African Journal of
Medicine, Surgery and Public Health Research

ISSN: 3434-3567

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJMSPHR.v2i2.5184

Assessment of HIV 1 GP120/CD4 Binding Inhibition Potential of Methanolic Extracts of Achyranthes Aspera Leaves

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Feb 17, 2024	Mar 1, 2025	Mar 13, 2025	Mar 18, 2025

Abstract

Achyranthes aspera (Amaranthaceae) is an important medicinal herb found as a weed throughout Nigeria. Though almost all of its parts are used in traditional systems of medicines, leaves, seeds, roots and shoots are the most important parts which are used medicinally. The present article gives an account of updated information on its phytochemical and pharmacological properties. The Human immunodeficiency virus (HIV) infection is still contributing significantly in morbidities and mortalities in the world today. The drugs normally used to treat the infection are costly, toxic, and less effective due to resistance by HIV. Thus, the assessment of gp120-CD4 binding inhibition potential of Achyranthes aspera leaves extract was conducted using gp120-CD4 capture ELISA kits. Aqueous, methanol, and petroleum ether extracts were prepared at 1000, 500 and 250 µg/ml and tested for gp120-CD4 binding

inhibition. Sub-cute toxicity assay was done using albino rats; Biochemical parameters including alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) as well as bilirubin for liver and urea, electrolytes and creatinine for kidney functions were evaluated. The results of the effect of crude aqueous, methanol and petroleum ether Achyranthes aspera leaves extract against gp120-CD4 showed inhibition ranging from 1.4 – 17.2 %. with 1000 µg/ml showing highest percentage of inhibition. There was no significant difference (P = 0.862) in terms of percentage inhibition between the three concentrations tested. Methanol extract demonstrated the highest percentage inhibition of gp120-CD4 bindings (17.2 %). No significant difference (P = 0.124) between the three extracts against gp-120-CD4 bindings was observed. The results of the sub-acute toxicity study have shown that, there were no physical changes in animals treated with 500 mg/kg of all the extracts. The result of liver function test revealed that, ALT, AST and ALP were within the normal range (12U/L) for both the high and low concentrations of the extracts including the control. Also result for total protein, albumin, globulin for the test albino rats and that of the control rat were found to be within the normal range 5.1-6.1, 4.2-5.3, and 3.1-3.8 g/dl respectively. For serum electrolyte level, sodium and potassium ions for the various concentrations of the extracts tested and control were also found to be within normal range. The result of kidney function test revealed that, urea, creatinine and direct and total bilirubin of the rats tested and control were all found to be within normal range. In conclusion, ether Achyranthes aspera leaves extract possessed some levels of HIV-1 gp120-CD4 binding inhibition potentials and the extracts was found to be non-toxic at 250 and 500mg/ kg body weight. It can be recommended that, the bioactive compounds should therefore be isolated and tested for gp120-CD4 binding inhibition activity.

Keywords: Assessment, HIV1, GP120/CD4, Binding, Inhibition, Potential, Methanolic, Extracts, Achyranthes Aspera, Leaves

INTRODUCTION

Human Immunodeficiency Virus (HIV) persists to be a significant public health issue worldwide. In 2018, 37.9 million people are living with HIV globally; out of which 36.2 million are adults and 1.7 million are children less than 15 years old. There were 1.7 million new infections and 770,000 people died from AIDS related illness worldwide [1]. The current strategy for the treatment of HIV infection is Highly Active Antiretroviral Therapy (HAART), based on combination of inhibitors of reverse transcriptase and protease.

Although HAART has considerably reduced deaths from AIDS related disease, it often has side effects and not well tolerated especially in persons undergoing long term treatment and maintains the risk of developing multidrug resistance [2]. Moreover, HAART is an expensive regime for underdeveloped and developing countries where the drugs are inaccessible to the HIV infected patients.

Thus, there is a need for the discovery of novel therapeutic strategies, which identify new anti-HIV compounds from natural sources particularly from medicinal plants. Natural sources provide a large reservoir for screening of anti-HIV agents with novel structure and antiviral mechanism due to their structural diversity.

The HIV virion enters macrophages and CD4 T Cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the target cell membrane and the release of the HIV capsid into the cell [3, 4].

The first step in fusion involves the high affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine receptor binding domains of gp120 and allowing them to interact with the target chemokine receptor. This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane [5,6].

There are two related but distinct types of HIV: HIV-1 and HIV-2 [7]. HIV-1 is the most pathogenic and causes over 99 % of HIV infections [8]. HIV-2 is also known to cause AIDS but is much less prevalent; being present in fewer and isolated geographic locations such as West Africa, therefore, most research is done on HIV-1 [9]. About 13 million people were infected with HIV worldwide in 1993 and the number has increased to about 21 million in 1996.

The World Health Organization (WHO) recommended that traditional healers be included in national responses to HIV/AIDS. As early as 1989, WHO had already voiced the need to evaluate ethno medicines for the management of HIV/AIDS. In this context, there is need or the systematic evaluation of the elements of traditional medicine, particularly medicinal plants and other natural products that might yield effective and affordable therapeutic agents [9]. Plants are important source of drugs; especially in traditional medicine [10].

Although there are reports on traditional uses of plants to treat various diseases, knowledge of herbal remedies used to manage HIV/AIDS are scanty, impressionistic and not well documented [11, 12]. Thus, it is important to search for novel anti-HIV agents that can be added to or replace the current arsenal of drugs against HIV [13]. Achyranthes aspera Leaves is traditionally used in the treatment of some ailments in human and animals such as Whole plant is used for nephroprotective, hypolipidemic activity. While the roots for anti-oxidant, spermicidal, activity and the leaves for anti-oxidant, anti-fertility, anti-depressant, anti-cancer, as well as anti- microbial activity. The Aerial parts is used for hepatoprotective activity and the seeds for anti- microbial activity [14–22]. Therefore, the study was aimed at assessing the HIV 1 GP120/CD4 Binding Inhibition Potential of methanolic extracts of Achyranthes aspera Leaves

MATERIALS AND METHODS

Sourcing of Plant Materials Achyranthes aspera Leaves were collected at Michika local government Area Adamawa State

Extraction of Phytochemical Constituents:

The phytochemical constituents Achyranthes aspera Leaves was extracted using Soxhlet extraction technique as described by Tariq et al. [23].

Preparation of Achyranthes aspera Leaves Extract

were washed, shade dried and finely powdered. The powder 50g, was suspended in 500 ml of sterile distilled water as described by Tariq et al. [23].

Phytochemical Analysis of the Extracts

The extracts were analysed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, cardiac glycosides and reducing sugars as stated Adetuyi and Popoola [24]; Salehi et al. [25].

Gp 120 Binding Inhibition Assay as described by Rege et al. [26]

Components A (plate holder with solid phase capture ligand CD4). B (Wash buffer), C (blocking/diluents) was brought to room temperature, while the wash buffer (component B) was diluted 1:10 with deionizer water. Then, the plate was removed from shielded bag using scissors. Subsequently, two hundred micro liters (200 µl) of wash buffer was added to

each well and was allowed to stand at room temperature until ready for the next step. Equally several dilutions of HIV gp120 (component D) was made in comparable tubes and label accordingly

Preparation of the Test Concentrations and Screening for Anti gp120-CD4 Binding Activity as described by Rege et al. (2009)

Three (3) different concentrations (1000 µg/µl, 500µg/µl, and 250µg/µl) of the plant extracts were prepared in three (3) separate test tubes using diluents buffer (component C) and labelled accordingly. The content of the wells was dumped and pad dried to remove the wash solution and placed on a grid.

Equally the grid was labelled 1 – 3 horizontally representing three different extracts tested (1=aqueous fruit extract 2 = methanol fruit extract 3=petroleum ether fruit extract) while the grid was label A-H vertically for the control and different concentration of the test extracts (A and B are for the control, C and D for the 1000 µg/µl in duplicate, E and F for the 500 µg/µl in duplicate, G and H for the 250 µg/µl in duplicate).

One hundred micro litters (100 µl) of Gp120 positive reference was mixed with 100 µl each of the different concentrations of the test extracts and the control in a separate sterile microlitre plate and allowed to stand for ten (10) minutes. These were subsequently dispensed into the appropriate CD4 coated microlitre wells as labelled. The plate was covered and allowed to stand at room temperature for an hour. The contents of the wells were dumped and washed three times with wash buffer (300µl/well), the wells were pad dried.

Detector reagent (component E) was diluted 1:100 in diluents buffer (component C) and 100 µl of the detector reagent was added to each of the well and allowed to stand at room temperature for one hour. Subsequently the wells were washed three times using washing buffer (component B) and pad dried. One hundred micro liter (100 µl) of 3.3', 5.5'-Tetramethylbenzidine (TMB) substrate (component F) was added to each of the wells. Blue colour developed within 10 minutes.

$$\text{Percentage (\%)Inhibition} = \frac{\text{Optical Density of the sample}}{\text{Optical Density of the control}} \text{x } 100 \text{ Eqn ….1}$$

Colour development was stopped by adding 100 µl of stop solution (Component G) to each well. The plate was read at 450 nm using ELISA reader within 5 minutes. The set up

was duplicated, so that average values were taken. Percentage inhibition was calculated using formula: [27].

Sub-Acute Toxicity Study as Described by Adeyomo And Makinde [27, 28]

Three groups of four animals (Albino rats) were set up with each group horizontally representing three different labelled as aqueous, methanolic and pet ether extracts. In each group, 2 animals were administered with 250mg/kg and the other 2 animals were given 500mg/kg extracts daily for 4 weeks. A control group of 2 animals that were only given 1ml of distilled water for the same period was set up. The body weight changes were monitored throughout the experimental period on weekly basis, while the animals were monitored for manifestation of toxicity and mortality.

Biochemical Analyses Liver Function Tests

The following tests were conducted to investigate derangements in the liver function of animals used for sub-chronic toxicity studies: Serum total bilirubin, serum total protein and albumin. Serum globulin level was calculated as the difference between serum total proteins and Albumin. Albumin/Globulin (A/G) ratio was estimated from the values obtained for the albumin and globulin.

Serum AST and ALT activities were determined using Reitman-Frankel [27] method, Chesbrough, [28]. Alkaline phosphatase activity was estimated in the serum by the nitrophenyl phosphate method of Bassey, [28].

Renal Function Tests

Serum creatinine, urea, sodium and potassium ions were evaluated as described by Reitman et al. [27], Chesbrough [28, 29].

Statistical Analysis

The result was analysed using Two-Way Analysis of Variance (ANOVA). The level of significance used was $P < 0.05$.

RESULTS

Phytochemical Physical Characteristics of Achyranthes aspera Leaves Extract. The physical characteristics of Achyranthes aspera Leaves extracts and the results are shown in Table 1,

in which the color ranges from Green, Dark Green, Light Green, while the texture was pastry after extraction and the pH ranges from 6.2– 6.3 (Table 1).

The results of qualitative phytochemicals analyses of Aqueous, Methanol and petroleum ether fruit extracts revealed the presence of some phytochemical components such as saponins, flavonoids, steroids, and polysterols (Table 2).

Anti-binding Effect of the Extract against gp120 – CD4. The results of the activity of crude aqueous, methanol and petroleum ether Achyranthes aspera Leaves extracts against gp120-CD4 (Table 3) showed inhibition at various concentration ranging from 1.4 – 17.2 %. The highest inhibition was 17.2 %, at 1000 µg/ml of methanol followed by 15.4 %, and 14.3 % in both 1000 and 500 µg/ml of petroleum ether and methanol respectively (Table 3).

Result of Sub-Acute Toxicity of Achyranthes aspera Leaves extract Results showed that, oral administration of aqueous, methanol and petroleum ether of Achyranthes aspera of 250 and 500mg/kg did not produce any sign of sub-acute toxicity (Such as, hair loss, weight loss, etc.) or instant death in any of rat group tested (Table 4).

Table 1: Physical Characteristics of the Achyranthes aspera Leaves extract

Extracts	Colour	Texture	Odor	Solvent	PH
Aqueous	Green	Powder	Odourless	DMSO	6.2
Methanol	Dark Green	Powder	Odourless	DMSO	6.4
Pet. ether	Light Green	Powder	Odourless	DMSO	6.3

Table 2: Qualitative Phytochemical Analysis of the Aqueous, Methanol and Petroleum ether Achyranthes aspera Leaves extract

Photochemical Components	Aas L Aqueous	Aas L Methanol	Aas L Pet Ether
Alkaloid	+	+	+
Saponin	+	+	+
Tannins	-	-	-
Flavanoids	+	+	+
Steroids	+	+	+
Trepenoids	-	-	-
Phenols	-	-	-
Antraquinone	+	+	+
Cardialglycoside	-	-	-
Phytosterol	+	+	+
Anthocyanin	+	+	+

Key: + = positive, - = not detected; Aas L Aqueous = Achyranthes aspera Leaves extract Aqueous, Aas L Methanol = Achyranthes aspera Leaves extract Methanol, Aas L Pet Ether = Achyranthes aspera Leaves extract Pet ether

Table 3: Mean Optical Density of Fruit Extracts of Achyranthes aspera Leaves extract against HIV1 GP120-CD4 Binding Inhibition at Various Concentrations in (µg/ml) %

Extracts	**Concentrations in (µg/ml) % Inhibition**		
	1000	500	250
Methanol	17.2	14.3	11
Aqueos	1.4	1.4	11.2
Petrol Ether	15.4	13.2	9.7
Control	100	100	100

P-value - 0.003022

Table 4: Mean Percentage Weight Gain and Physical Effect of the Achyranthes aspera Leaves extract

Extracts	Conc. of the Extract Kg/BW	Initial Weight (g)	Final weight (g)	weight Gain	Percentage (%) weight Gain	Hair Loss	Diarrhoea
Aqueous	250	102	104.8	3.6	3.3	N	N
	500	100.6	105.2	3.9	3.8	N	N
Methanol	250	100.7	107.3	6.5	6.4	N	N
	500	104.6	107.4	5.8	5.3	N	N
Pet. ether	250	99.4	103.7	502	4.6	N	N
	500	99.2	102.9	4.7	4.3	N	N
Control	-	99.8	104	-	4.02	N	N

Table 5: Liver Enzymes Activities (U/l) of Rats Treated with Sub-Acute Oral Doses of Aqueous Achyranthes aspera Leaves extract

Extracts	Dose (mg/kg)	Parameter (Activity U/I)		
		AST	ALT	ALF
Control	0	13	12.5	8.0
AHC	500	8.2	8.3	13
ALC	250	12.5	12.5	13
MHC	500	12.3	12.2	8.0
MLC	250	12.3	4.2	13
PHC	500	8.2	12.4	8.2
PLC	250	12.4	8.4	8.4

KEY: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline, phosphatase; NB: Normal Range; AST = Up to 12U/I, ALT = Up to 12U/I, ALP = Up to 12U/I,

Table 6: Serum Electrolytes Level of Rat Treated with Achyranthes aspera Leaves extract

Extracts	Dose (mg/kg)	Parameter (Activity U/I)		
		Na	K	HCO_3
Control	0	136.26	4.00	21.12
AHC	500	155.57	3.57	22.77
ALC	250	148.56	3.66	21.10
MHC	500	148.38	3.60	21.54
MLC	250	147.98	3.50	221.34
PHC	500	150.23	4.21	21.73
PLC	250	136.14	3.57	22.03

Key: Na+ = Sodium ions, K+ = Potassium ions, HCO_3, - = Hydrogen trioxocarbonate, NB: Normal Range (mEq/L); Na+=135-155, K+=3.4-5.3, HCO3 -=20-32; AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC =Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC =Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Table 7: Serum Protein Level of Rat Treated with Achyranthes aspera Leaves extract

Extracts	Dose (mg/kg)	Concentration of Parameter (Activity U/I)			
		Total protein	Albumin	Globulin	A: G
Control	Water	6.123	4.634	2.552	1.811
AHC	500	5.832	4.523	3.122	1.766
ALC	250	5.675	4.455	3.899	1.523
MHC	500	6.554	4.742	3.412	1.477
MLC	250	5893	4.235	3.475	1.345
PHC	500	5.563	5.245	3.611	1.511
PLC	250	5125	5.344	3.121	1.734

NB=Normal Range, Total protein=5.2-9.1g/dl, Albumin= 3.5-5.0g/dl, Globulin = 2.0 – 3.5g/dl, A: G = 0.8 – 2.0 **Key:** Water= Control, LC=Low concentration, HC=High concentration, AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC = Methanol Extracts High Concentration, MHC = Methanol

Extracts Low Concentration, PHC = Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Table 8: Some Kidney Function Parameter of Rat Treated with Achyranthes aspera Leaves extract

Extracts	**Dose (mg/kg)**	**Parameter (Activity U/I)**	
		Urea(mmol/L)	Creatinine(mg/dl)
Control	Water	8.134	3.327
AHC	500	3.511	1.923
ALC	250	4.142	8.652
MHC	500	7.112	7.145
MLC	250	7.223	1.261
PHC	500	2.124	3.435
PLC	250	5.974	3.951

Key: Water = Control, LC = Low concentration, HC = High concentration, NB: Normal Range, Urea=1.7-9.1mmol/L, Creatinine=Up to 20mg/dl, AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC =Methanol Extracts High Concentration, MHC = Methanol Extracts Low Concentration, PHC =Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Table 9: Serum Bilirubin Level of Rats Treated with Achyranthes aspera Leaves extract

Extracts	**Dose (mg/kg)**	**Concentration of parameters (mg/dL)**	
		Total Bilirubin	Direct Bilirubin
Control	0	0.098	0.154
AHC	500	0.199	0.187
ALC	250	0.165	0.211
MHC	500	0,145	0.188
MLC	250	0,203	0.231
PHC	500	0,186	0.188
PLC	250	0.178	0.167

Key: AHC = Aqueous Extracts High Concentration, ALC = Aqueous Extracts Low Concentration, MHC =Methanol Extracts High Concentration, MHC = Methanol Extracts

Low Concentration, PHC =Petroleum ether High Concentration, PLC = Petroleum ether Low Concentration

Biochemical Parameters for Liver Marker Enzymes

Liver marker enzymes of AST, ALT and ALP. The AST, ALT and ALP are all found to be within the normal range (Table 5).

Serum Electrolyte Parameters

Results of serum electrolytes parameters (sodium ion, potassium ion and bicarbonate ions) were all within normal ranges for both high and low concentration of aqueous, methanol and petroleum ether extracts (Table 6).

Serum Protein Level

Results of serum protein level, Albumin and globulin of rats treated with Achyranthes aspera Leaves extract were found to be within the normal acceptable range (Table 7).

Creatinine and Urea Level

The serum creatinine and urea concentrations were found to be within the normal range in all the various concentrations of the extracts tested including the control (Table 8).

Total and Direct Bilirubin of Serum

Results of total and direct bilirubin of the serum of the rats treated with high and low concentration of aqueous, methanol and petroleum ether Achyranthes aspera Leaves extract and control group were within the normal range (Table 9).

DISCUSSION

The phytochemical screening of Achyranthes aspera Leaves extract revealed the presence of Alkaloid, Saponin, Flavonoids, Antraquinone, Phytosterol, Anthocyanin, Steroids. This phytochemical most especially alkaloids, steroids, Anthocyanin, saponins, and phenols. The compounds that blocked HIV-1 infection were flavonoids and anthocyanidins [30]. Several chemical compounds were found to interfere with HIV entry into cells. BanLec, a jacalin-related lectin that binds to glycosylated viral envelopes blocked HIV-1 entry into cells [31]. cyanovirin, an 11 KDa protein isolated from Nostoc ellipsosporum, targeted gp120 proteins and blocked fusion of HIV-1 to lymphocyte membranes [32]. glycoprotein

complexes from Ganoderma mushrooms inhibited HIV-1 gp120 binding to CD4 cells [33]; a code-named compound, PJ-S21, from Punica granatum inhibited the binding of gp120 to cells expressing CXCR4 receptors [34]. Other active constituents included: diterpene lactones [35] and a coumarin named wedelolactone [36] inhibited cell-to-cell transmission of HIV-1; prostratin, a 12-deoxyphorbol, inhibited HIV-1 entry into lymphocytes [36, 37]; and rosmarinic acid isolated from Melissa officinalis inhibited fusion of HIV-1 to cells [36].

The oral administration of aqueous, methanol and petroleum ether of Achyranthes aspera Leaves of 5000mg/kg did not produce any sign of sub-acute toxicity or instant death in any of rat group tested. This suggested that, the extract has a low toxicity of 5000mg/kg body weight. This work was in agreement with the work of on the evaluation of Achyranthes aspera Leaves extract. They reported that, the extract did not produce any mortality even at the dose of 1500 mg/kg. All the doses (5, 50 and 300 mg/kg,) of Achyranthes aspera were thus found to be non-toxic. Any substances with a toxicity ranged between 2000-5000 mg/kg body weight given orally could be considered of low toxicity.

In the assessment of liver damage, certain biomarkers of hepatotoxicity are measured and such biomarkers are enzyme levels such as AST, ALT, ALP, total bilirubin and GGT because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; thus, measurement of these enzymes in serum gives an indication of the health status of the liver.

High levels of AST indicate liver damage, as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. It is known that an increase in the enzymatic activity of ALT, AST and GGT in the serum directly reflects a major permeability or cell rupture, and thus ALT is more specific to the liver, and is thus a better parameter for detecting liver injury [37].

The serum marker enzymes such as AST, ALT, ALP and total bilirubin are all within the normal range. And this is in agreement with the research by Ryan, [30] plants extract against CCI4-Induced Hepatic Damage in Rats" revealed that, the rat treated with the extract along with toxicant showed sign of protection against these toxicants to a considerable extent as evident from the formation of normal hepatic cards and absence of necrosis and vacuoles. Also decrease in serum bilirubin after treatment with the extract in

the liver damage indicates the effectiveness of the extract in normal functional status of the liver, [30].

CONCLUSION

It can be concluded that Achyranthes aspera Leaves extract possess some gp120-CD4 binding inhibition potentials with methanolic extracts having higher binding inhibition potential. Moreover, the plant extracts were found to be non-toxic to the animals used for the study. It is recommended that pure compounds should be isolated and tested for anti-gp120-CD4 binding activity in vitro should also be considered.

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