FORMULATION OF VACCINES FROM TRADITIONAL PLANTS EXTRACT FOR HIV-IMMUNE BOOSTING CHARACTERISTICS

A.K. Asiagwu
Department of Chemistry, Delta State University Abraka, Nigeria.
E-mail: drasiagwu@yahoo.com

ABSTRACT

Traditional herbs are the purest form of natural medicine and they all have therapeutic values, whether important or not. This research work involves formulation of vaccines or drugs from traditional herbs extract for HIV- Immune boosting characteristics. Five laboratory animals of different weights were infested with HIV virus and left for two weeks. After which five different compositions of four plant extracts were inoculated to these animals according to their corresponding numbers for four weeks. The weight and CD4 counts of the animals were taken before infection and four weeks after inoculation. Animal sample 5 showed a more responsive attitude than the other four. This indicates that extract composition 5 is a more potent HIV-Immune booster.

Keywords: *Local plant extract, drug formulation and anti-viral screening.*

1. INTRODUCTION

Most developing countries of the world especially Nigeria and other West African countries depend on plants for their traditional forms of Medicine, Harbourne, (1998). Medicine plants as they are called are used in treating and preventing specific ailment and diseases and as such are considered to play a beneficial role in health care, Tomas-Baberan, (1995).

It has been stated that hundreds of plants species are recognized as having medicinal valve and four out of every of those plants are collected from the wild forest, while most are from the floras of the developing countries, Soejarto and Farnsworth, (1991). Further, it has also been stated that medicine properties may be present in one or all the plant parts, root, stem bark. Leaf, flower fruit or seed. Three compounds may be found in a particular part of the plant or all over the body and they are often localized in glands, Fellows, (1991). Kham and co-workers observed that a striking characteristics of plants is that different chemical substances are obtained in members of even the same species in different areas, Srivatave, Lambert, and Vietemeyer (1996).

The continuous evolution of virus resistant to currently available anti retroviral drug and its attendant high cost has necessitated that search for an effective and affordable antiviral compounds. Efforts in this regard have focused on plants because of their use historically and that of a good portion of the World's population. Particularly in the developing countries, rely on plants for the treatment of infections and non-infectious diseases. Asuzu, (1986).

In 1985 scientist discovered the Human Immune Deficiency Virus (HIV) the causative agent of the Acquired Immune Deficiency Syndrome (AIDS) is a virus which damages the immune system and the body becomes susceptible to illness and infection. Culied, (1982). It can be transmitted from person to person through the exchange of body fluids such as blood semen, breast milk and vaginal secretion. They are two types of HIV (Human Immune Deficiency Virus). They are HIV – 1 and n HIV-2, with each having its own subtype or genotype M:HIV-1 has a global epidemic while HIV-2 remains almost within West Africa. Kharn, Ndu Alio, Nkunga, Wever and Sawheny, (1985).

Recent therapeutic option for this disease are grossly limited either as a result of rapid development of resistance, toxicity to host or their cost as a result, HIV agents especially from medicinal plants has increased tremendously, Sofowara, (1980). Thus this work is hereby focused on the determination of the anti-viral characteristics and immune boosting ability of drug formulations from local plant extracts.

2. MATERIALS AND METHODS

Four fresh bulbs of our local plants were obtained from their original source and examined physically for any abnormality due to bacteria viral or fungi infection nor infection due to insects or rodents. They were then washed, peeled and cut into pieces. Each herb was soaked with 0.5litres of ethanol and left for 18 hours. In order to obtain the active extract the solution was then decanted and the liquid boiled in order to evaporate the ethanol. After the solution has been heated, the extract was allowed to cool before making different composition of the extract as in table 1. Cummingham, (1993). The extract were then examined for their macro and micro properties. A clear plant and absence of particulate matter was observed. With a clean grease slide, a drop of the fluid was made and covered with cover slip. The slip was mounted under the microscope and was examined using x10 objectives lens. No abnormalities of any kink capable of causing bacterial/viral infection were found in the fluid. The fluids were

introduced into the inoculation medium with the use of a well sterilized wire loop. After the introduction of the plants extracts, the wire loop was planned at interval and separated on the plates starting from the points of inoculation. Commingham (1993). The plants extracts were inoculated in an incubator at 370c – 450c for 18 hours. The following day, the samples were examined and no bacteria morphology or structure which may regarded as a growth of bacteria was observed. The culture was done in the following media MacConkey Agar, Blood Agar and Nutrient Agar.

The samples were still introduced to Fibroid Dextrose Agar (PDA) in other to rule off the presence of Fungi. Cummingham (1997). In order to ensure that extracts do not contain any strains of viruses before being administered to individuals/person for immune boosting properties, the samples were introduced into rapid strip reagents for Human Immune deficiency Virus (HIV) and Viral Disease Research Laboratory (VDRL). While Ag strip reagent was used for Ag = hepatitis Agar (Hbs) as recorded in Table 2.

Furthermore five rabbits bought were examined for viral or bacteria infection. Then their CD4 counts were taken, their respective weights were also determined. The animals were then infected with the HIV and left for 2-weeks. After two weeks their weights and CD4 count were taken. Five samples of varying extract composition were administered to the different animals bearing their corresponding numbers. For 4 weeks 5ml of these extracts were given daily to the animals and their weights and CD4 count were recovered as in table 4. Cowan, (1999).

3. RESULTS AND DISCUSSION

Table 1: Composition of the four extract into five different forms:

Extracts (100mm/s)	**A**	**B**	**C**	**D**
1	20	30	20	30
2	30	20	30	20
3	50	10	15	25
4	15	25	40	20
5	5	50	30	15

Table 2: Results of the five different composition when tested with VDRL, HIV and HBS – AS

Reagent	**1**	**2**	**3**	**4**	**5**
HBS-AS	Negative	-ve	-ve	-ve	-ve
VDRL	Negative	-ve	-ve	-ve	-ve
HIV	Negative	-ve	-ve	-ve	-ve

Table 3: Original weight, CD4 count, current weight and CD4 count on the 5 laboratory animals before and after infection with HIV-Virus.

Sample/Test	**1**	**2**	**3**	**4**	**5**
Original Weight(kg)	6	6.5	6.2	7.1	6.8
Original CD4 count (mm3)	700	800	600	710	755
Current weight	4.5	5.3	4.1	5.6	5.1
Current CD4 (mm3)	390	500	375	410	470

Table 4: Weight and CD4 count of the infested when administered with 5m.s daily for 4 weeks.

Sample/Test	**1**	**2**	**3**	**4**	**5**
Weight(kg)	5.3	5.9	4.9	6.3	6.1
Original CD4 count (mm3)	490	620	550	550	560

4. DISCUSSION OF RESULTS

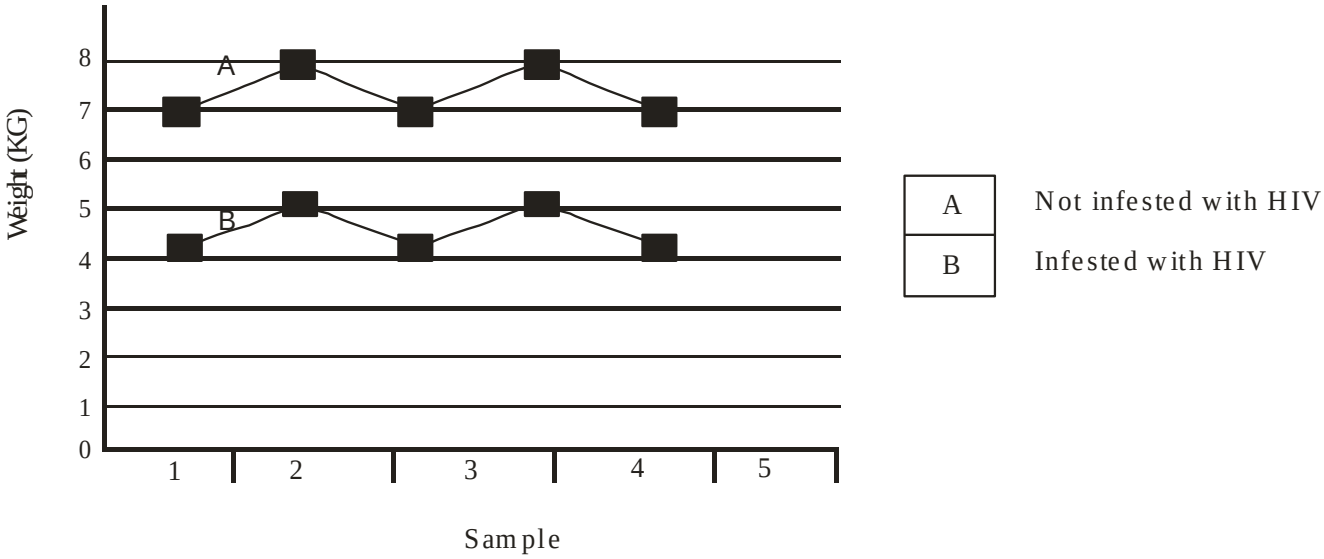
Taking a look at table 1, the four extracts gotten from our plants were made into 5 different compositions. This is in a bid to know which composition will have more effect than others. In sample 1, the ratio of

A:B :C :D is in the ratio of 20:30:20:30, while that of sample 2 is 30:20:30:20. Sample 3 is 50:10:15:25, while sample 4 is 15:25:40:20. Finally sample 5 was compounded in a ratio of 5:50:30:15. All these composition were measured in milliliters (m/s) and sum up to 700m/s.

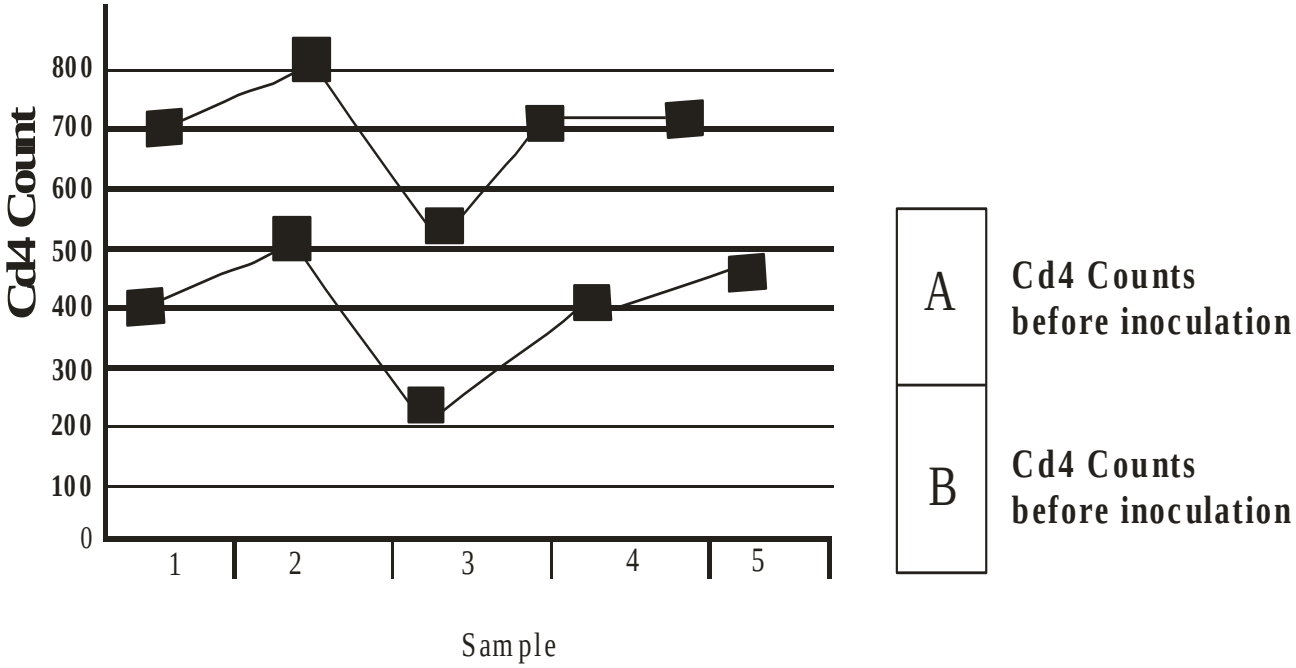
Table 2 gives a result of what happened when these 5 sample compositions were tested with HIV strip virus, VDRL strip and Hbs, Ag strip. This is to ascertain whether these extract carry any virus or bacteria which is or will be harmful to the consumers. A look at the results above show negative for all those testing media, which shows that

the 5 samples were free from contaminations. Table 3 is made up of free of two parts the weightiest/count and the CD4 test/count.

Individually these two parameters has its former (before infection) and latter (after infection) values. The animals were weighed on CD4 counts collected before and four weeks after inoculation. A graph at weight (kg) against samples gives us an ideal curve of which an animal lose weight more than others.



The graph clearly shows that sample 3 lost more weight than any other, it lost a total of 2.1kg in 2 weeks. Line A is the line graph made when the animals were not infected with HIV virus, while line B is a new line for their current weight after two weeks of being carrier. If a graph of CD4 count against example animals is drawn, we will know which animal is most affected at the T-cells. The graph is shown below.



Line A shows the former CD4 count before inoculation, while line B shows that current CD4 count after inoculation. A close look at this figures and graph you would find a variation from the graph of weight against the samples. The former graph reports that sample 3 is more physically affected, loosing a total of 301mm3 of CD4 cells, followed by sample 5 which lost 305mm3 yet sample 3 lost the least number of cells loosing 225mm3. this is to say that physical looks are not actual perimeter to know the most affected of all.

Table 4 give us the final verdict of what really happened to or with the weight and CD4 cells of the animals when inoculated wit the 5 samples, in accordance to the animal bearing the corresponding number for four weeks.
From table 2, we can see that physically saying sample 5 had more effects, than all other samples, this is because it gained 1kg at the end 0.4kg, 0.5kg, 0.8kg and 0.7kg respectively.
The primal animal sample 5, gained more CD4-cells than any other sample. It gained a total of 190 cells out of 305 cell it lost, while sample 3 followed by gaining 170 out of 235 cells, sample 4 gained 140 out of 300, also sample 2 gained 120 out of 300, and finally sample gained 100 out of 310 CD4 cells. Now we can say that extract 5 had more effect i.e. positive than any other extract. The other of potent action of the extract is summarized below 5>3>4>2>1.

5. CONCLUSION

After proper experimental analysis of the five sample extracts, we discovered that extract 5 is more potent than the other samples. Although this extracts has no effect on the HIV virus, that is to say that it did not remote the virus, but it had an effect on the immune system. The SC4 count of the laboratory animals were found increase after being administered with the drug. Since CD4 is the chief immune cell of the body, and very drug increase the SC4-T cell count, therefore we can say conclusively that this very drug is an immune booster.
In the light of the foregoing, I hereby make the following recommendation

1. Future analysis based on this plant extracts be prolonged and allowed to last for one or two years as to determine if it can cure or eradicate HIV virus.
2. This drug should be taking as a food complement.
3. Testing laboratories with adequate facilities herbs and establishing dosage norms for the most efficacious use of herbal extracts whether in tablets, capsule, powder, syrup or other form should be set up by government.
4. Government should establish the necessary institution and finance support to promote the potential role of herbal medicine and the establishing of local botanical gardens for the preservation of essential herbal plants in different parts of the country, in order to ensure a sustainable supply of safe, effective and affordable medicinal plants or herbs.

6. REFERENCES

[1]. Asuzu, I. U. (1986). Pharmacological Evaluation of follow of sphenosfylia slenocarpa. Journal of Ethno Pharmacology. 16:236-264.
[2]. Cowan, M.M. (1999), Plant products as antimicrobial agents, clinical microbiology reviews 12:PP564-583.
[3]. Culied (1982). Methodology for analysis of vegetable drugs. Practical Manual of the Industrial Utilization of Medical and aromatic Plant. Bucharest Office of Joint UNIDO.
[4]. Cummingham, A. B. (1993). African Medical Plants setting priorities of the interface between conservation and primary healthcare. Working paper LUNESCO Paris.
[5]. Cummingham, A.B. (1997), An African-wide overview of medical plant harvesting, conservation and healthcare. Non-wood forest products II, medical plants for forest conservation and health care FAO, Italy.
[6]. Esimone, R. Omabuwojo, Sowemimo and Proksch (2002), A research on single cycle vector based antiviral screening assays for high throughout evaluation of potential anti-HIV medicinal plants: a pilot study of some Nigerian herbs. Pp 3-53.
[7]. Fellows, L.E. (1991). Pharmaceuticals from Traditional Medical plants and others: future Prospects. A paper presented at symposium "New Drugs from National Sources sponsored by I.B.C. Technical Services Ltd. London. Pp 2-3".
[8]. 8 Harbourne, J.B. (1998) Photochemical Methods: A guide to modern techniques of plants analysis 3rd edition. Chapman & Hall, London. PP 1-36, 203-205
[9]. Kharn, M.R., Ndu Alio, G., Nkunga, M.H.H., Wever, H.and Sawheny, A.N. (1985).Studies on African Medicinal plants for Antibacterial activity African Journal of medicinal Science.15:65 69.
[10]. Soejarto, D.D. and Farnsworth, N.R. (1991). Global Importance of Medical Plants in: Akerele O. Heywood V. and Synge H(Eds) Conservation of Medicinal Plants: University Press, Cambridge PP231-240.
[11]. Sofowara, A.E. (1980). Guidelines for Research Promotion and Development in Traditional Medicine, Nigeria Journal of Pharmacy. 11(1):1-18
[12]. Srivatave, J., Lambert, J. and Vietemeyer, N. (1996). Medical Plants. An expanding Role in Development. World Bank Technical paper No 370, Washington D.C. 20433, U.S.A. Pp 1-2.
[13]. Tomas-Barberan, F. A. (1995). Photochemical Analysis. Academic Press, London. 6,177.