

## An In-Vitro Evaluation of A Triple Herbal Therapy Used in Kenya For HIV/AIDS Management.

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**Background:** HIV/AIDS continues to be a major health and economic problem worldwide. By end of 2012, the national prevalence for Kenya was 5.6%. A number of interventions are in place including but not limited to upscale of antiretroviral (ARVs). Though these interventions have worked to improve the quality of life for the infected, there are challenges to be overcome owing to the high cost of the drugs, lack of proper distribution infrastructure, side effects leading to poor adherence and ultimately drug resistance to the three classes of the available ARVs. There have been concerted efforts to exploit the potential of herbal medicine in managing the pandemic. However this has been accompanied by many invalidated claims of cure by promoters of herbal medicine. In many countries, Kenya included, Complementary and alternative medicine (CAM) is largely poorly regulated. In Kenya legislation is underway with an aim of integrating traditional medicine into the country's health care delivery system. Currently herbal pharmacies are doing thriving business and the remedies are widely available in drug stores, health shops as well as in major retail outlets. In the last couple of years three herbal products have been available over the counter (OTC) for people living with HIV/AIDS (PLWHA) in Kenya. The products are; Aloe vera, medicinal mushroom (Ganotech) and Hypoxis hemerocallidea and are offered as a triple HIV herbal therapy with claims of reversing HIV/AIDS symptoms including lowering the viral load. It is imperative that systems be established to validate such products for both safety and efficacy.

**Methods:** The purpose of this study was to screen Aloe vera, medicinal mushroom and Hypoxis hemerocallidea sold as anti HIV/AIDS medicine by evaluating their in vitro anti-HIV properties and or interactions against standard treatment, HAART using two HIV strains HIV-1<sub>025RTA</sub> a clinical isolate and HIV-1<sub>IIIb</sub>. CD4 cell line C8166 was used for the assay and syncytium induction and P24 antigen ELISA used to determine virus inhibition.

**Results:** The three herbal compounds under study did not show any anti viral activity in vitro whereas the ARVs used as a control were effective in inhibiting HIV-1 replication with EC<sub>50</sub> ranging from 0.002 to 0.2 µg/ml. The herbal products actually inhibited the ARVs when assayed within the span of their EC<sub>50</sub>.

**Conclusions:** The three herbal products studied did not exhibit any anti HIV-1 activity in vitro despite being sold as treatment for HIV/AIDS, furthermore they inhibited the ARVs anti HIV activity invitro an indicator of their potential risk owing to concomitant use by PLWA.

**Key words:** HIV/AIDS- antiretroviral - natural health products (NHPs) - efficacy

### I. Background

Trade in herbal medicine is gaining acceptance globally and is now a lucrative business generating lots of revenue partly attributed to the unavailability of healthcare facilities and affordability of conventional medicine (1, 2). The Kenyan Pharmacy and Poisons Board (PPB) is involved in the registration of herbal and complementary products (the medicinal products that have been formulated in commercial manner) (3). Most of these products are imported from Asia, with the bulk coming from India China and South Africa. Insufficient data exist for most plants to guarantee their quality, efficacy and safety.

Traditional medicines are employed for the treatment of AIDS in all WHO regions. In Africa many patients with a hospital diagnosis of AIDS seek alternative treatment among traditional practice African herbal medicines in the treatment of HIV (4). *Hypoxis hemerocallidea* is a popular species in South Africa for its use in African traditional remedies and is commonly used for its immune-boosting properties in HIV patients, despite the lack of scientific evidence of clinical efficacy (5).

Even though there is widespread anecdotal evidence of openly marketed AIDS 'cures', little quantitative research has been done to assess the use of unproven treatments by PLWHA or the overlapping or concomitant use of ART and traditional medicines. One survey of PLWHA in India found that 64% had heard about a traditional treatment to cure HIV. Of those who had heard of a 'cure', 78% had tried the treatment (6). By contrast, a study in Gabon of current patients at a medical clinic found that traditional healers were consulted

first after the onset of HIV symptoms only 5% of the time, but 17% of patients sought concomitant therapy with traditional medicine and 3% sought faith healing (7). Ethnographic research in Uganda (8) and South Africa (9) suggest a complex and chaotic process of treatment seeking on the part of PLWHA. Many Kenyans believe in the potency of herbal medicine, even when they can access modern medicine. In many cases they would choose to combine both herbal and modern medicine, especially if they are afflicted with chronic ailments such as HIV/AIDS, hypertension, infertility, cancer and diabetes (10).

## II. Methods

### Test compounds and viruses.

The three herbal compounds studied were commercially sourced from herbal pharmacies in Nairobi. Each had a user instructions label on it and this was followed during the assay re formulation. Zidovudine (AZT) Abacavir (ABC), Efavirenz (EFV), Lopinavir/ritonavir (LPV/r) and Nevirapine/Lamivudine/Stavudine (NVP/3TC/d4T) were sourced from the Liverpool VCT Care and Treatment (a comprehensive care and treatment centre) in Nairobi in tablets form. Working stocks were prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) and these were stored at -20°C. Compounds were added to the cultures so that the resulting concentrations of DMSO did not exceed 0.5% by volume (to guard against its deleterious effect on cells). Laboratory adapted strain HIV-1<sub>III B</sub> and clinical isolate HIV-1<sub>025 RTA</sub> (*Gene bank ascension number JF829689/D*) were sourced from the Centre for Virus Research HIV laboratory strains bank.

### Drug susceptibility assay

**Drug susceptibility:** Antiviral assays were performed in 96 well micro titer plates (*Nunc, USA*) as previously described by Yao and Wainberg (11) with some modification. Briefly, into each well was added C8166 cells growing in the logarithmic phase ( $10^5$ /ml) and both virus added at a multiplicity of infection (MOI) of 0.001 50% tissue culture infective dose of the III B virus strain and five fold serial dilutions in cell culture media of Zidovudine (AZT) Abacavir (ABC), Efavirenz (EFV), Lopinavir/ritonavir (LPV/r) and Nevirapine/Lamivudine/Stavudine (NVP/3TC/d4T). All drug dilutions were made in Dimethyl sulfoxide (DMSO) and added to the culture. Five fold serial dilutions (range; 5µg/ml to 0µg/ml). Control wells containing cells and virus but no test compounds were co incubated on each plate. Each virus strain used in the assay was pre titrated to determine its TCID<sub>50</sub> to confirm the amount of virus per assay and enable comparison and standardization. After a 7- to 10-day incubation at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air, the supernatants were tested for viral growth using an enzyme-linked immunosorbent assay specific for the p24 antigen of HIV-1 (*Vironistika, Biomereux, France*) as described by the manufacturer. The percentage inhibition of viral growth compared with control wells without drugs was calculated. The IC<sub>50</sub> was determined from the dose response curves generated. All experiments were performed at least twice and mean percentage inhibition determined by the formulae;

$$\% \text{ Suppression} = \frac{\text{Control P24 (pg/ml)} - \text{Test P24 (pg/ml)}}{\text{Control P24 (pg/ml)}} \times 100$$

### 2. Screening assay for herbal products

*Aloe vera*, *Ganotech* and *Hypoxis hemerocallidea* sold as anti HIV/AIDS medicine in herbal pharmacies were prepared following herbal pharmacy prescription instructions. Aqueous solutions were made and five fold dilutions made with concentrations ranging from 0.2 to 2µg/ml. Briefly, in each well was added C8166 cells growing in the logarithmic phase ( $10^5$ ) and HIV<sub>III B</sub> added at a multiplicity of infection (MOI) of 0.01 and five fold serial dilutions in cell culture media and added to the culture. Five fold serial dilutions (range; 5µg/ml to 0µg/ml). Control wells containing cells and virus but no test compounds were co incubated on each plate. Each virus strain used in the assay was pre titrated to determine its TCID<sub>50</sub> to confirm the amount of virus per assay and enable comparison and standardization. After a 7 to 10-day incubation at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>, the supernatants were tested for viral growth using an enzyme-linked immunosorbent assay specific for the p24 antigen of HIV-1 (*Vironistika, Biomereux, France*) as described by the manufacturer.

### 2. MTT assay for cytotoxicity

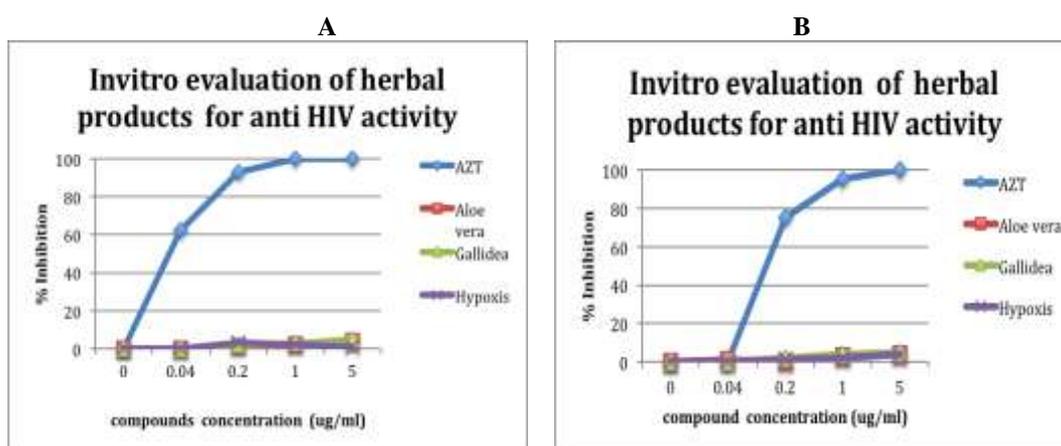
The cellular toxicity of herbal products and ARVs on C8166 cells was assessed by MTT colorimetric assay as described previously (12). Briefly, 100ul of C8166 cells were seeded on a 96 well microtiter plate in a concentration of  $1 \times 10^5$ /ml, 100ul of various concentrations of the test compounds were added and incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> for 72 h. 100ul of the cell suspension was discarded from each well and 20ul of MTT reagent (Roche, Indianapolis USA) was added and incubated for 4 h, after the incubation 100ul of solubilization buffer was added to dissolve the formazan crystals. After the formazan crystals were dissolved completely, the plates were read on an enzyme-linked immunosorbent assay (ELISA) reader (Lab

systems, USA) at 540nm. The results are shown as absorbance values and the percentage cell viability (CV) calculated manually using the formula:

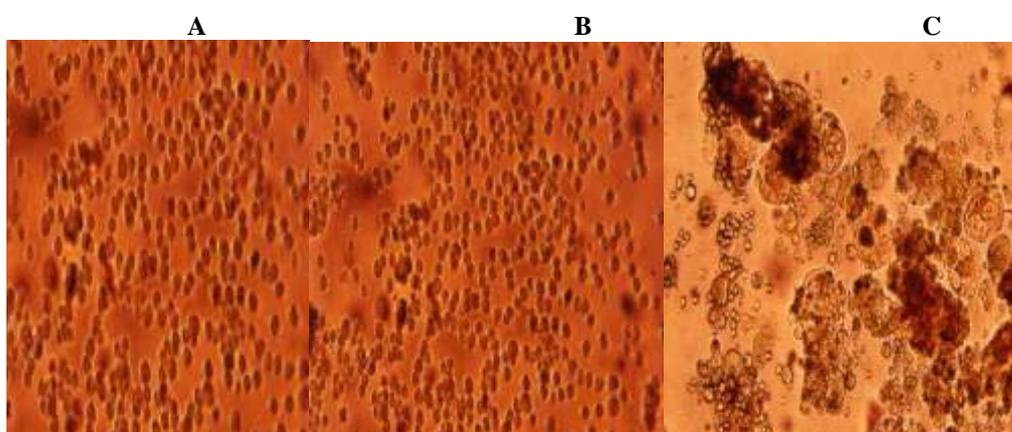
$$CV = \frac{\text{Average absorbance of duplicate drug wells}}{\text{Average absorbance of control wells}} \times 100$$

### III. Results

The three herbal compounds under study did not show any anti viral activity in vitro (figures 1 and 2) neither did they exhibit cytotoxicity on C8166 cells even at higher concentrations. These findings suggest that the herbal compounds sold as anti HIV/AIDS treatments in Nairobi Kenya have no activity against HIV and therefore cannot be used as a substitute for HAART. Contrary to showing synergy with ARVs the herbal compounds actually inhibited anti viral activity of the ARVs did not show any anti viral activity in vitro whereas the ARVs were effective in inhibiting HIV-1 replication with EC<sub>50</sub> ranging from 0.002 to 0.2µg/ml (data not shown save for AZT). These findings suggest that the three herbal compounds sold as anti HIV/AIDS treatment have no activity against HIV and cannot be used as a substitute for HAART, however on a brighter note the herbs did not show cytotoxicity even at higher concentrations.



**Figure 1:** Drug susceptibility curves for three herbal products *Hypoxis*, *Aloe Vera* and *Gallidea*. A 5-fold serial dilution of the products was carried out in DMSO and tested in a concentration ranging from 0 to 5µg/ml and tested against HIV III B (A) and HIV 025RTA (B) in C8166 cell line. AZT and other ARVs were tested as control. Each dilution of the compound was tested in duplicate; results represent the means of two independent experiments. For the herbal products the concentrations tested were as high as 200µg/ml.



**Figure 2:** C8166 cells were plated in 96-well plates at a concentration of  $1 \times 10^5$  /ml in RPMI1640 culture medium and infected with HIV-1 025RTA at a MOI of 0.001 and various concentrations of ARVs ranging from 0 to 5µg/ml. The microplates were incubated for 3 to 5 days and cells observed daily for CPE under inverted microscope. A and C show inhibition of cytopathic effects (CPE) in C8166 cell lines by the ARVs at a concentration of 5µg/ml and mock infected control respectively. At 0.04µg/ml drug concentration (B) a typical CPE of giant multinucleated cells is seen as there was no inhibition of CPE. Magnification x 400.

#### **IV. Discussion**

The use of natural health products (NHPs) as traditional medicines (TMs) among people living with HIV/AIDS is widespread, although their effects on the pharmacokinetics of antiretroviral medicines (ARVs) have not been established. Furthermore most of the herbs and natural products are categorized as foods therefore do not have to follow the rigorous safety and efficacy regulations that are expected from prescription drugs including ARVs.

There are reports that *Hypoxis hemerocallidea* received strong support from the South African Ministry of Health for use as an immune booster for HIV-positive patients, and is purported to be one of the bestselling NHPs in South Africa (13). The cure for HIV/AIDS have been falsely promoted since the first cases were identified and claims of cures continue in spite of the development of ARVs, which can dramatically slow the progression to AIDS but do not rid the body of HIV. The uncertainty and fear engendered by this stigmatizing and life-threatening disease make people easy prey to promises of cures (14). The idea that herbal drugs are safe and free from side effects is false as there is very little data documenting the global use of herbal remedies, nutritional supplements, and traditional medicines, though believed to be widely used by PLWHA to enhance their wellbeing. Unproven AIDS 'cures', on the other hand are marketed not just to enhance wellbeing but to specifically eradicate HIV. Despite these products being promoted as 'traditional' or 'herbal', many supplements, 'cures', and treatments are produced using modern mass manufacturing techniques and with industrial chemicals that could pose certain health risks (14). The most common response by health authorities to the claims of unproven AIDS treatments has been inaction. Due to the initial unavailability of ARV drugs, many governments did not promote ART as effective and life saving and warn that other treatments are unproven and potentially dangerous (14).

Globally the examples of unproven AIDS 'cures' and treatments are widespread and have been reported in the United States, Zambia, Mexico, South Africa Thailand, India, Zimbabwe, and elsewhere. In the US an orthopedic surgeon promoted an intravenous product containing *Aloe vera* to treat AIDS and cancer, netting as much as US\$18,000 for each 2-week treatment (15). Remedies with unsubstantiated claims of healing are attractive to patients with serious health conditions because they speak in certain terms about their successes, emphasize the uncertainties and side-effects of conventional medicine and offer unrealistic hope (16) Among PLWA, unsubstantiated claims by marketers of 'immune boosting products' and fake cures are seen as credible and evoke interest in their use (17). Unproven cancer treatments promoted online, for example, cause great concern and have led to measures that counter their use (18). In South Africa courts have banned the promotion of vitamins to cure AIDS (19) after years of government support for fake AIDS treatments (20) In Tanzania there was a huge exodus of PLHA for the herbal cure and belief in the ability of alternative treatments to cure HIV was observed to be high with implications for potentially sidelining effective HIV prevention and treatment initiatives (21). One of the major factors contributing to the increasing popularity of herbs in developed countries and the sustained use in developing countries is the perception that herbal remedies are efficacious, and in some cases more so than physician-prescribed allopathic medicines. This favourable level of perceived efficacy would support continued use, and in a significant number of patients, concomitant use with conventional allopathic medicines. This scenario, of concomitant herb-drug use, raises the growing public health concern of potentially harmful interactions (22).

There is no doubt that some herbal products may have therapeutic benefits as examples from history and the recent past have provided us with effective anti-malarials (20) and cancer treatments(23). Furthermore some of the earliest forms of protease inhibitors were derived from natural products (24, 25, 26). Even though the three herbal products have previously been reported to have immune modulatory activity the three have been falsely promoted as cure for HIV/AIDS Efforts should be directed at evaluating the therapeutic efficacy of these remedies as well as the possibility of interactions through systematic in vitro studies and clinical trials.

#### **V. Conclusion**

- Despite efforts by biomedical scientists/ research community and traditional health practioners towards search for HIV/AIDS cure, ART currently remains the only form of lifesaving treatment.
- The global community and governments have committed billions of dollars to increase access to effective medicine to fight AIDS, however much needs to be done to address the problems of unproven AIDS drugs/products.
- The current study though limited showed that the herbal compounds interfered with the antiviral activity of ART. It is imperative that more research be carried out to determine the active ingredients and document the potential interactions involving the herbal products used by PLHA. The extent to which this failure represents actual harm is as yet largely unquantified, but the existence of it is an unfortunate testament to the limitations of relevant stakeholders to fulfill their mandates to protect from harm and realize the right to health of those living with HIV/AIDS.

- The three herbal products studied did not exhibit any anti HIV-1 activity in vitro despite being sold as treatment for HIV/AIDS and therefore join a long list of unproven cures for HIV/AIDS.

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