* (Jascq) *Rumex stendelii* A.Rich		Antibacterial activity against Streptococcus pyogenes and Staphylococcus aureus, antiviral activity against Coxsackie virus B3 and anti-inflammatory activity (Gatie et al.., 2003). Anti-helminth activity (Jaya and Elias, 2010) Antifertility effect on rats (Endalk *et al*.., 2005)
Gentianceae	*Anthocleista vogelii* Planch.	Saponins, cardiac glycosides, flavonoids, terpenes , alkaloids, and steroids (Labari et al.., 2014)	Anti-plasmodial activity in albino mice (Gboeloh, Okon, & *t al*., 2014). Hypoglycaemic activity (Abuh*et al*., 1990)
Mimosaceae	*Entada abyssinica(A. Rich*	Alkaloids, flavonoids, Tannins, Saponins and cardiac glycosides; (5S,6R,8AR) -5-(carboxymethyl) -3,4,4a,5,6,7,8,8a – octahydro – 5,6,8a – trimethylnaphalene-carboxylic acid ; methyl 3,4,5 –	In vitro antibacterial activity against Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Shigella flexneri,, Salmonella typhi, Escherichia coli, Enterococcus facalis,

		trihydroxybenzoate(methyl galate) ; benzene – 1,2,3 –triol (Pyrogallol); and 2,3 – dihydroxypropyltriacontanoate. Lipids; Hexadecanoic acid, 9-Octadecenoic acid and Octadecanoic acid	Staphylococcus aureus, Some strains of Candida species. High in vitro antioxidant activity (Teke et al.., 2011; Tchana et al.., 2014)
Guttiferae	*Garcinia buchanii*		*In vitro* inhibition of gastrointestinal motility (Balemba *et al..*, 2010)
Apocynaceae	*Plumeria alba* L.	Leaves; Terpenoids, Flavonoids, Alkaloids, Glycosides and Phytosteroids (Radha et al.., 2008), Flowers; Steroids, Flavonoids and Alkaloids (Zaheer et al. 2010)	*In vivo* antimalarial activity (Boampong et al. 2013). *In vitro* antifungal activity against *Candinda albicans Aspergillus nige*r and *Penicillum chrysogenum* (Kumari et al.., 2012), *In vitro* antibacterial activity against Escherichia coli, Staphylococcus saprophyticus, Proteus vulgaris and Serretia marcescens (Syakira and Brenda, 2010), *In vitro* and *In vivo* anti- cancer activity (Radha and Sivakumar, 2009)
Caesalpinaceae	*Caesalpinia decepetala (Roth.I. Alston)*	6'-hydroxy-epoxy-propane)-2',3'-(1"β-hydroxy-2carbonyl-cyclobutane)-1,1'-diphenyl; Octacosyl 3,5-dihydroxycinannamate; 2'4,4'-trihydroxychalocone; bonducellin; 7,3'5'-trihydroxyflavanone, daucosterin and β-sitosterol (Zhang et al.., 2008), Cassane diterpenoid (spathulenol; 4,5-epoxy-8(14)-caryophyllene,; squalene; lupeol; trans-resveratrol; quercetin; astragalin and stigmasterol (Kiem et al.., 2005),	*In vivo* analgesic, anti-inflammatory and antipyrexia (Parveen*et al*., 2014),*In vivo* anit-hyperglycaemic, nephroprotective, hepatoprotective and hypolipidaemic (Hussain et al.., 2014),antioxidation activity (Gallego et al.., 2015; Pawar and Surana, 2010), Selective toxicity against cancerous cellines (Modh, 2012). Ethomedical use for gonorrhea (Erasmus et al.., 2012)

		lupeol acetate; lupeol; oleanoic acid; pentacosanoic acid 2,3-dihydroxypropylester; 1-(26-hydroxyhexacosanoyl)-glycerol; stigmasterol; beta-sitosterol (Li et al.., 2002)	
Asteraceae	*Ageratim conyzoides*	Precocene (Bayala *et al*..,2014)	*In vitro* toxicity against prostate cancer and gliobastoma cell lines (Bayala *et al*..,2014), ethnobotanical use for digestive disorders (Choudhury*et al* ., 2015), ethnomedical use for skin diseases (Sharma et al..,2014),*In vivo* renal and hepatic toxicity, and hematological diorders (Diallo *et al*.., 2014)
	Microglossa pyrifolia (Lam)O.Kutze	Dihydrobenzofurans (methyl 2-(5-acetyl -2,3-dihydrobenzo[beta]furan-2-yl)propenoate; methyl 2-(6-acetyl-5-hydroxy-2,3-dihydrobenzofuran-2-yl)propenoate; 6-acetyl-5-hydroxy-2-(1-hydroxy-2-propenyl)-3-methoxy-2,3-dihydrobenzofuran. Triterpenes 3 beta-acetoxy-25-hydroxydammara-20,23-diene; 3beta-acetoxy-24-oxo-dammara-20,25-dien; 17beta-hydroxy-3,16-dioxo-	*In vivo* anxiolytic and antipyretic activity (Bum et al.., 2011), *In vitro* antimicrobial activity (Dickson et al..,2006)

		28-norolean-12-ene; 17beta-hydroxy-3,11,16-trioxo-28-norolean-12-ene (Schmidt et al.., 2003)	
Liliflorae	*Gloriesa superba (L.)*		Antimicrobial activity against *Candida albicans, Candida glaberata, Trichophyton lonifusus, Microsporum canis, Staphylococcus aureus* (Khan et al.., 2008), inhibitor of psoriasis associated marker (K17) (Pattarachotanant et al.., 2014),*In vitro* antiproliferative activity on liver cancer cells(Manosroi et al.., 2015), large consumption causes hair loss, acute renal failure, gastroenterocolitis, pancytopenia, cardiotoxicity and hematological abnormalities (Khanam et al..,2015; Premaratna *et al..*, 2015; Mendis, 1989)
Compositae	*Erlangea tomentosa (Oliv. & Hiern). S.Moore*		Ethnomedical use for hernia and caugh (Kibuka and Anywar, 2015; Namukobe et al.., 2011)

Appendix 13: Abstract of published paper 1



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Types of Herbal Medicine Used for HIV Conditions in Vihiga County, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOR designed the study, wrote the protocol, collected data, performed the statistical analysis, managed the literature searches and wrote the manuscript drafts. Authors MK, AOM and FMT critically reviewed all the stages of this work. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To identify types of herbal medicine used for HIV conditions in Vihiga county, Kenya.
Study Design: Qualitative ethno botanical survey.
Place and Duration of Study: Hamuyundi sub-location, west Sabatia location, Sabatia Sub County, Vihiga County – Kenya. The study was carried out in December 2014.
Methodology: Information was obtained by interviewing Community health workers (CHW), as key informants using an interview schedule. Hamuyundi community was selected on basis of having the highest number of long serving CHW. All the 11 CHW were interviewed.
Results: Thirty six plant species belonging to 26 families were identified as medicine. The plant species with most consensus for specific conditions were *Cassia occidentalis* L. for malaria/fever at

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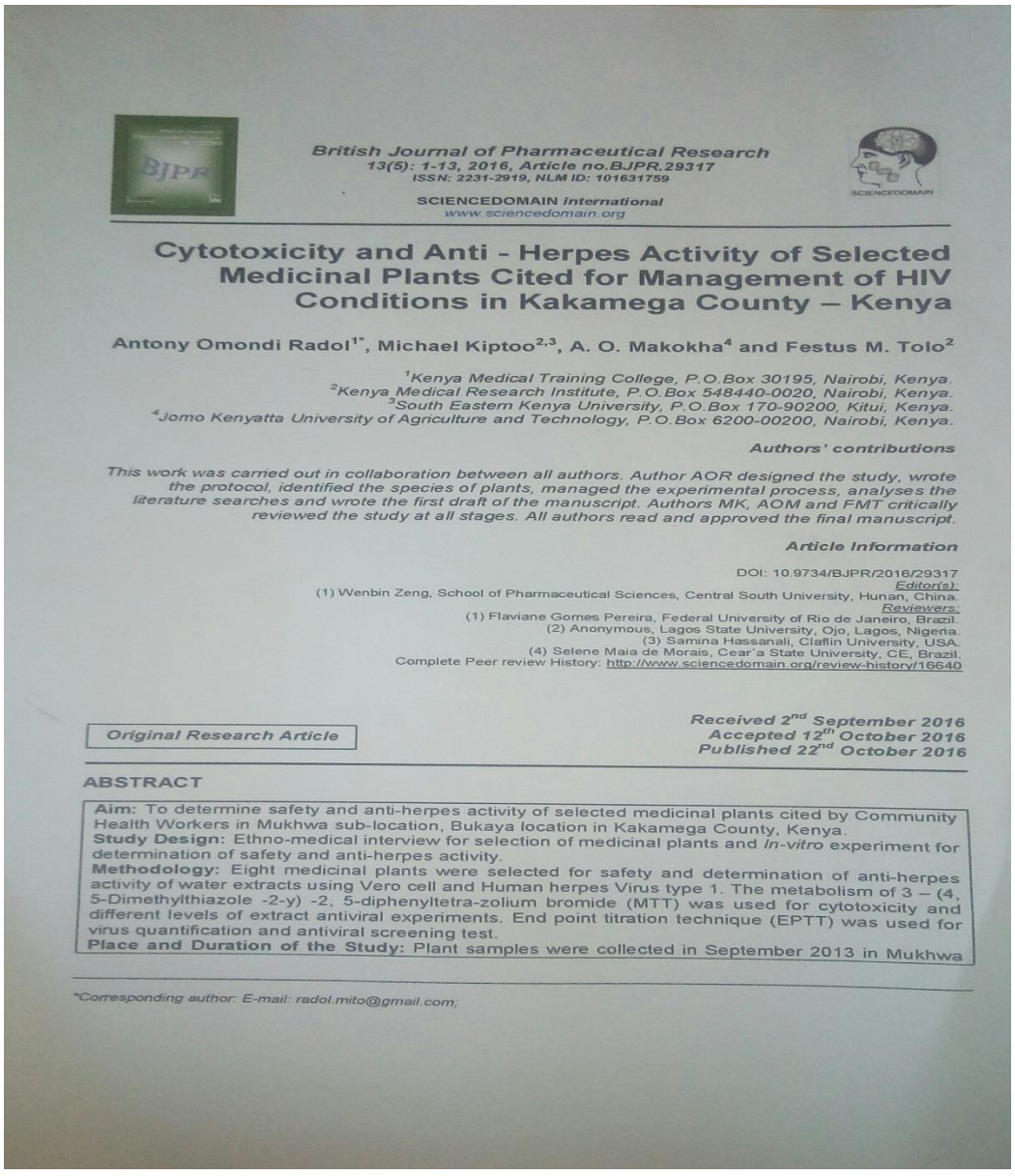
Appendix 14: Abstract of published paper 2



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Cytotoxicity and Anti - Herpes Activity of Selected Medicinal Plants Cited for Management of HIV Conditions in Kakamega County – Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOR designed the study, wrote the protocol, identified the species of plants, managed the experimental process, analyses the literature searches and wrote the first draft of the manuscript. Authors MK, AOM and FMT critically reviewed the study at all stages. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To determine safety and anti-herpes activity of selected medicinal plants cited by Community Health Workers in Mukhwa sub-location, Bukaya location in Kakamega County, Kenya.
Study Design: Ethno-medical interview for selection of medicinal plants and *In-vitro* experiment for determination of safety and anti-herpes activity.
Methodology: Eight medicinal plants were selected for safety and determination of anti-herpes activity of water extracts using Vero cell and Human herpes Virus type 1. The metabolism of 3 – (4, 5-Dimethylthiazole -2-y) -2, 5-diphenyltetra-zolium bromide (MTT) was used for cytotoxicity and different levels of extract antiviral experiments. End point titration technique (EPTT) was used for virus quantification and antiviral screening test.
Place and Duration of the Study: Plant samples were collected in September 2013 in Mukhwa

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sub-location, the processing and biological experiments were carried out between March 2014 and October 2015 at the center of traditional medicine and drug research of Kenya Medical Research Institute, Nairobi.

Results: Safety profile: *Tithonia diversifolia* (Whole root) gave maximum nontoxic extract concentration (MNC) at 20 µg/mL, extract concentration killing 50% of cells (CC_{50}) was 460 µg/mL. *Schkuhria pinnata* (Leaves); MNC <20 µg/mL, CC_{50} 90 µg/mL. *Entada abyssinica* (Stem bark); MNC 20 µg/mL, CC_{50} > 500 µg/mL. *Garcinia buchanii* (Stem bark); MNC 40 µg/mL, CC_{50} >500 µg/mL. *Croton macrostachyus* (Stem bark); MNC 40 µg/mL, CC_{50} >500 µg/mL. *Vernonia adoensis* (Whole root); MNC 20 µg/mL, CC_{50} 470 µg/mL. *Plumeria alba* (Leaves); MNC <20 µg/mL, CC_{50} 120 µg/mL. *Caesalpinia decapetala* (Whole root); MNC 20 µg/mL, CC_{50} 500 µg/mL. Anti-herpes activity: The best anti-herpes activity was obtained from *G. buchanii* (Stem bark), giving an extract concentration inhibiting 50% of virus activity (IC_{50}) at 20 µg/mL) and *C. decapetala* (Whole root) giving IC_{50} at 80 µg/mL. Therapeutic index of *G. buchanii* was > 25 and that of *C. decapetala* was > 6.

Conclusion: Majority of the medicinal plants selected for anti-herpes activity have narrow none-toxic limits. Of all the selected medicinal plants, *G. buchananii* and *C. decapetala* are the most promising for selective anti-herpes activity.

Keywords: Cytotoxicity; anti-herpes; medicinal plants.

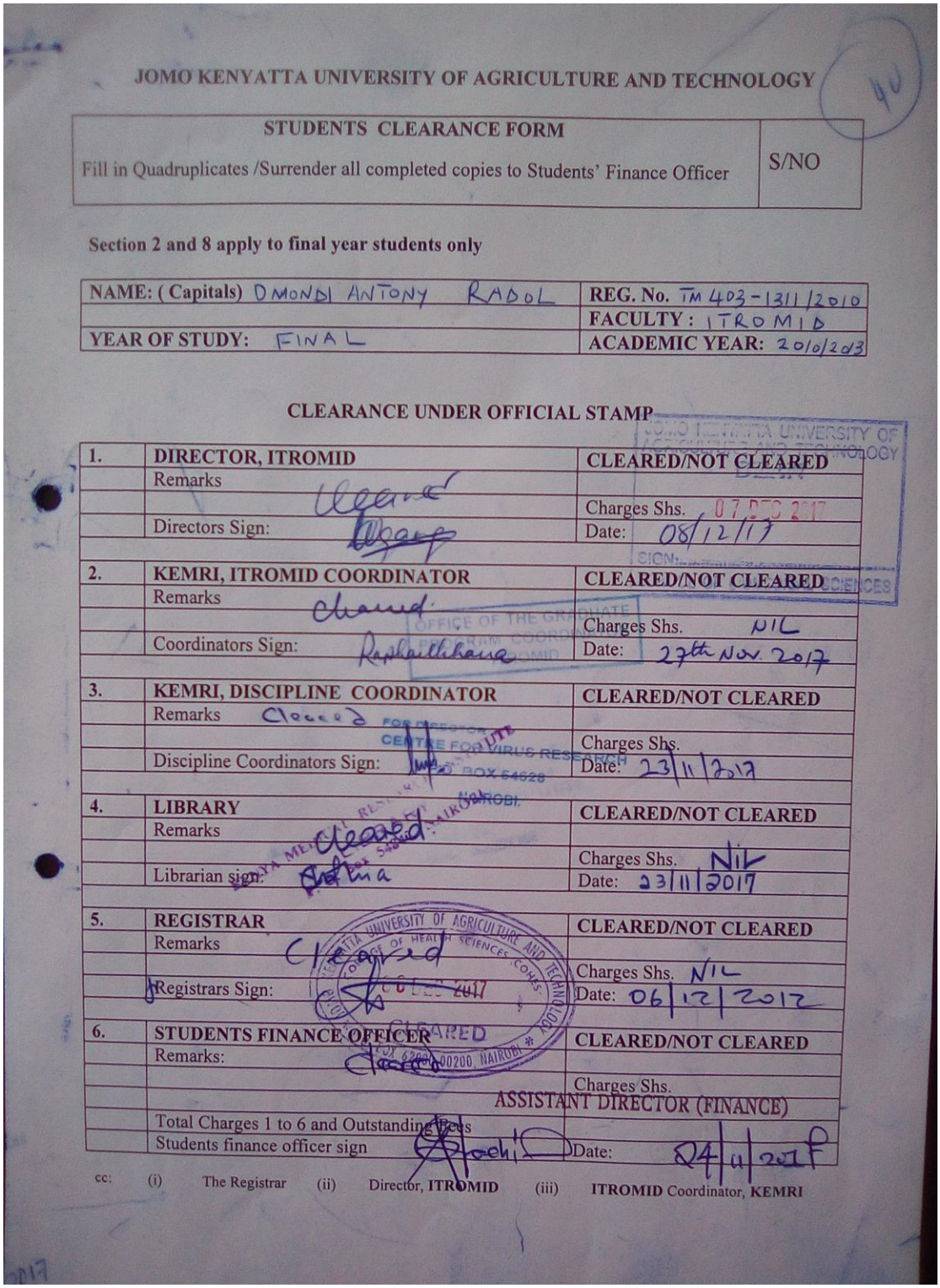
1. INTRODUCTION AND BACKGROUND INFORMATION

Kakamega County is one of the 47 counties in the Republic of Kenya. It is located to the western side of the country, bordering Vihiga, Siaya, Bungoma, and Nandi counties. It covers an area of 3050.3 km^2 and has an altitude between 1,240 meters and 2000meters above sea level [1]. According to Kenya National AIDS Control program [2], the HIV prevalence estimate was 5.9% by 2012, being generally the same as the national average of 6%. The cited priority service areas on HIV included strengthening of linkage to care for individuals testing positive, thus addressing the statistic showing that only 37.4% were linked to the services [3]. Further, the report indicated that the proportion of eligible clients started on ART was 81%, meaning that about 19% were either seeking care to non-reporting facilities or using self-administered care, with possibility of taking traditional medicine. Although statistics on HIV deaths for the county was not indicated, the national statistic of 5% [4] points to gaps in HIV care. Previous studies elsewhere have indicated a need for improved care for People living with HIV (PLwHIV), with reports indicating emergence of resistant HIV strains [5], unsustainable donor driven free public sector provision of Antiretroviral treatments (ART), inhibited access to treatment due to factors such as lack of confidentiality, lack of transport to hospitals, shortage of health workers, long queues, and lack of adherence due to adverse drug reactions [6,7]. Some HIV patients resort to use of ethno-medicinal plants as an alternative or concomitant use with ARTs in an attempt to overcome these challenges [8].

Medicinal plants have been cited as key ingredient of medication therapy in traditional medicine [9]. In its report, the International Bioethics Committee (IBC) of UNESCO highlights issues of uncertain benefits and risks associated with the use. On the other hand, studies on medicinal plants selected from the community have shown that the materials have potential to solve human health challenges. For instance, HSV has been shown to respond very well to plant species such as *Euphorbia continifolia*, *Euphorbia tirucali* [10] and *Carrisa edulis* [11]. However, the health value of the majority of the medicines are still mainly anecdotal and the utilization seem to be increasing and dynamic, fueled by problems of HIV and other chronic conditions [6,8].

The group of viruses referred to as Herpes belong to the family, *Herpesviridae*. They are morphologically identical and biologically closely similar. Their replication in the nucleus results in inflammatory response and destruction of host cells [12]. The agents are host specific and establish lifelong latent infection, with occasional reactivation. Infection sometimes results in malignant forms of host cells [13]. Viruses within the family includes Varicella zoster Virus (VZV), Epstein - Barr virus (EBV), Human cytomegalovirus (HCMV), HSV and Human herpes viruses [14]. Herpes Simplex Viruses are classified in the sub family *Alphaherpesvirinae* together with VZV. The subfamily characteristics include relative rapid replication cycle *In vitro*,

Appendix 15: Clearence certificate 1



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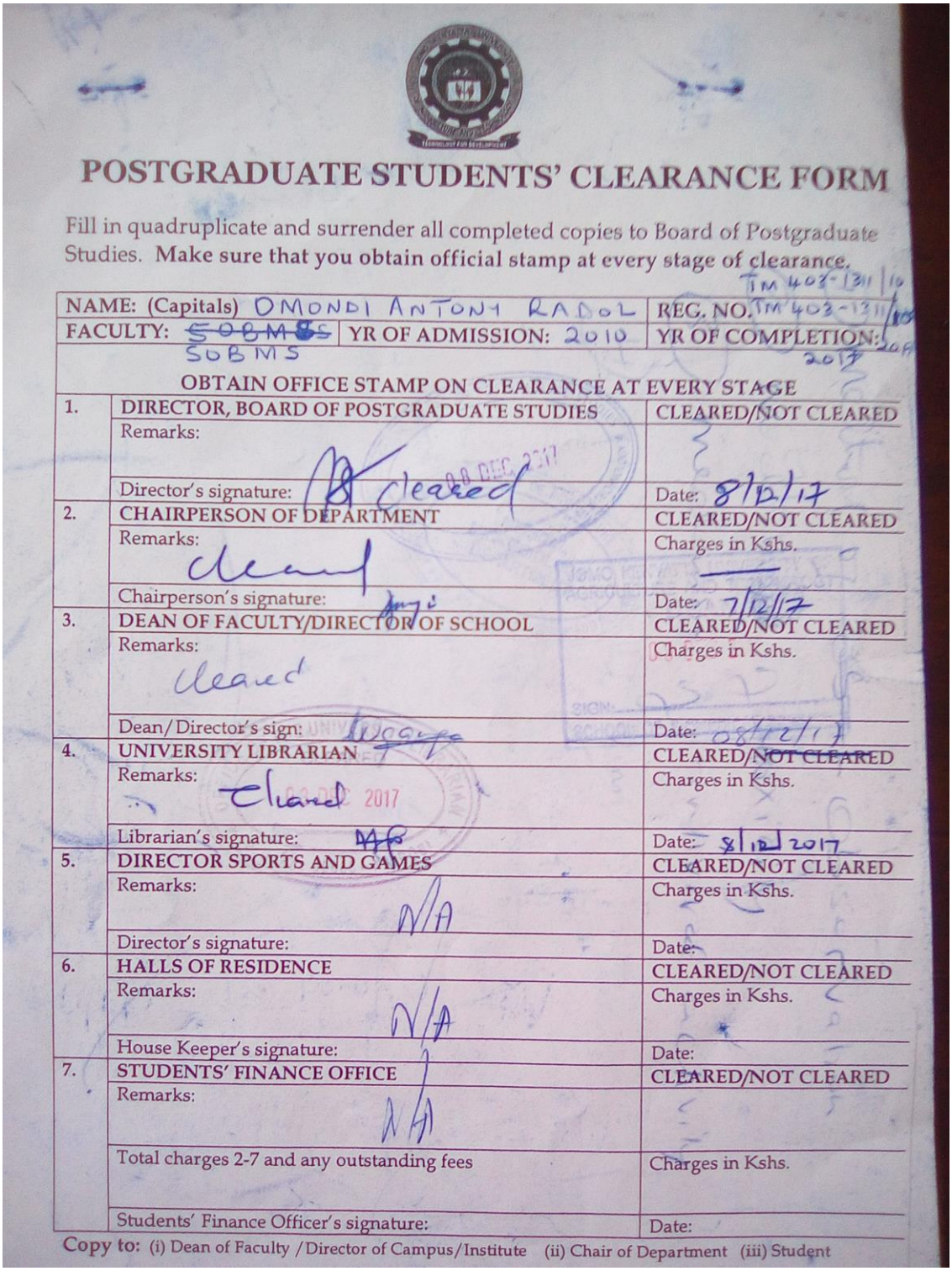

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