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The Effects of a Mixture of Extracts from Indigenous Herbs on HIV/AIDS Patients Employing CD4+ T Lymphocyte Counts and Viral Load Reductions as Assessment Indices

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MKE and CICO designed the study, authors MKE, CICO and EN performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MKE, FC, FOE, EA and EN managed the analyses of the study. Author MKE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Human Immunodeficiency Virus (HIV) infection is treated in some part of Africa using different herbal combinations. The study aimed at determining the effects of herbal extracts on HIV/AIDS patients and to access its antioxidant effects *in vitro*. The extract was made from selected fruits, leaves and mixed with honey. The herbal mixture was employed based on the claims by indigenous Biotechnologist (Medical Botany Practitioners) that such extracts were effective in HIV/AIDS management. A total number of 95 volunteers' from Jos North, Jos South and Mangu Local Government Areas, Plateau State, Nigeria were therefore chosen for this study. All the study subjects were adequately diagnosed prior to the study to ascertain their HIV/AIDS positivity status, CD4 counts and viral load baselines. They were further divided into age brackets to find out the age

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brackets that responded best to the treatment. The response of the patients was monitored using the CD4 counts increase and viral load reductions as indices at 6th, 12th, 18th and 24th-month intervals. Significant improvements were recorded in viral load reductions and CD4 counts in all the age groups after the 18th month except for the age group 20 – 29 years. The active age group (20 – 49 years) had a cumulative incidence of 88% of the total study population. The highest percentage adherence of 83% was recorded in 40 – 49 years volunteers while the highest non-adherence was recorded as 64% in 50 – 59 years volunteers. The herbal mixture possesses good *in vitro* antioxidant effect when compared with Butylated hydroxyl toluene (BHT). The present study showed that herbal therapy could reduce the viral loads and increase CD4 counts of HIV/AIDS patients. The herbal treatments were therefore effective. However, it should act as a supplementary treatment for patients and should not replace the conventional Anti-retroviral therapy.

Keywords: Adherence rate; antioxidant status; CD_4 increases; herbal treatment; HIV/AIDS; viral load reductions.

1. INTRODUCTION

The Human Immunodeficiency Virus (HIV) belongs to the retroviridae family. They are a family of virus, which affects the immune system of the human host. They destroy the ability of a human immune system to fight invading organisms and diseases effectively. HIV-1 and HIV-2 are the two types of HIV that have been successfully characterized. HIV-1 is more virulent and infectious when compared with HIV-2. It's also the primary cause of HIV infections globally, whereas HIV-2 is primarily confined to the West African region. Untreated HIV leads to the disease condition termed Acquired Immune Deficiency Syndrome (AIDS). Despite the breakthroughs in the development of effective antiretroviral drugs, a resistance of HIV to some used antiretroviral drugs poses the challenge to therapeutic failures in people treating HIV/AIDS [1]. Several treatment regimens may thus be needed in the effective treatment of HIV, making it a challenging task. Conventional HIV/AIDS management therefore among the methods are widely employed. The antiretroviral administration, including the conventional options, could have significant impact in the reduction of morbidity and mortality that stem from the HIV infection.

Several alternative therapies have been employed in the management of HIV/AIDS [2] as supplements to the widely used conventional Anti-retroviral Therapy (ART). Given the chronicity and the impact of HIV-related diseases on quality of life of patients with HIV/AIDS, and in view of the fact that the virus mutates very easily, it has become imperative to seek alternative therapies [2,3]. Thus, herbal medicines have been used globally to seek for alternative treatments to HIV/AIDS and could be effective in the management of the disease [4].

Garlic has been found to be effective against the opportunistic infections of HIV/AIDS patients. Such opportunistic diseases include herpes, tuberculosis and sexually transmitted infections [5]. Moringa leaf has been identified as a valuable nutritional component, which enhances the immune system [6]. Moringa leaves have also been reported to possess powerful anti-oxidants that can help prevent or delay some complications arising from AIDS [7]. *Artemisia Annua* improves the general condition of the patients living with HIV/AIDS, improving their appetite, weight gain, and healing of opportunistic infections associated with AIDS [8].

It has been reported that more than 70% of HIV-positive people prefer alternative medicine as a better way of management of the AIDS virus [9]. Some patients have resolved to alternative methods of treatments of HIV/AIDS infection instead of the standard and widely used conventional methods. The aim of this study was therefore designed to find out the effects of administration of extracts of some indigenous herbs for HIV/AIDS patients employing viral load reduction and increase in CD4 counts as assessment indices, as well as assessing its antioxidant potentials.

2. MATERIALS AND METHODS

2.1 Study Area/ Sample Size

A total number of 95 HIV/AIDS positive volunteers' were selected at guided random after meeting the inclusion criteria. They are from Jos North, Jos South and Mangu Local Government Areas of Plateau State, Nigeria (Fig. 1). This sample size was arrived at by considering the reports of Daniel [10] and Araoye [11] as well as the frequency of HIV infections in the study area. The volunteer was chosen because they

voluntarily refused conventional method of HIV/AIDS treatment and opted for the conventional treatment with the consent that no other ARVs will be combined during the treatment period, which was also confirmed during the various visitations (Fig. 2). They were screened with the aid of Serial Algorithm using antibody testing. A semi-structured questionnaire was used to collect some data which include parameters like the sex of patient, age, hospital/laboratory number and designation of blood sample collection. Samples were then aseptically taken to Our Lady of Apostles (OLA) Hospital located in Jos North Local Government Area of Plateau State, Nigeria.

2.2 Ethical Clearance

Ethical clearance was obtained from the Ethical Committee of OLA Hospital and consent from the individual participants. They were assured of full anonymity and all pieces of information obtained were handled confidentially and exclusively for the purpose of this study.

2.3 Preparation of Herbal Combinations

Fresh *Ananas comosus* juice (500 ml), *Citrullus lanatus* juice (2.2 L) and *Citrus medica* juice (200 ml) were extracted using a blender (Model BL330, Kenwood, Hong Kong, China) and suspended in coconut oil (250 ml). Aqueous extracts of zobo (*Hibiscus sabdariffa*) was prepared by extracting 100 g in 500 ml of distilled water from which 200 ml was mixed in the previous preparation. Garlic (7000 mg), *Moringa oligofera* (250 g) and *Artemisia annua* leaves (250 g) were mixed with the whole preparation and then suspended in 1L of Tualing Honey. The herbal combination was dispensed in sterile plastic containers and refrigerated. The volunteers were asked to take two tablespoons thrice daily (approximately 10 ml).

2.4 Sample Collection/Analysis

The infected patients were chosen for the study after their consents were obtained. Firstly, their HIV status was confirmed using the commercially available test kit (Determine-Alere, Unigold-Trinity, and Stat-pak- Chembio). Their blood samples were collected with the aid of sterile (EDTA) containers. Their baseline viral loads and CD4 cell counts were determined (Eppendof amplification and Emax detection using Amplicor-Monitor techniques, Partec SL3, flow cytometer). Those with high viral loads and low CD4 counts and who were not on antiretroviral treatment (conventional) were included in the study. Their blood samples were again collected in EDTA containers at six (6) month intervals for two years after the commencement of the Herbal treatments. The blood samples were subjected to CD4 cell counts and viral load determination to assess the responses of the patients to the herbal treatments.

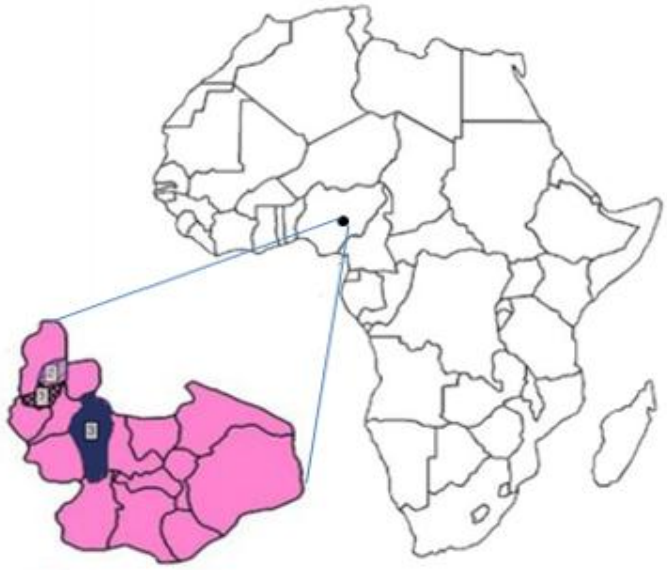

Fig. 1. Map of Africa showing Nigeria, plateau state and the local government where the study was conducted

1. Jos South, 2. Jos North, 3. Mangu

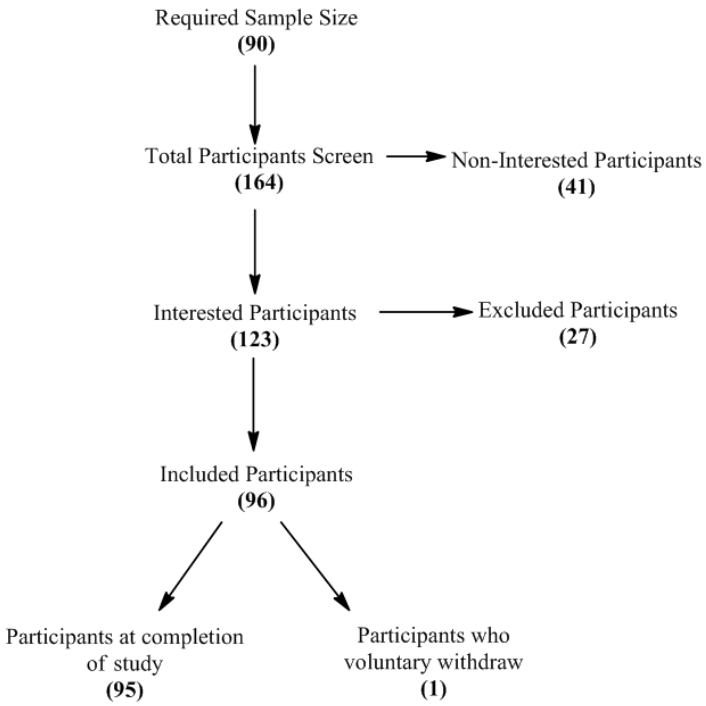

Fig. 2. Flow chart showing how participants were screen based on inclusion and exclusion criteria

2.5 Viral Load Assessments

The Viral Load assessment was performed using the Ultra-Sensitive specimen preparation procedure. Briefly, the HIV viral particles in plasma of collected blood samples were concentrated by high-speed centrifugation, followed by lysis of the virus particle with a chaotropic agent and precipitation of the HIV RNA with the aid of alcohol. A standard RNA molecule was then introduced into each specimen with the lysis reagent. This was followed by reverse transcription, amplification and detection steps for the quantitation of HIV RNA in the test specimens.

2.6 CD4 Cells Count

CD4-PE fluorescence was used in the analyses of CD4 cells count on a Partec SL3, flow cytometer(manufacture by Partec) with an excitation light source of 488 nm (blue or green solid-state laser). 840 µl blood sample was prepared with appropriate dilution factor of 42 and transferred to Partec flow cytometer where the counting analysis was carried out. Counting result was displayed automatically as $CD4^+$ T-cell per µl whole blood.

2.7 DPPH Radical Scavenging Assay

The scavenging activity of the herbal combination against DPPH radical was determined according to the method of McCune and Johns [12]. 500 µl of 0.11M methanolic DPPH was added to 500 µl of different concentration of the herbal combination and Butylated hydroxyl toluene (BHT) and incubated at room temperature in the dark for 10 min. The absorbance of the blank (A_b) and samples (A_s) was measured at 517 nm (Spectrophometer by Newlife-England). manufacturer). DRSA was calculated using the formula as

$$[(A_b - A_s)]/A_b \times 100$$

2.8 Evaluation of Total Antioxidant Capacity (TAC)

The total antioxidant capacity was evaluated using the phosphomolybdenum assay (Prieto et al. [13]. Briefly, 0.1 mL of extracts was combined with 1 mL reagent solution (0.6 M tetraoxosulphate (VI) acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95ºC for 90 min. After cooling to room temperature the absorbance was measured at 695 nm.

2.9 Ferric Reducing Antioxidant Assay (FRAP)

The FRAP of the herbal combination was determined according to the method reported by Girgih et al. [14]. To 250 µL of plant extracts or BHT was added 250 µL of 0.2 M phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferricyanide solution. The mixture was incubated at 50ºC for 20 min. After incubation, 250µL of 10% aqueous trichloroacetic acid (TCA) was added. Then, to 250 µL of the extract/TCA mixture was added 50 µL of 1.0% of $FeCl_3$ and 200 µL distilled water and allowed to stand at room temperature for 10 min. The mixture was then centrifuged at 1000 g for 20 min. After that, absorbance was measured at 700 nm.

2.10 Phytochemical Screening

Qualitative phytochemical analysis of the herbal combination and the individual components carried out using the method described by Sofowora [15] and Evans [16], for assessing the presence or absence of alkaloids, tannins, saponins, flavonoids, steroids and phlobatannins. Quantitative phytochemical analysis were reported following the methods of Harborne [17] and Pearson [18].

2.11 Flavonoid Determination

The flavonoid content of the herbal mixture was determined by the gravimetric method as described by Harborne [17]. Briefly; 5 g of the powdered sample was placed into a conical flask and 50 ml of water and 2ml HCl solution was added. The solution was allowed to boil for 30 minutes on hot plate. The boiled mixture was allowed to cool before it was then filtered through Whatman filter paper (No 42). 10ml of ethyl acetate extract which contained flavonoid was recovered while the aqueous layer was discarded. A pre-weighed Whatman filter paper (Whatman®, Swastik Scientific Company, India) was used to filter second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60ºC. It was cooled in a desiccator until a constant weight was obtained.

2.12 Alkaloid Determination

The concentration of alkaloid in the herbal mixture was determined using the alkaline precipitation gravimetric method described by Harborne [17]. Briefly; 5 g of the powdered sample was soaked in 20 ml of 10% ethanolic acetic acid. The mixture was allowed to stand for four (4) hours at room temperature. After that, the mixture was filtered through Whatman filter paper (No 42). The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60ºC for 30 minutes, cooled in a dessicator and reweighed. The process was repeated two more times and the average was taken. The weight of alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed.

2.13 Determination of Saponins

The saponin content of the herbal mixture was determined by double extraction gravimetric method (Harborne [17]). Briefly; 5 g of the powered sample was mixed with 50 ml of aqueous ethanol (20-80) solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55ºC; it was then filtered through what man filter paper (No42). The residue was extracted with 50 ml of 20% ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90ºC and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re extraction by partitioning was repeatedly done until the aqueous layer becomes clear in color. The saponins were extracted with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was then dried at 60ºC in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample.

2.14 Steroid Determination

The steroid content of the herbal mixture was determined using the method described by Harborne [17]. Briefly; 5 g of the powdered sample was hydrolysed by boiling in 50 ml hydrochloric acid solution for about 30 minutes. It

was filtered using Whatman filter paper (N042) the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100ºC for 5minutes in a steam bath (CDWR Intl North America). It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed with Whatman filter paper (N042). The dry extract was then cooled in a dessicator and reweighed. The process was repeated two mere times and an average was calculated.

The concentration of flavonoid, alkaloid, saponins and steroid was determined and expressed as a percentage using the formula:

$$\%\ \text{Phytochemical} = \frac{W2 - W1}{Weight\ of\ sample} \times \frac{100}{1}$$

Where:

W1 = Weight of evaporated dish
W2 = Weight of dish + sample

2.15 Phenols Determination

The concentration of phenols in the herbal mixture was determined using the folin-cio Caltean colorimetric method described by Pearson [18]. Briefly, 0.2 g of the powdered sample was added into a test tube to which 10 ml of methanol was added to it, shaken thoroughly and allow to stand for 15 minutes before being filtered using Whatman (No42) filter paper. 1 ml of the extract was placed in a text-tube, 1 ml folin-cio Caltean reagent in 5 ml of distilled water was added, and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave. The process was ensured in three times and the average taken.

2.16 Tannin Determination

The tannin content of the herbal mixture was determined using the Folin Dennis spectrophotometric method described by Pearson [18]. Briefly: 2 g of the powered sample was mixed with 50 ml of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered and the filtrate used for the next step. 5 ml of the filtrate was measured into 50 ml volume flask and diluted with 3 ml of distilled water. Similarly 5 ml of standard tanuric acid solution and 5 ml of distilled was added separately. 1 ml of Folin- Dennis reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760 nm with the reagent blank at zero. The process was repeated two more times to get an average.

Percentage phenol and tannin content was calculated using the formula:

% tannin = 100/W x AY /AS x C/100 x VF/VA x D

Where,

W= weight of sample analysed, AY=Absorbance of the standard solution, C= Concentration of standard in mg /ml, VA= volume of filtrate analysed and D= Dilution factor.

2.17 Statistical Analysis of Data

The results obtained were subjected to statistical analysis which include one-way analysis of variance (ANOVA) using SPSS 20.0 computer software package (SPSS Inc., Chicago, U.S.A) where applicable. Differences at $p<0.05$ were considered significant.

3. RESULTS

The age group 30-39 years had the highest incident rate in the study followed by the age groups 40-49, 20-29 and 50-59 years respectively, as shown in Table 1. Also, female participants (60%) were higher than male participants (40%) in the study. Participants reported no major side effect during treatment with herbal combination and no single death was recorded during this study period.

Table 1. Age distribution of HIV/AIDS patients on herbal combination treatment

Age (years)	Frequency	Percentage (%)
20-29	19	20
30-39	42	44
40-49	23	24
50-59	11	12
Total	95	100

The viral loads significantly decrease at 12th month in 50-59 age groups while 30-39, 40-49 age groups had significant decrease at 18th

month after combined herbal treatment (Table 2). The CD4 counts similarly improved at the 24th month after combined herbal treatment except in 20-29 age group which show no significant difference (Table 3).

The adherence and non-adherence rates monitored during the study revealed that the highest percentage adherence rate was in age groups 20-29, 30-39 and 40-49 years while the non-adherence rate was in age group 50-59 years (Fig. 3).

Table 2. The effects of combined herbal treatments on viral loads at 6-month intervals

Age (years)	Viral load (×10^3 copies/mL)				
	Base line	6 months	12 months	18 months	24 months
20-29	5.29±2.76^a	2.87±2.33^a	2.37±1.97^a	2.34±1.95^a	1.49±1.19^a
30-39	6.93±2.24^a	4.77±1.78^a	3.17±1.29^{ab}	2.29±0.96^b	1.98±0.89^b
40-49	4.35±1.67^a	2.93±1.37^a	2.15±0.94^{ab}	1.53±0.74^b	1.02±0.57^b
50-59	51.54±40.37^a	37.59±20.25^a	4.86±3.84^b	5.80±4.82^b	3.11±2.91^b

NOTE: Values are mean of group ± SEM, Values with different superscript (a – b) are significantly different (p<0.05) across the row

Table 3. The effects of combined herbal treatment on CD4 counts at 6 months intervals

Age (years)	CD4 (×10^2 cell/mm^3)				
	Baseline	6 months	12 months	18 months	24 months
20-29	6.62±1.90^a	7.02±1.40^a	7.82±1.22^a	8.88±1.34^a	9.74±1.31^a
30-39	4.09±0.56^a	4.51±0.72^a	5.40±0.69^{ab}	10.33±4.69^b	6.58±0.89^b
40-49	3.67±0.64^a	4.81±0.63^{ab}	5.28±0.74^b	6.30±0.72b^c	8.09±1.36^c
50-59	1.72±0.42^a	2.13±0.57^a	2.99±1.01^{ab}	3.77±1.90^{ab}	4.87±1.96^b

NOTE: Values are mean of group ± SEM, Values with different superscript (a – c) are significantly different (p<0.05) across the row

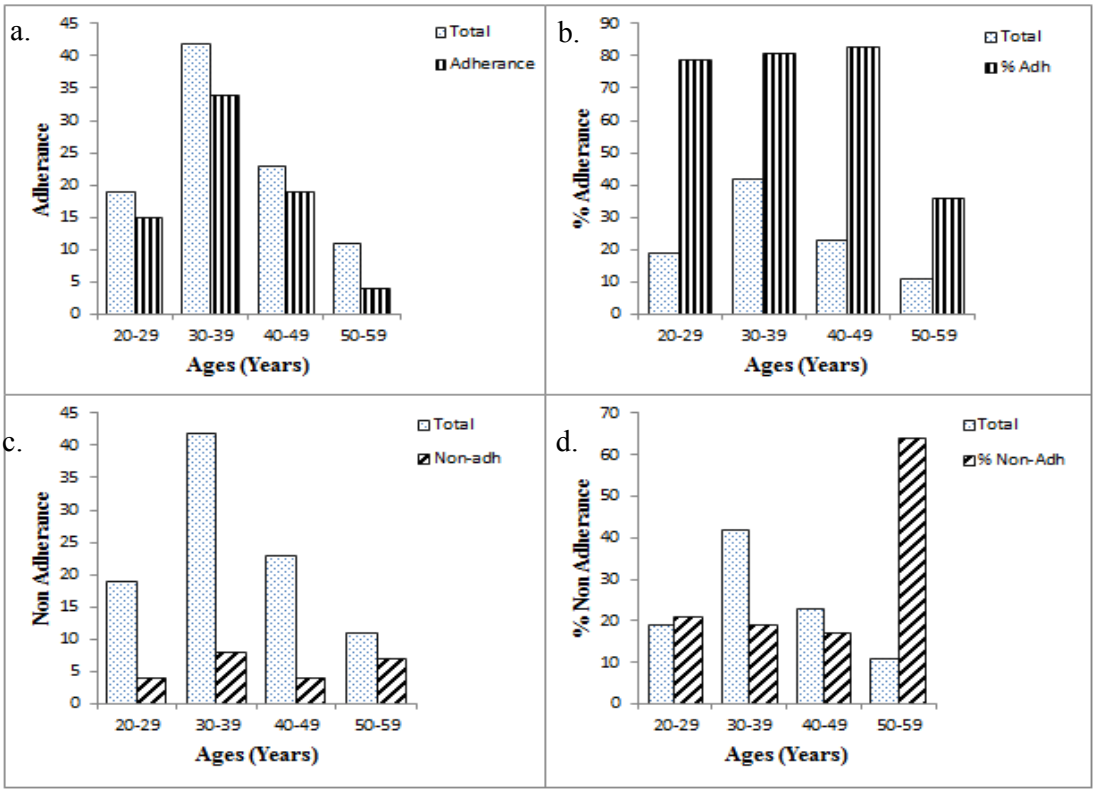

Fig. 3. Adherence and non-adherence rate of HIV/AIDS patients after 24 month of combined herbal treatments

The DPPH scavenging effect of the herbal mixture was superior to that of reference BHT and vitamin C (Fig. 4), with IC_{50} values of 1.42, 2.94 and 2.55 mg/ml respectively (Table 4). However, the extract combination and BHT has similar Total Antioxidant Capacity (TAC) with BHT slightly higher than the herbal combination (Fig. 5). Vitamin C had a better Ferric ion reducing effect compared to herbal combination and BHT at the concentration used (Fig. 6).

The presence of alkaloids, flavonoids, saponins and steroids were detected in the herbal combination while phlabotannins and tannins where not detected (Table 5). Flavonoid and saponins were detected in moringer and artemesia while other individual components such as watermelon and orange contain flavonoid, alkaloids, steroids and tannins. Only saponin was detected in galic. Quantitatively, alkaloids was found to be highest (16.96%), followed by Saponins (11.98%), Flavonoid (9.21%), Steroid (2.48%), Tannin (0.59%) and Phenols (0.46%) (Table 6).

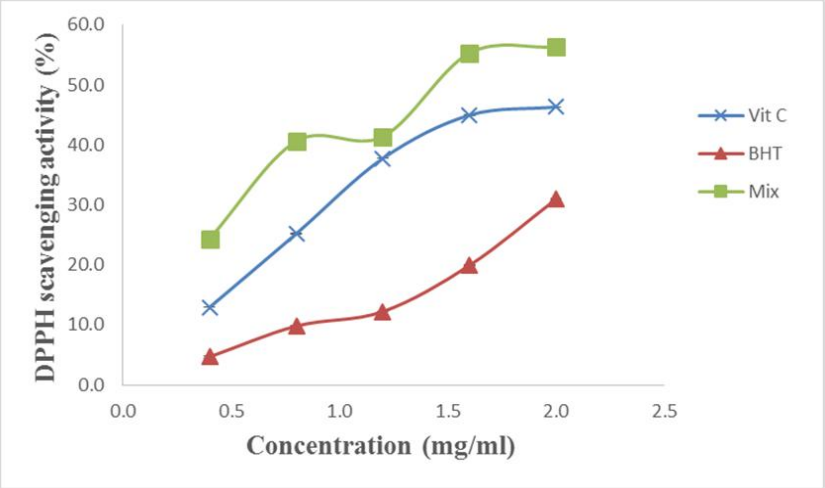

Fig. 4. DPPH scavenging activities (%) of herbal combination
Values are means ± SEM of triplicate determination
Vit C = Ascorbic acid, BHT = Butylated hydroxyl toluene, Mix = Herbal combination

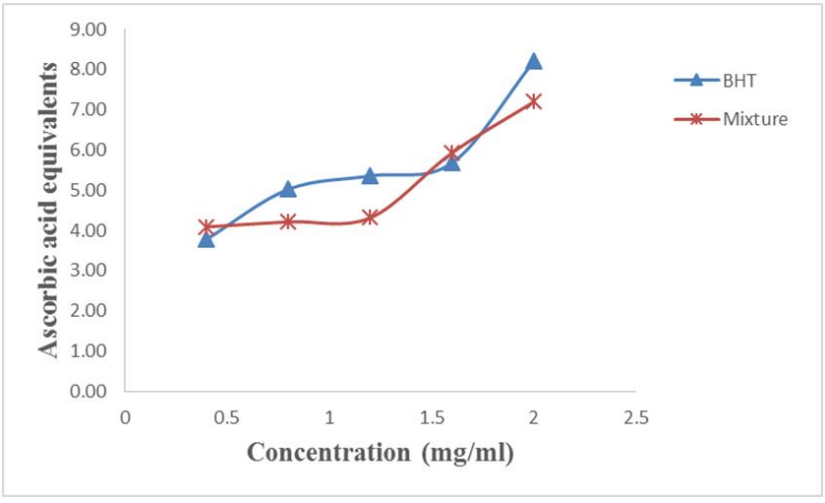

Fig. 5. Total Antioxidant Capacity (TAC) of herbal combination
Values are Means ± SEM of triplicate determination
BHT = Butylated hydroxyl toluene, Mix = Herbal combination

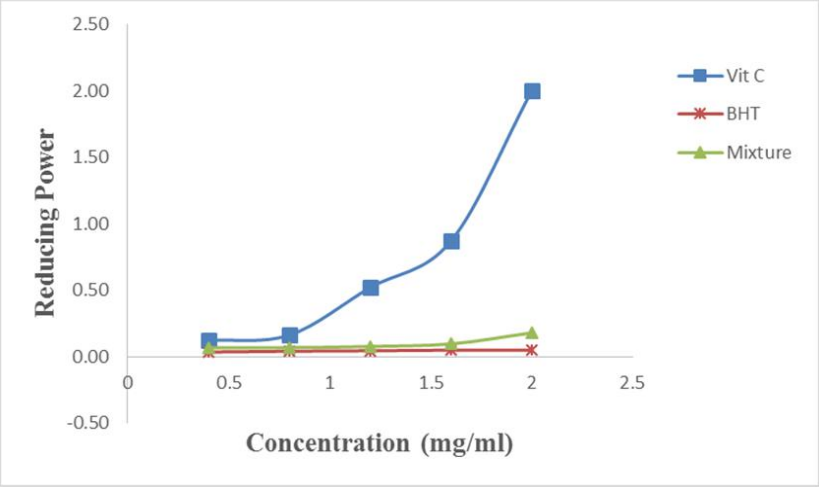

Fig. 6. Ferric ion reducing effect of herbal combination
Values are means ± SEM of triplicates determination
Vit C = Ascorbic acid, BHT = Butylated hydroxyl toluene, Mix = Herbal combination

Table 4. EC_{50} for DPPH scavenging activities of herbal combination

	EC_{50}
Compounds	**mg/ml**
Vit C	2.49
BHT	2.55
Mixture	1.42

EC_{50}= Half maximal effective concentration
Vit C = Ascorbic acid, BHT = Butylated hydroxyl toluene, Mix = Herbal combination

4. DISCUSSION

HIV/AIDS is a global phenomenon that affects all sexes, and that is not limited to any age group. Sexual intercourse including oral and anal, blood transfusion, and sharing of sharp objects are some of the means in by which HIV is transmitted. In Nigeria, the prevalence of HIV among young women of age group 15 – 24 years is estimated to be three times higher than among men of the same age [19]. In term of age distributions, the study showed that the age group 20-29 had 20% frequency while the highest percentage frequency (44%) of HIV infected group was recorded in 30 – 39 years of age. The least frequency of 12% was recorded in the age group 50 – 59 years (Table 1). This could be because the active sex age is between the age group 20 – 39 years. An earlier report by Elujoba et al. [20] has also reported a higher percentage of HIV/AIDS infected patients in the age group 30 – 39 years with the least from selected age groups 50 – 59 years. More females than males were willing to participate in the study as indicated by the data.

In an earlier report also by Elujoba et al. [20], anti-retroviral (ART) drug such as Nevirapine (NVP), Efavirenz (EFV), Zidovudine (AZT) and

Table 5. Qualitative phytochemical status of herbal combination and some individual components

S/No	Component	Mixture	Moringer	Galic	Artemesia	Watermelon	Orange
1.	Flavonoid	+	+	-	+	+	+++
2.	Alkaloid (Wagners)	+	-	-	-	-	+
	Alkaloid (Mayers)	+	-	-	-	+	++
3.	Saponins	+	++	+	++	-	+
4.	Steroid	+	-	-	-	++	+++
5.	Tannin	-	-	-	-	+	++
6.	Phlobatannins	-	-	-	-	-	-

Note: - = Not detected, + = present, ++ = slightly present, +++ = highly present

others was shown to reduce the viral load of HIV/AIDS patients after 6 months of treatment. However, the study with herbal combination therapy, a combination of some fruits and leaves with acclaimed anti-HIV activities suspended in honey showed a similar reduction in Viral Load after 18 months of treatment in all age groups except the age group 20 – 29 years (Table 2). The Viral load reduction rate recorded could be due to certain plant constituents with potent immune-system stimulator and antiviral components. Many herbal remedies have been documented to be useful in HIV infection. They have been found to inhibit one or more steps in HIV replication [21,22].

Table 6. Quantitative phytochemical status of herbal combination

S/No	Component	Mixture (%)
1.	Flavonoid	9.21
2.	Alkaloid	16.96
3.	Saponins	11.98
4.	Steroid	2.48
5.	Phenols	0.46
6.	Tannin	0.59

The combined herbal treatment had no significant ($p>0.05$) effect on the CD4 counts of volunteers from the age groups of 20 – 29 years while the best effect of the herbal treatment was recorded in the age group 40 – 49 years (Table 3). The non-significant improvement in CD4 count of age group 20 – 29 years could be contributed by non-adherence rate of the groups which was 21%. The two age groups of 30 – 39 and 40 – 49 years showed an impressive adherence rate of 81% and 83% respectively (Fig. 3).

Individual components of the combined herbal treatment could be increased for better performance as there are no particular restrictions to variation in the herb combination. Extracts included in the study showed significant improvement at the latter stage of the study. Several herbal components have been reported to be of great benefit in HIV/AIDS. For example, Garlic may help strengthen the immune system in HIV. Acemannan, one of the constituents of the herb *aloe vera*, has shown some promise in test tube and animal studies for stimulating immunity and inhibiting the growth of viruses, including HIV [23-25]. Patients living with HIV/AIDS have a weakened immune system due to the effect of the virus. They also lost appetite. Some conventional medicines change the taste of food and reduce appetite. Herbal medicines and spices have been found to improve digestion and stimulate appetite [26-27].

The prevalence of HIV/AIDS varies from state to state in Nigeria. Despite the availability of free Highly Active Anti-Retroviral Therapy (HAART), Nigerians still go in search of alternative treatment/management options for the HIV by visiting herbal medicine practitioners. This has paved the way for the documentation of different herbal remedies with active antiretroviral properties. As stated earlier, some of this herbal remedies has been found to inhibit one or more steps in the replication of the virus [21-22]. Examples include tropical liana plant (Ancistrocladus korupensis) which inhibit reverse transcriptase and HIV induced cell fusion [28]. Also, coumarins from Calophyllum lanigerum was found to be a potent non-nucleoside reverse transcriptase inhibitor [29-30]. Other herbal remedies used in HIV are aimed at treating opportunistic infections that are occasioned in HIV [31], while other actively boost the immune system by increasing CD4 lymphocyte count in HIV [31-32]. Several other plant with one or more beneficial effects in the treatment of HIV/AIDS has been documented [33].

Phytochemicals, can also referred to as phytonutrients. They are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds and are classified according to their chemical structures and functional properties. Research in the field of phytochemistry has demonstrated that foods and beverages rich in phytochemicals may help prevent diseases. The richness of the herbal mixture in the various phytochemical could be responsible for the reduced viral load and CD4 lymphocyte counts. The herbal mixture contains all the phytochemicals tested for except Tannin and Phlobatannins (Tables 5 and 6). This was also from the richness of the individual constituents that makeup the herbal mixture as seen from Tables.

Benefits of antioxidant in HIV replication has been demonstrated *in vitro*. HIV replication was promoted by Hydrogen peroxide, which a strong oxidant. The infected cells also release gene-stimulating cellular proteins called nuclear factor kappa B (NF-kB). The effect observed with the strong oxidant was however counteracted by the addition of N-acetylcysteine which is a known antioxidant [34]. The strong antioxidant nature of the herbal mixture could be one of the effect

observed in increased CD4 counts and reduced viral load replication over the period of study. Vitamin C is one of the components of the herbal mixture used in the study. It is a know and widely used antioxidant. It has been reported to play a significant role in reducing HIV replication. it blocks some specific enzymes that HIV requires to complete its lifecycle [35-36]. Ascorbic acid lowers HIV reverse transcriptase activity by over 99% and a type of antigen known as p24 antigen by 90% [37].

5. CONCLUSION

The present study showed that combined herbal therapy could bring the reduction in the viral load and increase CD4 counts of HIV/AIDS patients. It similarly expresses good antioxidant activities *in vitro* when compared to the commercial reference BHT. Herbal treatment should be encouraged. It should act as the supplementary treatment for patients and should not replace the conventional ART therapy. Increases in adherence rate should also be advocated for a better response as non-adherence to the treatment regime may present a decrease in CD4 count and an increase in viral load.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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