THE PHYTOCHEMICAL COMPOSITION, EFFICACY AND SAFETY OF HERBAL FORMULATIONS USED FOR MANAGEMENT OF HIV/AIDS IN MOMBASA COUNTY, KENYA

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DECLARATION

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

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To my family; my children Ruth Pahe, Faith Jumwa, Warren Mwavita, Sandrah Comfort Chizi, David Mwavita, Andrew Matano. Who was my inspiration at conceptualizing this study.

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ABBREVIATIONS AND ACRONYMS

3TC	Lamividine
ABC	Abacavir
AIDS	Acquired Immunodeficiency syndrome
ALT	Alanine aminotransferase
ARF	Acute Renal Failure
ART	Antiretoviral treatment
ARVs	Antiretroviral drugs
AST	Aspartate aminotransferase
AZT	Zidovudine
bDNA	Branched DNA
BMI	Body mass indexes.
BUN	Blood urea nitrogen.
CDC	Centers for Disease Control and Prevention
D4T	Stavudine
DDC	Zalcitabine
DDI	Didanosine
EDTA	ethylene diamine tetra-acetic acid
ESR	Erythrocyte sedimentation rate
FTC	Entricitabine
GALT	Gut-associated lymphoid tissue
GGT	Gamma-glutamyltransferase
GLA	Gammalinoleic acid
HAART	Highly active antiretroviral therapies
HB	Hemoglobin
IMB	immune boosting herbal formulation
KDHS	Kenya demographic and health survey
LFTs/LFs	Liver function tests,
NATs	Nucleic acid-based tests,

NRTIs	Nucleoside reverse transcriptase inhibitors
NtRTI	Tenofovir;
PIs	protease inhibitors
PLWHA	People Living With HIV and AIDS.
PRC	Polymerase Chain Reaction
RBC	Red blood cells
RT	Reverse transcription step mediated by the enzyme
TCM	Traditional Chinese medicine
TLC	Thin layer chromatography
TMPs	Traditional Medicine Practitioners
UNAIDS	United Nations Program on HIV/AIDS
VIRAD	Antiretroviral herbal formulation
WBC	White blood cells
WHO	World health organization

ABSTRACT

Kenya has the fourth largest HIV epidemic in the world with adult prevalence of 5.9% (2016) (UNAIDS). Currently 80% of HIV patients access ARVs, while the rest do not due to stigma and traditional beliefs. In the coast of Kenya some patients seek alternative treatments to manage HIV/AIDS. Pwani herbs clinic in Mombasa is a popular clinic that dispenses herbal medicines to manage HIV and AIDS. Unfortunately, these medicines have not been subjected to formal clinical research to ascertain their efficacy and safety. This study's objective was to isolate the components of the herbal formulations VIRAD (Antiretroviral herbal formulation) and IMB (immune boosting herbal formulation) and to determine their efficacy on immunological parameters and safety on liver and kidney parameters in the treatment for people living with HIV and AIDS (PLWHA) in Mombasa County. Herbal formulations were analyzed for phytochemical components by qualitative and quantitative screening, mineral compositions by Atomic absorption spectrophotometer (AAS) Model: 210VGP. Patients that met the inclusion criteria and for whom written/oral informed consent was obtained and were on treatment with these herbal formulations were purposely sampled as they visited the herbal clinic. Patients were evaluated for anthropometric screening including phenotypic indices, hematological, immunological parameters CD4 /CD8 and viral load tests, liver function tests, endocrine related; were screened for a period of 12 months; including HIV disease progression, quality of life and adverse side effects. Results revealed Phytochemical components (mg/100g) Virad; Phenols 84.4mg, Saponins 531mg, Tannins 324mg, Alkaloids 2304mg, Flavonoids 2173mg, Protein 442mg and Lipids 2444.2mg. Phytochemical components (mg/100g) IMB; Phenols 75.1mg, Saponins564mg, Tannins 51mg, Alkaloids 1531mg, Flavonoids 2533mg, Proteins 544mg, Lipids 2553.8mg, characterised as metabolites antioxidant, anti-inflammatory anticholinesterase, immune boosting and antiviral agents. Trace elements in VIRAD Mineral levels (µg/g) K 12922 ± 103 Na 475 ± 4 Ca8861 ± 71 Mg547 ± 1 Fe361 ± 4 Cu 98.6 ± 1.5 Zn43.7 ± 0.9. Trace elements in IMB Mineral levels (µg/g) K 12085 ± 87 Na 598 ± 11 Ca 6047 ± 46 Mg 545 ± 1 Fe348 ± 4 Cu 5.9 ± 0.4 Zn25.3 ± 0.6. Patients with subclinical deficiencies of trace elements would be at risk of impaired immune function. A total of 188 patients were purposely recruited into this longitudinal study. They were followed every two months for a period of one year. At months 2, 4, 6, 8 and month 10, 23/188 was lost to follow-up. Out of these three 3/23 died while twenty 20/23 developed other complications such as tuberculosis and cancer. Those who developed complications were referred for specialized treatment. Others were advised to use conventional antiretroviral drugs. At baseline were two peaks for CD4 levels 300-350 and 200-250. At completion of study weight gained by average 17kg, BMI increase 4.85, CD4 raise 126, CD8 declined 15, CD4/CD8 raise 0.19, Viral load drop 864, Hemoglobin Hb raise 3g/dl, RBC raise 1.3, WBC raise 1.6, ESR drop 21. Toxicity on kidney Urea drop 0.1g/dl, Liver GGT, AST, ALT drop0.3g/dl. Flavonoids, Alkaloids and lipids were of highest concentrations in these formulations. Immunological factors CD4/CD8 were raised; viral burdens reduced therefore improved immunity and were efficacious. Liver and kidney functions were not adversely altered; these phytochemicals were not toxic and therefore safe for use.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The human immunodeficiency virus (HIV) infection has been a pandemic for more than three decades and has always been concentrated in Sub-Saharan Africa. In 2013 an estimated 35.0 million people were living with HIV worldwide. Sub-Saharan Africa is home to only 12% of the global population, yet accounts for 71% of the global burden of HIV infection. Ten countries, mostly in southern and eastern Africa, *viz.* South Africa (25%), Nigeria (13%), Mozambique (6%), Uganda (6%), Tanzania (6%), Zambia (4%), Zimbabwe (6%), Kenya (6%), Malawi (4%) and Ethiopia (3%), account for almost 80% of all people living with HIV [Joint United Nations Programme on HIV/AIDS (UNAIDS, 2015).

Acquired Immunodeficiency Syndrome (AIDS), is a threat to economic, social and human development for many developing countries. Many people living with HIV or at risk for HIV do not have access to prevention, care, and treatment, and there is still no cure (Risley *et. al.*, 2012).

In the late 1990s, HIV prevalence in Kenya recorded the highest peak, however this dramatically reduced to about 6.2 percent (UNAIDS, 2012). This was presumably due to an increase in education awareness, and also from high death rates (UNGAS, 2008). There are challenges for some that are not being reached with HIV prevention and treatment services. Seventy two percent of adults in 2009 received treatment and in 2011 an additional 200,000 patients were put on treatment (UNAIDS, 2012). Unfortunately, the proportion of eligible children in many sub-Saharan Africa receiving antiretroviral treatment is much lower.

The management of HIV and AIDS has enormous challenges coupled with the adverse effects presented by the antiretroviral medicines currently in use in the country (WHO, 2005). In resource-limited settings, illness can impose a major financial burden on patients and their families (Kumarasamy *et al.,*2007). Highly active antiretroviral therapies (HAART) are supposed to improve overall health status. However, the side effects resulting from the antiretroviral drugs (ARVs) occasionally lead to poor adherence to the drug regimens. These include; loss of appetite, nausea, vomiting, diarrhea, loss of taste, anemia and constipation (Heath *et al.,* 2002). The prominent signs of fat redistribution include facial and limb wasting, localized adiposity and visceral fat accumulation, insulin resistance, cardiovascular risks and birth defects. This is common in individuals who take nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) (Heath *et al.,* 2002).

In Africa, combination of herbs is a fundamental philosophy. Herbal therapies ideally should be standardized for their active constituents to ensure clinical reproducibility (WHO Traditional Medicine Strategy, 2005). The use of herbal medicines in the coastal areas of Kenya is on the raise as many practitioners use the available media to advocate for their use. Pwani herbs clinic in Mombasa is a popular clinic that dispenses anti-retroviral herbal formulation (Virad) and immune booster (IMB) to manage HIV. These herbal medications packaged in 500mg capsules and in tablet form; dispensed as alternative to the conventional ARVs. Virad is a herbal combination of herbal components assumed to have effects to combat viral infections. The immune booster IMB is a combination of herbal components and mineral agents alleged to boost immune system. It was important to investigate these herbal remedies in order to determine their composition, efficacy and safety.

1.2 Problem Statement

HIV and AIDS is degenerative disease affecting livelihoods of many families with patients, many of whom cannot access ARVs and other drugs to manage HIV related ailments. Patients using herbal treatments are limited by costs of drugs and the daily nutrition demands required when taking the allopathic medications. Conventional drugs are associated with side effects including visceral fat accumulation. These patients turn to unconventional treatments to cure their ailments. The herbalists administer herbal therapies that have not been scientifically investigated before being used on human subjects. Virad and IMB are herbal formulations currently being used to manage HIV, but the phytochemical composition, efficacy and safety of these formulations are not known. This study sought to evaluate the patient characteristics, phytochemical composition, efficacy and safety of herbal antiviral formulation (Virad) and herbal immune booster (IMB) used to treat people leaving with HIV/ AIDS in Mombasa.

1.3 Justification

Herbal medicines have for many years been used to treat both acute and chronic ailments in Africa. They have also been the source of therapeutic materials in allopathic medicines for example Cinchona and Artemisia for malaria, Digoxin for heart treatment without toxicities. The emergence of HIV and AIDS has seen many patients turn to herbal products because of belief, or fear of possible side effects associated with conventional ARVs. The empirical evolutions for each study participant receiving herbal treatments warranted the use of the self-controlled case series (SCCS) method as an alternative study method for investigating the association between a transient exposure and an adverse event. The method uses only cases; no

separate controls were required as the cases act as their own controls. Each case is given observation time; is divided into control and risk periods defined during or after the exposure (Whitaker *et al*., 2006) comparisons are made within individuals. Previously comparable studies included; when should case-only designs be used for safety monitoring of medical products? (Maclure *et al*., 2012); Use of the self-controlled case series method in vaccine safety studies: review and recommendations for best practice, Epidemiology and Infection and Self-controlled case series with multiple event types, computational statistics and data Analysis (Weldeselassie *et al*., 2011, Weldeselassie *et al*., 2017). Self-controlled case series (SCCS) were originally developed for evaluation of vaccine safety, but have since been applied in a range of settings where exact information on the size of the population at risk is lacking or identification of an appropriate comparison group is difficult, for studies of adverse effects of drug treatments (Weldeselassie *et al*., 2011).

1.4 Null Hypothesis

The herbal formulations Virad and IMB used in management of HIV/ AIDS are neither effective nor safe for human consumption.

1.5 Research questions

1. What is the phytochemical composition of Virad and IMB formulations?
2. Is there any efficacy on immunological factors for PLWHA?
3. Are there any toxic effects on the liver and kidney during treatment?

1.6 Objectives

1.6.1 Broad objective

To determine the phytochemical compositions, efficacy and safety of herbal formulations used by people living with HIV/ AIDS at the coast of Kenya.

1.6.2 Specific Objectives

1. To isolate the phytochemicals of Virad and IMB used for treatments

2. To determine efficacy for immunological parameters of PLWHA

3. To determine any toxic effects on the liver and kidneys during treatment

1.7 Significance of the Study

The results of this study provided information that the phytochemicals obtained were of benefit to human health. They were not toxic and could be improved to make a drug formulation that could be used to manage immune suppression and HIV/AIDS.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of HIV

Human immunodeficiency virus is a Retroviridae of genus Lent virus (International Committee on Taxonomy of Viruses, 2014). These viruses have a long incubation period responsible for long-duration illnesses. Upon entry into the target cell, the viral RNA is converted into double-stranded DNA in a reaction catalyzed by reverse transcriptase enzyme. The viral DNA is then integrated in to the cellular DNA for transcription of other viral particles. Once the virus has infected the cell, two pathways are possible: either the virus becomes latent and the infected cell continues to function, or the virus becomes active and replicates and a large number of virus particles are liberated that can then infect other cells.

Human immune deficiency Virus (HIV) leads to acquired immunodeficiency syndrome (AIDS), a condition in which the immune system is highly compromised, exposing an individual to opportunistic infections (Coffin *et al.,* 2010). Human immune deficiency Virus (HIV) can occur as either a free virus or a virus within the immune cells that are infected. It is spread by various methods including blood transfer and its products, semen, vaginal fluids, pre-ejaculation or breast milk (Joint United Nations Program on HIV/AIDS, 2006).

There are two major types of the human immunodeficiency virus. Human immune deficiency Virus HIV-1 is the most widespread type worldwide; it was discovered first and is most virulent type that rapidly mutates. The strains of HIV-1 are classified

into four groups: group M, group O and N and P. These four groups cause immunodeficiency into humans (Parry *et al.,* 2003).

Human immune deficiency Virus HIV-2 is 55% genetically different from HIV-1. It is less prevalent, less pathogenic type, and found principally in western Africa (Parry *et al.,* 2003). Genetic difference brings distinction between HIV-1 and HIV-2 antigens. Tests that are sensitive to both types of virus have been developed (Dougan *et al*, 2005, Parry *et al.,* 2003). Human immune deficiency Virus HIV-2 is becoming more common in India. Some cases have also been reported in Portugal, France, and other European countries including the UK and America, largely in individuals of West African origin. A person presenting with clinical signs of HIV infection but does not test positive to screening that is sensitive only to HIV-1, should be screened specifically for HIV-2 (Parry *et al.,* 2003).

2.2 Transmission

There are three main transmission routes identified for HIV. The first is sexual transmission including genital, oral, or rectal mucous membranes of an infected individual (Coovadia, 2004). The second is by intravenous drug users and hemophiliacs usually acquire infections through Blood or blood product routes; sharing of needles also can spread HIV (Daar *et al.,* 2001). Scarification procedures by people, who give and receive tattoos, piercing, are also at risk of infection. The third is by HIV positive Mothers that could transmit infections to their unborn children in the uterus, during pregnancy or during delivery (Coovadia, 2004). In the absence of treatment, the transmission rate between the mother and child is around 25-40%. Breast-feeding by infected mothers may also risk infection for the baby.

2.3 Diagnosis of HIV

The diagnosis of Human Immunodeficiency virus (HIV) could be carried out by ARCHITECT HIV Ag/Ab Combo Assay. This is an immunoassay for micro particles intended for use in the diagnosis of HIV-1 and HIV-2 infection. This essay could be used to diagnose earlier, acute phase infection, specifically detection of the HIV-1 p24 antigen, as well as antibodies to HIV-1 groups M and O, and antibodies to HIV-2 (FDA, 2010). It could also be used for children as young as 2 years of age, and pregnant women. It takes earlier than 7 days to detect viral infection prior to the emergence of antibodies produced by the infected patient, effectively reducing the window period compared to 3rd generation enzyme immunoassay antibody tests. Most tests in the diagnostic setting used currently detect only HIV antibodies (FDA, 2010). Although the assay was not intended for routine screening of blood donors, it was approved as a donor-screening assay for HIV-1/HIV-2 infection in urgent situations.

Earlier screening methods for Human immune deficiency Virus HIV-1; involved enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV-1. Specimens which were reactive to ELISA were tested in duplicate (CDC- P, 2001), while Specimens which were nonreactive from the initial ELISA were considered HIV-negative. If the result of either duplicate test was reactive, the specimen under-went confirmatory testing with a more specific supplemental test (Western blot or, an immunofluorescence assay (IFA). Specimens that were reactive by ELISA and positive by IFA or reactive by Western blot were HIV-positive and indicative of HIV infection. There were indeterminate specimens occasionally from Western blot result, either from an incomplete antibody response to HIV or nonspecific reactions in an

uninfected person (Celum *et al.,* 1991). Although IFA was used to confirm infection in these cases, the assay was not widely used because it required a second specimen to be collected more than a month later and retested for persons with indeterminate Western blot results.

2.4 Clinical Course of HIV Infection

During the clinical latency phase of infection, large amounts of HIV virus trapped within lymphoid organs remain active in the follicular dendritic cells (Burton *et al.,*2002). Cell-mediated immunity is lost when CD4+ T cell numbers decline below a critical level, and a variety of opportunistic microbial infections appear (Clapham and McKnight, 2001). There are three main mechanisms which HIV infection depletes levels of CD4+ T cells: direct viral killing of infected cells; increased rates of apoptosis in infected cells; and killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.

The initial symptoms include unexplained weight loss, recurring respiratory tract infections, skin rashes, and oral ulcerations. Opportunistic infections and tumors that are normally controlled by CD4+ T cell-mediated immunity start to affect the patient. If not treated, an individual may get Acquired Immunodeficiency Syndrome (AIDS) and die (Coffin *et al.,*2010).

Following loss of immunity and AIDS there is increased susceptibility to oral candidiasis (thrush) and tuberculosis respectively; herpes simplex eruptions, shingles, Epstein-Barr virus-induced B-cell lymphomas, or Kaposi's sarcoma (Clapham and McKnight, 2002). These are tumors of endothelial cells that occur when HIV proteins

interact with Human Herpesvirus-8. A common and fatal fungal Pneumonia caused by Pneumocystis jirovecii also occurs. In the final stages of AIDS infection, individuals acquire cytomegalovirus or *Mycobacterium avium*, however not all patients with AIDS get these infections or tumor (Clapham and McKnight, 2002), there are other tumors and infections that are less prominent but still significant.

2.4.1 HIV Oxidation mechanisms

Human immunodeficiency virus (HIV)-infected individuals are deficient in antioxidant micronutrients and are therefore oxidatively stressed (Mgbekem *et al.,* 2011). Development of AIDS is aggravated by oxidative stress, which is a principal mechanism in expression of HIV (Alexander *et al*., 2016). At some past AIDS research, three unrelated studies hypothesized and agreed that oxidative mechanisms played a critical role in HIV expression and AIDS development (Doyle et *al.,* 2002). Oxidative stress in AIDS patients would lead to cellular anomalies, including lymphocytes, resulting in opportunistic infection, immunological abnormalities and neoplasia (French *et al*., 2007). Kaposi's sarcoma (KS) is one of the first indicators attributed with AIDS that may not directly be caused by HIV, but a precursor of oxidative stress, accepted by researchers at the Center for Disease Control CDC (Doyle *et al.,* 2002).

2.4.2 Effects on CD4 and CD8 T -cells

Human immunodeficiency virus (HIV) infections target CD4 white blood cells, and deplete them as HIV progresses. CD4 count, percentage, and CD4/CD8 ratio indicate the risk of complications and debilitating infections (American Association of Clinical Chemistry, 2012). They may be used to track the progression of HIV and evaluate

infection and disease. Evaluating CD4 counts combined with viral load testing is an effective monitoring process for the progression of HIV. Centers for Disease Control and Prevention (CDC) established; any person with HIV and with CD4 count below 200 or a CD4 percentage below 14% is considered to have AIDS (DHHS, 2009). A decreased CD4 count, in combination with higher numbers on a viral load test, has an increased risk of getting sick from opportunistic diseases (American Association of Clinical Chemistry, 2012).

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Public health guidelines state, any infected person that registers CD4 count below 200cells /mm^3 of blood, even when they have no symptoms should be started on preventive therapy,   regardless of whether they have any signs or symptoms (Conference reports, 2015). Acute ill-nesses, such as pneumonia, influenza, or herpes simplex virus infection, can cause the CD4 count to decline.

2.4.3 Viral load

Severity of a viral infection is measured by the amount of virus in a body fluid. Viral load is reported as copies of HIV and given in RNA copies per milliliter of blood plasma. This tells the amount of virus circulating in the blood. The higher the viral load value, the more viral elements present in tissues and in circulating blood and other body fluids. Tracking viral load enables the monitoring of therapy during chronic viral infections (Puren *et al.,* 2010).

Viral load also tells how quickly the disease will progress. A viral load greater than 100,000 copies/mL of blood within six months of sero conversion indicate a greater likelihood of developing AIDS within five years. High viral load in the blood is an

indicator of the likelihood of transmitting HIV to another (Patel *et al.,* 2010). Treatment guidelines recommend that anyone with a viral load greater than 100,000 copies/mL of blood should begin treatment (Cohen *et al.,*2010).

2.5 Conventional treatments against HIV and AIDS

When prophylaxis is given shortly after exposure, it reduces the risk of adverse infections (Fan *et al.,* 2005). The current interventions include use of highly active antiretroviral therapy (HAART), (DHHS, 2005). Since the introduction of protease inhibitor-based HAART in 1996 and made available, many HIV-infected individuals have benefited (Martinez et *al.,* 2006). The current antiretroviral agents belong to two main classes; these include two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI) (DHHS, 2005). Highly active antiretroviral therapies (HAART) stabilize patient's symptoms and viremia.

Highly Active antiretroviral agents are not for complete cure, they become resistant once treatment is stopped, and it would take a lifetime for HIV infection to be completely eliminated from the body tissues (Blankson *et al.,* 2002, Dybul *et al.,* 2000; Martinez *et al.,* 2000). Despite this many HIV-infected individuals have experienced improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed world (Chene *et al.,* 2003). The US treatment guidelines in 2006 projected an average life expectancy for an infected individual that started ARVS treatment when CD4 count was 350/µL to be 32.1 years (Schackman *et al.,* 2006).   In the absence of HAART,

HIV progressions to AIDS occur at between nine to ten years and the survival time after developing AIDS is only 9.2 months (Morgan *et al.,* 2002).

There are reasons that make HAART medication ineffective and most individuals fail to benefit, these include medication intolerance or side effects, infection with a drug-resistant strain of HIV, non-adherence and non-persistence with antiretroviral therapy (Becker *et al.,* 2002). The reasons for non-adherence and non-persistence with HAART are varied. They include psychosocial issues, such as poor access to medical care; inadequate social supports, psychiatric disease and drug abuse, these contribute to non-adherence. Complexity of these HAART regimens, whether due to pill number, dosing frequency, meal restrictions or other issues along with side effects, create intentional non-adherence adding to this problem (Kleeberger *et al.,* 2001, Nieuwkerk *et al.*, 2001). Other side effects include lipid and fat movements, insulin resistance, an increase in cardiovascular risks and birth defects (Montessori *et al.,* 2004, Saitoh *et al.,* 2005).

Kenya national guidelines state that treatment should be started when CD4 count falls to 350 or CD4 percentage falls below 15% (Wood *et al.,* 2003, Wang *et al.,* 2004). World health organization (WHO, 2006) states patients with stage III or IV disease should be offered treatment. There are challenges facing most of the infected individuals of the world, they do not access treatments because HAART are expensive (Ferrantelli *et al.,* 2004). Research should be conducted to make treatments available for many infected individuals as well as reduce toxicities and side effects of current drugs, improve adherence, and to determine the best sequence of regimens to manage drug resistance.

2.5.1 Herbal Management of HIV and AIDS

It is considered that there are some ten stages in the replication of the HIV virus that could be targeted in the search for effective drugs. One such stage of critical importance is the reverse transcription step mediated by the enzyme RT and many compounds have now been shown to have HIV- RT inhibitory properties. Few of the compounds showing activity in the initial screens reach the stage of clinical testing, mostly being of low potency, too cytotoxic or, as with the tannins and flavonoids, and being non- specific in their action (McKee *et al.,* 1997).

Bell and colleagues, while working on possible pesticidal nonprotein amino acids, discovered the seeds of Castanospermum australe (leguminosae), an alkaloid of the tetrahydroxyindolizidine group (Mathee *et al.,* 1999). Castanospermine was found to exert its biological effects on insect larvae by inhibiting the carbohydrase enzymes that are essential for the elaboration of the oligosaccharide side chains on glycoproteins,. This led to the testing of the alkaloid against HIV on the basis that the compound inhibits α-glucosidase I and II, which control the formation of glycoprotein in the viral coat, then without the essential envelope structure the virus would be unable to infect healthy white blood cells. The antiviral tests proved positive and various 0-acyl derivatives have since been shown to be up to 20 times more active than castanospermine itself (Vlietinck *et al.,* 1998).

One significant discovery relates to a series of coumarines, the calanolides. In 1991 Zhou and others researched on the leaves and twigs of the tropical rainforest tree *Calophylum lanigerum* (Guttiferae). They isolated calanolide A and B that possessed anti HIV activity (Zhou *et al.,* 2000). The structures of the active (+) - calanolide A

and (-)- calanolide B were established. Another drug formerly used for its stimulant and antispasmodic properties, Sumbul root (Ferula sumbul, Umbelliferae). Studies on this plant isolated at least 27 coumarins that possessed anti-HIV activity. Sometimes, more than one phytochemical class with anti- HIV agents may occur in the same plant such as in Licorice e.g. coumarins, flavonoids and pentacyclic triterpenoids.

The roots of Tripterygium Wilfordii (Celastraceae) possess salaspermic acid, a pentacyclic triterpene that inhibits of HIV reverse transcriptase and resists HIV replication in Human Gonadotrophic (HG) lymphocyte cells (Zhou *et al.,* 2000). Tripterygium wilfordii plant is a toxic liane that is known for its pesticide properties and has been shown to possess a number of other biological actions.

There are several genus species of gourds (Trichosanthes, Cucurbitaceae) in Asia that contain a toxic protein trichosanthin (Mackee *et al.,* 1997). Preparations based on this compound appeared to have ribosome- inactivating properties and selectively kill cells infected with HIV. Although the roots of T. kirilowii have traditional medicinal uses in China, Tai-wan and Korea a number of AIDS sufferers in the US who took this preparation on their own initiative suffered side effects including death, illustrating the necessity for adequate testing of such materials (Mackee *et al.,* 1997).

Most of the African medicinal plants and natural products have yielded compounds with anti- HIV activity, these could provide treatments for AIDS and develop biotechnological understanding of plants with antiviral activity.

About 120 plants are reported to possess antiviral activity, these include Diospyros, Spondias, terminalia species, while others have immunomodulation properties such as the Aloe and Zingiber species (Hostettmann, 1991). A broad range of anti-HIV activity was also discovered in Ancistrocladaceae (Ancistrocladus korupensis) that contained alkaloids castanospermine, michelin A-C and B (Mathee *et al.,* 1999).

A Clinical trial (1991) discovered Glycyrrhiza glabra that possessed anthraquinones hypericin with antiviral activity and also Flavonoids i.e. Glycyrrhizoflavone, Isolicoflavonol and licochalcone that showed similar action to liquorice coumarines. Other species of Glycyrrhiza glabra possessed Pentacyclic triterpenoids, Glycyrrhin, and Coumarins Calanolides A and B, Lycopyranocoumari. These Glycycoumarin showed inhibition of cell blastism formation in HIV infected cell cultures. Asymptomatic HIV carriers experienced delayed development of AIDS symptoms (Mathee *et al.,* 1999).

Gossypium species seeds possessed Gossypol with Dimeric sesquiterpenes that showed inhibitory effect on HIV replication (Vlietinck *et al.,* 1998). Ipomea cairica (Convolvulaceae) possess Lignans (-) – Trachelogenin, these suppressed the integration of proviral DNA into cellular genome (Vlietinck *et al.,* 1998).

Various Chinese herbal medicines including Viola yedoensis (Violaceae); Prunella vulgaris (Labiatae); Alternanthera philoxeroids (Amaranthaceae), these possess Sulphated polysaccharides that had invitro inhibitory activity against HIV (Vlietink *et al.,* 1998).

Commercial Tannic acid possessed tannins tetragallolquinic acids that had HIV reverse transcriptase inhibitors (Mathee *et al.,* 1999, Vlietinck *et al.,* 1998).

Abrus precatorius (Leguminosae) well-known to African and Asian societies and widely used to treat a variety of medical complaints, possess a number of indole alkaloids. The seeds contain bases isolated with two new saponins, shown to have HIV protease and HIV- induced cytopathogenicity (Aslam and Shaw, 1991). The seeds contain the glycoprotein abrin, which resembles ricin. It is not particularly poisonous when taken orally but is extremely toxic when injected.

2.5.2 Plant sources with Anti-oxidant effect

Aloe species: such as Aloe Vera contain C-glycosides and resins; Acemanan, Aloin A and B that are potent immune stimulating compounds beneficial in AIDS therapy, (Manitto *et al.,* 1990, Rauwald *et al.,* 1989). Astralagus (huang qi) (Tragacanth): gum yielding mucilaginous plants with polysaccharides tragacanth and bassorin that possess immune boosting properties and broad-spectrum virastatic activity; and have antiviral activity against a wide range of viruses (Manitto *et al.,* 1990). Blessed thistle (Cnicus benedictus): contains silimarins, which are compounds that have anti –HIV activity. Echinaceae (coneflower): These are immune stimulating herbs; E.angustifoli, E. pallida, and E. purpurea possess caffeic acid, chicoric acid and echinacin. They have antiviral properties similar to interferon, the body's own antiviral compound and increase properdin that help infection fighting white blood cells to reach the infected areas of the body (Gonda *et al.,* 1992).

Elderberry (Sambucus nigra) has reputation as remedy for viral infections being studied for activity against HIV (Gonda *et al.,* 1992). Evening primrose (Oenother biennis) possess oil rich in gammalinoleic acid (GLA) omega 3 fatty acids. Studies carried out in Tanzania showed life expectancy of people with positive HIV was improved (Wagner, 1999). Garlic (Allium sativum); possess phytochemicals Ajoene, Allicin; disulphides such as allicin and allylpropyldisulphide, these are effective against several opportunistic infections of AIDS including herpes and pneumonia, and inhibit the spread of HIV within the body (Gonda *et al.,* 1992). Sweat marjoram (Labiatae): Origano vulgare and Prunella vulgaris possess volatile oils that inhibit free oxygen molecules and have antioxidant effect that maintain body immune functions, (Gonda *et al.,* 1992). Turmeric: possess polysaccharides curcumin, dihydrocurcumin, the new cid glycans designated ukonan A, B, C which have immunologic activity with reticulo-endothelial system potentiating properties (Gonda *et al.,* 1992).

There is general acceptance that most herbs rely for their effects on a variety of constituents and there occurs synergy within and between them. It is not well documented whether they act in a truly synergistic way, or by additive effects, yet it is important to develop methods of standardization and to further our knowledge of mechanisms of action (Izaddoost and Robinson, 1987). Clinical evaluation is also more difficult without knowing the extent to which synergism occurs within the herbal preparation and it should further be investigated for all these reasons. Meanwhile evidence is accumulating to show that synergism does occur in extracts and mixtures and that we should continue to use our whole extracts with confidence, rather than assuming that a single chemical entity is responsible and which should be extracted and used alone (Duke and Bogenschutz, 1999).

Synergy as evidenced in herbal medicine, may lead to improved products with increased efficacy, reduced toxicity, and in the case of antibiotic action, less likely to lead to microbial resistance. It is vital to ensure that extracts are standardized properly for their active principles known at the time and that any known synergistic interactions are taken into account (Izaddoost and Robinson, 1987, Wagner, 1999).

Mixtures of chemotherapeutic agents are now being used to treat AIDS and cancer and historically many people, even if contrary to medical advice, have always instinctively practiced polypharmacy, whether herbal or not (Wagner, 1999). It is expected that in future, many untested natural products isolated from African plants will be put through a variety of biological tests (Hostettmann, 1991).

2.6 Tissue Function Assessments

The liver stores glycogen, processes fats and proteins, digests foods and processes proteins that are essential for blood clotting. Many medicines that are taken into the body are detoxified in the liver thereby removing poisons and toxins from the body system (Lee and Mary, 2009).

2.6.1 Liver Function Tests

The liver has its normal functions processing Total protein (aPTT), albumin, bilirubin, alkaline phosphate and alanine aminotransferase/ aspartate aminotransferase and other products (Lee and Mary, 2009). (AST/ALT (SGOT/SGPT) are biomarkers of liver injury in a patient who has an intact liver function. Diseases that affect liver functioning are of crucial importance (McClatchy and Kenneth, 2002, Mengel *et al.*, 2005).

2.6.2 Kidney Function Tests

Urea nitrogen forms when protein in the body breaks down (Pagana and Pagana, 2010). Many drugs affect blood urea nitrogen (BUN) levels, in turn affecting how the kidney functions. Kidney function tests evaluate how well the kidneys are working. The normal result is generally 6 - 20 mg/dL (Fischbach and Dunning, 2009). When the readings record higher values it is indicative of possible Congestive heart failure, excessive protein in the gastrointestinal tract, gastrointestinal bleeding, heart attack, kidney disease and acute tubular necrosis, kidney failure, shock, or urinary tract obstruction. When the readings are lower-than-normal levels may be due to liver failure, low protein diet, malnutrition, and over-hydration (Chernecky and Berger, 2008, Pagana and Pagana, 2010). Measuring creatinine level is necessary in order to monitor kidney functioning that indicates how well the kidneys are working; a high creatinine level means the kidneys are not working properly (Chernecky and Berger 2008).

If kidney function is abnormal, creatinine levels will increase in the blood. A BUN-to-creatinine ratio guides clinicians to check for problems, such as dehydration (Pagana and Pagana, 2010), because creatinine is entirely removed from the body by the kidneys (Fischbach and Dunning, 2009). Higher than normal levels indicate acute tubular necrosis, dehydration, diabetic nephropathy, eclampsia, glomerulonephritis, kidney failure, muscular dystrophy, preeclampsia, pyelonephritis, reduced kidney blood flow (shock, and congestive heart failure), or urinary tract obstruction. While lower than normal levels may be due to late stage muscular dystrophy or myasthenia gravis (Fischbach and Dunning, 2009). Females usually have a lower creatinine than males, because they usually have less muscle mass (Pagana and Pagana, 2010). A

normal result is 0.7 to 1.3 mg/dL for men and 0.6 to 1.1 mg/dL for women (Chernecky and Berger, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This study was carried out on People Living with HIV/AIDS (PLWHA), who were attending clinic at Pwani herbs and were on treatment using Virad and IMB. Their physiological and immunological parameters were measured; CD4/CD8 counts, Viral Load and toxicity on Patients kidney and liver were assessed. This study adopted the statistical procedure by Whitaker *et al* (2006). The self-controlled case series (SCCS) method was an alternative study method for investigating the association between a transient exposure and an adverse event. Patients were purposively recruited and screened at inception, then at every two months during the course of this study to monitor their evolutions. Every sample was its own control from baseline.

3.2 Study area

This study was done in Coast province of Kenya in Mombasa County. Medicinal formulations Virad and IMB were dispensed to HIV/AIDS patients at Pwani herbs clinic Mombasa. Patients were recruited for this study as they attended the clinic. Laboratory analyses were done at Bomu Medical Clinic Laboratories in Mombasa.

3.3 Study Population

This study obtained 188 patients. Adult males (64) and female (124) confirmed HIV positive living with HIV/AIDS in Mombasa County. They were recruited as they came to the clinic. These were the patients that had preferred to treat their HIV/ AIDS statuses with Virad and IMB.

3.3.1 Inclusion Criteria

Male and Female Patients with Human Immunodeficiency virus (HIV) aged 18 years up to 60 years, that gave Informed Consent and had preferred to take herbal treatments.

3.3.2 Exclusion Criteria

Patients who did not wish to be included in this study. Patients that were at late stage HIV and AIDS disease. Patients with co infections like cancers, diabetes and Tuberculosis.

3.4 Sample size

The prevalence of HIV in coast province stood at 7.9% (KAIS, 2007). Conditioning on the number of events ni observed for individual i during the observation period, the log likelihood is multinomial: The number of events required to detect a given relative incidence in general depends on age effects and the age-distribution of the exposure. The following expression for the sample size applies when age effects can be ignored.

Let β denote the true log relative incidence associated with exposure, r the ratio of the risk period to the observation period, and write π

$$\pi = \frac{re^{\beta}}{re^{\beta}+1-r}.$$

Assume that a proportion p of individuals in the population are exposed during the observation period (note that p relates to the total population, not just cases), and let α denote the significance level and $1-y$ the power required, $z_{a/2}$ and z_y the $100(1-\frac{\alpha}{2})$ and $100(1-y)$ percentiles of the standard normal distribution. Then the number of

events required achieving the stated power to reject the null hypothesis $\beta = 0$ in a 2-tailed test at the stated significance level is: -

$$n = \frac{C}{A}\left(z_{a/2} + z_y\sqrt{B}\right)^2 \qquad Where: - \quad A = 2\{\pi\beta - \log(re^\beta + 1 - r\},$$

$$B = \frac{\beta^2\pi(1 - \pi),}{A}$$

$$C = 1 + \frac{1 - p}{p(re^\beta + 1 - r)}$$

In the formulae outlined above, the:

- Significance level (a) to be used is 5% which corresponds to an inverse

  normal of 1.96.

- Power to detect meaningful effects = 80% corresponding to an inverse normal

  of 0.84.

- p (HIV prevalence in Mombasa= 7.9%, ref).

- $r = \frac{1}{11}$, where risk period = 1 month and observation period = 11 months.

- β is used to mean the incidence rate ratio of experiencing mortality during the

  risk period as also defined in Musonda (2005). This is an estimated average

  from studies reporting early mortalities in Africa. Table xx summarises the

  proportions of early mortalities by study:

Assuming the patients would be enrolled and put on medication immediately after

enrolment such that no mortality would occur (100% survival), then the anticipated

incidence rate ratio would be $(100\%/62.6\%) = 1.59$.

Therefore, using the parameter values as defined above:

$$\pi = \frac{re^\beta}{re^\beta + 1 - r} = \frac{\frac{1}{11}e^{1.59}}{\frac{1}{11}e^{1.59} + 1 - \frac{1}{11}} = 0.33$$

$$A = 2\left\{\pi\beta - \log(re^\beta + 1 - r)\right\}$$

$$= 2\left\{0.33 \times 1.59 - \log(\frac{1}{11}e^{1.59} + 1 - \frac{1}{11})\right\} = 0.44$$

$$B = \frac{\beta^2 \pi(1-\pi)}{A} = \frac{1.59^2 \times 0.33 \times (1-0.33)}{1.27} = 1.27$$

$$C = 1 + \frac{1-p}{p(re^\beta + 1 - r)} = 1 + \frac{1 - 0.079}{0.079 \times (\frac{1}{11}e^{1.59} + 1 - \frac{1}{11})} = 9.58$$

Hence the derived sample size is approximately:

$$n = \frac{C}{A}\left(z_{\alpha/2} + z_y\sqrt{B}\right)^2 = \frac{9.58}{0.44}\left(1.96 + 0.84 \times \sqrt{1.27}\right)^2 \approx 182$$

3.5 Sampling technique

This was a purposive random sampling method, where study participants were recruited as they attended clinic for treatment. Patients were registered in individual files with study numbers allocated to each. They were weighed and their heights were measured at the clinic. The Body Mass Index (BMI) was then computed for each patient.

3.5.1 Anthropometric assessments

Anthropometric screening was carried out through measurements of weight and height. The changes in body mass were recorded. The Body Mass Index (BMI) was calculated from weight in kilograms divided by square of the height in meters. This

test was to evaluate physiological parameters, provide nutritional status and for assessing the impact of illness on the study subjects.

3.5.2 Blood Collection

The blood sample was collected from each study participant using a 10ml syringe and dispensed into two tubes of 2ml with EDTA anti-coagulant. The samples were labeled appropriately, packaged then transported to Bomu Medical clinic laboratories for analysess. This was repeated every two months until the end of the study period.

3.5.3 Hematological assessments

This was carried out by determining the total white blood cell count (T wbc), differential counts, hemoglobin concentrations (HB) and Red blood cell count (RBC) Two 2mls of whole blood was sampled for the complete blood count, 2mls of venous blood in anti-coagulant was used for these tests. This was carried out with the use of Beckman Coulter Flow cytometry, using the procedure of Ormerod M. (2000). Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scatter gram plots the cells based upon the measurements of these three parameters.

3.5.3.1 Determination of Hematological parameters by Coulter Counter

Ten microlitters (10μl) Isoton II Diluent was filled with Coulter solution and Primed. Another 10μl Isoton II Diluent added with 50μl of the test cell suspension for

counting was also primed. After completion of priming, the remaining Isoton II Diluent was discarded and counting done. This counting procedure took ~15 seconds. Readings were taken when the numbers of counted particles were between 500–5000, corresponded to cell concentrations between $2 \times 10^5 - 2 \times 10^6$ per ml (Ormerod M, 2000).

3.5.3.2 Determination of Erythrocyte sedimentation Rate

This was carried out following the procedure by Dhurba Giri (2015) (Procedure 14-8, 2010). This method used Wintrobe's tube, a narrow glass tube closed at the lower end only. The Wintrobe's tube has a length of 11 cm and internal diameter of 2.5 mm. It contained 0.7-1 ml of blood. The lower 10 cm are in cm and mm. The marking was 0 at the top and 10 at the bottom for ESR. The ant coagulated blood was mixed thoroughly. By using Pasteur pipette, the Wintrobe's tube was filled up to '0' mark avoiding bubbles in the blood. The tube was placed vertically in ESR stand and left undisturbed for 1 hour. At the end of 1 hour, the results were read.

3.5.4 Determinations of CD4/CD8

This was carried out following a procedure by Wallace 2003. Venous Blood samples were collected in EDTA vacutainer tubes for the enumeration of T-Cells CD4/CD8, (Wallace, 2003). Whole blood was added to the fluorochrome labeled reagent that bound specifically to leukocyte surface antigens. The laser beam scattered and fluoresce signals were detected by the instrument and provided information about the cell's size, internal complexity and relative fluorescence intensity. In this Process, 0.5mls of blood was collected into EDTA collection tube; each patient serum was labeled with an identification number alongside the control sample, and stored at

room temperature (20-25 C). These were later stained within 48 hours and analyzed within 24 hours. Into each of the labeled tubes, was added 20uL of multitest reagent (CD3/CD8/CD45/CD4), and test was repeated for the multitest reagent (CD3/CD16CD56/CD45/CD19). 50uL of anti-coagulated whole blood were added into the tube, caped and vortexed to mix gently. It was incubated for 15 minutes in dark at room temperature (20-25C) and ready to be analyzed on the flow cytometer.

3.5.5 Viral load Assessment

This was carried out by use of Polymerase Chain Reaction (PCR) following the procedure by Todd (2012). Briefly, A 96 well plate was placed into an ice bucket as a holder for the 0.2 ml thin walled PCR tubes. PCR reagents were added into the cold 0.2 ml thin walled PCR tubes to prevent nuclease activity and nonspecific priming. These PCR reagents included (Sterile Water, 10X PCR buffer, dNTPs, $MgCl_2$, primers, and template DNA). These were added following specific order into a 0.2 ml thin walled PCR tube (The Master Mix was set as a control in a 1.8 ml microcentrifuge tube). All the reagents except template DNA for a negative control were added into separate 0.2 ml thin walled PCR tubes. In addition, another reaction containing a positive control using template DNA and or primers previously known to amplify under the same conditions as the experimental PCR tubes. Taq DNA polymerase required gentle mixing of the PCR reagents by pipetting up and down at least 20 times (Taq DNA polymerase is typically stored in 50% glycerol solution and for complete dispersal in the reaction mix). The micropipette was set to about half the reaction volume of the master mix during mixing, and care was taken to avoid introducing bubbles. Caps were put on the 0.2 ml thin walled PCR tubes and placed into the thermal cycler. Once the lid to the thermal cycler was firmly closed the

process started. When the process finished, the 0.2 ml thin walled PCR tubes were removed and stored at 4 °C. The polymerase chain reaction (PCR) products would be detected by loading aliquots of each reaction into wells of an agarose gel then staining DNA that had migrated into the gel following electrophoresis with ethidium bromide. If a PCR product were present, the ethidium bromide would intercalate between the bases of the DNA strands, allowing bands to be visualized with a UV illuminator (Todd, 2012).

3.6 Liver function tests (LFTs or LFs)

The following tests were carried out to determine the integrity of the liver. The parameters to analyze were Total protein, albumin, bilirubin, alkaline phosphate and alanine aminotransferase (ALT) /aspartate aminotransferase (AST). These tests were done following protocols by STAT FAX, (2017) (Technology STAT FAX 4500 Chemistry Analyzer). The principal for this process: AST –aspartate amino transferase or SGOT –serum oxalate acetate transferase catalyses the transfer of an amino acid group from an alpha amino ./acid to an alpha keto acid to form an alpha amino acid /L- glutamate and an alpha keto acid/ oxalateacetate.. Alternatively ALT – alanine Amino transfarese or SGPT – serum glutamate pyruvate transaminase catalyses the transfers of an amino acid group from an alpha amino acid/ alanine to an alpha keto acid to form an alpha amino acid/ L- glutamate and alpha keto acid/ pyruvate. The equipment required for Start fax chemistry analyser, micropippetes, tips, cuvettes, test tubes, rack and Reagents Buffer and Substrate (STAT FAX, 2017).

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3.6. 1 Specimen processing Procedure

Substrate Stat: A cuvette was labeled as test, and 1 ml of buffer was put in. 100ul of serum was added and mixed. This was incubated at 37^0C for 5 min. 250ul of substrate was added and mixed and read at 340nm immediately against 1ml reagent blank.

3.6.2 Procedure 2 Sample Start

 Two milliliters of substrate was added into 8 ml buffer and mixed to make the working reagent. Two cuvvetes were labeled as test and blank. One milliliter of the working reagent was put into each of the 2 cuvettes. One hundred microliters of serum was added and mixed and read against the reagent blank at 340nm immediately.

3.6. 3 Procedure for Plasma bilirubin determination

This was done following procedures by Stat fax (Beckman Coulter Synchron Clinical Systems Chemistry Information Manual, 2007). The reagents required TBR, TNR, DBR, and DNR as provided by the manufacturer.

3.6. 3.1 Determination of Total Bilirubin

Two cuvettes were labeled as blank and test. One microliter of TBR was put in all the cuvettes. One drop or 40 µl   of TNR was added to the entire cuvettes labeled test and mix. This was left to stand at room temperature for 5 min and then 100ul of test serum was added to both cuvettes and mixed. This was incubated at room temperature for 10 − 30 min. This was read and results recorded at 546nm.

3.6. 3.2 Determination of Direct Bilirubin

Two cuvettes were labeled as blank and test. Into the cuvettes 1 ml DBR was put added and a drop of DNR added to the cuvettes labeled test followed by mixing. This was left to stand for 2 min at room temperature after which 100ul of test serum was added to both cuvettes followed by mixing. The cuvettes were incubated at room temperature for exactly 5 min and read then at 546nm.

3.6. 3.3 Determination of Plasma Albumin

Bromocresol green forms with albumin in citrate buffer a colored complex. The absorbance of this complex is proportional to the albumin concentration in the sample. The START FAX (Laboratory Procedure Manual, 2008) protocol was used to measure Albumin. Three cuvettes were labeled as Blank, Standard and Test. One milliliter of the color reagent was put into each one of the 3 cuvettes. One hundred microliters of standard was added to the tube labeled standard and mixing followed. One hundred milliliters of the serum sample was added to the tube labeled test and was mixed. These were incubated at room temp for 5 min. Then the Standard and Test were read against reagent blank at 540nm filter.

3.7 Kidney Function Tests

These tests were done to evaluate the functions of the kidney and to monitor any possible danger following uptake of the herbal medicines.

3.7.1 Determination of Blood Urea Nitrogen BUN

The determination of was done by analyzing Blood Urea Nitrogen (BUN) that forms when protein breaks down. Blood samples are collected through Venipuncture and

the amount of urea nitrogen in the blood is measured (Logan McLennan, 2010). The procedure: 2 ml of the patient sample was put in to 60mls (pyrex) glass and stoppered. Another 2 ml of the reagent Blank bottle was set as control. 8.5 ml of distilled water was added to each bottle and the solutions were well mixed. 8.5 ml of diacetyl monoxime solution was added to both bottles with constant rotating of the bottle to mix. After thorough mixing; 8.5 ml of arsenic sulfuric acid solution was added and placed in a temperature bath of 100 ^{0}C for exactly 20 minutes. The bottles were left to cool in air for 15 minutes, and further cooled in 25^{0}C water bath for 15 minutes. The absorbance of the yellow solution read on a spectrophotometer, against the reagent Blank at 475 mm (Coleman Universal spectrophotometer, 13 mm square cells was used). Results were recorded in milligrams per 100 milliliters BUN from a graph.

3.7.2 Determination of plasma Creatinine

This was carried out following procedure by John (2015). Blood was collected without using any anticoagulant such as heparin or citrate. To obtain serum plasma the blood was centrifuged at 700 – 1000 x g for 10min at 4°C; the yellow plasma layer was pipetted off without disturbing the white buffy layer. Plasma was kept frozen at – 80°C, in this procedure; plates were designated (Tube A-H) and 15µl of creatinine standard was added into each designated well. 15 µl of Test sample was added into at least two of the wells on the plate followed by 100 µl of creatinine reaction buffer added to all the wells on the plate. 100µl of creatinine color reagent was added to all the wells that were used and timing of reaction was immediately started. Readings were recorded at one minute and at seven minutes, the absorbance 490 -500nm. Standard plasma range for creatinine concentrations is 0.5 – 1.5mg/dl.

3.8 Phytochemical analysis

Pwani Herbs provided raw materials for analysis of the herbal formulations Virad and IMB. These were powdered mixtures stored in air tight bags for analysis . Each package was a mixture of up to six different herbs that made the formulation for either VIRAD or IMB.

3.8.1 Digestion of herbal mixtures for analyses (Wet oxidation)

Each package sample was brought to solution by wet oxidation. In each case, samples weighing 1g were transferred into 100ml Pyrex beakers and to each beaker; 10ml of concentrated nitric acid was added, and then allowed to soak thoroughly. 10ml milliliters of per chloric acid (60% HCL04) was added to each beaker, and then warmed on a hot plate slowly, until frothing ceased. Heating was then intensified until all nitric acid was evaporated. When charring occurred, the mixture was cooled, 10ml of nitric acid was added and heating continued until white fumes of per chloric acid were observed. The final solution was then quantitatively transferred into 100ml volumetric flask by filtering through Whitman filter paper No. 42. The solutions were then made to volume and shaken well to allow proper mixing before the contents were transferred to plastic sample bottles. For each sample, digestion was done in duplicate. The samples were analyzed immediately whenever possible, otherwise kept in refrigerator at -200C waiting analyses.

3.8.2 Laboratory scale extraction

Four grams of Herbal materials were placed on filter paper and inserted into the extractor (Quality control methods, 1998). Solvent was placed in the flask and brought to boiling point. Its vapors passed into the upper part of the drug and then

condensed and dropped back on to the drug. During its percolation, it extracted the soluble constituents. When the level of the extracts reached the top level of syphon tube, the whole of the percolates syphoned over into the flask. The process was continued until the drug was completely extracted and the extract in the flask was then processed.

3.8.3 Hot extraction

About 4.0 grams of powdered material was placed in a conical flask, and 100 ml of water was added. The total weight of the herbal sample including the flask was recorded. This was well shaken and left to stand for 1 hour. A reflux condenser was attached to the flask and boiled for 1 hour, cooled and weighed. It was well shaken and filtered through a dry filter. The filtrate (25 ml) was transferred to a flat-bottomed dish, evaporated at 105 °C for 6 hours to dryness and cooled in a desiccator for 30 minutes, and weighed. The extractable matter was calculated in mg per g of air-dried material (Quality control methods, 1998)

3.8.4 Determination of extractable matter by Gas chromatography

To process this Technical Procedure for Gas Chromatography Version 7 the method of Agilent 6890 Gas Chromatograph (Raleigh Laboratory) was employed (Caroli *et al*., 2013). Briefly – a 10μL syringe injection with the auto sampler was used, from each wash bottle eight solvent washes were performed. 1.0μL sample was injected with a fast plunger speed, three sample washes, and three sample pumps. – Trace Unit Effective Date: 12/11/2015 Issued by Physical Evidence Section Forensic Scientist Manager Page 3 of 8 All copies of this document are uncontrolled when printed. 5.3.1.2 Inlet – Run in split mode at 240 °C with a 50:1 split ratio. Carrier gas –

Hydrogen. Oven –Run a temperature program starting at 80.0°C for 0.00 minutes. Then ramp at 20.0 °C per minute until 280°C is obtained. Hold at 280°C for 2.00 minutes. Column – Use a HP-5MS or DB -5MS column which is 0.25mm in diameter that is approximately 30m long with a 0.25μm film thickness. The column shall be kept at a constant flow of 1.6 mL/min. Flame Ionization Detector –The detector temperature shall be set at 300°C. The flow rate for hydrogen is 30.0 mL/min, for air is 400.0 mL/min, and the make-up flow of nitrogen is 25.0 mL/min.

3.8.5 Qualitative Phytochemical screening

To test for the presence of Tannins, the methods by Jigna *et al.* 2007 was employed. In this process, 2 ml of aqueous extract was added 2 ml of 5% $FeCl_3$. Formation of yellow brown precipitate indicates the presence of tannins. To test for the presence of Alkaloids, the methods by Oguyemi (1979) were employed: 1 gram of powder was extracted with 10ml methanol for 15 min on water bath. The mixture was filtered and filtrate was evaporated to 2ml of methanolic filtrate. 1.5 ml of 1% HCl was added to 2 ml methanolic filtrate, and heated in water bath, and 6 drops of Mayors reagents/Wagner's reagent/ Dragendroff reagent was added. Formation of orange precipitate indicates the presence of alkaloids.

To test for the presence of Saponins, the methods by Sofowora (1993) were employed: 2 grams powdered drug in aqueous solution was extracted for 15 min and subjected to frothing test; persistence of Frothing indicates presence of saponins. Latter the froth was mixed with few drops of olive oil and formation of emulsion indicates the presence of saponins.

To test for the presence of Cardiac glycosides the methods by Trease and Evans (1989) were employed: 1 gram powdered drug was extracted with 10ml methanol on water bath for 15 min. This was filtered and filtrate was evaporated to 2ml of Methanolic filtrate. 1 ml of glacial acetic acid and 1-2 drops of $FeCl_3$ were added to the 2 ml Methanolic filtrate, followed by 1 ml of concentrated H_2SO_4. Appearance of brown ring at the interface indicates the presence of cardiac glycosides. A violet ring may also appear below the brown ring (Trease and Evans, 1989). To test for the presence of Terpenes, the methods by Harborne (1973) were employed: 5 ml of chloroform was added 2 ml of aqueous extract, 2 ml of acetic anhydride and 2 ml of concentrated H_2SO_4 to form a layer. The formation of a reddish brown coloration at the interface indicated presence of terpenes.

To test for the presence of Flavonoids, the methods by Jigna *et al*. (2007) were employed: 2 grams plant material was extracted in 10 ml ethanol, and few drops of concentrated HCl was added to 2 ml of the filtrate, 0.5 g of zinc or magnesium turnings was added. The formation of magenta red or pink colors after 3 minutes indicated the presence of flavonoids. To test for the presence of Phenols, the methods by Martinez (2003) were employed: 1ml of 1%ferric chloride was added to 2mls of aqueous ethanol extract; formation of a blue green color indicated presence of phenols.

3.8.6 Quantitative phytochemical screening

To screen for quantitative examination of Alkaloids, the methods by Obadoni and Ochuko (2001) and Edeoga *et al.* (2005) were employed: 2.5 grams of the powder was extracted using 100 ml of 20% acetic acid in ethanol for 15 min in a water bath.

It was covered and left standing for 4 hours. After filtration the filtrate was evaporated and concentrated to 25 ml. Ammonium hydroxide was added to obtain precipitates. The precipitate was washed with dilute ammonium hydroxide and finally filtered. The filtrate was discarded and the pellet obtained was dried and weighed

The quantitative examination of Saponins, were done using the methods of Edeoga *et al.* (2005) and Okwu and Josiah (2006): 10 g of powder was mixed with 100 ml of 20% aqueous ethanol. The mixture was put on water bath shaker at 55° C for 4 hours. The extracts were concentrated to 40 ml over water bath at 90°C and 10 ml of diethyl ether was added. The aqueous layer was separated and ether layer was discarded, n-butanol was added to the aqueous layer and the whole mixture washed with 10 ml of 5% aqueous NaCl. The upper part was heated in water bath until evaporation. It was dried in oven to a constant weight.

The Phenolic acids were screened for quantitative examination using the methods of Malick and Singh (1980): 1 gram of powder was extracted in 10ml of 80% ethanol and supernatant was evaporated to dryness. It was then dissolved in 5mls of water. 0.1 to 1ml aliquots were put in test tubes and made to 3mls using distilled water. Into each tube 0.5 ml folin's reagent was added and 2mls of 20% Na_2CO_3 added respectively. The tubes were votexed and kept in boiling water for 1 minute. Readings were recorded at an absorbance of 650nm against the blank. Standard curve was prepared using 100mg% catechol.

To screen for the quantitative examination of Tannins, the methods by Graham (1992) were employed: 2g of plant powder was extracted thrice in 70% acetone. After

centrifuging the drugs mixture, the supernatant was removed. Different aliquots of the solutions were taken and final volume was adjusted to 3 ml by distilled water. The solutions were vortexed and mixed with 1 ml of 0.016M $K_2Fe(CN)_6$, followed by 1 ml of 0.02M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept for 15 min. 5 ml of stabilizer (3:1:1) ratio of water, H_3PO_4 and 1% gum arabic was added and revortexed. The absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001M gallic acid.

To screen for the quantitative examination of Carbohydrates, the methods by Krishnaveni *et al.* (1984) were employed: 0.5 grams of powder was extracted in 80% ethanol. The extract was dissolved in 10 ml water, and different aliquots were prepared. The final volume was made to 1 ml by distilled water. 5 ml of 96% of concentrated H_2SO_4 was added followed by shaking and incubation for 40 min at room temperature. 1 ml of 5% phenol was added to each cuvette and the absorbance was taken at 490nm. The standard curve was made using different concentrations of 25 mg% glucose.

To screen for the quantitative examination of Proteins, the methods by Lowry *et al.* (1951) were employed: 1gram of powder was extracted using 10 ml water added with few drops of triton X- 100. The supernatant was extracted in acetone and resultant pellets dissolved in 0.1 M, NaOH. Aliquots with 1ml distilled water. 5 ml of copper reagent was added to each tube, mixed well and incubated for 10 minutes. 1 ml of folin's reagent was added to the tubes and mixed. The tubes were incubated for 30 min at room temperature and the absorbance reading was taken at 700 nm. Standard curve was prepared using 50 mg % BSA.

To screen for the quantitative examination of Lipids, the methods by Ganai *et al.* (2005) were employed: 1gram of powder was dissolved in ether and mixed with stirring for 1 hour. The mixture was centrifuged, dried and dissolved in ethanol. Test tubes were labeled as follows; 0.1 ml alcohol as the blank, olive oil as the standard and test sample as the unknown, respectively. To each of the test tubes was added, 2 ml of concentrated H_2SO_4, 5 ml of phosphovanillin reagent and were mixed well, then incubated for 30 min. Absorbance was read at 540nm.

3.9 Determination of Mineral Composition of the plant materials

3.9.1 The Atomic absorption spectrometry (AAS)

For the determination of mineral compositions of plant materials, the methods by Hagen, (2005) was used to determine the quantity of specific heavy metals, especially of Magnesium, Chromium and Vanadium present in the plant extracts. Atomic Absorption Spectrophotometry (AAS) Model: 210VGP works on the principal that Atoms in the ground state absorb light of a specific wavelength, each particular atom radiates specific wavelength when the light passes through and enables each element to be determined (Hagen, 2005).

3.9.1.1 Analysis for Chromium, Vanadium and Magnesium

The wet digests of the plant materials were analyzed for Cr, V and Mg. The sample solutions for analysis of magnesium were prepared by withdrawing 1ml of the digested sample solution, into 100ml volumetric flasks. Five milliliters of lanthanum solution was added in each flask and the mixture diluted to volume using distilled deionized water. However, for analysis of Cr and V, the digested sample solutions were analyzed without further dilution. After setting the Atomic Absorption

spectroscopy (AAS) instrument to the right conditions for each element the respective standards and sample solutions were aspirated into the flame in turns to determine their respective absorbance. At least four standard solutions were aspirated between 6-10 samples to monitor the stability of the working conditions. Distilled deionized water was always flushed into the flame to re-establish the zero absorbance. For each element, the above procedure was done in duplicate for each sample. The mean absorbance for each sample solution and standard solutions were calculated and recorded. The concentration values obtained were corrected by multiplying with the respective dilution factors.

3.9.2 Preparation of Standard stock solutions for AAS

For the determination of the presence of Chromium and magnesium standard stock solutions of 1000 ppm for AAS were supplied by the manufacturers (Aldrich Chemical Co., Inc). 1.7852g of vanadium pent- oxide was dissolved in minimum amount of conc. sulphuric acid and heated to dissolve completely, then cooled. The solution was made to 1 liter in a volumetric flask with distilled deionized water. This gave 1000 ppm of the stock solution. Immediately after each standard solution was prepared, the flask was thoroughly shaken for mixing and the contents transferred into a clean plastic bottle and kept in a refrigerator.

3.9.2.1 Preparation of working standards solutions for use with AAS

Suitable aliquots of standard stock solutions of each element were taken in a series of 100ml volumetric flasks. The solutions were diluted to volume using distilled and de-ionized water, mixed thoroughly and transferred into plastic beakers. This procedure was done for each element when its analysis was due. During each analysis fresh working standards were prepared. For each element, working solutions were prepared within a given range where the relationship between the concentration and absorbance was linear. In case of magnesium, 2ml of 5% lanthanum solution was added to each series of working standards before diluting the standards to volume. In addition, standard blank reagents for Mg, V and Cr were prepared by adding all the used reagents, except the target element being determined.

3.9.3 Preparation of Lanthanum solution for use with AAS

Lanthanum solution (50 mg/ml) was prepared by dissolving 12.6263g of lanthanum chloride in distilled deionized water. The solution was diluted to volume using distilled deionized water in 250ml volumetric flask. After mixing thoroughly, the solution was kept in clean plastic bottle and used during the determination of magnesium in the plant materials.

3.9.3.1 Analyses of Trace elements by Total Reflections X-ray Fluorescence

For the analysis of trace elements the methods by Hagen (2005) were used. The Total Reflection X-Ray Fluorescence Analysis (TXRF) is an established technique for trace element analysis in various sample types. TXRF consists of an extremely low-angle x-ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd109 (half-life, $T1/2$ = 453 days and activity = 10mCi) was

incident on the sample that emits the characteristic X-rays. The fluorescence photons emitted from the surface atoms was characteristic of the elements present. To enhance binding 25 mg of cellulose was mixed with the ground plant material. Total Reflection X-Ray Fluorescence (TXRF) system was used to determine the content of manganese, iron, nickel, copper, zinc, arsenic, lead, potassium, titanium, vanadium, bromine, and calcium in the Virad and IMB herbal samples. The spectral data for analysis were collected using personal computer based Canberra S-100 multi-channel Analyzer (MCA). The acquisition time applied in the TXRF measurement was 1000 seconds. For data analysis, the X-ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS, 1992) that is based on the fundamental parameters method (FPM). The composition of Virad and IMB sample was extrapolated by its fluorescence X-ray intensity of each element. The results were expressed in parts per million (ppm).

3.10 Statistical Analysis

The statistical analysis package used was R version 3.0.2 software. The data obtained were not normally distributed and varied in structure. These would be best presented with medium values. Here, the linear structures of time (month) with AIC values of 8268.88 and 4791.43 were considered best fitting for weight and BMI linear mixed effects models. Both of the models were fitted with unstructured variance covariance structures. The need for random slopes was tested by the use of a mixture of chi squares which resulted in test statistic values of 2.52 and 3.01 for weight and BMI models which were less than 3.84.

First the Average and individual level changes for phenotypic, immunological, and hematological and biochemical parameters explored using trend graphs. (Raw score graphs). Secondly the Components of parameters were further analyzed using linear mixed effects model. (How independent variables affected the outcome of trends). Then the random intercepts and random slopes (R. intercepts started at different points. R. slopes acknowledge healing patterns) as each individual had a different changing pattern. Finally the mean structures for each of the outcomes were explored using polynomials (structure for the own trends) and unstructured variance (data followed their own structures) and for all the variables. Polynomials are used when the lines in the evolution graphs are nonlinear so quadratic and some of the trends are best described by quadratic trends. The polynomial with the least Akaike Information Criterion was considered the best for describing the mean structure (Model selection criterion).

3.11 Ethical Approval

Approval was obtained from the secretariat of Kenya Medical Research Institute National Ethics Review Committee, from reference letter Reference KEMRI/RES/7/3/1. Informed consent was obtained from patients who were willing to participate in the study. They were required to read the consent form and sign after understanding its contents.

CHAPTER FOUR

RESULTS

4.1 Study participants at baseline

A total of 188 patients were recruited into this study. During the follow up 23 out of 188 patients dropped out of the study; 3 died while 20 developed other complications such as tuberculosis and cancer. They were referred for specialized treatment at other conventional hospitals. Out of 23 patients that fell out, 14.8% had CD4 count below 150 and had developed other complications including tuberculosis, cancer, and severe diarrhea. Some were discouraged by skeptics of herbal medication. Two of the patients died from cancer. One of the patients developed severe migraine headache and died, while another became anxious following fluctuating CD4 counts and resolved to change to ARVs. Majority of those who fell out because of skeptics were females, while most males that discontinued treatment were due to TB infections (Table 4.1).

Table 4.1: HIV/AIDS participant's reasons for fallout from the study

Gender	CD4 levels of the HIV/AIDS patients							
	25-49	50-99	100-149	150-199	200-249	250-299	300-349	>350
M	2(1.1)	1(0.5)	0	0	2(1)	0	1(0.5)	0
F	5(2.6)	4(2.1)	4(2.1)	6(3.2)	3(1.6)	1(0.5)	0	1(0.5)
Total	7(3.7)	5(2.6)	4(2.1)	6(3.2)	5(2.6)	1(0.5)	1(0.5)	1(0.5)
Reasons	2, 3, 7	2, 3, 4	2, 5, 1	1	2, 1	1	6	5

Log: Numbers in Brackets are percentage expressions of the number of patients at that CD4 level. 1-skeptics of the study, 2-Tuberculosis, 3-Cancers, 4- Severe Diarrhea, 5- Self-Volition, 6- Migraine head ache, 7- Death

*Skeptics = Persons who oppose the use of herbal medicines.

4.1.1 Study participants by age, sex and CD4 counts

The age range for the participants of this study was 18 and 65years. The modal age for both male and female participants was 31 and 40 years; mean age of the participants was 37.22(SD 9.31) years with median age of 37 years. Females were double [124 (66%)] the number of males [64(34% males)]. There were two main pick frequencies, first was at 44.2% for ages (31 to 40 years) and the second was at 27.13% ages (41 to 50 years). Majority of the participants were aged above 31 years. There was significant association (χ^2 = 105.298; df = 4; P = 0.0001) between the age of HIV/AIDS patients and the gender (Table 4.2).

Table 4.2: HIV/AIDS participants by Age and gender

Sex	Age of HIV/AIDS patients					Total	p-value		
	<18	19 – 30	31 – 40	41- 50	>50	<18 to >50	χ^2	df	p
F (%)	4 (2.1)	33(17.6)	55(29.3)	24(12.8)	8 (4.2)	124(66)	105.1	5	0.001
M (%)	1 (0.5)	4 (2.2)	28(14.9)	27(14.4)	4 (2.1)	64 (34)			
Total	5 (2.6)	37(19.7)	83(44.1)	51(27.1)	12(6.4)	188(100)			

The numbers in brackets n % number of patients over the total 188 participants

Majority 142 (75.3%) of the patients had CD4 cells/ml below 350 cells/ml and presented with HIV related symptoms. They also had two main CD4 peak frequencies, the first was at 300 to 349 CD4 (25%), the second was at 200 to 249 CD4 (19.2%) stage. A total of 85 (32.9%) of female patients had CD4 count below 250cells/ml. While 39 (20.6%) of male patients had CD4 count below 400 cells /ml.

In total 172 (99%) of patients had CD4 count below 500 cells/ml and 14 (7.4%) of the patients had CD4 counts below 100 cells/ml and presenting with HIV/AIDS related complexities. The study showed that there was significant association ($\chi^2 = 105.298$; df = 4; P = 0.0001) between the sex of participants and the CD4 values (Table 4.3).

Table 4.3: HIV/AIDS participants by CD4 staging and gender

CD4	Gender				p-value	
	Female	Male	Total	χ^2	df	p
0-49	3(1.6)	3(1.6)	6(3.2)	19.149	1	0.001
50-99	7(3.7)	1(0.5)	8(4.2)			
100-149	13(6.9)	1(0.5)	14 (7.4)			
150-199	7(3.7)	0	7(3.7)			
200-249	32(17)	4(2.1)	36(19.1)			
250-299	23(12.2)	1(0.5)	24(12.7)			
300-349	30(16)	17(9)	47(25)			
350-399	4(2.1)	12(6.4)	16(8.5)			
400-449	2(1)	11(5.9)	13(6.9)			
450-499	1(0.5)	4(2.1)	5(2.6)			
500-549	1(0.5)	0	1(0.5)			
550-599	1(0.5)	0	1(0.5)			
>600	0	0	0			

4.2 Phytochemical compositions of Herbal drugs (Virad and IMB)

The components isolated in Virad and IMB were phenols, saponins, tannins, alkaloids, flavonoids, proteins and lipids, while cardiac glycosides were absent in both. The biochemical components alkaloids (2.3g %), flavonoids (2.7g %), and lipids (2.4g %) were present at highest concentrations in Virad and in IMB. Tannins and alkaloids were more in Virad compared to IMB. Flavonoids were more in IMB than in Virad. Phenols and saponins had the least concentrations in both Virad and IMB (Table 4.4).

Table 4.4: Phytochemical composition of Virad and IMB

Drugs	Phenols	Saponins	Tannins	Alkaloids	Flavonoids	Cardiac glycosides	Proteins	Lipids
Virad	0.08± 0.02	0.53± 0.00	0.32 ± 0.01	2.30 ± 0.01	2.17 ± 0.03	0	0.44± 0.03	2.44± 0.02
IMB	0.08± 0.02	0.56± 0.00	0.05± 0.00	1.53± 0.02	2.53 ± 0.00	0	0.54 ± 0.00	2.55± 0.02

Key: Bioactive Agents Quantity/100g of plant material (g %), Mean ± SD of triplicate determination on the basis of dry weight.

4.2.1 Mineral composition of Virad and IMB

All mineral components isolated in Virad and IMB were below the daily recommend allowance when compared with the total mineral consumed per day, except for chromium and vanadium. The total calcium taken was 17931µg while, magnesium consumed was 1365µg. Total iron consumed was 883ug/day, while zinc was 81.7 µg/day (RDA for zinc is $1.1 \ x10^{4}$). Total copper consumed was 107.5µg, and total Iron consumed was 8.83mg, (RDA, Iron = 15 mg). The hard metal Lead was also present and merits concern for its possible cumulative effect (Table 4.5).

Table 4.5: Mineral components of VIRAD and IMB and the quantity orally administered daily to each patient

Element	Mineral levels(µg/g)		Daily mineral intake (µg)		RDA (ug/day)
	VIRAD	IMB	VIRAD (1g/daily)	IMB (1.5g/daily)	
K	12922±103	12085±87	12922±103	18127.5±154.5	2×10^6
Ca	8861±71	6047±46	8861±71	9070.5±69	1×10^3
*Na	*475±4	*598±11	*475±4	*897±6	5.0×10^5
Ti	45.7±2.5	17.3±1.5	45.7±2.5	26.0±2.3	0.015
*P	*71±2	*63±2	*71±2	*94.5±3	7.0×10^5
V	6.0±1.6	5.8±1.1	6.0±1.6	8.7±1.7	0.064
*Cr	*89±23	*57±24	*89±23	*85.5±36	0.01
Mn	120±3	131±2	120±3	196.5±3.0	2.3×10^3
*Mg	*547±1	*545±1	*547±1	*817.5±1.5	150
Fe	361±4	348±4	361±4	522±6.0	8.0×10^3
Ni	BDL	BDL	BDL	BDL	
Cu	98.6±1.5	5.9±0.4	98.6±1.5	8.9±0.6	1.5×10^3
Zn	43.7±0.9	25.3±0.6	43.7±0.9	38.0±0.9	1.1×10^4
As	11.2±0.4	1.4±0.2	11.2±0.4	2.1±0.3	-
Br	13.6±0.4	9.6±0.3	13.6±0.4	14.4±0.5	0.08
Pb	2.8±3	6.7±0.4	2.8±3	10.1±0.6	-

Key: Results are expressed as Mean ± Standard Deviation (SD) for three replicates, Dosage of VIRAD is 1000mg once daily while IMB is 500mg three times a day, *Values with asterisks as superscript were determined using the AAS while those without were determined using TRXF. BDL is below detectable levels.

4.3 Changes in Weights and BMI for the study participants

The Empiric individuals had different weights and Body Mass Indices as they first came to the clinic for treatment at baseline. After they took Virad and IMB medications, weight increase was not significant (p − value >5%), but Body Mass Indices increased with time (0.43 Kg/m^2 per month), this pattern did not differ for different ages and gender. At baseline, males and females patients were underweight. As the Patients took medications, phenotypic changes were evident. Weight for male and female increased from with average median weight of 60.5 kg to 76.0 kg at end of the study. Body mass index (BMI) rose with average median 17.8 to 22.6 kg/m^2. Body Mass Index (BMI) of males increased from 17.9 to 22.5. Females also increased in average weight from 56kg to 74 kg and BMI from 17.8 to 22.7. There was increase in weight and Body Mass Index, weight increased by 65 kg (P= 0.05) and BMI by 17. This gain was observed at first 1st month to the 6th month before leveling out, then weight decline between months 11th to month 12th. The Body Mass Index for both male and female increased steadily from first month to the end of the study (Figure 4.1a, 4.1b, Table 4.6 Appendix).
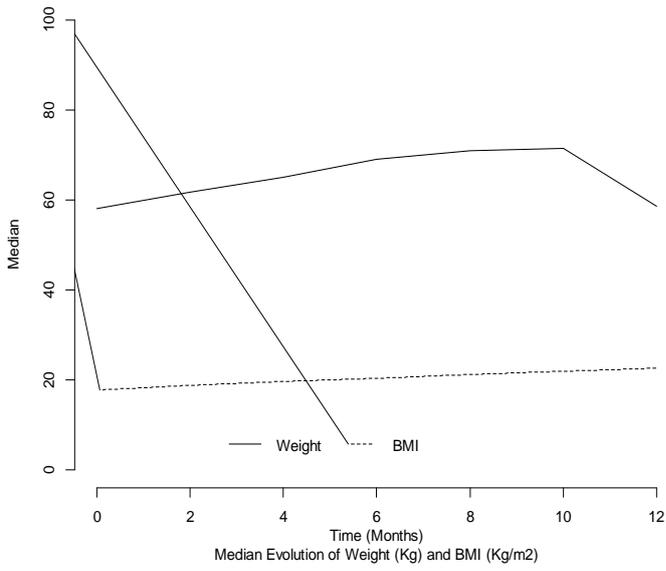
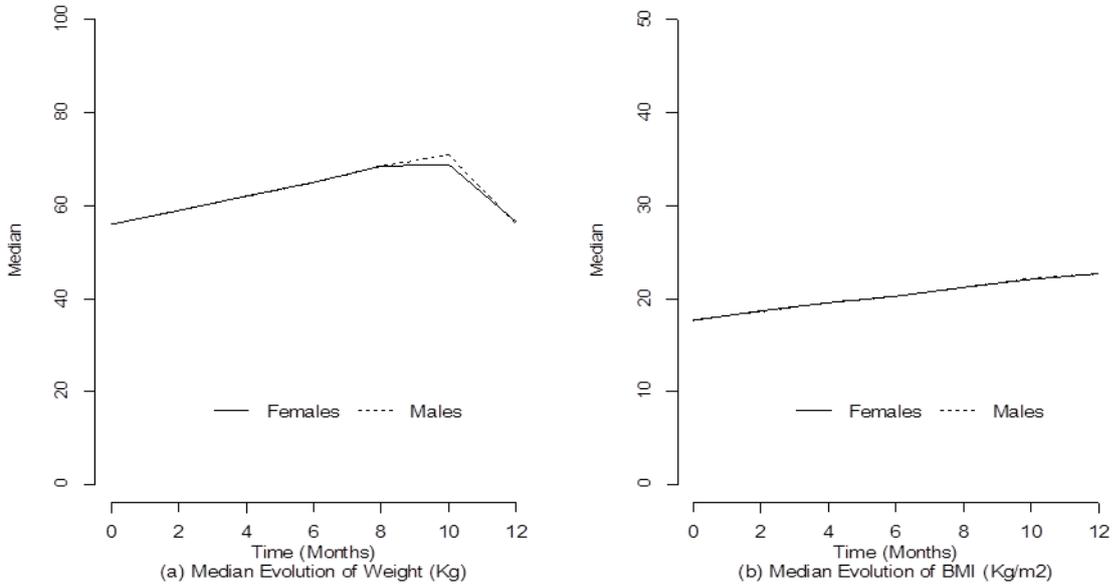**Figure 4.1a: Evolution changes in median Weight & BMI for participants**

Figure 4.1b: Evolution changes for weight and BMI of females and males

4.4 Effects of Virad and IMB treatment on Hematological Parameters

The haematological parameters; Haemoglobin (Hb), Red Blood cells (RBC) and White Blood cells (WBC) increased by 0.27 g/dl, 0.11 x 10^{12}/L and 0.23 x 10^9/L respectively each month. While Erythrocyte Sedimentation Rate (ESR) fell by 3.13 mm/ hr. Hemoglobin levels for study increased from baseline 11.1 g/dL to 14.4g/dL. The normal hemoglobin concentration for males is 10.6 to 15.6 g/dL, and that of females is 8.1 to 14.2 g/dL. The hemoglobin of male and female increased from baseline 10.1g/dL to 13.2g/dL with a difference of 3.1g/dL. The graph had gradual increase between months zero to 4th month, then by a sharp raise at month 4th and 6th. From baseline, Red blood cells (RBC) improved from 4.6 x 10^6 to 5.9 x 10^6cells/µl by RBCs ($p<0.05$). The normal for males is 4.3 x10^6 and 6.5 x10^6 cells /µl that of females are 3.4 x10^6 and 5.7 x10^6cells /µl. There was increase from month zero to month 12th for each individual participant (Figure 4.2, Table 4.7 Appendix).

White blood cells (WBCs) increased from baseline to the end of study. For both male and female participants, there was average increase from 5.4 to 7.2 x 10^3cells/µl making total difference of 1.8 x 10^3 cells. The normal for males is 2.5 and 7.4 x 10^3 cells /µl that of females are 3.3 and 9.7 x 10^3 cells /µl. The average erythrocyte sedimentation rates (ESR) at baseline was 59 mm/hr for both male and female participants; this decreased progressively with time; The Erythrocyte Sedimentation Rate (ESR) presented hyperbolic decrease curve from 58 to 36 mm/hr. The total decrease in males was by 20mm/hr and in females by 22mm/hr. There was no difference for gender in this parameter (ESR). In both male and female participants, ESR reduced significantly ($p<0.05$) improving the quality of the blood (Figure 4.2, Table 4.7 Appendix).
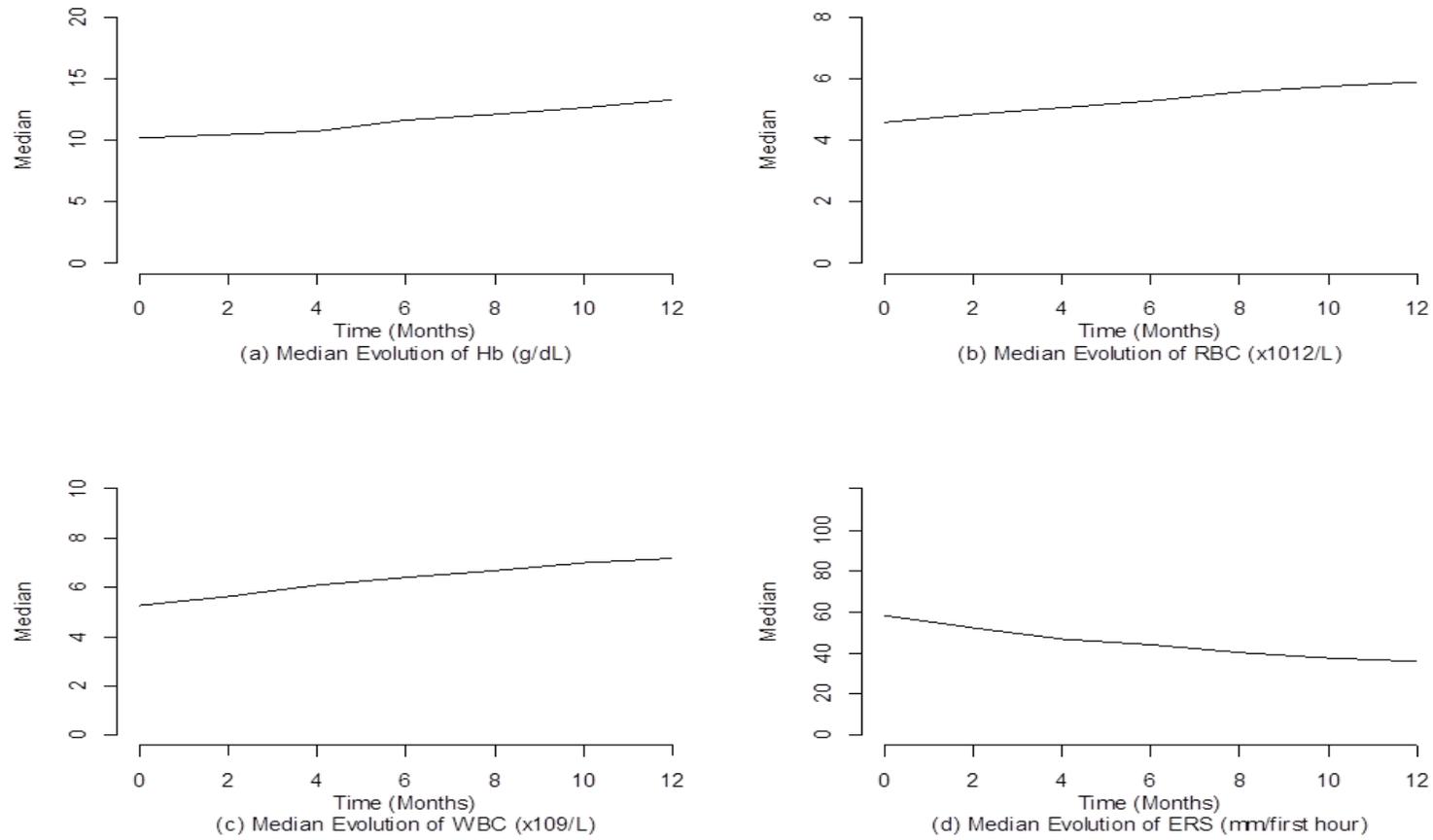
Figure 4.2: Haematological parameters (Average progression Evolutions)

4.5 Effects of Virad and IMB treatment on Immunological Parameters

The trends of CD4 and CD4/CD8 ratio increased while viral load decreased significantly ($p < 0.005$). In this study CD4 cells for both male and female participants elevated from 290 to 420 cells / mm^3, this was by a total of 130 cells at end of the study. Empirically CD4 counts for each patient showed different characteristic at baseline, a unit difference in age resulted in a difference of 0.22 cells /mm^3 of CD4 cells; older patients had higher counts compared to younger participants. Female participants had higher CD4 cell counts with difference of 82.56 cells /mm^3 compared to their male counterparts. The normal range for CD4 cells is 800 and 1200 cells/mm^3 (Figure 4.3, Table 4.8 Appendix).

The baseline for CD8 cells was 796; there was overall reduction of CD8 cells to 781, a difference of 15 cells / mm^3. In this study CD8 cells for both male and female participants reduced from baseline to 0.81 cells /mm^3. There was no difference seen with gender and age. The normal ranges are (211 to 1078) cells / mm^3. CD4/CD8 ratio at base line ratio was 0.40, this rose to 0.60 at the end of study. The ratio CD4/CD8 increased by 0.02 per month. The normal range for CD4/CD8 is 0.8 - 1.2.

The baseline count for viral burdens was 2814 copies/ml. This reduced over time to 1876 copies/mL. There was a total reduction by 223.63 copies / mL per month. Viral load decrease was not different for age and gender. Empiric Individuals had different slopes of viral load changes (there were different reduction rates of viral load for different patients). Overall both male and female patients had the viral load reduction by 864 copies / mL ((Figure 4.3, Table 4.8 Appendix).
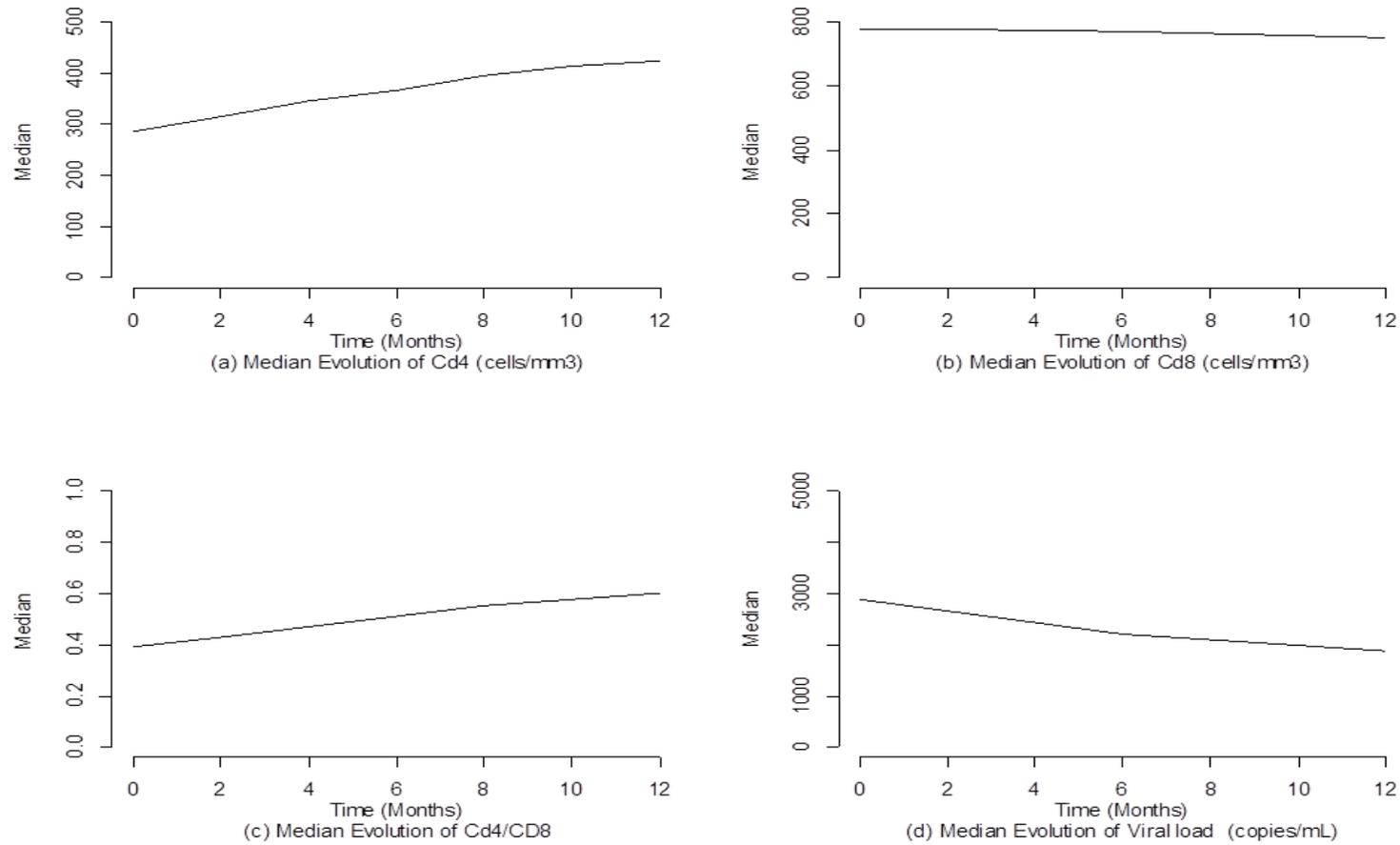
Figure 4.3: Immunological parameters (Average Evolution e– h)

4.6 Effects of Virad and IMB treatment on Liver and Kidney parameters

The kidney and liver parameters urea, creatinine, gamma guanidine amino transferase (GGT), Aspartate aminotransferase (AST) and Alanine amino transferase (ALT) did not show any increase over time. Empiric Individuals had different readings at baseline. AST measurements were different for the gender while ALT were different for both age and gender. The normal ranges of ALT for males are 10 to 55µ/l, females 7 to 30u/l (figure 4.4, Table 4.9 Appendix).

4.6.1 Effects of Virad and IMB treatment on Liver Parameters

Liver transaminases such as Aspartate transferase biomarkers of liver injury in a patient with some degree of intact liver function. The individuals Gamma guanine Transferase (GGT) (U/L) profile showed universal progression with very minimal fluctuation between the 4th and the 8th months. Some individuals had a sharp reduction at month 2nd to the 4th months followed by a gradual decline to the 12th month. GGT for Male and female patients displayed minimal decline. The average changes for GGT (U/L) exhibited a leveled profile parallel to X axis with very slight drop.

The individual Aspartate amino transferase AST (IU/µl) did not show any significant change ($p > 0.05$) from month zero to month 12th. There was no change during the treatments. Normal AST for Males is 10 to 40 IU/l, and female is 9 to 25 IU/µl. Male patients AST values decreased by 0.8u/l compared to that of females that decreased by 1.2 IU/µl. The AST for men was 44.5 IU/µl (normal 10 to 40 IU/µl), that of women was 40.3 IU/µl (normal 9 to 25 IU/µl). Male patients in this study had an average Alaninine amino transferase (ALT) reduction from 31.2 to 30.6 IU/µl. While females in this study had ALT values decrease from 28.3 to 27.9 IU/µ. Alanine amino

transferase (ALT) levels for both male and female participants did not show significant changes. The ALT levels in Males reduced by 0.6 IU/µl, while that of Females reduced by 0.4 IU/µl. The normal range for males 10 to 55 IU/µl; while normal range for females is 7 to 30 IU/µl (figure 4.4, Table 4.9 Appendix).

4.6.2 Effects of Virad and IMB treatment on Kidney Parameters

During treatments, there were no significant changes, but there was overall reduction of 0.2mg/dl. Normal range of Urea in male gender is 36 to 85 µmol /l, and Females is 23 to 66µmol/l. The average urea level for both sexes was 15mg/dl to 14.8mg/dl. The individual Urea profiles (mM) showed no changes for the majority of participants (p=0.05). The average profile of UREA (mM) showed a more leveled to the lower axis with very minimal fluctuation at months 6th to 8th, it stabilized toward the end of study at the 12th month. The average changes of UREA did not exhibit notable changes. The average change of Creatinine (mM) profile exhibited a universal slight reduction with very minimal changes paralleled to X axis. Normal values for Creatinine in Men are 70 to 123 µmol/l; in female is 53 to 106 µmol/l (figure 4.4, Table 4.9 Appendix).
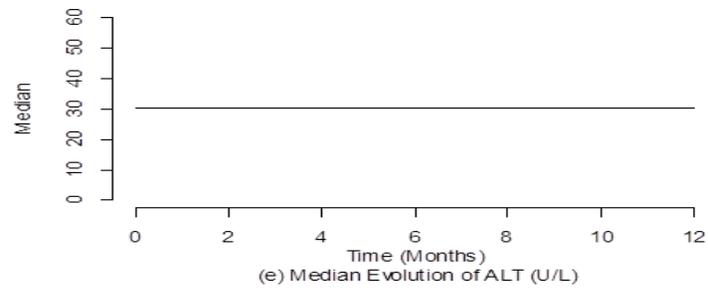
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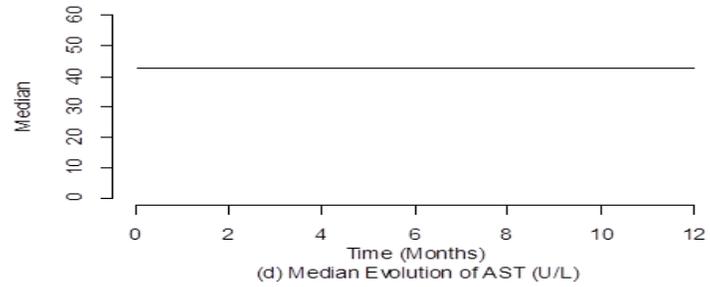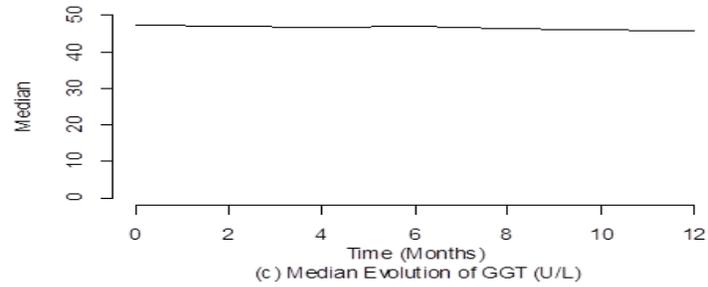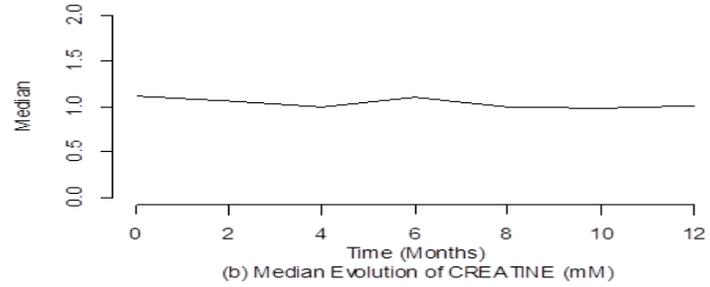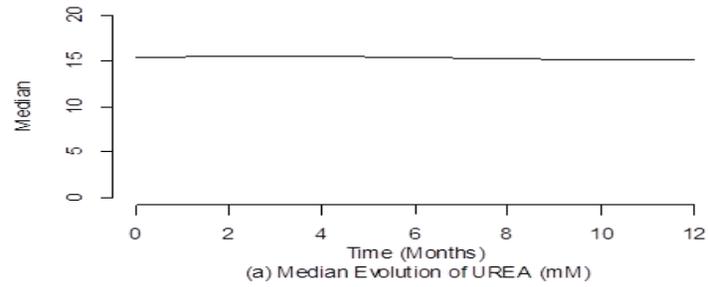
Figure 4.4: Biochemical parameters (Average Evolutions)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMENDATIONS

5.1 Discussion

This study provided information on characteristics of patients living with HIV/AIDS, their phenotypic body mass indexes (BMI), Hematological and Immunological properties, liver and Kidney parameters, their changing features as the participants took herbal formulations Virad and IMB to mitigate viral complications. The representative sample (n= 188) of patients from 18 years to 65 years both male and female from Mombasa county.

Patients seeking herbal treatments Virad and IMB were evaluated for changes in the parameters for weight, BMI, hematological factors and liver and Kidney functions tests, following uptake of the herbal formulations for a period of 12 months. Sixty six percent (66%) were female and 34% were male. Females were double the number of males, and their modal age range was 31 and 40 years with median of 37 years. This was consistent with the study by Stephen and Nancy (1999) that reported increasing infection rates in females, where more women than males were dying of HIV/AIDS. Also consistent with argument by UNAIDS (2010) this was prime age forming the bulk of Kenyan work force and most active sexually and probably formed the age group that carried the highest burden of HIV infection and with highest rate for HIV infection and transmission.

There were also two age group peaks; the first at 44.2% aged (31 and 40 years) and the second at 27.1% aged (41 and 50 years). Majority of the participants were aged above 30 years (P=0.0001). Middle aged people in the age group of 41 to 50 were equally affected by HIV. This

was consistent with the study by UNAIDS (2010) that middle aged persons were infected after caring for family members infected with HIV and orphaned children. According to a study by UNAIDS (2010), millions of middle aged men and women care for sons and daughters who are living with HIV and sometimes care for orphaned grandchildren. Gender analysis forms the basis for the changes required to create an environment in which women and men can protect themselves and each other. Based on the ratios defining numbers of male and female participants, it could be possible females were more vulnerable and likely to seek medication compared with males.

At baseline, there were two CD4 stage peaks, the first one at 25% (CD4, 300 and 349) and the second at 19.2% (CD4, 200 and 249). According to WHO, (2007) these stages would be described as immune compromised at stage II and stage III where patients presented with minor mucocutaneous manifestations and recurrent upper respiratory tract infections, to those with advanced symptoms that included chronic diarrhea and associated bacterial infections. These stages were also consistent with system of HIV/AIDS (2012), where HIV treatments would have been started to manage the patients with ARVs.

Ninety eight percent (98%) of participants for this study were at stages II and III. The complications associated with these stages and related psychological issues could have influenced HIV patients to seek treatments from herbal practitioners and would most likely suggest patients were anxious and required psychological assurance. It is on this anxiety basis that groups of HIV + persons would be encouraged to join up with groups that would assure prolonged life.

The analysis of Virad and IMB obtained spectra of phyto-components including phenolic acids, saponins, tannins, alkaloids, flavonoids, protein and lipids. These components were at different proportions in the herbal formulations Virad and IMB. The combinations of active components in both Virad and IMB could have provided the patient improvements as would be suggested by Gonda (1992); who described that herbs relied for their effects on a variety of constituents and the idea of synergy within them was gaining acceptance. Their additive or synergistic effects could have contributed to therapeutic evidence seen in the patients of this study. This also agrees with a study by Wagner (1999), who argued "Cocktails" of chemotherapeutic agents were now being used to treat AIDS and cancer. It also concurs with studies of DHHS (2005), who discussed that current HAART options were cocktails consisting of at least three drugs belonging to classes of anti-retroviral agents. Based on this information, it is important to understand bioactive constituents within the herbal formulations are various phytochemicals that may be useful for treatment for HIV and AIDS.

The total Phenolic acids in both Virad and IMB were (159.1 mg/g). According to Sofowra (1993), plants synthesize phenolic acids (hydroxybenzoic and hydroxycinnamic acids) as a preventive mechanism for infectious agents that cause diseases. These phenolic acids have health benefits in human beings as well and they possess properties such as antiappoptosis, antiaging, anticarcinogenic, anti-inflammatory, antiatherorosclerotic, cardiovascular protection and improvement of endothelial function. They also inhibit cell proliferative activities. In agreement with findings of Han *et al.* (2007), phenolic acids provide stamina to the head as brain tonic and remove general body weakness. HIV/AIDS is a highly oxidative mechanism and the anti-

apoptosis potentials of phenolic acids may have played a therapeutic role in patients of this study.

The other phytochemical group isolated was saponins (531mg/g). The studies of Okwu and Josiah (2006) described saponins as able to stop bleeding, treat wounds and ulcers and helped in red blood cell coagulation. Another study by Aslam and Shaw (1991), described saponins to possess inhibitory effect on HIV replication as HIV protease enzyme inhibitor, helped in red blood cell coagulation and inhibited viral DNA synthesis. An additional study by Gonda *et al.* (1992), described ursane type saponins as being capable of interfering with the formation of capsidal proteins. This could help explain their effective role in Virad and IMB as they worked against several opportunistic infections of AIDS including herpes and pneumonia, and inhibited the spread of HIV within the body.

The other phytochemical group isolated was Tannins at a concentration of 375 mg/g. Tannins were higher in Virad compared to IMB. A study by Vlientinck *et.al.* (1998) described Tannins to have properties of astringency and HIV reverse transcriptase inhibition. Astringent medicines caused shrinkage of mucous membranes or exposed tissues and when used internally they check discharge of blood serum or mucous secretions. This happens with a sore throat, hemorrhages, diarrhea, or with peptic ulcers. Also, Tannins have styptic effects preventing apoptosis and lysis of infected cells crucial in progression of HIV /AIDS (Vlientinck *et al.*, 1998).

Another phytochemical group isolated was Alkaloids at a concentration of 3835 mg/g. This was probably the most dominant component in Virad and IMB and could suggest the most

contributory component in therapy for HIV/AIDS in this study. According to Mathee *et al*. (1999), alkaloids such as of castanospermine contain Michelin A-B and C, which have a broad range of anti-HIV activity. In another similar study by Aslam and Shaw (1991): Alkaloids found in gossypol and Tylosema also present in Virad and IMB provided HIV protease and HIV-induced cytopathogenicity. Periwinkle indole alkaloids possess antineoplastic effects, antiluekaemic and vasodilator and antitumor properties and inhibit development of malignant conditions (Aslam and Shaw, 1991) which are a common phenomenon in HIV/AIDS compromised cases.

Another phytochemical group isolated was Flavonoids. These were at high concentrations in IMB compared to Virad. According to a study by Del-Rio *et al*. (1997), flavonoids increased appetite for human nutrition and health. At a similar study by Okwu (2004) and by Zhou (2000), flavonoids contain hydroxyl functional groups, responsible for antioxidant effect and inhibit HIV reverse transcriptase and HIV replication in HG lymphocyte. They inhibit giant cell formation in HIV infected cell cultures. Other studies by Marjorie (1996) and Mathee (1999) discussed that asymptomatic HIV carriers on Flavonoids and liquorice coumarines experienced delayed development of AIDS symptoms.

The other phytochemical group isolated was Protein (986 mg/g) and was within RDL. According to a study by Hermann and Janice (2003), plant proteins are nutrients that build and repair damaged body tissues, especially muscles, they act as fuel particularly under starvation conditions and support life (Brosnan, 2003). Another study by Genton and colleagues (2010), described Plant proteins synthesized essential amino acids required by the human body. In yet

another study by Williams *et al.* (2003), loss of Protein was associated with decreased lean body mass, which was strongly associated with disease progression and death in HIV positive patients.

Antioxidant micronutrients and trace elements present in Virad and IMB were useful for treatment of patients in this study. The presence of potassium, sodium, calcium, magnesium, iron, zinc and copper micronutrients supplemented the therapeutic potentials in this study. Consistent with studies by Papadopoulos (1988), patients that had subclinical deficiencies of trace elements and vitamins were at risk of impaired immune function and hence an increased risk of infection. When a patient suffers deficiencies in elements such as potassium (K) and sodium (Na), they may suffer from neurological disorders. When calcium (Ca) and magnesium (Mg) micronutrients are deficient, patients develop muscular dysfunctions. When iron (Fe) mineral is deficient, patients develop hematological dysfunctions. Deficiency in zinc (Zn) and copper (Cu) micronutrients affect immune factors and were essential for cell cytoplasm development according to a study by Cunningham (2000).

Following the administration of Virad and IMB on PLWHA, there was evidence of changes in phenotypic parameters. The average weight and BMI for patients increased from 60.5 kg to 76.0kg, (total improvement by 15.5kg) and BMI increased from 17.8 kg/m^2 to 22.6 kg/m^2 toward normalcy. This could have been the contribution of flavonoids (Del-Rio *et al.,* 1997) that increased appetite and improved nutritional uptake and that of Proteins (Brosnan, 2003) which built body tissues and repaired tissue damage, especially muscles. Much as there was no control over what patients took for their nutrition, there was evidence of increase in weight and BMI for

the patients taking Virad and IMB. Before the uptake of Virad and IMB, 98% of the patients had BMI that was less than 18kg/m^2 a highly significant independent predictor of mortality.

At baseline 61.5% of patients had abdominal hyperacidity with ulcers known to limit food uptake and affecting the general nutritional status of patients, and this affected weight and BMI (IRIN, 2009). This agrees with findings of Cunning-ham (2000), that weight loss was a common problem in HIV and patients were frequently found to have abnormalities of plasma mineral and trace element concentrations, especially of zinc, selenium and magnesium. A similar study by Shenkin (1995) discussed that weight loss was a result of a number of interacting factors in response to infection including loss of appetite, decreased absorption, diarrhea and the effects of redistribution from plasma to tissues. Another study by Williams and colleagues (2003) showed evidence that malnourished people were less likely to benefit from antiretroviral treatment and were at a higher risk of faster progression to AIDS. HIV /AIDS infection affects Body weight and Body mass index (BMI) of patients.

Following uptake of Virad and IMB, weight and BMI gained significantly (P < 0.05) with difference of 16kg in weight and BMI by 4.8kg/m^2 and this prolonged patient's survival. This study compares with that in Gambia (1992) where the median survival time of those presenting with a BMI less than 16kg/m^2 were 0.8 years in contrast to those with a baseline BMI greater than 22kg/m^2 that were 8.9 years. HIV/AIDS in Kenya is prevalent among poor people and access to food faces challenge affecting the general diet of many sufferers. According to the argument of IRIN (2009): Poverty levels are high in Kenya and food shortages frequent, people living with HIV are often unable to eat a healthy balanced diet. However, in contrast to the

above circumstances, patients taking Virad and IMB exhibited improved appetite, antiulcer potentials and antioxidant effects that resulted to increased weight and BMI for both male and female study patients. There was total weight increase of 15.5 kg from baseline to end of study. Females had better prognosis in weights and body mass indices (BMI) compared with males probably because of resilience, adaptation, treatment and access to nutrition.

In this study hematological factors improved. At baseline 27.7% of patients had less than 4.1×10^{12} Red blood cells. The combined uptake of Virad, IMB and vitamin C increased red blood cells (RBCs) and also increased the levels of Hemoglobin (Hb) by 3g/dL. There was also increase of white blood cells (WBCs) and there a significant reduction in sedimentation rate ESR by -21mm/hr. These results agree with Larrañaga *et al.* (2003) and Haissman *et al.* (2009) that HIV could cause inflammation and raise Erythrocyte Sedimentation Rate (ESR) due to inflammatory cytokines and endothelial markers. Although it was reported to course inflammatory reactions, the results of this study showed that ESR was mitigated, probably because of the antioxidant potential in the components of Virad and IMB, plus the effects of mega doses vitamin C that maintained proper immune function. According to Savarino *et al.* (1999) vitamin C supports proper development and functioning of many parts of the body, and maintains proper immune function, also increases the absorption of iron when taken at the same time.

Where HIV infection is fully established, lower hemoglobin levels were shown to correlate with decreasing CD4+ cell counts according to another similar study by Lau *et al.* (2005). This study found an association between anemia during established infection and a faster progression to

AIDS and death, especially where HIV infection was established. The immune booster IMB contained neem leaf extracts as part of its content and consistent with the study of Udeinya *et al.* *(*2003) which noted that neem leaf extracts improved the condition of patients, increasing hemoglobin, platelet count, CD4+ cell count and body weight. A similar study by Derdemeziz *et al.* (2010), found neem leaf extracts contained omega -3 fatty acids and triterpenes that have sterols and stanols. These affect physiological inflammatory markers, coagulation parameters, as well as platelet and endothelial function. It is possible that neem leaf components present in IMB played may have played a role in the therapeutic effects improving hematological factors.

Majority (70.7%) of Patients in this study at baseline were likely to suffer impairment in hematological factors. This was comparable with study by Kreutzer and Rockstroh (1997) who found that anemia was well described in established HIV-1 infection, with an estimated prevalence ranging from 10% in asymptomatic HIV-infected patients to 92% in patients with AIDS. According to studies of Lau *et al.* (2005); anemia results from the impairment of red cell production in the bone marrow due to the release of inhibitory substances (cytokines) and inappropriately low levels of the hormone erythropoietin common among people with advanced HIV infection, and signals a more rapid progression toward AIDS. Another study by Lanzillotti and Tang (2005) showed that Low blood concentrations of many micronutrients were common in HIV-positive individuals and were associated with disease progression and in-creased mortality. Virad and IMB contained spectra of antioxidant micronutrients including zinc which could have contributed to the therapeutic effects seen in this study.

There was mitigation in blood sedimentation rate (ESR). Consistent with studies by Kris-toffersen *et al.* (2008), Sfikakis *et al.* (1995) and Wolf *et al.* (2002), ESR was shown to be elevated in HIV positive patients. In contrast to these findings, treatments with Virad and IMB significantly reduced the values of ESR and registered improvement of the hematological factors ($P < 0.05$).

There was evidence of improvement in immunological factors. CD4 counts increased as CD8 counts decreased. Therefore CD4/CD8 ratio increased as the viral load reduced significantly ($p < 0.05$). According to The American Association (AACC, 2001 – 2012); CD4/CD8 ratio can tell how strong the immune system is and help predict the risk of complications and debilitating infections. HIV progression and associated immune activation are closely linked to the presence of CD8 T cells and play a critical role in prevention and control of viral infections.

A study by Egger *et al.* (2002) argued that patients with high HIV viral loads also had faster decline in CD4 count and progression to AIDS-related illnesses, this also could tell how long an individual remained healthy or how quickly the disease would progress to AIDS. The higher the viral load the more viral elements are likely to be in tissues and in circulating blood and other body fluids (Egger *et al.,* 2002). Coffin *et al.* (1986) reasoned that HIV- infected people were known to be oxidatively stressed; this lead to cellular anomalies and inflammatory process in human tissues, and resulted to lowered immunity through viral induced lysis of cells, apoptosis and CD8 cytotoxicity hence contrasting with the results of the current study. Patients of this study displayed improvement in immune status, female patients had better prognosis compared

with male counterparts. The combined treatment of Virad and IMB improved quality of blood and a significant drop in viral burdens.

It is understood that viral burdens and long term use of ARVs affect Liver and Kidney parameters and may result to toxic effects in these organs. It is also assumed that herbal treatments are safe and may not affect internal organs negatively. According to Shaninian *et al.* (2000) people living with HIV/AIDS risk developing kidney disease because of the progression of the AIDS virus and the side effects of the medicines they may be taking to slow this progression, although it still ranked relatively low in the order of individual causes of death.

In this study the liver parameters gamma glutamyltransferase (GGT), Aspartite amino transfarase (AST) and Alanine amino transfarase (ALT) were all reduced by 0.3 U/L. In both male and female participants liver parameters, gamma glutamyltransferase (GGT) reduced. The presence of silimarin (Flavonoids) present in IMB known to fortify the liver could have protected the liver from possible damage. According to Kren *et al.* (1998), Morazzoni and Bombardelli (1995) Flavonolignans found Silimarin compounds exert antihepatotixic activity preventing galactosamine induction of cell lesions, hence could be hepato-protective and antagonized liver degenerative mechanism. Despite the presence of Flavonoids known to mitigate ailments of the liver, the values were not significant ($p=0.05$), but more important, it could be concluded these flavonoidal silimarins safeguarded the liver cells from damage. Mata-Marín *et al.* (2009) described HIV infection to be associated with direct inflammation of hepatocytes leading to liver damage. Antiretroviral therapy, co-infection with other hepatotropic viruses, tumors, parasitic infestation and non-antiretroviral therapeutic drugs may cause considerable hepatic damage in HIV infection. Another study by Jennifer *et al.* (2010) described that Liver disease among HIV-

infected individuals as a common and an important cause of non-AIDS-related morbidity and mortality. Similar studies by Casula *et al.* (2005) and Jacotot *et al.* (2000) attributed Liver destruction to apoptosis, mitochondrial dysfunction either by decreasing mitochondrial DNA in various cells or by alteration in mitochondrial membrane by HIV proteins that in turn stimulated inflammatory response.

At the close of this study, it was evident that Urea levels were not significantly reduced in the participants (0.1 Mm). There was also no significant decrease in Creatinine values both male and female participants. It is possible the presence of phenolic acids protected the kidneys from injury. Much as phenolic acids were known to be active in curing kidney and stomach problems and helpful as anti-inflammatory in action, in this study phenolic acids were not able to change the status of the kidney (Marjorie, 1996). It could be concluded there were no adverse effects at using these herbal therapies for the period of this study. It could also be true that presence of Phenolic acids protected the kidney from injury and dilapidating effects of the HIV/AIDS viral progressions. According to Shaninian *et al.* (2000): Kidney disease was an important cause of morbidity and mortality affecting about 30% of people with HIV and causing more than 10% of HIV-related deaths. An additional study by Moro (2011) showed that renal disease was a relatively common complication in patients with HIV.

Therefore, the combinations of phytochemicals and trace elements in Virad and IMB could have played a role in providing efficacy and safety for study participants in this study. This agrees with Duke and Bogenschutz (1999) that synergism could have occurred in extract mixtures. Whole extracts were responsible for the therapeutic effects, rather than assuming that a single

chemical entity could be extracted and used alone. The outcome of this study, Virad and IMB provided pharmacological and clinical effects. Further research may be required to make these materials available for use in conventional medicine practice. There were limitations in this sturdy; Skeptics' of this study hindered participation of patients denying progress, study was self-sponsored and had financial limitations.

5.2 Conclusions

i. Flavonoids, Alkaloids and lipids were of highest concentrations in these formulations.

ii. Immunological factors CD4/CD8 were raised; viral burdens reduced therefore improved immunity and were efficacious.

iii. Liver and kidney functions were not adversely altered; these phytochemicals were not toxic and therefore safe for use.

5.3 Recommendations

i. Virad and IMB should be considered for development and subsequent inclusion in management of HIV/AIDS.

ii. More studies are required to assess long term effects of treatment using these herbal medications.

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APPENDICES

Appendix I: Ethical clearance letter

KENYA MEDICAL RESEARCH INSTITUTE

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

KEMRI/RES/7/3/1   January 14, 2011,

TO: MR. AMOS LEWA MWAVITA,
             PRINCIPAL INVESTIGATOR

THRO':   DR. SAMUEL KARIUKI,
             THE DIRECTOR, CMR,
             NAIROBI

RE: SSC 1772 (*RE-SUBMISSION*): THE SAFETY AND EFFICACY OF
             HERBAL FORMULATIONS USED FOR THE MANAGEMENT OF
             HIV/AIDS IN COAST PROVINCE, KENYA

Make reference to your letter dated January 6, 2011 received on January 7, 2010. Thank you for your response to the issues raised by the Committee. This is to inform you that the issues raised during the 183rd meeting of the KEMRI/ERC held on October 12, 2010, have been adequately addressed.

Due consideration has been given to ethical issues and the study is hereby granted approval for implementation effective this **14th day of January 2011,** for a period of twelve (12) months.

Please note that authorization to conduct this study will automatically expire on **13th January 2012.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **15th September 2011.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the ERC prior to initiation. You may embark on the study.

Yours sincerely,

Caroline Kithinji,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICS REVIEW COMMITTEE

In Search of Better Health

Appendix II: Notes about Virad

Virad is an herbal combination of multiple active constituents for polyvalent action with overall synergistic effect to combat viral infections. The broad-spectrum antiretroviral activity includes HIV reverse transcriptase inhibition; Inhibition on HIV replication, inhibition of giant cell formation in HIV infected cell cultures; regression of AIDS symptoms development; suppression of the integration of proviral DNA into cellular genome.

Virad Therapy; Oral presentation for treatment of retroviral HIV infections; virastatic to a wide range of classes of viruses of the Baltimore classification single strand & double strand: (reverse RNA) HIV; Hepatitis B; Influenza virus; Reo-virus; Herpes virus; Pox virus; Papilloma virus. Dosage and Administration

Usual dosage for treatment of oral indication; Therapy is started and continued with oral preparations:

Adults and children over 12 years: 1000mg once daily

Children 5 – 10yrs: 5 –7.5mls a day: Virad may not be recommended for neonates and infants. Take together with Immune Booster (IMB).

Dosage caution:

Use Virad with caution in patients on blood thinning anticoagulants; It may intensify blood-thinning effect of the medications. Over use may lead to dangerously low levels of potassium in the body. Virad may neutralize digitalis medicines, for patients on cardiac glycosides. Zinc in Virad may make antibiotics on patients less effective and should be taken at least two hours after antibiotic therapy. Excessive consumption leads to hypertension and hypokalaemic alkosis.

Pharmacological activity

Pharmacological contents include: phenolic glycosides group; flavonoids; coumarins and isoprenoids; Glycyrrhizin; Azadiractin limonoids; indol alkaloids with saponins and sesquiterpene stress compounds. These constituents synergistically have astringent and styptic effects with HIV reverse transcriptase inhibition. They have ant gastric effects; with pronounced activity on reticuloendothelial system characterized as representative polysaccharides with immunological activity. They have anti-inflammatory and antiviral effects; inhibit giant cell

formation in HIV infected cells. Treat rheumatoid arthritis, Addison's disease and various inflammatory conditions and give symptomatic relief from peptic ulcer pain.

They have antiplasmodial activities and with anti-inflammatory and antibacterial potency. Antibacterial tetranoterpenoid add to Virad pharmacological activity. Some polysaccharides present show anti-inflammatory and antitumer activities suggesting powerful antioxidant effect. Virad glycoprotein that posses' HIV- protease and HIV- induced cytopathogenicity and related aldehydes with various pharmacological properties including inhibition of viral replications; mucilaginous polysaccharide with trace elements and iodine are polyphenols that inhibit viral replication. Indole antineoplastic alkaloids with vasodilator potency and antitumer activity inhibit development of malignant conditions, these acts as transport agents for the combination. The added Mineral agents play crucial role for proper functioning of the immune system and enables body to fight colds, flu and other infections hence stabilizing immune system. Virad also aids the treatment of rheumatoid arthritis, lupus, and fibromyalgia, multiple sclerosis such as AIDS associated with improperly functioning system.

Contraindications: Virad may not be very useful at very late stages of HIV & AIDS. Avoid taking during Pregnancy, it may increase blood pressure; do not exceed recommended dosages.

Do not mix allopathic antiretroviral therapy with Virad, unless with consultation. Virad may interfere with recommended dosage allowances (RDA) for the antiretroviral. It may also intensify effects of the antiretroviral therapy leading to toxic levels. It may lower plasma levels concentrations of protease inhibitor indinavir. Refrain from alcohol.

Possible side effects

Virad is relatively safe with few side effects. For patients with deep gastrointestinal erosions, hyperacidity and ulcers; they may present with emesis and or severe diarrhea that lasts for three days (Self-limiting: BUT could be fatal for very weak patients; require careful or medical supervision). Virad may cause mild gastrointestinal irritation leading to diarrhea but this is self-limiting. If diarrhea persists beyond five days. Discontinue administration.

Over dosage

Cases of over dosage with Virad are unlikely to occur, if encountered gastrointestinal symptoms and disturbances of the fluids and electrolyte balances may be evident. They may be treated symptomatically with attention to water electrolyte balance.

Appendix III: Notes about IMB -Herbal Immune booster

IMB is a combination of multiple active constituents for polyvalent action with overall synergistic effect to have overall effect on boosting immune system. The broad-spectrum immune boosting action includes stimulation of immune system, repair of oxidized body tissues and fights weight loss; improve survival rates in AIDS related cancers and other malignancies. Cures peptic and Oral gastrointestinal ulcers resulting from HIV related hyperacidities and augment appetite.

IMB Therapy

Oral presentation for boosting immune system; IMB phytoflavonoids have broad spectrum anti-inflammatory; antiallergic effects; antithrombotic and vasoprotective properties for inhibition of tumor promotion and as a protective for the gastric mucosa; biflavonoids in IMB have recently been show to have activity against influenza A virus, HSV-1 and HSV-2 viruses (Planta Medica, 1999, 65, 120).

Dosage and Administration

Oral indication; Therapy is started and continued with oral preparations: Adults and children over 12 years: 500mg three times a day for a total of 20 days initially.
Children 5 – 10yrs: 5 –7.5mls three time's day: Administration may continue for 5 months with monitoring.

Pharmacological activity

Since HIV/ AIDS is an oxidative mechanism resulting from viral blasters of immune cells, IMB phytomedicines are combined to produce synergism as well as antagonism where that apply to reduce undesirable effects. Anthraquinones mucilaginous gel possesses anti-inflammatory properties, gastrointestinal activity, and antidiabetic activity, anticancer, antibacterial activity and radiobiological protection. Interferon components included as an immonomudulator and for adjuvant therapy and prophylaxis of recurrent infections of the upper respiratory tract and of urogenital tract. Flavonolignans that exert antihepatotixic activity preventing galactosamine induction of cell lesions, hence hepatoprotective; included in IMB to antagonize possible liver

degenerative mechanism.IMB diglucopyranisiduronic acid and deoxycorticorticosterones for treatment of Addison disease and various inflammatory conditions; flavonoid components possess antimicrobial properties; also exert spasmolytic and antiulcerogenic activity.

IMB has antiretroviral activity and has recently been shown to have activity against influenza A virus, HSV-1 and HSV-2 viruses. Geranylgeranyl diphosphates with provitamin A with anticancer potency.IMB has allicins, allylpropyldisulphide which have been shown to be active in humans and animals; antithrombitic, hypolipidaemic, hypoglycemic, hypotensive, diaphoretic, expectorant and antibiotic medicinal properties; vinblastine and vinchristine and vincamine indole antineoplastic alkaloids with antiluekaemic alkaloids; vasodilator potency and antitumer activity; inhibit development of malignant conditions. These have anticancer properties as well as those exhibiting anti -aging and antiviral possibilities.

Dosage caution

Use IMB with caution in patients on blood thinning anticoagulants; may intensify blood-thinning effect of the medications. Over use may lead to dangerously low levels of potassium in the body. May neutralize digitalis medicines, for patients on cardiac glycosides.

Contraindications: Avoid taking during Pregnancy; do not exceed recommended dosages. Consult your Doctor before adding conventional antidepressant medicaments. The combination may lead to serious adverse reactions. Take care when using Thiazide diuretics; IMB may cause loss of potassium and intensify the potassium – depleting effects of medications when over used and may increase the potency of blood pressure medications or neutralize the blood pressure – lowering effect of all antihypertensive; IMB is best avoided during pregnancy and when breast-feeding. Refrain from alcohol.

Possible side effects

IMB is relatively safe with few side effects. Laxative effects in combined mixture may cause slight diarrhea in some patients. Slight nausea in some cases but overall IMB is safe to use taken at the rightful doses. Some cases may have slight dizziness.
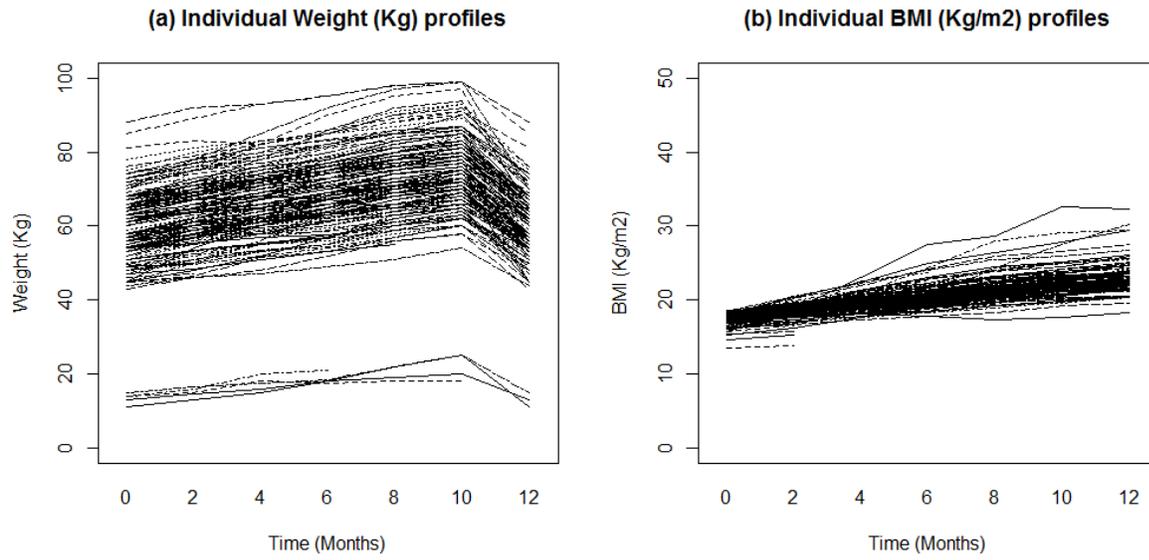
Figure 4.5 : Phenotypic Parameters (Individual Profiles a – b) for weight and BMI

(a) Individual Weight (Kg) profiles

(b) Individual BMI (Kg/m2) profiles

Table 4.6: Selection of mean structure (AIC estimates) for Weight and BMI.

Model Phenotypic Parameters (Individual Profiles a – b)	Variables	Weight	BMI
1	Age,Gender, Month	8268.88	4791.43
2	Age,Gender,Month, $Month^2$	8269.56	4800.59

Estimates of linear mixed models.

Coefficient	Weight	BMI
Fixed effects		
Intercept	32.90[23.91,41.88]*	18.03[17.09,18.98]*
Age	0.70[0.54,0.85]*	-0.01[-0.04,0.01]
Gender		
Female	7.05[3.96,10.14]*	0.02[-0.46,0.51]
Male#		
Time		
Month	0.52[-0.47,1.51]	0.43[0.41,0.46]*
$Month^2$	-	-
Random effects	Standard Deviation	Standard Deviation
Random intercept	9.86	1.46
Random slope	5.42	0.06
Residual	5.19	1.47

- reference level; * - significant at 5%

Table 4.7: Selection of mean structure (AIC estimates) for Hematological Factors

Model	Variables	HB	RBC	WBC	ERS
1	Age, Gender, Month	3325.59	1548.20	2032.11	7116.99
2	Age, Gender, Month, $Month^2$	3336.16	1554.34	2041.17	7104.44

Estimates of linear mixed models

| Coefficient	HB	RBC	WBC	ERS
Fixed effects				
Intercept	9.88[8.77,11.00]*	4.73[4.23,5.24]*	5.35[4.49,6.21]*	53.13[43.01,63.25]*
Age	-0.01[-0.04,0.02]	-0.01[-0.02,0.00]	0.01[-0.01,0.03]	0.29[0.02,0.56]*
Gender				
Female	1.61[1.04,2.18]*	0.69[0.43,0.96]*	0.09[-0.36,0.54]	-1.40[-6.65,3.85]
Male#				
Time				
Month	0.27[0.23,0.30]*	0.11[0.10,0.12]*	0.13[0.11,0.15]*	-3.13[-3.28,-2.97]*
$Month^2$	-	-	-	0.10[0.09,0.12]*
Random effects	Standard Deviation	Standard Deviation	Standard Deviation	Standard Deviation
Random intercept	1.83	0.84	1.45	17.13
Random slope	0.18	0.04	0.02	0.00
Residual	0.70	0.34	0.39	2.98

- reference level; * - significant at 5%
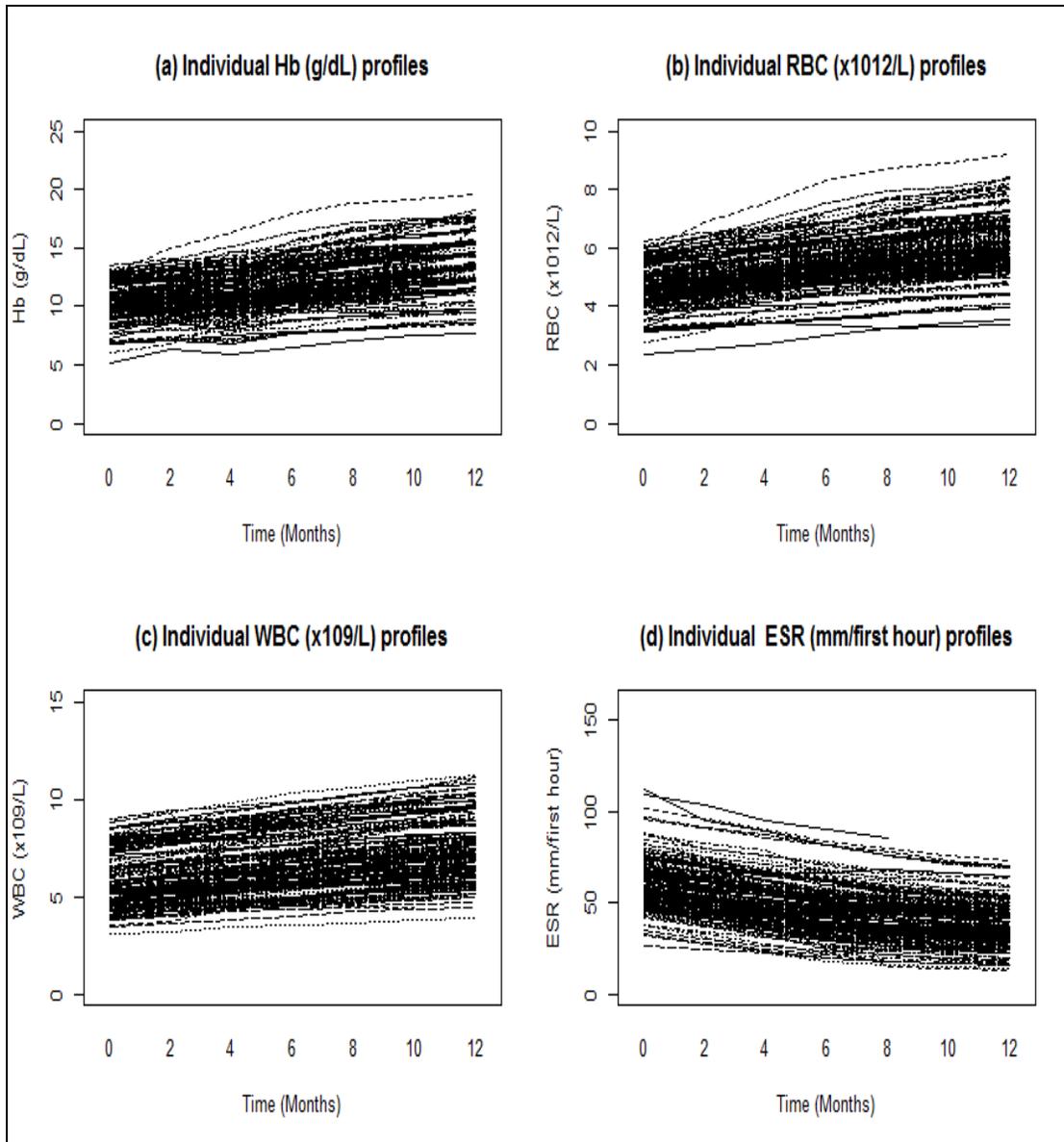
Figure 4.6 Haematological parameters (Individual Profiles a – d)

Table 4.8: Selection of mean structure (AIC estimates) for Immunological Factors.

Model	Variables	CD4	CD8	CD4/CD8	Viral load
1	Age,Gender, Month	11660.57	12478.56	-3531.75	9403.73
2	Age,Gender,Month, Month^2	11656.07	12484.51	-3522.83	9398.22

Estimates of linear mixed models.

Coefficient	CD4	CD8	CD4/CD8	Viral load
Fixed effects				
Intercept	234.43[172.07, 296.84]*	608[431.78,785.46]*	0.55[0.43,0.66]*	4632.52[1637.18,7627.97]
Age	0.22[-1.43,1.87]	3.41[-1.27,8.10]	0.00[-0.01,0.00]	0.35[-79.00,79.70]
Gender				
Female	82.56[50.21,114.90]*	38.26[-53.57,130.10]	0.07[0.01,0.12]*	-1088.54[-2643,465.91]
Male#				
Time				
Month	15.67[13.88,17.46]*	-0.81[-1.17,-0.46]*	0.02[0.01,0.03]*	-223.63[-287.55,-159.94]*
Month^2	-0.38[-0.52,-0.24]*	-	-	10.34[5.23,15.46]*
Random effects	*Standard Deviation*	*Standard Deviation*	*Standard Deviation*	*Standard Deviation*
Random intercept	105.45	300.09	0.19	5053.60
Random slope	2.32	0.00	0.01	4853.50
Residual	19.92	24.79	0.04	876.70

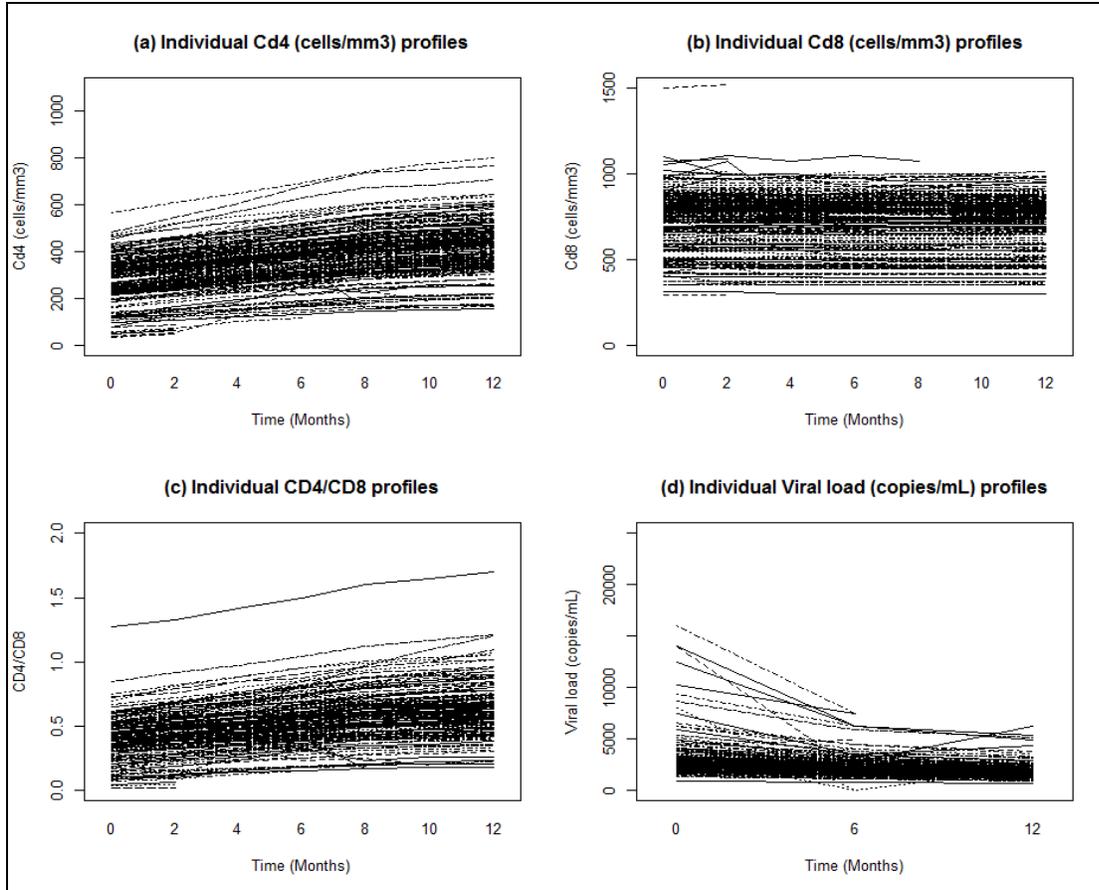# - reference level; * - significant at 5%

Figure 4.7:Immunological parameters (Individual Profiles a – d)

Table 4.9: Selection of mean structure (AIC estimates for Kidney and Liver Parameters)

Model	Variables	UREA	CREATINE	GGT	AST	ALT
1	Age,Gender, Month	5596.83	106.14	6600.47	-	-
2	Age,Gender,Month,	5607.48	121.03	6609.67	-	-

Estimates of linear mixed models for Kidney and Liver Parameters

Coefficient	UREA	CREATINE	GGT	AST	ALT
Fixed effects					
Intercept	12.31[8.82, 15.80]*	1.08[0.80,1.36]*	37.73[28.86, 48.60]*	36.22[32.67,39.77]*	24.31[21.96,26.66]*
Age	0.06[-0.04,0.15]	0.00[-0.01,0.01]	0.18[-0.10,0.47]	0.11[-0.01,0.21]	0.10[0.04,0.16]*
Gender					
Female	1.04[-0.77,2.85]	0.04[-0.10,0.19]	2.58[-3.07,8.22]	3.00[1.16,4.84]*	1.98[0.67,3.29]*
Male#					
Time					
Month	0.01[-0.02,0.03]	-0.01[-0.01,0.00]	-0.04[-0.08, 0.01]	0.00[0.00,0.00]	0.00[0.00,0.00]
	-	-	-	-	-
Random effects	*Standard Deviation*	*Standard Deviation*	*Standard Deviation*	*Standard Deviation*	*Standard Deviation*
Random intercept	5.88	0.47	18.43	6.01	4.27
Random slope	0.00	0.01	0.00	0.00	0.00
Residual	1.74	1.19	2.32	0.00	0.00

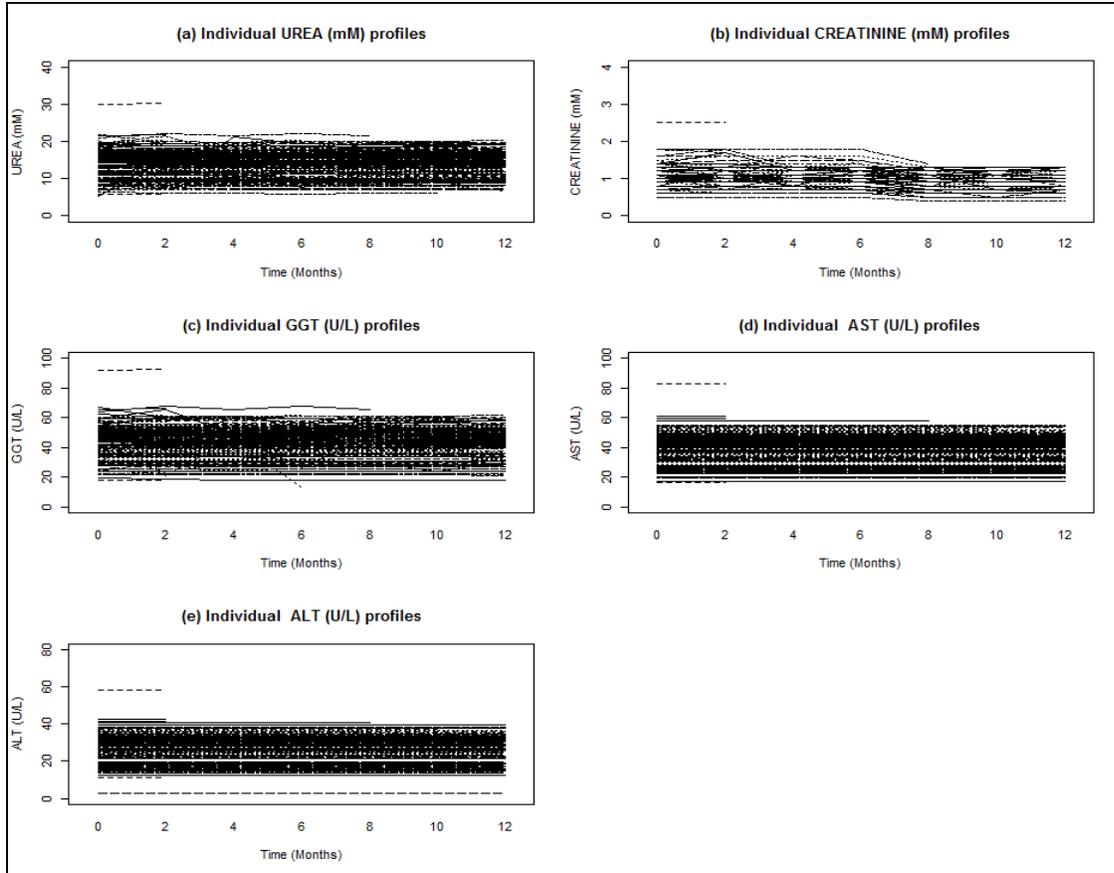# - reference level; * - significant at 5%

Figure 4.8 Biochemical parameters (Individual Profiles a – e)

Appendixx: Consent Form

THE PHYTOCHEMICAL COMPOSITION, EFFICACY AND SAFETY OF HERBAL FORMULATIONS USED FOR MANAGEMENT OF HIV AND AIDS IN MOMBASA COUNTY

Conducted by the Kenya Medical Research Institute, and the Kenyatta University Nairobi

Researcher	Institution	Study Role
Mwavita Amos Lewa	KEMRI & Kenyatta University, Nairobi	Principal Investigator
Dr. Margaret Muturi	Kenyatta University Nairobi	Principal Supervisor
Dr. Gabriel Mbugua	Kenya Medical Research Institute,	Supervisor
Prof. Eliud Njagi	Kenyatta University, Nairobi	Supervisor

Researchers' Statement

This study is being conducted by researchers from the Kenya Medical Research Institute (KEMRI), and Kenyatta University. The purpose of this consent form is to inform you so as to help you decide whether to be in the study or not.

You are allowed to ask questions about the purpose of the research, what happens if you participate in the research, the possible risks and benefits, your rights as a volunteer, and anything else about the research. When we have answered all your questions, you can decide to be included in this study or not. This process is called 'informed consent.' We will give you a copy of this form for your own records.

1. What Is This Study About?

The researchers listed above are conducting a study to learn the safety and efficacy of the herbal medicines used by people living with the HIV virus (PLWHA). We are asking patients who receive this care to understand their clinical benefits and possible other effects of these medicines. This study, aims to determine the acceptance, safety, and efficacy of herbal formulations used in the management of HIV and AIDS in Kenya. We are asking you to participate by allowing us to sample your blood and monitor your progress and development so that we can advise you better as you continue with medication. You are being asked to take part

in this study because you are an adult of ages >= 18 who is HIV-infected and receiving this care at this Herbal facility.

2. How Many People Will Take Part In This Study?

Approximately 200 people will take part on the study.

3. What Will Happen If You Decide To Be In This Research Study?

If you agree to participate in this study, the following things will happen: The researcher will conduct the interview in your preferred language of choice. During the interview, the researcher will take you through a Questionnaire. After that we will want to measure your weight and height, then will collect venous blood sample for analysis in Bomu Medical laboratories situated at Mombasa. You are free to access your analysis results and maintain your copy of the same. You will be expected to be investigated again every two months for the period of six months initially. You will also receive benefits of other interventions incase of need as you proceed with medication for these six months. The interviews will take place in a private area at the herbal health facility. Both interviews and the sampling will take place on the same day.

4. Will Any Parts Of This Study Hurt Or Have Other Risks?

Participation in this study is voluntary and declining to participate will not affect your care at any hospital. You will receive a study number and your record shall be stored as numeric however your name shall also appear with the study form and the potential risk may be loss of privacy because your name appears on this form. We will do our best to make sure that the personal information gathered for this study is kept private by storing this form in a locked cabinet.
Some procedures such as collecting blood samples from your veins may slightly hurt but be assured it is done by medical professionals. For more information about risks, ask one of the researchers.

5. Benefits

There will be no direct benefit to you from participating in this study. However, the information we finally get from investigations will help researchers in this country to appreciate the role of herbal medicines in the management of HIV and AIDS in Kenya and other parts of Africa.

6. Costs

There will be no costs to you as a result of taking part in this study.

7. Reimbursement

Since you will receive copies of your investigations as you progress and possibly other medications to assist; there will not be any reimbursement after the study.

8. What If You Have Questions?

In case you wish to ask more or have concerns about participating, please call any of these cell phone numbers 0723976988 or 0722721435 or 0727046761. You may also contact the Ethical Review committee, Kenya Medical Research Institute at Tel. 020-2722501.

9. What Are Your Choices?

Your decision to participate in research is voluntary. You are free to decline participation in the study and you can withdraw from the study at any time. If you decide not to take part in this study, there will be no penalty to you. You will not lose any of your regular benefits, and you can continue to receive health services from health facility including those participating in the study.

 You have the right to decline to be in this study, or to withdraw from it at any point without penalty or loss of benefits to which you are otherwise entitled.

.Do you provide consent to participate in this study?

☐ Yes   ☐ No

Signed by participant: Name _____Date_____

If participant is illiterate please put the right thumb print above

Witnessed by:

Name_____Signature_____Date_____

Consent obtained by:

_____

Name of staff person   Designation   Signature   Date

_____