FINAL REPORT

PROJECT TITLE: **OBSERVATION AND EVALUATION FOR EFFICACY AND SAFETY OF TRADITIONAL HERBAL REMEDIES USED IN THE MANAGEMENT OF HIV/AIDS IN TANZANIA.**

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Executive summary

1.0. BACKGROUND

The first cases of AIDS in Tanzania were reported in Kagera region in 1983 (1). Subsequently, other regions also started reporting cases and by 1985 all regions in Mainland Tanzania had documented HIV/AIDS as an emerging public health problem. By end of 2003, it was estimated that about 1.8 million Tanzanians were living with HIV/AIDS and about 700,000 cumulative AIDS cases had occurred (1). The epidemic in Tanzania is caused mainly by three HIV-1 subtypes, including subtypes A, C and D (2), and to a limited extent by recombinant strains (2). Generally, it is estimated that in Tanzania the major subtypes A, C and D occur at frequencies of 40%, 40% and 20%, respectively (1-2). Transmission in Tanzania is mainly heterosexual, hence the highest prevalence and incidence is among the sexually active age groups. All sectors of the society have been affected, and HIV/AIDS has had profound health and socio-economic impact in Tanzania like in other endemic areas of the world.

Following infection, the virus establishes a persistent infection in cells of the haemopoietic system as well as the nervous system. $CD4^+$ T-lymphocytes, which have the CD4 receptor for HIV, are the cells most affected by HIV. A variable period of chronic asymptomatic infection is established. Some individuals remain asymptomatic for up to 10 years (usual progressors), while others (fast progressors) show signs of disease progression within a few years after seroconversion (3). A small group, less than 5% of infected individuals (long-term survivors) remain without clinical symptoms for more than 10-15 years (3). Ultimately, a progressive HIV infection leads to depletion of $CD4^+$ T-lymphocytes in the majority of cases. Since $CD4^+$ T-lymphocytes play a central role in mediating immune responses, their depletion leads to profound immunodeficiency. The severe immunodeficiency characteristic of AIDS is associated with various opportunistic infections, malignancies and neurological disease. Opportunistic infections and reactivated latent infections seen in HIV infected persons vary from region to region according to the different types of endemic pathogens found in these regions. However, the most commonly encountered opportunistic infections in developing countries include bacterial infections (e.g. tuberculosis, salmonellosis, pneumonia), viral infections (e.g. infections with human herpesviruses, cytomegalovirus, human papillomavirus) and fungal infections (e.g. Candidiasis, Cryptococcosis). Of the opportunistic malignancies, Kaposi's sarcoma and non-Hodgkins lymphomas are quite common.

In the early phase of the HIV/AIDS epidemic, management of patients focused mainly on the treatment of opportunistic infections, nutritional care and attempts at immunological reconstitution. During recent years, a number of anti-retroviral (ARV) drugs have been developed, and their use, especially in combined regimens e.g. highly active anti-retroviral therapy (HAART) has led to prolonged survival and better quality of life of people living with HIV (4). Use of ARV is associated with improved immune status, fewer episodes of opportunistic infections and tumours, and lower risk of mother – to –

child transmission of HIV (4). Since the introduction of HAART in 1995-96, death rates and the number of diagnosed cases of AIDS have decreased in areas where the therapy is available (4). However, HAART is very expensive, and therefore not an absolute realistic option for care for HIV/AIDS patients in developing countries where the majority of HIV infected people lives. Long term toxicity, complicated regimens that require stringent monitoring and emergence of ARV drug resistant strains are additional limitations for use of HAART (5-6). Newly developed antifungal and antibacterial drugs have made it possible to treat most opportunistic infections effectively.

The optimism arising from use of HAART regimens and effective remedies for opportunistic infections have stimulated interest from national and international partners in the fight against HIV/AIDS, to develop a strategy of accessing care to the majority of people living with HIV/AIDS in developing countries. In line with this initiative, Tanzania has developed National guidelines for clinical management of HIV/AIDS (7). These guidelines provide recommendations and criteria for use of ARV drugs and treatment of opportunistic infections. The Ministry of Health has also developed a National HIV/AIDS treatment plan which became operational since 2003. This plan provides for free ARV treatment to people living with HIV/AIDS. The drug access initiative intends to find mechanisms of procuring ARV drugs at a relatively cheaper cost and sustaining their use where indicated. However, there are valid concerns regarding sustainability beyond the donor-funding period. Therefore, affordability to the majority of affected individuals will remain a major drawback.

This project was done as part of realization that HIV/AIDS is a significant public health problem in Tanzania and that there is not yet a cure or effective vaccine. The available ARV drug regimen retard disease progression and improve quality of life of patients with HIV/AIDS but they are too expensive for most of the patients in resource-poor communities and they have serious side effects, especially among the malnourished that constitute a significant group.

BROAD OBJECTIVE

The main objective of the study was to evaluate efficacy and safety of traditional medicines that are being used by traditional health practitioners for the management of HIV/AIDS.

The specific objectives of the study were:

(i) To determine socio-anthropological aspects associated with the selection of remedies for treatment of HIV/AIDS

(ii) To monitor the anti-HIV efficacy of the herbal preparations prescribed by traditional health practitioners (THPs) in patients

(iii) To monitor safety and/or toxicity in patients being treated for HIV/AIDS by the traditional health practitioners.

(iv) To determine safety and/or toxicity of the herbal preparations using laboratory animals

(v) To determine chemical profile of the herbal extracts

EXPECTED OUTCOME

- The primary expected endpoint was lowering of viral load and increase of absolute CD4 counts within research subjects
- Documentation of traditional herbal remedies that can alleviate secondary HIV/AIDS associated infections
- Documentation of perceptions and beliefs associated with HIV/AIDS aetiology and understanding of the process involved in the identification of traditional therapies for the treatment of HIV/AIDS conditions
- Documentation of medicinal plants with leads for: antiviral, antifungal, antibacterial, antiprotozoa, anticancer and immunomodulating activity
- Identification of possible toxic plant extracts through use of laboratory animals
- Documentation of chemical profile of effective herbal extracts

METHODOLOGY

1. Recruitment of Collaborating Traditional Health Practitioners.

Structured (open and closed) questionnaires were used to interview several THPs both in Arusha and Dar es Salaam urban centres. Based on the interview, THPs were scored for knowledge on HIV/AIDS (19). A total of 33 TPHs, from Dar es Salaam were recruited in 2003 and 8 TPHs from Arusha in 2004. In both cases, a series of seminars/workshops were conducted so as to educate TPHs on issues concerning collaborative modalities with Biomedical Researchers, Intellectual Property Rights (IPR), and general ethical conduct of TPHs.

2. Recruitment of Patients

Patients attending traditional therapies were interviewed for their willingness to participate in the study and only consenting patients were recruited. They signed two copies of consent forms of which one copy was retained at the Institute and one was given to the patient or guardian.

3. Patient evaluation and monitoring

The primary aim of the study was to observe, monitor and evaluate HIV/AIDS-infected patients undergoing traditional herbal therapy, by selected reputable THPs, for changes in their immunological status, quality of life and incidence of opportunistic infections. This clinical monitoring study assessed 277 HIV-seropositive adults attending herbal therapy in DSM and Arusha. The patients were initially evaluated using ELISA for HIV-1 antibody status, viral load counts, CD4 and CD8 counts. They were then followed-up using the same laboratory indicators, quality of life and incidence of opportunistic infections for a period of two years. The HIV/AIDS patients were physically assessed at predetermined time points as they continued to use herbal therapy during the whole follow-up period. The collaborating traditional health practitioners were advised to administer the treatments themselves and monitor compliance. Monitoring of patients included the following parameters.

a) Safety
   - Renal function tests (serum creatinine)
   - Liver function tests (ALAT and ASAT)
   - Haematological indices (F B P, Platelet count, bleeding and clotting times)
   - Allergic reactions

b) Clinical progression
   - Serial physical examinations
   - Body weight
   - Documentation of illness episodes

C) Virological and immunological indices
   - Viral load
   - Absolute counts of $CD4^+$ and $CD8^+$-T-lymphocytes

These parameters were taken initially on the starting date, then on a weekly basis during the first month, then monthly for the next three months and subsequently every six months. A change by 5% of the upper limits of normal ALAT and serum creatinine or the lower limits of the haematological parameters was considered as evidence of toxicity.

STUDY FINDINGS

Recruitment of collaborating traditional health practitioners

The entry point and characteristics of traditional health practitioners

Three traditional health practitioners ' associations namely Tanzania Traditional Healthcare Practitioners Association (TATHEPA) in Arusha and Dar-es-Salaam Municipalities, African Traditional Medicine Men (ATME) – in Dar-es-Salaam and Chama cha Utafititi wa Magonjwa Sugu (literally meaning an association that investigates chronic diseases/illnesses) Tanzania (CHAUMUTA) in Dar-es-Salaam were involved in the consultative meetings as well as in the project. In both Dar-es-Salaam and Arusha consultative meetings took two days in each association. The key concerns and issues arising during consultative meetings by leaders of traditional health practitioners (chairman, secretary and executive committee) included: what the benefit of the project to traditional healers would be; which referral system would be used, disclosure agreement and patent rights; terms of reference of the collaboration and in contracts; and uncontrolled massive exploitation of medicinal plants by biomedicine prospectors.

In Arusha, the leaders of TATHEPA were in the fore front of uniting traditional healthcare practitioners by making them agree to participate in the study as well as in the collaboration for the benefit of both the traditional health practitioners and the Institute of Traditional Medicine. Further they were involved in mobilizing traditional health practitioners to come out and be interviewed. A similar response of harmonising traditional health practitioners to appear for interview was noted in Dar-es-Salaam.

In Arusha, with the help of leaders of traditional health practitioners ' associations, a total of 132 traditional health practitioners were interviewed; of which 30.3% (40) were females. In Dar-es-Salaam, on the other hand, a total of 60 traditional health practitioners were interviewed and of these 25% (15) were females. In all study areas the ages of the respondents were between 25 and 60 years. Female traditional health practitioners tended to cluster between age 31 to 50 years. The analysis of the findings show that most of them had primary education (70%), few had secondary education (5%) and the rest were illiterate. Further, in the study areas nearly all ethnic groups of Tanzania were represented; and hence different cultural backgrounds of the country were captured. During the first year of the project 33 traditional health practitioners were engaged as collaborators whose patients were monitored and evaluated. Among these only 18 THPs, 11 in Dar es Salaam, and 7 in Arusha qualified, based on patient performance, to continue with the study up to the reporting time. The results that are presented in this report are results that emanate from the 18 THPs. The remaining THPs did not get patient acceptance and as a result some had no patients at all, while others remained with too few patients to qualify for statistical analysis.

Traditional Health Practitioners knowledge on HIV/AIDS

On the question of awareness of HIV/AIDS, respondents were rated poor if they could not mention any symptoms of HIV/AIDS, those mentioning one to two symptoms were rated fair, whereas those who mentioned more than three were rated good. The analysis of the findings showed that more than 75% of respondents could at least mention one key symptom as defined by WHO [3] like diarrhoea and persistent cough for more than one month, weight loss, general body weakness and repeated fevers. Most of the healers were rated average (49%) on the knowledge of HIV/AIDS (see table 3). Further, the analysis showed that 40% of the respondents have attempted to treat HIV/AIDS patients. Nevertheless, there was some misinterpretation in the management of HIV/AIDS. For example, 10% of the total

respondents claimed to have cured HIV/AIDS patients. The indicators to the respondents who claimed to have cured AIDS patients were HIV positive patients becoming negative, giving birth to a healthy child, the patients becoming healthy after the remedies and gaining weight.

One of the problems which the research team encountered in this study was traditional health practitioners ' unwillingness to mention medicinal plants used in the management of HIV/AIDS. They were willing to mention the number of medicinal plants that composed their remedies. For instance, 50% of the respondents reported that their remedies were composed of more than ten medicinal plants; and about 10% of the respondents reported that their remedies were composed of more than 100 medicinal plants and other ingredients. In addition, there was a problem of keeping records. Only 30% of the respondents had records of the patients they have treated.

Handling cultural aspects like rituals in healthcare

The research team learned that about 10% of the respondents believed that there was man made HIV/AIDS (caused by witchcraft, sorcery or evil eye or ancestral spirits) which had similar symptoms to actual HIV/AIDS; and it is only the traditional health practitioners who can identify such cases through *ramli* (divination); and were meeting such patients in their daily practice. The management of the 'man-made' HIV/AIDS required the use of rituals. The research team was sceptical on the man made HIV/AIDS because it is not in line with the theory of HIV/AIDS causation in biomedicine. This was one of the dilemmas the research team faced in associating the 'man-made' HIV/AIDS with the actual cause of HIV/AIDS.

Expectations of traditional healers from collaborations with researchers

All traditional health practitioners interviewed showed they would like to collaborate with the Institute of Traditional Medicine. This was further confirmed, during the educational seminars on issues raised by traditional health practitioners like how to handle HIV/AIDS patients when treating them, various modes of transmission of HIV/AIDS, hygiene, record keeping. Traditional health practitioners ' responses to the educational seminar were very good. Several questions were asked and mainly focused on; CD4 and CD8, Viral load and ESR, question of turning negative after treatment; health and giving birth to a healthy baby without HIV/AIDS as a sign to have treated HIV/AIDS and issues of mother to child transmission. Also issues of depletion of medicinal plants through biopiracy, fires and poor harvesting methods, particularly the rare and endangered species were raised. On the other hand the staff from the Institute of Traditional Medicine and some of the medical doctors who participated in the project were willing to collaborate with the traditional healers and become partners in the research project.

Recruitment of Patients (Research participants)

A total of 277 patients, 151 from Dar es Salaam and 126 from Arusha participated in the study, among which 155 (56%) completed the follow-up period of 2 years. Seventy five (27.1%) absconded/dropped out of the study, and 47 (16.9%) were reported to have died. In the case of the 75 patients who absconded, 6 (8.0%) had been transferred from their working place; 14 (18.7%) were advised to join ARV clinics due to deteriorating CD4 cell count and 55 (73.3%) had their reasons not revealed to the study.

Table 2. Socio-demographic characteristics of Patients at recruitment in Dar es Salaam and Arusha.

	Dar es Salaam (n = 151)		Arusha (n = 126)		Total	
	Number	%	Number	%	Number	%
Sex						
Male	32	22.2	26	20.6	58	20.9
Female	119	78.8	100	79.4	219	79.1
Age						
< 30	28	18.5	35	27.8	63	22.7
31 – 40	79	52.3	62	49.2	141	51
41 – 50	31	20.5	25	19.8	56	20.2
>51	13	8.6	4	3.2	17	6.0
Marital Status						
Single	28	18.5	12	9.5	40	14.4
Married	44	29.1	41	32.5	85	30.7
Divorced	20	13.2	31	24.6	51	18.4
Widowed	53	35.1	42	33.3	95	34.3
Cohabiting	6	4.0	0	0	6	2.2
Educational level						
No formal education	11	7.3	3	2.4	14	5.1
Primary education	91	60.3	103	81.7	194	70.0
Secondary education	43	28.5	19	15.1	62	22.4
College education	5	3.3	1	0.8	6	2.2
University education	1	0.7	0	0	1	0.4
Occupation						
House wife	60	39.7	40	31.7	100	36.1
Business	47	31.1	42	33.3	89	32.1
Employed	30	19.9	15	11.9	45	16.3
Small self activities	14	9.2	11	8.7	25	9.0
Peasant	0	0	18	14.3	18	6.5

As indicated in Table 2, the study was conducted in Dar-es-Salaam and Arusha. Patients that were recruited for this study consisted of females (78.8% and 79.4%) and males (22.2% and 20.6%), in Dar-es-Salaam and Arusha stations, respectively. Overall, the male population was relatively low compared to that of females (20.9% vs 79.1%). Majority of the registered patients ranged between the age of 30 and 50 years and their marital status ranged from single, married, divorced, widowed and a relatively small percentage of cohabiting (4%). Interesting to note is that 92.4% of all clients had either primary and/or secondary

education, whereas 2.6% had college/University education. Only 5.1% of all clients reported as having no formal education. In terms of economic status, the study population registered 36.1% participants as house wives, whereas 32.1% were engaged in business. In addition, 16.3% of all patients were employees and 9.0% were engaged in small scale self activities. Only 6.5% among all patients reported to be peasants.

Assessment of safety and/or toxicity of traditional herbal remedies

Monitoring for toxic effects was done on the starting date, then weekly during the first month, then monthly for the next three months and subsequently every six months. A change by 5% of the upper limits of normal ALAT and serum creatinine or the lower limits of the haematological parameters was considered as evidence of toxicity. Serum creatinine was measured as a marker to be able to follow renal function for all clients. The upper and lower limits for serum creatinine were considered based on the normal range, i.e., 62-97 µmol/L for males and 55-80 µmol/L for females.

Figures 1a and 1b indicate that overall, there was a slight upward trend in serum creatinine levels for patients seen by Traditional health Practitioners in Arusha, while creatinine levels for patients seen by THPs in Dar es Salaam remained stable.

Liver function performance as an indicator for toxicity

The levels of alanine transaminase lwhich was used as a marker for liver toxicity remained within the normal range as indicated in Figures 1a and 1b below.

Fig 1a. Liver function test (ALAT) among patients recruited in Dar es Salaam

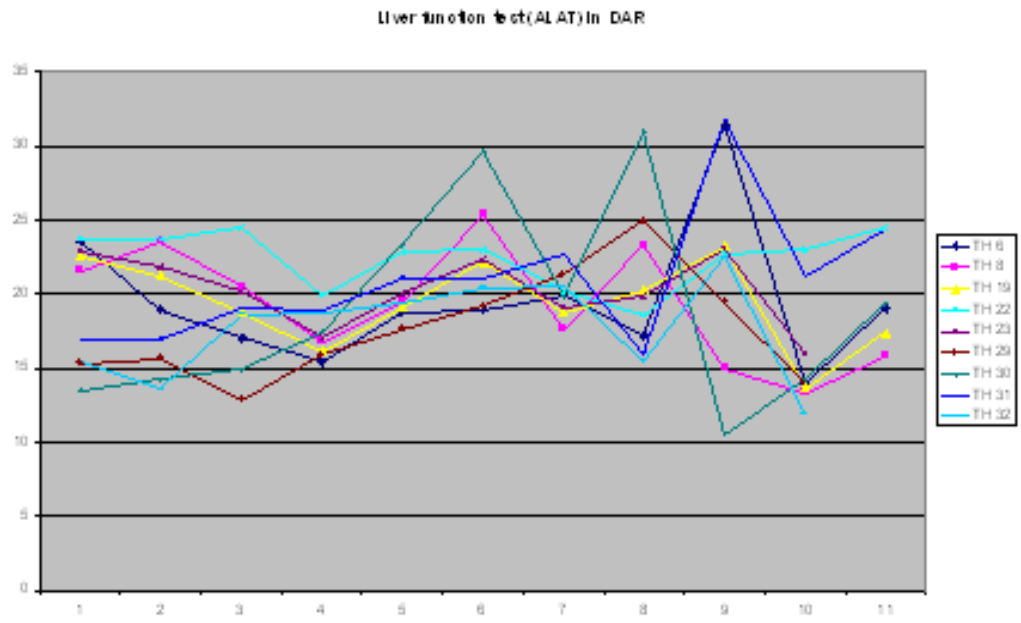
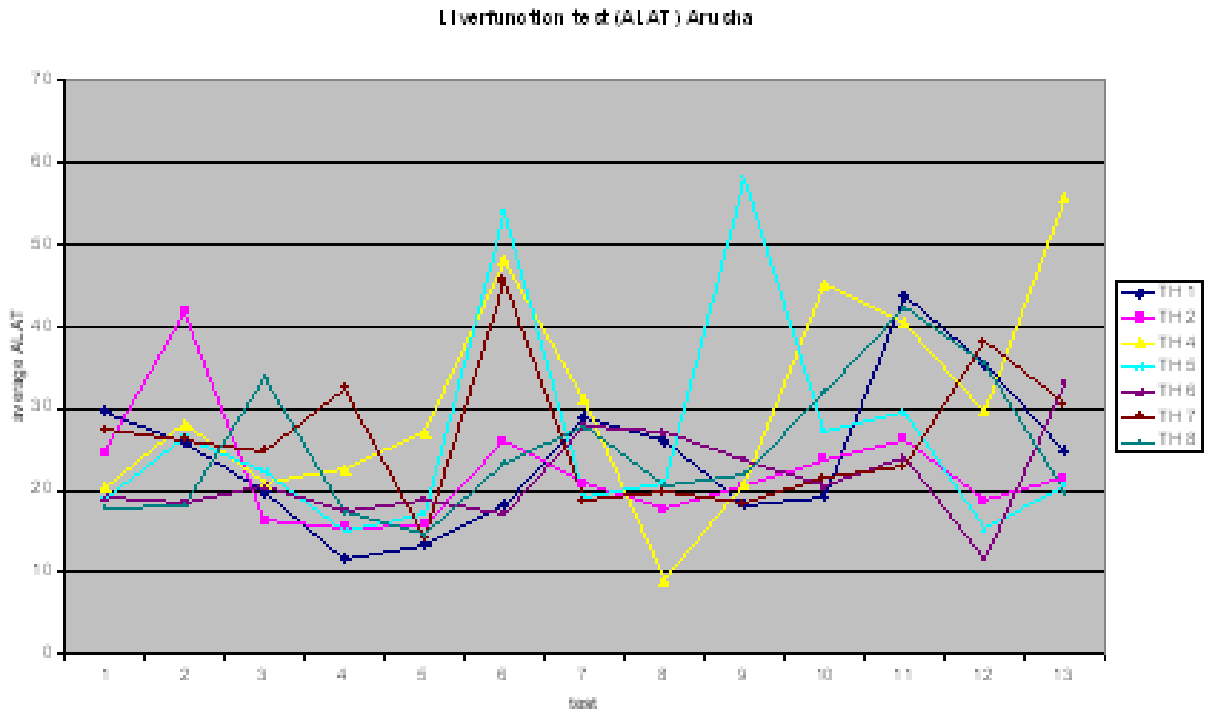


Fig 1b. Liver function test (ALAT) among patients recruited in Arusha



In the case of Haematological indices, white blood counts (WBC) weres determined, which results show that the levels remained within the normal range as indicated in Figures 2a and 2b.

Fig 2a. WBC among clients recruited in Dar es Salaam

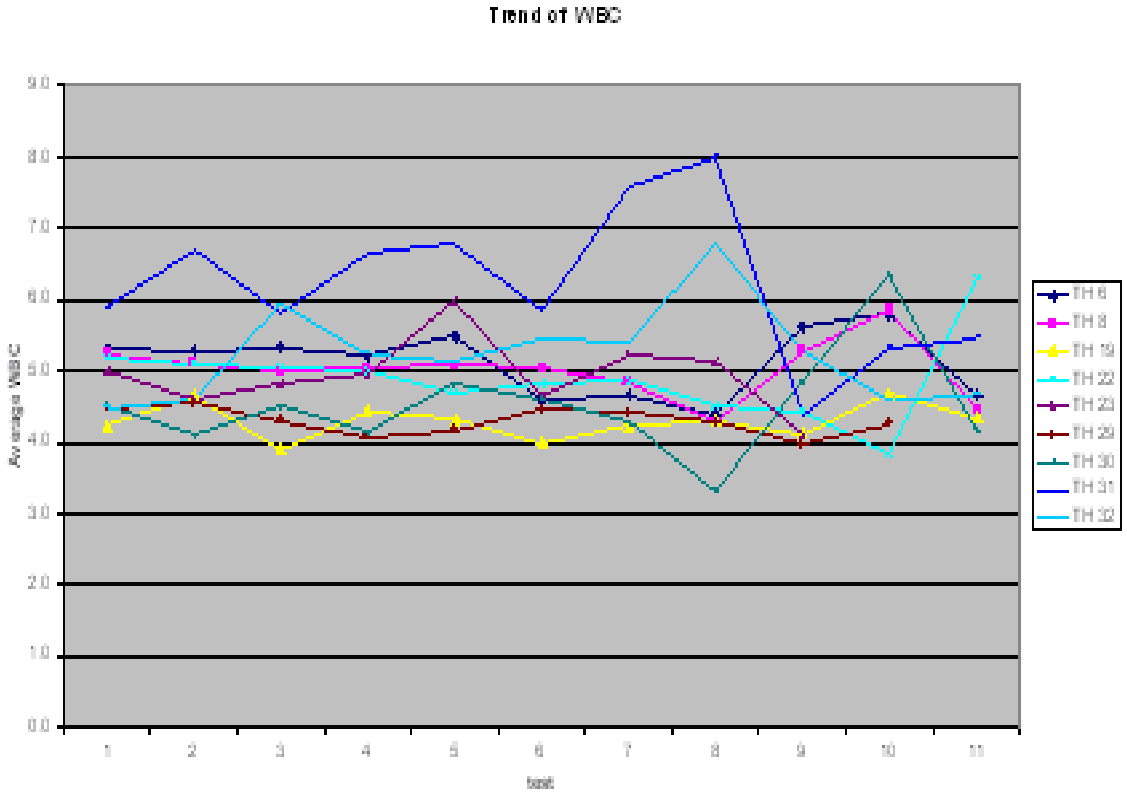
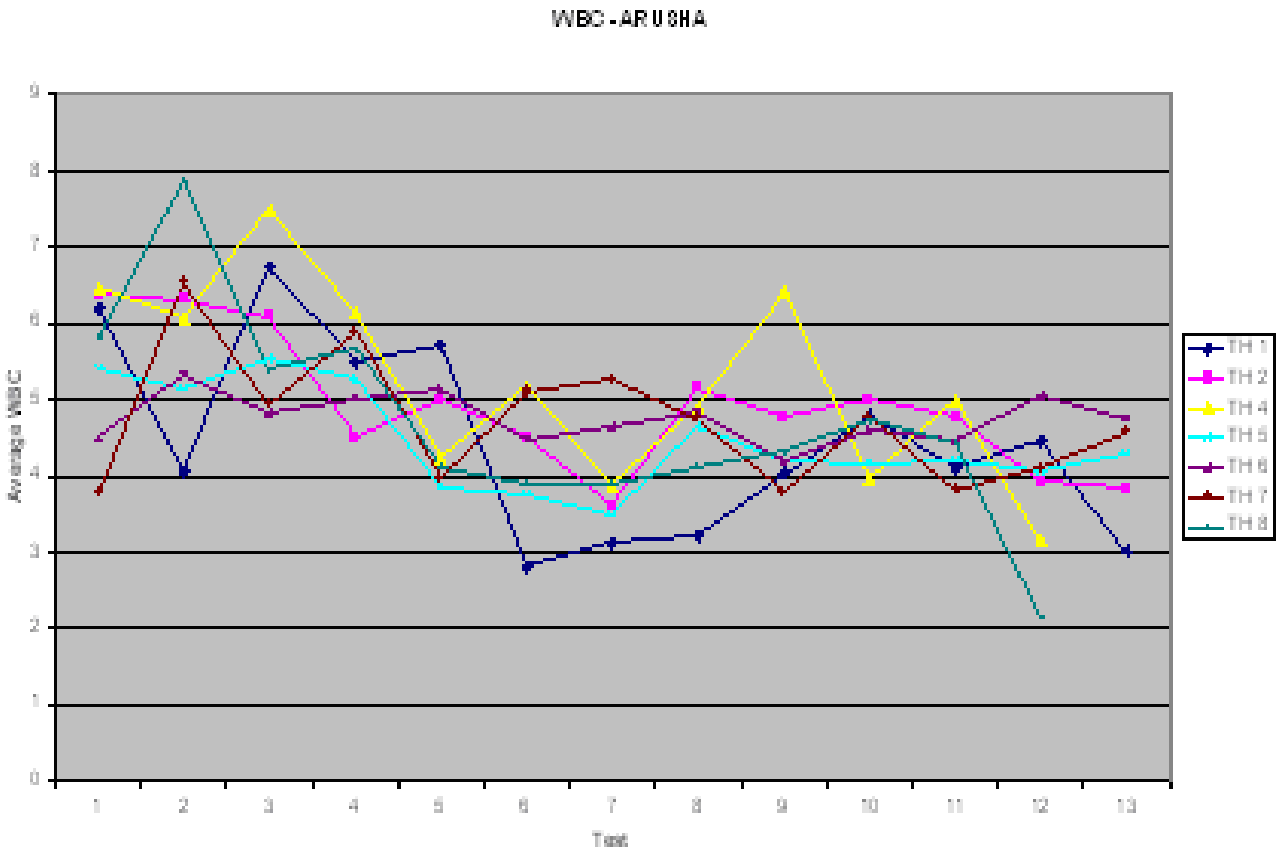


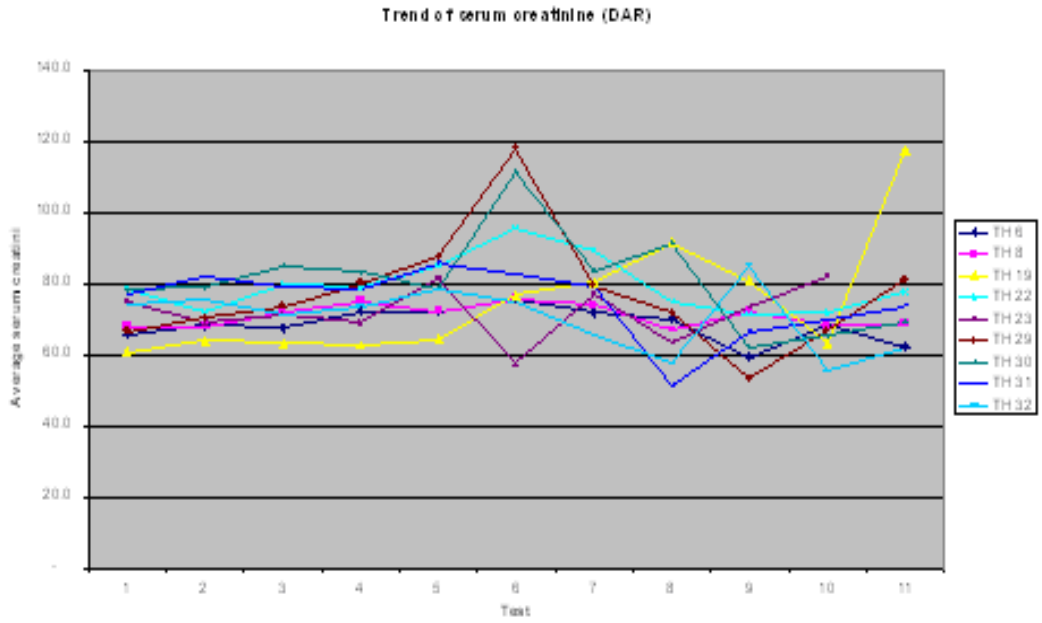
Fig 2b. WBC among patients, recruited in Arusha



Renal function test was assessed by measuring serum creatinine levels, which fluctuated minimally among the patients being managed by both the Dar es Salaam and Arusha Traditional Health Practitioners as indicated in figures 3a and 3b.

Fig.3a. **Serum creatinine among patients, recruited in Arusha**

Fig.3b. **Serum creatinine among patients, recruited in Dar es Salaam**



Patients were also monitored physically and in clinical symptoms as a way to detect any signs of toxicity or deterioration. Patients were monitored for symptoms and signs that might cause discomfort and hence induce unwanted conditions. Relevant conditions and symptoms considered for HIV/AIDS included headache, dizziness, abdominal pain, nausea /vomiting and diarrhea among others. All the mentioned conditions were reported by few patients as indicated in Tables 3 and 4 in the course of traditional therapies indicating good tolerance which might reflect signs of safety.

Table 3. Reported symptoms and signs of side effects in relation to TPHs in Dar es Salaam

	No of patient	Headache	Dizziness	Abdominal pain	Nausea/ vomiting	Diarrhea
TH 2	15	2	0	1	1	2
TH 6	11	0	2	1	0	0
TH 8	12	0	0	0	0	0
TH 19	29	2	0	1	1	2
TH 22	24	2	0	1	1	1
TH 23	6	0	0	0	0	0
TH 29	15	0	0	0	0	0
TH 30	10	1	0	1	0	0
TH 31	17	0	0	1	0	1
TH 32	12	0	0	0	0	0
Total	151(100%)	7 (4.6%)	2 (1.3%)	6 (4.0%)	3 (2.0%)	6 (4.0%)

Table 4. Reported symptoms and signs of side effects in relation to THPs in Arusha

	No of patient	Headache	Dizziness	Abdominal pain	Nausea/ vomiting	Diarrhea
TH 1	16	3 (18.8%)	4 (25.0%)	1 (6.3%)	2 (12.5%)	0
TH 2	19	2 (10.5%)	3 (15.8%)	2 (10.5%)	2 (10.5%)	1 (5.1%)
TH 4	15	7 (46.7%)	3 (20.0%)	2 (13.3%)	2 (13.3%)	1 (6.7%)
TH 5	15	0	1 (6.7%)	3 (20.0%)	3 (20.0%)	0
TH 6	18	4 (22.2%)	2 (11.1%)	3 (16.7%)	4 (22.2%)	2 (11.1%)
TH 7	19	2 (10.5%)	2 (10.5%)	4 (21.1%)	1 (5.3%)	1 (5.3%)
TH 8	22	4 (18.2%)	3 (13.6%)	3 (13.6%)	2 (9.1%)	1 (4. %)

ASSESSMENT OF CLINICAL CONDITION OF PATIENTS BEFORE AND AFTER TREATMENT

Cllinical condition for patients was assessed at recruitment and during each follow-up visit. Tables 5 and 6 show the prevalence of key clinical signs and symptoms of patients at recruitment compared to reported incidence of the conditions during the follow-up period. Overall, there was a decrease in occurrence of specific signs and/or symptoms following treatment. For example, in both sites there was observed a marked decrease in patients reporting persistent fatigue, fever, cough and body itching. There was however noted a slight increase in the percentage of patients reporting to have difficulty in swallowing and persistent diarrhea compared to magnitude at baseline.

Table 5. Clinical progression among 151 Patients recruited in Dar es Salaam

	Initial visit		During follow up	
	No of patient n= 151	Percentage (%)	Reported episodes n=3437	Percentage (%)
Fatigue	31	20.5	98	2.9
Persistent fiver	13	8.6	97	2.8
Oral ulcers	10	6.6	15	0.4
Oral thrush	4	2.6	45	1.3
Difficult in swallowing	2	1.3	69	2.0
Chronic Diarrhea	2	1.3	55	1.6
Cough for a month	20	13.2	55	1.6
Genital discharge	9	6.0	33	1.0
Genital ulcer	8	5.3	30	0.9
Skin rash	32	21.2	131	3.9
Body itching	33	21.9	158	4.7
Kaposi's Sarcoma	3	2.0	24	0.7

Table 6. Clinical progression among 126 Patients recruited in Arusha

	Initial visit		During follow up	
	No of patient n= 126	Percentage (%)	Reported episodes n = 1309	Percentage (%)
Fatigue	75	59.5	28	2.1
Persistent fiver	46	36.5	26	2.0
Oral ulcers	3	2.4	0	0
Oral thrush	28	22.2	0	0
Difficult in swallowing	9	7.1	22	1.7
Chronic Diarrhea	18	14.3	17	1.3
Cough for a month	40	31.7	3	0.2
Genital discharge	29	23.0	1	0.08
Genital ulcer	9	6.3	1	0.08
Skin rash	26	20.6	0	0
Body itching	48	38.1	0	0
Kaposi's Sarcoma	1	0.8	0	0

Change in body weight

Figure 4a and 4b show that the use of traditional herbal remedies was associated with stabilization or increase in body weight. Indeed for the Arusha cohort there was an overall upward trend in body weight increase.

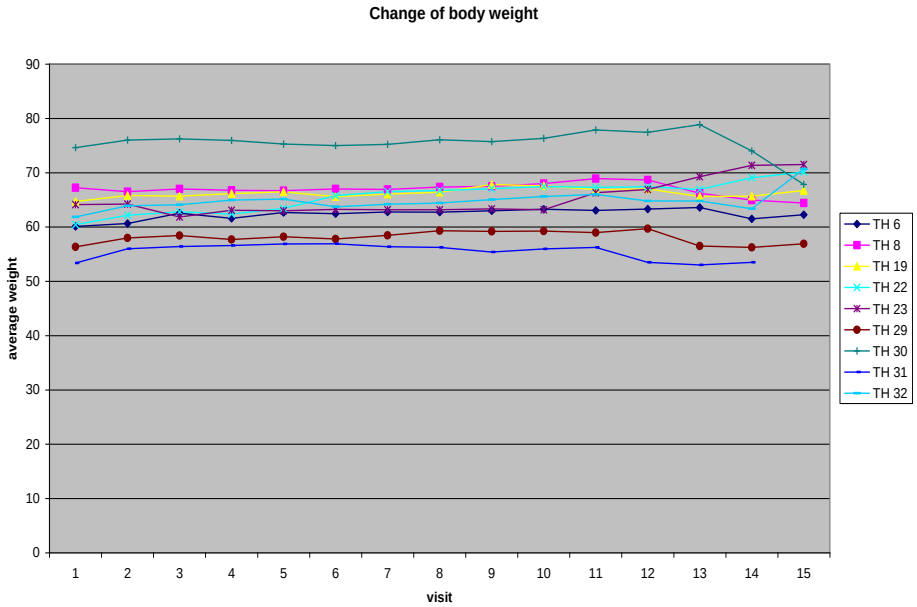


Figure 4a. Weight change among clients recruited in Dar-es-Salaam

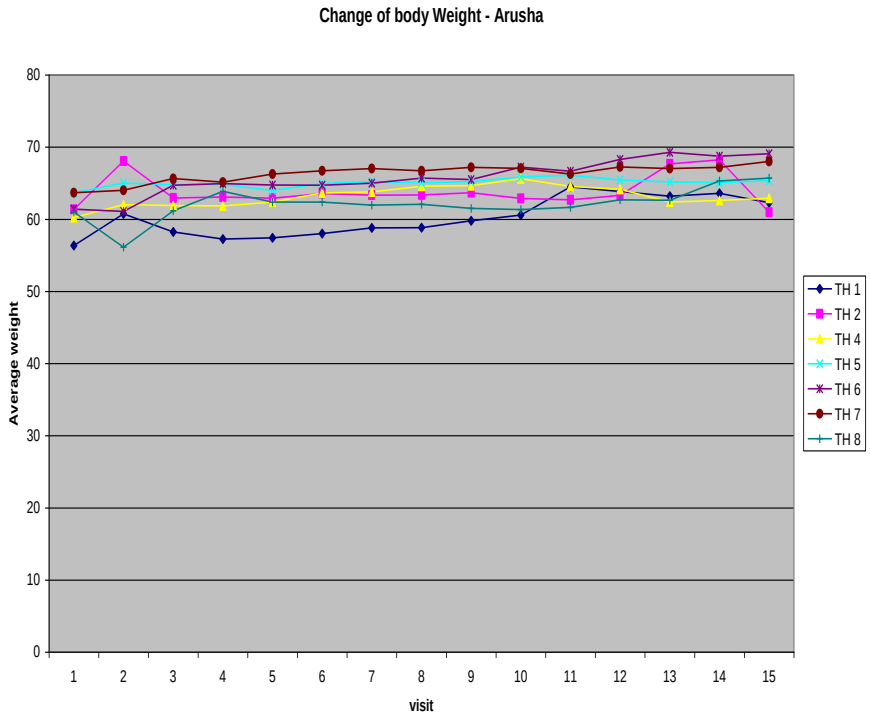


Fig. 4b. Weight change among clients recruited in Arusha

EFFECT OF TRADITIONAL MEDICINE ON THE PERFORMANCE OF IMMUNOLOGICAL PARAMETERS

Figures 5a and 5b show the CD4 profile among patients recruited in Arusha . The two figures show that there was an overall increase or stabilization of CD4 cell counts during the whole two year follow up period.

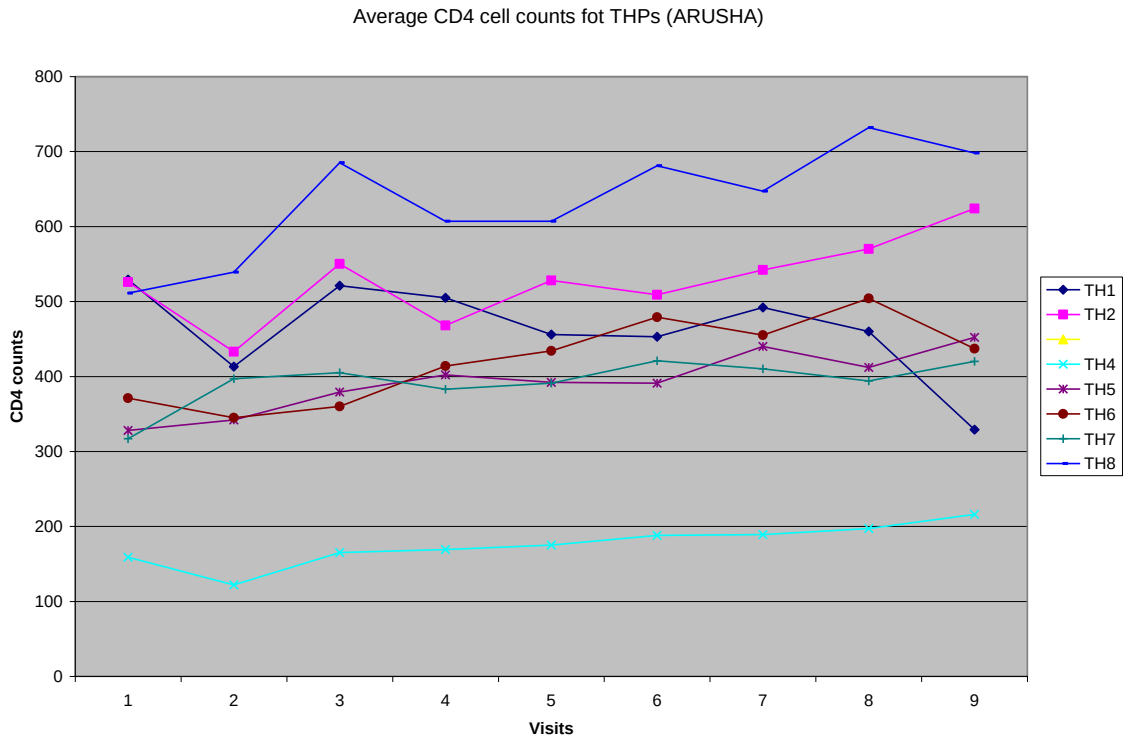


Fig 5a. CD4 profile of patients recruited in Arusha

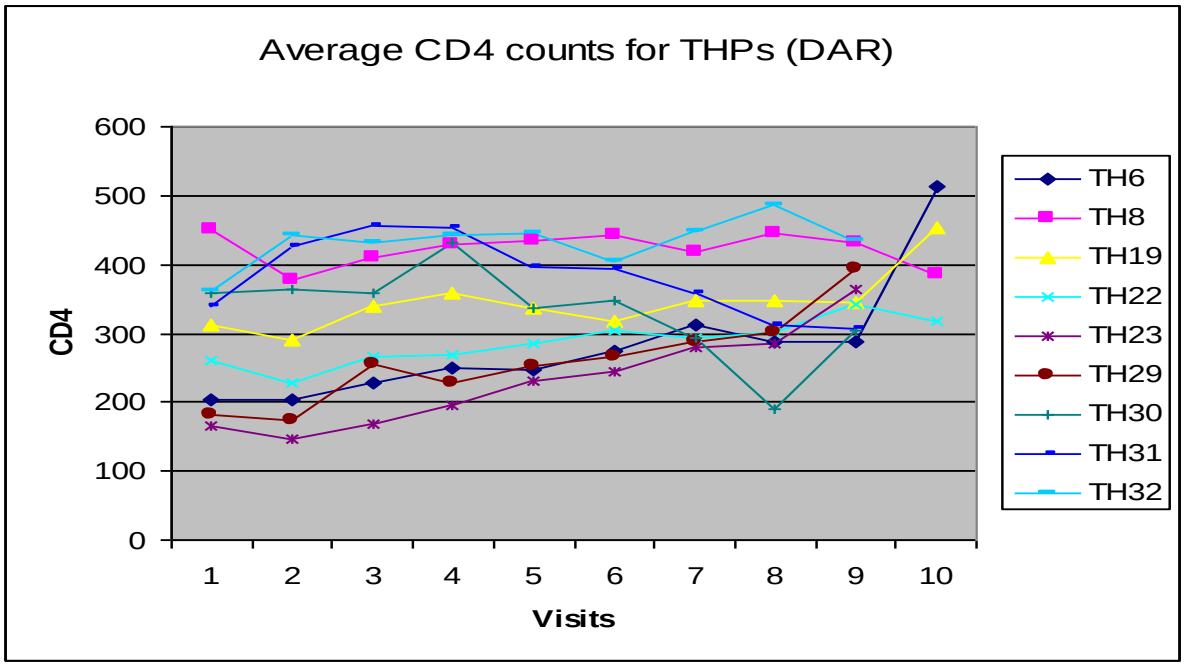


Fig 5b. CD4 profile of patients recruited in Dar es Salaam

Changes in Viral load levels.

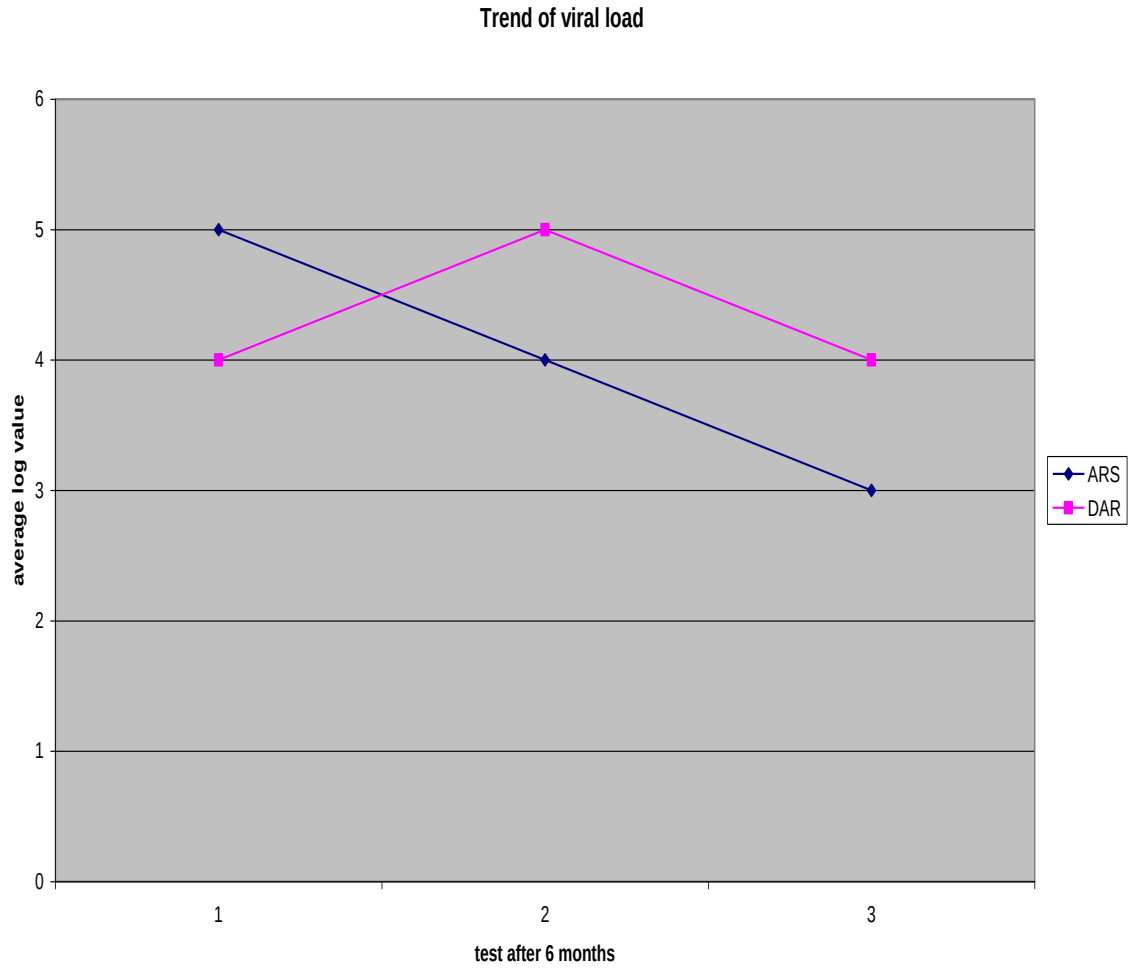


Fig 6a. Trend in viral load levels among patients recruited both in in Dar es Salaam and Arusha

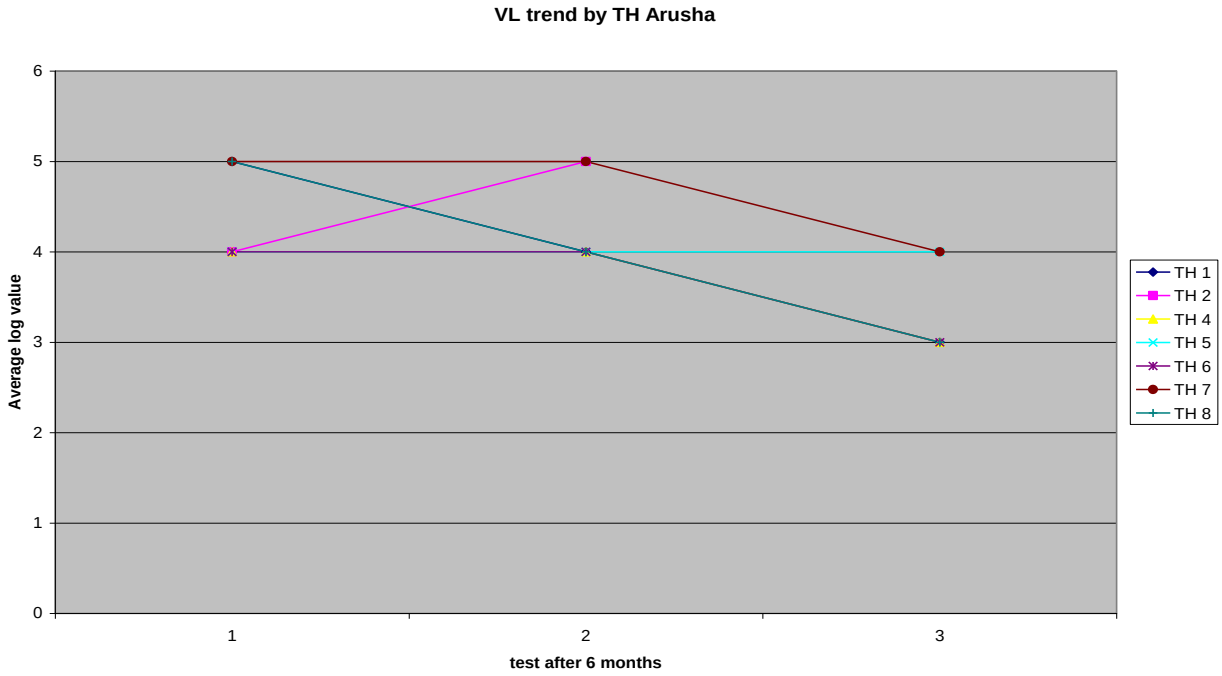


Fig 6b. Trend in viral load levels among patients recruited in Arusha

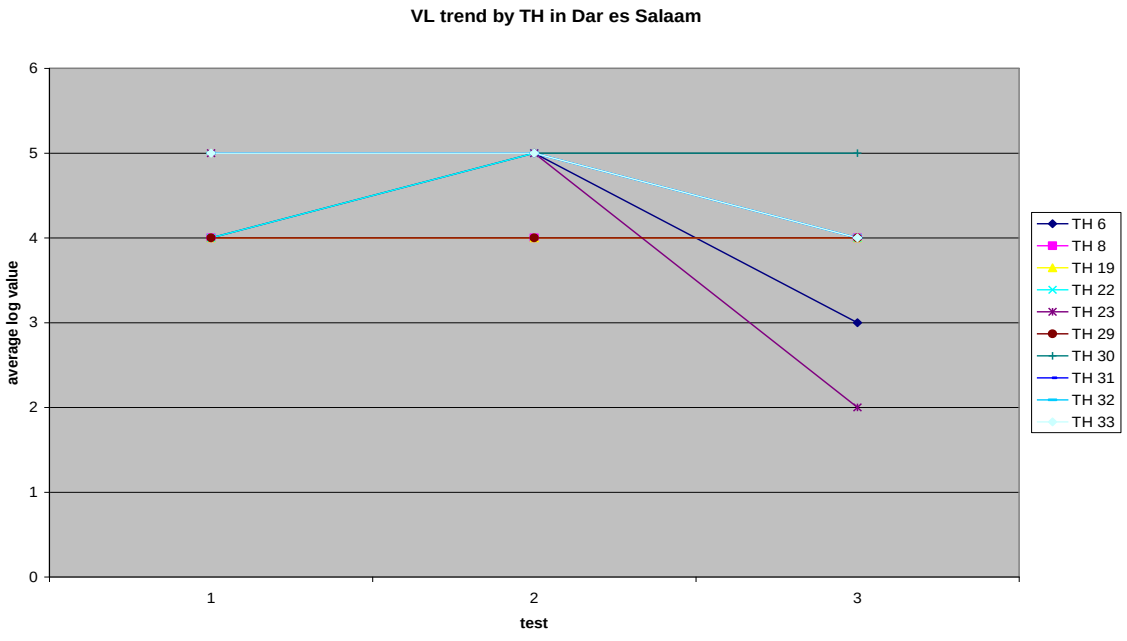


Fig 6c. Trend in viral load levels among patients recruited in Dar es Salaam

Documentation of traditional herbal remedies that can alleviate secondary HIV/AIDS associated infections

Alongside the clinical evaluation of patients on traditional medicines, interviews were done in Morogoro, Tanga, Bagamoyo and Singida to identify plants that are specifically used for the treatment of fungal infections. A total of 56 plant species were mentined by traditional health practitioners to be used in the treatment of different fungal infections. Aqueous methanol extracts of these plants were tested for antifungal activity against different Candida species, specifically, to identify candidate plants which may be useful for the management of Candida infections in HIV patients. Table 7 shows the antifungal activity of some of the plants.

Table 7: MICs and MFCs of plant extracts showing complete inhibition of fungal growth. MIC_0 and MFC in mg/ml.

Botanical name: Family, Species	**Plant part**	**C.a**	**C.g**	**C.p**	**C.t**	**C.k**	**Cr.n**
		MIC_0(MFC)	**MIC_0(MFC)**	**MIC_0(MFC)**	**MIC_0(MFC)**	**MIC_0(MFC)**	**MIC_0(MFC)**
ANACARDIACEAE 5 *Sclerocarya birrea Sond*	R	0.25 (-)	- (-)	0.125 (-)	0.063 (-)	0.063 (-)	0.25 (-)
BIGNONIACEAE 27 *Kigelia africana L.*	Fr	- (-)	- (-)	- (-)	- (-)	- (-)	1 (-)
CARYOPHYLLACEAE 56 *Drymaria cordata (L) A. Schult*	WP	- (-)	- (-)	- (-)	- (-)	4 (4)	- (-)
CELASTRACEAE 7 *Elaeodendron buchannanii (Lows)*	SB	- (-)	- (-)	- (-)	0.25 (-)	0.063 (-)	0.031 (-)
CELASTRACEAE 36 Elaeodendron schlechteranum (Lows)	SB	- (-)	- (-)	- (-)	- (-)	2 (-)	0.25 (-)
COMPOSITAE 13 *Tagetes minuta L*.	L	- (-)	- (-)	- (-)	0.5 (-)	1 (-)	0.5 (-)
CUCURBITACEAE 28 *Senecio deltoidea Less*	WP	- (-)	- (-)	- (-)	0.25 (0.5)	- (-)	- (-)
DENNSTRAEDIACEAE 42 *Pteridium aquilium (L) Kulm*	L	- (-)	- (-)	- (-)	0.5 (-)	0.5 (-)	- (-)
ERICACEAE 61 *Agauria salicifolia Oliv.*	L	- (-)	0.5 (4)	1 (-)	4 (-)	2 (-)	1 (-)
EUPHORBIACEAE 58 *Spirostachys africana sonder*	S	2 (2)	1 (2)	- (-)	- (-)	- (-)	1 (-)
EUPHORBIACEAE	WP	- (-)	- (-)	- (-)	4 (4)	- (-)	4 (-)

33 *Euphorbia heterophylla* L.							
EUPHORBIACEAE 25 *Jatropha multifida L.*	S	- (-)	- (-)	1 (-)	0.25 (0.25)	- (-)	1 (-)
EUPHORBIACEAE 44 *Jatropha multifida L.*	R	- (-)	- (-)	- (-)	- (-)	- (-)	4 (-)
MELIACEAE 40 *Khaya anthotheca C.DC*	SB	- (-)	- (-)	- (-)	- (-)	2 (-)	- (-)
MELIACEAE 14 *Turraea holstii Gurk*	L	0.5 (-)	- (-)	1 (-)	0.063 (0.5)	0.5 (-)	0.125 (-)
MIMOSACEAE 47 *Acacia nilotica (L) Willd ex Del.*	SB	- (-)	- (-)	0.031 (-)	0.063 (-)	1 (1)	4 (-)
MIMOSACEAE 43 *Acacia robusta subsp Usambarensis (Taub) Brenan*	L	- (-)	1 (-)	0.063 (-)	0.5 (1)	0.031 (-)	4 (-)
MYRSINACEAE 11 *Rapanea melanophloeus (L) Mez*	SB	- (-)	- (-)	- (-)	- (-)	1 (-)	0.5 (-)
RHAMNACEAE 57 *Ziziphus pubercens Oliv*	L	- (-)	- (-)	- (-)	- (-)	1 (-)	4 (-)
RUBIACEAE 38 *Hymenodictyon parvifolium Brig*	R	- (-)	- (-)	- (-)	- (-)	- (-)	0.125 (-)
RUTACEAE 37 *Clausena anisata Oliv*	SB	1 (1)	4 (-)	0.063(0.125)	0.125(0.25)	0.25 (0.5)	0.25 (0.25)
RUTACEAE 34 *Zanthoxylum chalybeum L.*	RB	- (-)	- (-)	2 (-)	2 (4)	2 (2)	2 (-)
SOLANACEAE 39 *Solanum incanum L.*	L	- (-)	- (-)	4 (-)	4 (-)	- (-)	- (-)
STERCULIACEAE 23 *Sterculia africana (Lour) Fiori*	L	- (-)	- (-)	1 (-)	0.063 (0.5)	0.25 (-)	1 (1)
VITACEAE 10 *Cyphostemma hildebrandtii (Gilg) Desc*	L	- (-)	1 (-)	1 (-)	0.25 (0.5)	1 (-)	0.5 (-)
VITACEAE 31 *Rhoicissus tridentata (Fresm) Placium*	Tub	- (-)	- (-)	- (-)	- (-)	0.125 (-)	- (-)

Where: **MIC_0** Minimum Inhibitory Concentration that inhibit growth by 95-100%
MFC Minimum Fungicidal Concentration

- No in vitro activity at concentration less than 4 mg/ml.

C.a *Candida albicans*
C.g *Candida glabrata*
C.p *Candida parapsilosis*
C.t *Candida tropicalis*
C.k *Candida krusei*
Cr.n *Cryptococcus neoformans*

L leaves, S Stem, ST Stem bark, Fr Fruits, Tub Tubor, WP Whole plant, R Root, RT Root Bark

Evaluation of cytotoxic, genotoxic and CYP450 inhibitory effects of Tanzanian plants traditionally used for treatment of fungal infections

The tests for antifungal activity in Table 7 revealed twelve extracts with potent antifungal activity. Although the plants may be good candidates for new treatment opportunities, they can be mutagenic or toxic and could cause pharmacokinetic interactions when used concomitantly with antiretroviral medications. Therefore, they were investigated for cytotoxicity, mutagenicity and cytochrome P450 (CYP) interaction potential.

Mutagenicity was tested by VITOTOX assay and Hoechst33342, Alamar Blue, Calcein-AM, glutathione depletion and O_2-assays were used for cytotoxicity testing. Inhibitory activities of the twelve extracts on substrate metabolism by CYP3A4, 2C9, 2C19 and 2D6 was tested with high throughput CYP inhibition screening. PXR inductive activities were tested using CHO cell lines expressing hPXR. Herbs exhibiting high hPXR activities were tested for CYP3A4 inductive activities with QPCR.

Mutagenicity was found for *Jatropha multifida, Sterculia Africana and Spirostachys Africana*. All plant extracts showed high cytotoxic effects in almost all tests. Potent inhibitory activities on CYP3A4, 2C6, 2C9 and 2C19 were found for 75% of the herbs. *Spirostachys africana* did not affect CYP2D6 and for *Sterculia africana* and *Turraea holstii* no effect on CYP2D6 and CYP3A4 (DBF) was found. Sixty seven percent (67%) of the herbs showed significant activation of hPXR, but only *Turraea holstii* and *Sterculia africana* significantly induced CYP3A4. These results indicate the possibility for cytotoxicity and potential of medicinal plant-ARV interactions.

Results

Cytotoxicity

The effects of the 12 medicinal plant extracts on mitochondrial activity, cellular proliferation, damage to the cell membrane, glutathione depletion and radical formation were tested with Alamar blue, Hoechst33342, Glutathione depletion, Calcein-AM uptake and O_2 assays, respectively. All plant extracts were tested in a concentration range from 8 to 500 µg/ml. This range was based on the minimal concentrations (MIC_0) of the herbs that inhibited growth of *Candida* sp. by 100%. In table 8 the results of the cytotoxicity assays are shown.

The effects on mitochondrial activity of the 12 medicinal plant extracts were tested with alamar blue assay and showed that all herbs, except *Pteridium aquillinum* and *Turraea holstii*, reduced the NADPH content. *Eleaodendron buchannanii* had the highest effect on mitochondrial activity with a MTD of 62,5 µg/ml, while *Pteridium aquillinum* and *Turraea holstii* showed no effect at concentrations up to 500µg/ml.

The effect on cellular proliferation tested with Hoechst 33342 for the 12 medicinal plant extracts showed that seven medicinal plant extracts (*Acacia nilotica, Acacia robusta, Agauria salicifolia, Cyphostemma hildebrandtii, Elaeodendron buchannanii, Jatropha multifida, spirostachys africanan, Sterculia Africana*) reduced DNA levels in the concentration range of 8 and 500 µg/ml. *Cyphostemma hildebrandtii* had the highest effect on cellular proliferation with a MTD of 62,5µg/ml, while again for *Pteridium aquillinum* and *Turraea holstii* no effects were found.

Glutathione depletion

The depletion of glutathione revealed that eleven of the twelve medicinal herbs induced glutathione depletion in the concentration range of 8 and 500 µg/ml. *Turraea holstii* had no effect on glutathione depletion.

Calcein-AM uptake

The calcein-AM uptake assay used to assess damage to the cellular membrane showed that nine medicinal plant extracts induced cellular membrane damage. *Jatropha multifida* had the strongest effect on the membrane integrity with a MTD of 31.3 µg/ml.

O2 test

The O2 test performed to test O_2 uptake/ release by HepG2 cells showed that except for *Sterculia africana* and S*pirostachys africana*, all herbs showed toxicity in this test. Highest effect was found for A*gauria salicifolia* with a MTD of 31.3 µg/ml.

Vitotox test

Mutagenic effects were found for *Sterculia africana,*, *Jatropha multifida* and *Spirostachys africana* with minimal mutagenic doses of 1.00, 0.5 and 1.00 mg/ml, respectively. No mutagenicity was measured. after metabolic activation (addition of S9) However, the results indicated also that many of the plant extracts were toxic according to the above mentioned criteria, and that this toxicity could mask the genotoxic response.

CYP450 inhibition

The IC_{50} values for the 12 medicinal plant extracts are shown in table 5.
All herbs showed inhibitory activity on the metabolism mediated by CYP2C9, CYP2C19 and CYP3A4 (7BQ and DBF) and CYP2D6 in a dose dependant manner. All herbs had potent inhibitory activities with IC_{50} values less than 100µg/ml against CYP2C9 and CYP2C19. The most potent inhibitor of CYP2C9 was *Acacia nilotica* (1.03µg/ml), while *Jatropha multifida* is the most potent inhibitor of CYP2C19 (4.26µg/ml). No inhibitory activity on CYP2D6 was found for *Spirostachys aficana*, *Sterculia africana* and *Turraea holstii*, while the IC50 values of the other nine herbs ranged from 2.96-79.4µg/ml. The most potent inhibitor of CYP2D6 was *Cyphostemma hildebrandtii* (2.96µg/ml). Except for *Sterculia africana* all herbs potently inhibited the activity of CYP3A4 when 7BQ was used as substrate. Furthermore, all herbs with exception of *Sterculia africana* and *Turraea holstii,* potently inhibited the activity of CYP3A4 when DBF was used as substrate. *Cyphostemma hildebrandtii* was also found to be the most potent inhibitor of CYP3A4 when DBF and 7BQ were used as substrate (1.26 and 2.69µg/ml, respectively).

PXR assay

Table 6 shows inductive activities of the Tanzanian medicinal plants on pregnane X receptor are shown. Significant PXR induction with induction factor > 2.00 was found for *Agauria salicifolia* (5.44), *Cyphostemma hildebrandtii* (2.41), *Elaeodendron buchannanii* (4.49), *Jatropha multifida* (5.9), *Pteridium aquillinum* (3.58), *Sclerocarya birrea* (3.33), *Sterculia africana* (2.16), and *Turraea holstii* (4.12). The highest inductive activity was found for *Agaura salicifolia* with PXR stimulation factor of 5.44 at dosage of 63µg/ml.

CYP3A4 induction

The concentrations of the medicinal plants that showed the highest PXR induction were also tested for CYP3A4 induction. Significant induction of CYP3A4 was only found for *Turraea holstii* and *Sterculia africana*, both at concentrations of 125µg/ml, while the lower dosage showed no significant induction (table 3).

Discussion

The VITOTOX data indicated that *Sterculia africana*, *Spirostachys africana* and *Jatropha multifida* were genotoxic and many herbs showed a toxic response. Furthermore, all herbs showed high cellular toxicity in one or more assays. The herb with the highest toxicity was *Acacia nilotica*, whereas *Turraea holstii* had the lowest toxicity. It was also revealed that almost all herbs potently inhibited CYP2C9, 2C19, 2D6 and 3A4. While 70% of the herbs potently induced PXR activity, only for *Turraea holstii* and *Sterculia africana* was high inductive activity on CYP3A4 found.

Genotoxicity of the twelve plant extracts was evaluated using the VITOTOX test. This test is based on *Salmonella typhimurium* TA104 recN2-4 that contains a *lux* operon of the luminescent marine micro-organism *Vibrio fischeri* under transcriptional control of the recN gene, which is part of the SOS system. When a genotoxic compound is added to the bacterial strain, the RecN promoter becomes derepressed resulting in expression of the *lux* operon. This expression results in light production as function of genotoxicity. Another control strain *S. typhimurium* TA 104*pr*1, that has a constitutively expressed *lux* operon, is used for detection of false positive responses (when compounds act directly on light production or enhance the metabolism of the bacteria, light is produced here) or of a toxic response (when compounds are bacteriotoxic and decrease light intensity because the micro-organisms are killed) (Verschaeve et al., 1999). Our test results indeed revealed that many of the herbs have a toxic response. The found toxicity is probably caused by bactericidal activity of the herbs against *Salmonella typhimurium*. In a separate study testing African plant extracts in the VITOTOX test found a high toxic response, and the authors proposed that herbal extracts might interfere with the *lux* operon, thereby affecting the results (Elgorashi et al., 2003). So although we found three herbs with mutagenicity, the toxic response found for the other plant extracts might mask the genotoxic effects. This may have been the case for *Acacia nilotica* (Arora et al., 2005)*, Clausena anisata* (Uwaifo, 1984) and *Pteridium aquillinum* (Bonadies et al., 2004; Castillo et al., 1998; Marrero et al., 2001; Potter et al., 2000; Rasmussen et al., 2005) for which previously genotoxicity has been reported (table 6).

For the cytotoxicity assays, the effects of the twelve herbal extracts on cellular toxicity were studied in human cell lines. These assays are a reliable method for high throughput screening of toxicity effects of potential medicinal compounds (Schoonen et al., 2005b; Schoonen et al., 2005a). We found high toxicity for almost all of the herbs at concentrations that were based on the antifungal activity of the herbs. This high toxicity may suggest that the potent antifungal activities of these herbs are a toxicity effect rather than a specific effect on the fungi. However, most of the herbs inhibited the growth of the *Candida* sp. by 100%, but had no killing effect. Furthermore, each plant extract had different antifungal effects on the diverse *Candida* species tested. For example all herbs potently inhibited *Candida krusei*, while *C. albicans* was only inhibited by *Clausena anisata*, *Sclerocarya birrea* , *Spirostachys africana* and *Turraea holstii*.

According to the cellular toxicity effects as measured in the five assays, the herbs were ranked from high to low toxicity in table 4. Overall, *Acacia nilotica* was shown to have the highest toxicity. It was previously reported that rats that were fed an 8% *Acacia nilotica* diet for up to 4 weeks their body weights were significantly reduced and serum cholesterol and serum total protein significantly decreased. Symptoms reversed one week after treatment termination, leading the authors to conclude

that *Acacia nilotica* had a low toxicity potential (Al-Mustafa et al., 2000). These findings illustrate that results obtained with *in vitro* tests can not easily be extrapolated to *in vivo* effects. In consistence with the *in vitro* data for *Clausena anisata* and *Pteridium aquillinum* toxicity in animal study was reported and for *Agauria salicifolia*, *Jatropha multifida* and also *Pteridium aquillinum* toxicity in humans has been reported (table 7). For *Acacia robusta*, *Sterculia africana* and *Elaeodendron buchananii* no earlier reports on toxicity were found.

In a recent review article potential interactions between commonly used herbs and antiretroviral agents have been described (Ref.). These interactions can be based on inhibition or induction of CYPs involved in ARV metabolism. Our results revealed that 75% of the herbs potently inhibit CYP3A4, CYP2C9, 2C19 and 2D6. A study on 30 Indonesian medicinal plants also revealed that 63% gave significant inhibition of CYP3A4 and CYP2D6 (Usia et al., 2005). In the aforementioned review it was shown that indeed many herbs can inhibit CYP enzymes. For example, 33% of the American 20 Top-selling herbs were shown to inhibit CYPs involved in ARV metabolism, and have potential for interaction with ARVs (van den Bout-van den Beukel CJ et al., 2006).CYP3A4 is the most important enzyme involved in the metabolism of all PIs and NNRTIs. Our results revealed that, with exception of *Sterculia africana* and *Turraea holstii* and when DBF is used as substrate, all herbs potently inhibited CYP3A4. Therefore, usage of these herbs concomitantly with ARVs may lead to high plasma levels and hence toxicity. However, potent inhibition of CYP2C9, 2C19 and 2D6 was also found and might be involved in interaction with atazanavir, nelfinavir and ritonavir, respectively.
Turraea holstii and *Sterculia africana*, were shown to have no inhibition on CYP2D6 and only *Turraea holstii* potently inhibited CYP3A4 when 7BQ was used as substrate. Remarkably, although 67% of the herbs showed PXR inductive activities, only these two herbs also showed CYP3A4 induction. Since PXR regulates the expression of CYP3A4 genes, we determined whether the found activation indeed led to CYP3A4 expression, which was, however, not the case for each herb. Thus, usage of *Turraea holstii* and *Sterculia africana* might lead to induction of CYP3A4 resulting in sub-therapeutic plasma levels and development of drug resistant HIV. To date, the only clinically significant interactions with ARVs were found for garlic, *Cyphosterma hildebrandtii* (SJW) and milk thistle and were suspected to be based on CYP3A4 induction (van den Bout-van den Beukel CJ et al., 2006).

Results of *in vitro* studies might however not correspond with the activity *in vivo*. For example, St John's wort (SJW) in some *in vitro* studies was reported to be a potent inducer of CYP2B6 (Goodwin et al., 2001b), CYP2C9, CYP2C19 (Zou et al., 2002a) and CYP3A4 (Goodwin et al., 2001a; Moore et al., 2000; Wentworth et al., 2000; Zou et al., 2002b). Contradictory, using cDNA-expressed enzymes, crude SJW extracts have been shown to inhibit the activities of CYP2D6, CYP2C9, CYP2C19 and CYP3A4 (Obach, 2000). While *in vivo* in HIV patients SJW was reported to lead to a 35% increase in the clearance of nevirapine (de Maat et al., 2001) and to decrease plasma concentrations of indinavir with 57% in healthy volunteers (Piscitelli et al., 2000). Both interactions are suspected to be caused by induction of CYP3A4.

Additionally, the preparation (oral or topical) of each herb influences the final *in vivo* toxicity and interaction potential. The potent inhibition of CYP might be of less importance for topical agents, but is very relevant when the herb is used orally.
Another important consideration remains that the *in vivo* concentrations of these medicinal plant extracts are unknown. The tested concentrations in the cytotoxicity and CYP450 interaction tests are however based on the found antifungal concentrations for these herbs.

In conclusion, the VITOTOX test seems not to be appropriate for testing of the genotoxicity of plant extracts so another test should be used for future testing. Furthermore, almost all herbs have been shown to have high cytotoxicity and high CYP inhibitory potential which may result in toxic ARV plasma levels. *Turraea holstii* and *Sterculia africana* were the only herbs found to significantly induce CYP3A4. Usage of these herbs by patients on ARVs might lead to sub-therapeutic plasma levels and

development of drug resistant HIV strains. These results indicate that more research on the interaction potential of (African) herbal medicines is urgently needed. Furthermore the awareness of HIV/AIDS health care workers and the general public for the risks of herb-ARV interactions needs more attention.

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List of abbreviations

ARV, antiretroviral agent; CYP450, cytochrome P450; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

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Table 8. Twelve most potent herbal plant parts used for treatment of fungal infections and other diseases in Tanzania[a]

Family	Species (voucher specimen No.)	Local name	Part used	Life form	Region collected	Preparation	Other uses reported[b]
Anacardiaceae	*Sclerocarya birrea* Sond (OH8)	Muongozi	Root	Tree	Morogoro	Topical	snake poison
Celastraceae	*Elaeodendron buchananii* (Loes.) (OH19)	Muhorachwi	Stem	Tree	Singida	Oral	Pneumonia
Dennsstraediaceae	*Pteridium aquilinum* (L.) Kuhn (OH41)	Shilu	Leaves	Herb	Mlalo	Topical	-
Ericaceae	*Agauria salicifolia* Oliv. (OH45)	Mwomboa	Leaves	Tree	Mlalo	Topical	-
Euphorbiaceae	*Jatropha multifida* L. (OH53)	Maugwamwipoli	Stem	Shrub	Coast region	Topical	-
	Spirostachys africana Sonder (OH54)	Ormotanga	Stem	Tree	Coast region	Topical	-
Meliaceae	*Turraea holstii* Gurk (OH37)	Muhenga	Leaves	Shrub	Mlalo	Oral	Convulsions
Mimosaceae	*Acacia robusta* subsp usambarensis (Taub.) Brenan (OH38)	Mkame	Leaves	Tree	Mlalo	Topical	Convulsions
	Acacia nilotica (L.) Wild ex Del (OH58)	Kloriti	Stem	Shrub	Coast region	Topical	-
Rutaceae	*Clausena anisata* Oliv. (OH6)	Mjavikali	Stem bark	Shrub	Morogoro	Oral	Convulsions, gonorrhea
Sterculiaceae	*Sterculia africana* (Lour.) Fiori (OH39)	Muhoza	Leaves	Tree	Mlalo	Oral	Convulsions
Vitaceae	*Cyphostemma hildebrandtiii* (Gilg) Desc. (OH14)	Damanyamwili	Leaves	Herb	Morogoro	Topical	-

[a] conducted from O. Hamza et al., 2005. [b] -:no other use reported.

Table 2. Summary of assay conditions and concentrations of enzyme, substrate, positive controls and buffer.

Enzyme	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP3A4
Enzyme/well (pmol/ml)	10	5	7.5	15	15
Phosphate buffer pH 7.4 (mM)	25	325	325	325	325
Substrate	DBF	DBF	AMMC	DBF	7-BQ
Substrate conc. (μM)	1	2	1.5	1	40
Positive control	Sulfaphenazole	tranylcypromine	Quinidine	ketoconazole	ketoconazole
Positive control conc. (μM)	0.1	0.1	0.1	0.1	0.1
Incubation time (min)	30	30	30	30	30
Excitation wavelength (nm)	485	485	390	485	409
Emission wavelength (nm)	538	538	460	538	530

Abbreviations: DBF, dibenzylfluorescein; AMMC; 3-[2-(N,N-diethyl-N-methylamino) ethyl] 7-methoxy-4-methylcoumarin; 7-BQ, 7-benzyloxyquinoline.

Table 3. CYP3A4 inductive activities of selected concentrations of medicinal plant extracts that showed high PXR activity.

Medicinal plants	**Tested concentration (µg/ml)**	**CYP3A4 Fold induction (mean ± sd) ***
5, *Sclerocarya birrea*	16 31	0,52 ± 0,12 0,61 ± 0,30
7, E*laeodendron buchannanii* leaves	31 63	0,95 ± 0,32 -
10 *Cyphosterma hildebrandtii*	31	0,37 ± 0,10
14, *Turraea holstii*	63 125	0,39 ± 0,04 **3,95 ± 1,15**
23, *sterculia Africana*	63 125	1,34 ± 0,06 **2,01 ± 0,96**
25, *jatropha multifida*	250 500	- -
37, *clausena anisata*	63 125	- -
61, *Agauria salicifolia*	31 63	0,34 ± 0,13 0,69 ± 0,39

* induction >2 is considered significant
- toxic concentration for HepG2 cells

Table 7. Overview of Minimal inhibitory concentrations (MIC_0), Efficacy (EFF in %), minimal toxic dosage (MTD, μg/ml) and cumulative index (CI=0-560) of 12 Tanzanian herbal medicines in cytotoxicity tests ranked from high to low toxicity.

			Alamar Blue			Hoechst 33342			Glutathion depletion			Calcein-AM uptake			O2		
Herbs (storage number)	Dose range wherein MIC_0 against *Candida sp.* were found (μg/ml)	Highest test dose	EFF	CI	MTD	EFF	CI	MTD	EFF	CI	MTD	EFF	CI	MTD	EFF	CI	M
Doxorubicine		$3.2x10^{-5}$ M	**100**	**282**	**$1.00x10^{-7}$**	**100**	**494**	**$1.00x10^{-8}$**	**100**	**252**	**$3.16x10^{-7}$**	**100**	**253**	**$3.16x10^{-7}$**	**100**	**196**	**1.x**
Acacia nilotica (47)	31-1000	500	**97**	**152**	**250**	**50**	**73**	**125**	**219**	**428**	**<7.81**	**93**	**106**	**250**	**116**	**272**	**62**
Clausena anisata (37)	63-4000	500	**112**	**134**	**250**	24	25	250	**137**	**200**	**<7.81**	**89**	**108**	**250**	**110**	**227**	**62**
Jatropha multifida (25)	250-1000	500	**90**	**119**	**250**	23	5	250	**114**	**204**	**<7.81**	**114**	**194**	**31.3**	**90**	**131**	**25**
Acacia robusta subsp Usambarensis (43)	31-1000	500	**95**	**78**	**125**	**95**	**75**	**500**	**174**	**453**	**<7.81**	**72**	**70**	**62.5**	**136**	**320**	**62**
Sterculia Africana (23)	63-1000	500	**111**	**225**	**125**	36	**69**	**125**	**171**	**409**	**<7.81**	**133**	**225**	**125**	20	0	25
Elaeodendron buchannanii leaves (7)	63-250	500	**107**	**215**	**62.5**	33	18	250	**193**	**355**	**62.5**	**98**	**97**	**250**	**119**	**196**	**12**
Agauria salicifolia (61)	500-4000	500	**91**	**125**	**250**	26	**51**	**125**	**111**	**117**	**125**	0	10	250	**97**	**156**	**31**
Cyphosterma hildebrandtii (10)	250-1000	500	**96**	**89**	**125**	65	**86**	**62.5**	**58**	**175**	**<7.81**	**70**	**50**	**250**	**66**	46	**25**
Spirostachys Africana (58)	1000-2000	500	**95**	**75**	**500**	**54**	34	**500**	**169**	**199**	**<781**	**100**	**92**	**250**	21	0	50
Pteridium aquillinum	500	500	-38	0	>500	18	0	>500	**56**	**122**	**<7.81**	-3	0	>500	**103**	**102**	**25**

(42)																	
Sclerocarya birrea (5)	63-250	500	**73**	**62**	**125**	34	14	250	**66**	**85**	**125**	**69**	**49**	**250**	**90**	**92**	**1**
Turraea holstii (14)	63-1000	500	-44	0	>500	14	0	>500	-3	0	>500	36	16	>500	**85**	**65**	**2**

MIC_0, minimal inhibitory concentration: concentration (µg/ml) that visually showed no growth and percentage growth less than 5% spectrophotometrically.

*Herbs are ranked according to the following system: The herb receives a score per assay; when 50 < CI < 100 the test score is ½ and when CI >100 the test score is 1. The sum of the test score defines the final rank of the herb.

A herb is considered toxic when EFF or CI is ≥ 50 (the values are marked bold). A herb has weak toxicity when EFF and CI are both > 20 (also the MTD was marked bold).

Table 5. IC_{50} values of the Tanzanian herbal medicines on CYP2C9, CYP2C19, CYP2D6, CYP3A4 enzymes.

Herbs (storage number)	Dose range wherein MIC_0 against *Candida sp.* were found (μg/ml)	IC_{50} value (μg/ml)[a] CYP2C9	CYP2C19	CYP2D6	CYP3A4 (7BQ)
Acacia nilotica (47)	31-1000	1.03	5.98	22.6	
Acacia robusta subsp. Usambarensis (43)	31-1000	3.78	9.42	29.1	
Agauria salicifolia (61)	500-4000	3.12	14.34	28.6	
Clausena anisata (37)	63-4000	19.93	39.82	5.02	
Cyphosterma hildebrandtii (10)	250-1000	4.37	6.09	2.96	
Elaeodendron buchannanii (7)	63-250	3.78	12.66	37.95	
Jatropha multifida (25)	250-1000	15.42	4.26	79.4	
Pteridium aquillinum (42)	500	4.98	12.06	70.5	
Sclerocarya birrea (5)	63-250	5.78	22.18	39.41	
Spirostachys africana (58)	1000-2000	20.00	32.22	>139	
Sterculia africana (23)	63-1000	22.75	66.96	>139	
Turraea holstii (14)	63-1000	35.58	6.30	>139	
Positive controls[b]		Sulfaphena-zole	Tranylcypro-mine	Quinidine	ketoconaz
		$4.66x10^{-7}$	$3.74x10^{-6}$	$3.19x10^{-7}$	4.45

[a]IC50 = concentration of herbal medicine (μg/ml) whereby CYP activity is inhibited with 50% in comparison to that of reference compound

[b] values in M

[c]ranking according to IC50 values.

Table 6. Inductive activities of Tanzanian medicinal plants on human pregnane X receptor (hPXR)

Herbs (storage number)	Dose range wherein MIC_0 against *Candida sp.* were found (µg/ml)	hPXR EC50 (µg/ml)[a]	hPXR Induction factor	Concentration showing highest PXR induction (µg/ml)
Acacia nilotica (47)	31-1000	250	0.11	-
Acacia robusta subsp Usambarensis (43)	31-1000	250	0.44	-
Agauria salicifolia (61)	500-4000	31.25	**5.44**	63
Clausena anisata (37)	63-4000	93.75	**3.91**	125
Cyphosterma hildebrandtii (10)	250-1000	15.63	**2.41**	-
Elaeodendron buchannanii (7)		46.875	**4.49**	63
Jatropha multifida (25)		62.50	**5.90**	500
Pteridium aquillinum (42)	500	93.75	**3.58**	250
Sclerocarya birrea (5)	63-250	39.1	**3.33**	16
Spirostachys africana (58)	1000-2000	250	0.27	-
Sterculia africana (23)	63-1000	31.25	**2.16**	125
Turraea holstii (14)	63-1000	31.25	**4.12**	63
"Tularik" Org 9572 (mol/L in 0.1%DMSO)		$1.00x10^{-6}$	6.41	$1.00x10^{-4}$

[a]EC50 = lowest plant extract concentration whereby hPXR activity is inhibited by 50% compared to reference compound.

[b] Stimulation factor is defined by means of maximal induction of the PXR activity at the most active concentration of the 11 tested concentrations with respect to the blanc signal.

Table 7. Overview of literature reports on toxicity and mutagenicity of 12 medicinal plants from Tanzania

Herbs (storage nr.)	Literature reports on toxicity and mutagenicity (first author, year)
Acacia nilotica (L) Wild ex Del (47)	In vitotox test no genotoxicity was found. In comet assay, significant DNA was found. (Arora et al., 2005) (Arora, 2005) In rats fed a 8% acacia diet for up to 4 weeks showed that body weight w cholesterol and serum total protein significant decreased. Symptoms rev termination. So Authors concluded that acacia nilotica has a low toxicity Mustafa, 2000)
Acacia robusta subsp Usambarensis (Taub) Brenan (43)	No reports
Agauria salicifolia (61)	Case report of a 28 year old female with grayanotoxin intoxication, with v bradycardia due to ingestion of herbal tea made of the leaves. (Martinet
Clausena anisata Oliv (37)	Intraperitoneal treatment with Chalepin (100 mg/kg), an furanocoumarin days caused death of 4 out of 10 rats within 48 hrs of treatment. Rats su changes in hepatic enzyme activity, reduced glutathione and DNA conce Ames test showed mutagenicity of imperatorin and marmesin with optima respectively. Microsomal activation was not required for mutagenicity in b Estragole: an acute toxic principle from the volatile oil of the leaves of Cl Okunade et al., 1987)
Cyphosterma hildebrandtii (Gilg) Desc (10)	No reports
Elaeodendron buchannanii (Lows) leaves (7)	No reports
Jatropha multifida L (25)	Intake of fruits caused toxicity in two children with symptoms vomiting an contain the toxalbumin, ricin wich causes sever vomiting and diarrhea, d imparment. Ricin also has cardiotoxic and hemolytic effects and several 2000) Also others reported toxicity of the seeds (Inman, 1967; Baruffa 1964)
Pteridium aquillinum (42)	Several reports on poisoning (intestinal and bladder tumors, chronic hem Hoque et al., 2002; Lioi et al., 2004; Marrero et al., 2001; Schrader et al., (Xu, 1992; Marrero, 2001; Schrader, 2001; Gava, 2002; Hoque, 2002; Tv Reduction in female rat fertility and weight gain during pregnancy was fo 30% of bracken fern. (Gerenutti et al., 1992) (Gerenutti, 1992) Associated with high incidence of stomach and esophageal tumors in hu as food (Ngomuo, 1996). No genotoxicity was found in mice after administration of quercetin at 20 (Ngomuo, 1996). Identified carcinogenic compound was ptaquiloside (Bonadies et al., 200 2001; Potter et al., 2000; Rasmussen et al., 2005) (Castillo, 1998; Marre Rasmussen, 2005) and mutagenic compound quercetin (Beniston et al., Fern spore extracts were shown to cause DNA damage in human cells in 2000) Ptaquiloside caused brine shrimp toxicity (LC50 62.5 μg/ml at 24 h and 7 (Castillo, 1998)

Sclerocarya birrea Sond (5)	Acute toxicity testing in rats showed a LD_{50} value of 1087 $\pm$ 41 mg/kg an methanolic and aqeous extract respectively. Author conclude that these plant is relative "safe" in mammals. (Ojewole, 2003)
Spirostachys africana Sonder (58)	No mutagenicity at concentrations up to 5000 μg/ml in Ames assay , alt concentrations of 100 and 500 μg/ml was found with micronucleus test
Sterculia africana (Lour) Fiori (23)	No reports
Turraea holstii Gurk (14)	No reports

NARRATIVE SECTION:

OBJECTIVE 1:

To determine socio-anthropological aspects associated with the selection of remedies for treatment of HIV/AIDS

The identification of remedies for treatment of conditions similar to HIV/AIDS manifestation was a result of knowledge obtained through past experience from ancestors and learnt experience through individual THPs.

This exercise was done cross-sectionally through a structured questionnaire (appendix 1).

A list of plants from families of *Combretaceae*, *Apocynaceae*, *euphorbiaceae, Fabaceae*, *Cluciaceae* and others have been mentioned as being used bt THPs in managing HIV/AIDS patients.

Several other medicinal plants mentioned by the collaborating THPs are protected under the MoUs that have been signed between the collaborating THPs and the Institute and hence could not be mentioned in this document.

Development of Products for use by HIV/AIDS Patients tat the Institute

Based of the above findings, some of the THPs have revealed the actual medicinal plants as a source of their remedies. The Institute have already formulated some products out of these medicinal plants. More efforts are underway to standardize these formulations while they are being used by few patients. This stage necessitates clinical trial as has also been highlighted on the section under the way forward.

DISCUSSIONS:

When infected with the HIV virus the body's defence system - the immune system - works harder to fight infection. This increases energy and nutrient requirements. Such needs will increase even further as the HIV/AIDS symptoms develop. HIV/AIDS reduces food intake as the illness and also the medicines taken for it may reduce the appetite, modify the taste of food and prevent the body from absorbing it. Symptoms such as a sore mouth, nausea and vomiting make it difficult to eat. Circumstances surrounding patients including tiredness, isolation and depression reduce the appetite and the willingness to eat regularly. This phenomenon leads to serious weight loss, a condition commonly known as wasting. Weight change is therefore an important marker in the course of evaluating clinical progression of HIV/AIDS patients. In our case as shown in Figures 4a and 4b above, utilization of herbal remedies is speculated to have influenced the association with stabilization or increase in body weight for patients under the study.

CD4 and CD8 and Viral load

DEVIATIONS:

Dual therapy: Whereas the study strictly wanted patients who were not using ARV, some of them were faithful enough to inform the staff that they have been using both treatment. They were however, stoped from the study since they did so willingly.

Traditional Healers ethical conducts…..

Adherence to clinic

Stigma ….

Data interpretation limited to a number of parameters only due to limited budget.

Number of patients based on conditions the THP do treat?

CONCLUSION

Based on this 2 years observational study, the following observations were deduced

- There was no obvious toxicity observed due to use of traditional herbal remedies as indicated by renal and liver functional tests (ALAT and ASAT) This observation implies safety upon use of traditional herbal remedies as provided by Traditional Health Practitioners.
- In the course of follow-up it was revealed that patients reported fewer incidences of illness episodes showing that traditional herbal remedies are effective against a number of HIV/AIDS –associated secondary infections.
- Based on immunological changes that were observed, the use of herbal medicines have been shown to relatively stabilize and in certain cases elevate CD4 cells in HIV/AIDS patients.

RECOMMENDATION and WAY FORWARD

Based on the findings from this study, the following are feasible recommendations

1) Continue the spirit of collaboration with Traditional Health Practitioners (TPHs) in the exploration of medicinal plants with antiviral, antifungal, antiprotozoal, antibacterial and immunostimulant activity.

2) Conduct clinical trial for specified herbal preparations from THPs whose recipes have shown promising leads against HIV/AIDS

3) Conduct intensive phytochemical analysis of medicinal plants whose herbal preparations have shown anti-HIV activity

4) Document all medicinal plants in the country that have been reported to having antiviral and/or antiinfective activity through use of literature.

5) Conduct toxicity study for all preparations earmarked for clinical trial

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