

SUMMARY

There has been a tremendous increase in modernity and sophistication of human life. Equally, the elixir of human life is plagued by diseases that are prevalent all over the globe or found only in certain pockets of the world. Such diseases can be listed as follows:

1. HIV/AIDS
2. Malaria
3. Diarrhoea
4. Tuberculosis
5. Swine flu
6. Chicken guinea

Of all these diseases, AIDS (Acquired Immuno Deficiency Syndrom) is one such disease that is prevalent all over the world with high intensity among the populace in African countries (Swaziland, Sub-Saharan countries and South Africa), India, Thailand, Bangladesh etc .AIDS is caused by HIV-1(Human Immuno Deficiency Virus Type-1). In spite of hectic, ceaseless and meticulous researches, no permanent cure is in sight by way of vaccine or specific drug with least side effects. Even in the case of administration of AZT (Zidovudine,Didanosine, Lamivudine, Tenofovir,Nevirapine, Sanquinavir and Enfuvirtide),mitochondrial toxicity has been reported. In many cases resistance of HIV to ART (Anti Retroviral Therapy) has been reported due to frequent changes (Mutation) in the coat protein. The present study also confirms changes in the coat protein by sequence analysis in the C2-V4 region of the HIV-1 env gene. This is one of the reasons why promising vaccine against HIV could not be developed so far.

There is some upsurge in medicinal plant research all over the world in recent times laying emphasis on the bioactive compounds of plants for many diseases (Jaundice of unknown etiology, common cold, fever, diabetes, hypertension etc.) prevalent in African and Asian countries. It is opined that there is hardly any resistance when the herbal medicines are employed either as crude extract or in the partially purified form. As a matter of fact, these medicinal herbs are available at cheaper cost and can be propagated on a large scale for commercial purposes. There are also sporadic reports that medicinal herbs can act as phytochemical therapy in the control of AIDS.

Therefore, in the present study the following medicinal herbs were employed to treat the AIDS patients (Asymptomatic:Symptomless, Viral load below one lakh and CD4 cells \approx 500) based on the survey of literature in Siddha and Ayurveda, which are complementary as popular medical practices established in India from time immemorable.

<u>Plant name</u>	<u>Family</u>	<u>Organ used</u>	<u>Bioactivity</u>
1.Phyllanthus niruri	Euphorbiaceae	Leaves	Antiviral
2.Withania somnifera	Solanaceae	Roots	Antiviral
3.Ocimum sanctum	Lamiaceae	Leaves	Antiviral
4.Curcuma longa	Zingiberaceae	Rhizome	Antiviral
5.Phyllanthus emblica	Euphorbiaceae	Fruits	Antiviral
6.Piper longum	Piperaceae	Fruits	Immunostimulant

The respective organ of the plant used as medicine was shade dried and powdered into fine particles using nylon mesh. The powdered materials from all the six medicinal herbs were mixed in equal proportions (1 gram each) and packed into commercially available gelatin capsules for easy consumption by the patients. Each capsule ('00'size) when fully packed weighed about 1 g. Six capsules were administered per meal totalling 18 capsules (18 g powder mix) per day.

In the present study asymptomatic patients were selected based on their own concurrence and absolute willingness to undergo the herbal treatment. A comparison has been made by choosing 14 patients from India and 16 from Madagascar (Upon Invitation), an Island in African Continent. In the present study, coercion could not be enforced for any sizable patient population, since willingness to undergo the treatment was the utmost concern with the case history of the patients maintained incognito. During the period of treatment, there was no food restriction enforced on the patients except prohibiting them from taking hot drinks and smoking. The patients belonged to the both the sexes and most of them were in the middle age group. The patients were treated with herbal capsules for 36 months. During the period of treatment, the following parameters were assayed to find out the efficacy of the medicinal herbs cumulatively, since these medicinal herbs independently did not reveal any amelioratory role on the patients:

1. Viral load (RNA)
2. P24 Antigen
3. Quantitation of reverse Transcriptase
- 4 .CD4 Count

5. CD8 Count

6. CD4/CD8 Ratio

7. IFN- γ

8. IL-2

9. Haemogram

At desired time intervals, serum was collected from the patients in India and Madagascar and assayed for viral load. There was a steady and steep fall in the viral load during the treatment period in both the Indian and Madagascar patients. In most of the cases less than detectable limit (LDL) was achieved in about 36 months treatment, although in some cases LDL was achieved even much earlier. Quantification of viral load, as a matter of comparison, when assayed by real time PCR and Roche cobas amplicor method, did not reveal any significant difference. A similar downward trend was observed in P24 antigen and reverse transcriptase as well.

On the contrary, CD4 cell count increased significantly at all levels of treatment suggesting an inverse relationship between the striking fall in the viral load and increased CD4 cell count. However, there was hardly any difference in CD8 cell count for the entire period of treatment. As matter of fact, CD4/CD8 cell ratio increased by almost two-fold which may be attributed to the only increase in CD4 cells. Therefore, it is suggestive from the present study that the medicinal herbs acted cumulatively as a phytochemical therapy in the control of AIDS virus (HIV-1) by suppressing the viral load and thereby viral antigen (P24), which incidentally led to increased in CD4 cells and thereby increased CD4/CD8 cell ratio. When interferon gamma, an antiviral compound and interleukin-2, a cell proliferating agent, were assayed in the serum of the

patients, their levels also increased significantly. The increased in CD4 cell population and increased levels of IL-2 and IFN- γ probably indicated triggering of the immune system by the phytochemicals employed in the form of herbal capsules.

Haemogram of the patients prepared before and after treatment revealed a significant increase in WBC followed by marginal increase in lymphocyte population and packed cell volume which might serve as another evidence of stimulation of the immune system in the AIDS patients, which, otherwise, got progressively suppressed as the disease progression continued.

From the above results it becomes evident that the medicinal herbs not only downloaded the intensity of HIV in the patient but also egged on the immune system and thereby rejuvenating the health of the patients. Therefore, antiviral compounds in the following plants were isolated and identified as given below.

1. Phyllanthus niruri-Limonene
2. Ocimum sanctum-Eugenol
3. Phyllanthus emblica-Ascorbic acid
4. Withania somnifera-Betasitosterol
5. Curcuma longa-Curcumin

As a matter of fact, different herbs collected either from irrigated or rainfed conditions under different kinds of soil did not reveal any significant difference in the quantitation of antiviral compounds. To confirm the antiviral activity of limonene, eugenol, beta-sitosterol, ascorbic acid and curcumin, animal cell culture assay was resorted to employing peripheral blood monocyte cells (PBMC) which were inoculated with HIV and the effect of the aforesaid antiviral

compounds (synthetic and purified chemicals from Sigma) was assayed either independently or cumulatively against HIV-1 RNA, P24 antigen and Reverse transcriptase. After 96 hours of incubation of the culture, the HIV-1 RNA, P24 antigen and Reverse transcriptase were significantly reduced.

To confirm the aforesaid results still further, herbal extracts were directly employed using peripheral blood monocyte cells inoculated with HIV-1. As observed in the case of authentic and purified synthetic antiviral compounds, HIV-1 RNA, P24 antigen and Reverse transcriptase were significantly reduced confirming that the plants exerted antiviral activity in the cell culture experiments which might be extrapolated to the human system as well, as it was observed in the present study.

A significant inference is that in both the synthetic chemicals and herbal extracts, the cumulative effect was much greater on HIV-1 RNA, P24 antigen and Reverse transcriptase, compared to the effect imposed by the compounds in isolation (Individual medicinal herb). As a measure of toxicity, likely to be inflicted cumulatively by these synthetic chemicals/plant extracts, PBMC, Jurkat cell line and Vero cell line cultures were employed. The measure of toxicity was observed through viable cell count employing tryphan blue. Whereas synthetic compounds reduced the viability of cells to some extent, the plant extract did not have any impact on the viability of cells suggesting the possible superiority of plant crude extract over these synthetic chemicals.

As an additional confirmatory test, the PBMCs were isolated from the patients, who were under treatment for 36 months, to find out the presence or absence of proviral DNA employing specific forward (BK1F) and reverse primers (BK1R). As evidenced by agarose

electropherogram, proviral DNA was absent in patients who received the phytochemical therapy (Herbal capsules). In untreated patients proviral DNA (≈ 250 bps) was present. Thus this is yet another proof wherein the medicinal herbs cumulatively exerted influence to control AIDS virus (HIV-1).

To surmise, the possibility of treating AIDS patients (asymptomatic) through phytochemical therapy (medicinal herbs) is highlighted. Of the six medicinal herbs employed, five (*Phyllanthus niruri*, *Ocimum sanctum*, *Withania somnifera*, *Curcuma longa* and *Phyllanthus emblica*) were antiviral in nature and the other one (*Piper longum*) was immunostimulant. As evidence, the antiviral compound has been isolated and quantified from the respective medicinal herb. The steep fall in HIV RNA, P24 antigen and Reverse transcriptase possibly indicated the antiviral nature of the medicinal herbs. The reappearance of CD4 cells, an increase in CD4/CD8 ratio coupled with IFN- γ and IL-2 and increased WBC and lymphocyte population might indicate boosting of the immune system by the phytochemical therapy (Herbal medicines). These observations were further confirmed by cell culture experiments employing PBMC culture, wherein a reduction in HIV-1 infected PBMC population was observed. Cumulatively not only the synthetic chemicals but also the plant extracts exerted a strong influence on the control of HIV-1 proliferation. Although it is subjected to further verification, it appears from the preliminary assays that the plant extract employed in the crude form possibly may not cause any secondary or deleterious effect on cell population. However, it may be construed that the bioactive compounds in the medicinal herbs worked in coordination rather than in tandem in bringing about restoration of deranged health in AIDS patients.

The present work is a novel method of approach and an answer to many of the diseases paralyzing the human life all over the globe. The phytochemical therapy is already catching up

with many countries and if proved unequivocally in many laboratories, the plant-based therapy might rule the roof one day or other at least in many developing countries as a viable alternate treatment for many diseases such as AIDS. Another point of interest emanating from the study is that this kind of phytochemical therapy (Herbal medicines) can be employed on any population in the world with success, as it has been authenticated in the case of Madagascar AIDS patients for the purpose of comparison. The possible mode of action of medicinal herbs with curative properties on asymptomatic AIDS patients is also briefly presented and discussed.