Original Article

Anti-HIV Activity and Cytotoxic Effects of *Aerva lanata* Root Extracts

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ABSTRACT

The aim of the present study was to evaluate the anti-HIV activity and cytotoxic effects of *Aerva lanata* root extracts. Extracts were prepared in hexane, chloroform, ethyl acetate, acetone and methanol solvents by sequential maceration method and the extract was filtered mass was obtained at low room temperature under pressure in a rotary vaccum evaporator. Anti-HIV activity of the all solvent extracts of *Aerva lanata* root done by Retro Sys HIV-1 RT activity kit (Innovagen, Sweden). Cytoxicity study was performed on all extracts by MTT assay using PBMCs isolated from whole blood. All extracts exhibited the most notable activity and the chloroform extract of *Aerva lanata* showing highest (91.0%) HIV-RT inhibition at 2mg/ml concentration, hexane, ethyl acetate and acetone extractions showed highest inhibition of HIV-RT at 2 mg/ml concentration (86.9, 85.2 and 77.5 respectively). While control drug (AZT) showing 91.7% at 2mg/ml concentration. IC_{50} value of all extracts determined below 40mg/ml. This result suggests that *Aerva lanata* root extracts contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various infectious diseases.

Keywords: HIV-RT, AIDS, PBMC's, Cytotoxicity, *Aerva lanata*.

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) in humans, which is caused by the human immunodeficiency virus type 1 (HIV-1) remains among the leading causes of death worldwide. Currently, over 35.3 million people are infected with HIV/AIDS and 95% of them live in the developing countries[1]. Although HAART has reduced HIV mortality significantly, adhering to the recommended drug schemes, significant toxicities experienced by treated patients, and the high mutation rate of the virus that seem to easily circumvent the action of these drugs emphasize the need for alternative treatment strategies. Medicinal plants are a good

source for the discovery of novel antimicrobial chemotherapeutic agents. Several plant extracts have been shown to possess activity against HIV by inhibiting various viral enzymes[2]. Various studies have shown anti-HIV properties of the extracts prepared from variety of plants[3-5]. Many plant products are being used by patients with AIDS in some countries without any scientific proof that they possess anti-HIV activity. Traditional healers are now offering their remedies for scientific evaluation, and a number of studies provide information on the inhibitory activity against HIV of selected plants[6-8].

The fight against HIV is a grand scientific challenge where knowledge and experiences stemming from many disciplines are needed. Novel methods implemented in early drug discovery could lead to a new and more potent generation of microbicides and anti-HIV drugs. It has been shown that plants that were selected using ethnobotanical information have provided more active leads than random screenings[9]. Therefore, it is essential to focus on isolation of novel anti-HIV therapeutics from natural resources which are well tolerated, convenient and relatively cheaper. HIV reverse transcriptase inhibitors are important drugs for the treatment of AIDS and many natural products from plants. Based on previous research work[10] the *Aerva lanata* plant selected to test anti-HIV activity and cytotoxic effects. It was therefore decided to analyze the anti-HIV activity of *Aerva lanata* root extracts and also evaluate it's cytotoxicity in PBMC cells.

MATERIALS & METHODS

Plant collection

Plant was selected for this study is based on its traditional medicinal use[11]. Roots of *Aerva lanata (Figure-1 and 2)* were collected from the Chintur mandal, Khammam district of Andhra pradesh, India, in the month of September 2012. The plant voucher specimen identification was done with the help of Prof. Vastsavaya. S. Raju Department of Botany Kakatiya University, Warangal and the same was deposited at Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal.

Preparation of plant extract

After collection of selected medicinal plant material sample was dried at room temperature until they were free from moisture. The selected part of plant subjected to size reduction to get coarse powder was then stored in a clean dry air tight container. The air dried powder was subjected to sequential maceration method used by different solvents (hexane, chloroform, ethyl acetate, acetone, and methanol etc ;) for seven days. The extract was filtered mass was obtained and it was finally dried at low room temperature under pressure in a rotary vaccum evaporator (Thermotech, buchi type model th-012).

HIV-1 Reverse Transcriptase Inhibition Assay

The HIV reverse transcriptase enzyme inhibition due to each extract was determined using HIV RT inhibition assay by using of Retro Sys HIV-1 RT activity kit (Innovagen, Sweden). To determining RT activity on inhibiting substances that are to be analysed are serially diluted. The diluted substances are then added to a plate with reaction mixture. After 30 minutes of pre-incubation at 33°C, the reaction is started by the addition of a standardised amount of RT. The RT will now incorporate BrdUMP depending on the level of inhibition. The product is quantified by the addition of the RT Product Tracer which binds to the incorporated BrdUMP. After removing excess tracer the amount of bound

tracer is determined by an alkaline phosphatase / pNPP colour reaction[12]. After correction for background signal, the measured residual RT activity for each substance dilution is calculated as a percentage of the measured RT activity in absence of inhibiting substances. Plot the percentage of residual RT activity against the concentrations of the substance dilutions for each of the tested substances. AZT (Azidothymidine) was used as control. The inhibitory effect of each substance is expressed by RT activity and is determined with the aid of the obtained graph. The percentage inhibition of HIV-1 RT was calculates as,

Inhibition (%) = [(A control-A sample) / A control] x 100.

Where, A is Optical Density (OD).

Preparation of Peripheral Blood Mononuclear Cells (PBMCs)

Aseptically 2.5ml of HiSep media transferred in to a15 ml heparin coated test tubes and overlay with 7.5ml diluted blood (blood sample from healthy volunteers were collected by venipuncture and blood sample were diluted at 1:1 ratio with PBS). Centrifuged at 1,000 x g for 30 minutes. During the centrifugation the PBMC's moved from the plasma and were suspended in the density gradient, isolating them from erythrocytes and granulocytes. The PBMC's layer was removed and then washed twice with PBS centrifuged at 400 x g. The supernatant was then removed and the PBMC's were resuspended in RPMI 1640 medium.

Cytotoxicity Screening by MTT assay

Cell viability was determined by the MTT 3-(4, 5 dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide) test method. MTT (5 mg/ml) was dissolved in PBS. PBMC Cells were cultured in 96-well plates containing 100 µl medium prior to treatment with different solvent extracts of *Aerva lanata*. To that, 100 µl DMSO solution containing various concentrations (0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0mg/ml) of extracts were added to each well, and incubated for 37°C for 24 h. Diluted extracted solutions were freshly prepared in DMSO prior to each experiment. The metabolic activity of each well was determined by the MTT assay and compared to those of untreated cells.

After removal of 100 µl medium, MTT dye solution was added (15 µl / 100 µl medium) and the plates were incubated at 37°C for 4 h. After that, 100 µl of DMSO were added to each well, and mixed thoroughly. The absorbance was measured at 570 nm with a reference wavelength of 630 nm. High optical density readings corresponded to a high intensity of dye colour that is to a high number of viable cells able to metabolize MTT salts. The fractional absorbance was calculated by the following formula.

$$\% \text{ cell inhibition} = 100 - [(A_t - A_b)/ (A_c - A_b)] \times 100$$

Where, A_t = Absorbance value of test compound
A_b = Absorbance value of blank
A_c = Absorbance value of control

The effects of extracts were expressed by IC_{50} values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells). Dose-response curves between percentage of cell inhibition and concentrations of *Aerva lanata* extractions were constructed. The IC_{50} value was determined from the plotted curve.

RESULT

Percentage of yield extract

The yield of sequential extracts *Aerva lanata* (g) is shown in (Table 1). The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 5.020 gm, 4.080 gm, 2.750 gm, 1.720 gm, and 3.750 gm respectively.

Anti-HIV activity of *Aerva lanata* root extracts

Inhibition of HIV-RT by *Aerva lanata* root extracts were presented in Figure 3. Chloroform and methanol extraction shows highest inhibition of recombinant HIV-RT (91.0% and 89.0% respectively) at 2 mg/ml concentration. Hexane, ethyl acetate and acetone extractions showed highest inhibition of HIV-RT at 2 mg/ml concentration (86.9, 85.2 and 77.5 respectively). While control drug (AZT) showing 91.7% at 2mg/ml concentration.

Cytotoxicity of *Aerva lanata* root extraction on PBMC cells

Cytotoxicity activity of *Aerva lanata* root extractions were carried out against PBMC's at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay. Results of different concentrations of *Aerva lanata* extractions including 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0mg/ml graphically represented in figure 4. MTT assay of *Aerva lanata* extractions shows significant effect on PBMC's in different concentration. The highest cytotoxicity of hexane extraction against PBMC's was found in 2.0 mg/ml concentration with 66% of cell growth inhibition while methanol, ethyl acetate, acetone and chloroform extractions showing 61.5%, 59%, 57.2% and 52% at 2mg/ml respectively and control drug (AZT) showing 78% of cell growth inhibition. It was found that the percentage of growth inhibition to be increasing with increasing concentration of test compounds. The standard drug showing IC_{50} value at 0.25mg/ml and IC_{50} value of chloroform assay was 0.40mg/ml.

DISCUSSION

In the present study, the assay was optimized and standardized with respect to various experimental parameters and then applied to test the HIV-RT inhibitory activity of the different extracts. Most studies considered inhibition > 50% as significant[13,14]. Previous investigations established that different medicinal plant extracts inhibit HIV reverse transcriptase in non-specific manner[15,16]. The number of studies provide information on the inhibitory activity against HIV of selected plants[17, 18]. (Kalvatchev *et al*., 1997 and Mlinarie *et al.,* 2000). At the concentration of 0.5 mg/ml to 2 mg/ml all extractions of *Aerva lanata* shows significant inhibition of recombinant HIV-RT. The results obtained in the present investigation indicated that *Aerva lanata* roots with chloroform extraction shows highest inhibition activity (91% at 2mg/ml) against HIV-RT when compared to other extractions, while control drug (AZT) shows 91.7% at 2mg/ml concentration. In the present study, the cytotoxic effect of *Aerva lanata* extractions on PBMC's was evaluated by MTT assay. Different concentrations of extractions show more than 50% cell viability from 0.0625mg/ml to 0.5mg/ml. This finding suggests that phytochemicals were present viz; alkaloids, favonoids, phytosterols etc: good enough to reflect its importance.

CONCLUSION

The results obtained in the present investigation indicated *Aerva lanata* as a rich source of secondary metabolites. The root of *Aerva lanata* can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Therefore it is necessary to

purification, identification and characterization of the active compounds from *Aerva lanata* crude.

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Table 1. Extractive values of different extracts of *Aerva lanata* root

	Solvent	Color of extract	Yield of the extract (in gm)	Percentage yield(%w/w)
1	Hexane	White	5.020	2.51%
2	Chloroform	Light brown	4.080	2.04%
3	Ethyl acetate	Light brown	2.750	1.37%
4	Acetone	Light brown	1.720	0.86%
5	Methanol	Dark brown	3.750	1.85%



Figure 1. *Aerva lanata* plant with roots.



Figure 2. *Aerva lanata whole plant*.

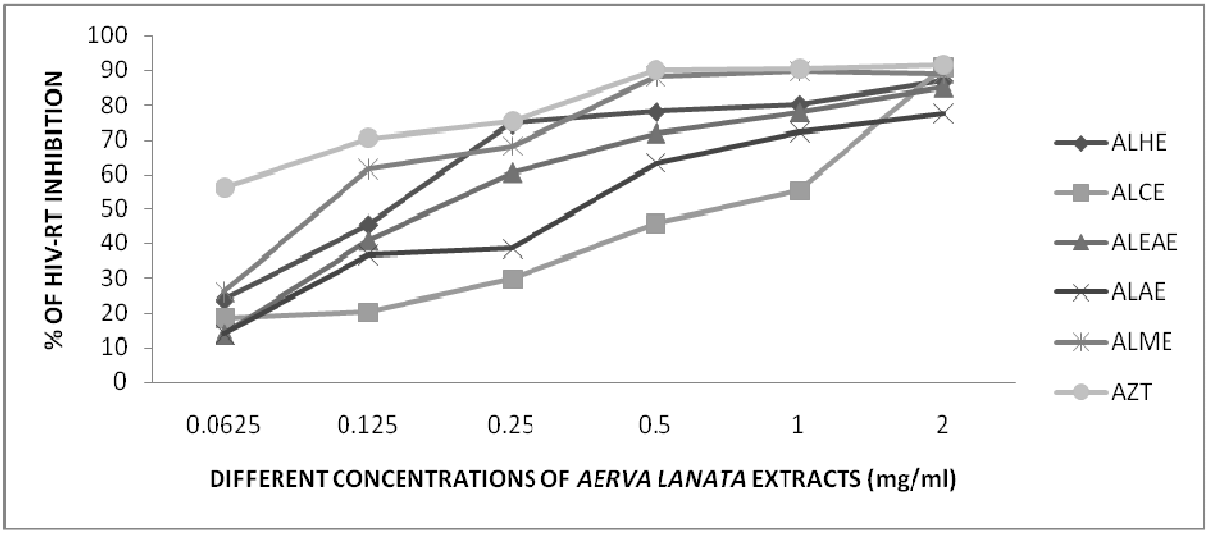


Figure 3. *In vitro* HIV-RT inhibitory activity of *Aerva lanata* root extractions.

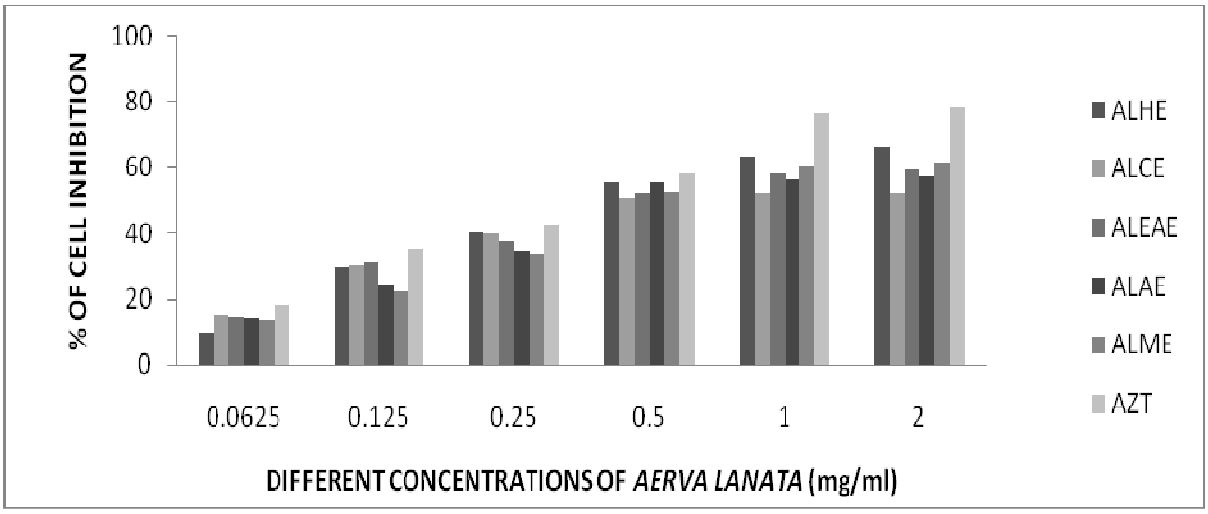


Figure 4. Effect of *Aerva lanata* root extraction on PBMC cells.

ALHE - *Aerva lanata* hexane extract
ALCE - *Aerva lanata* chloroform extract
ALEAE- *Aerva lanata* ethyl acetate extract
ALAE - *Aerva lanata* acetone extract
ALME - *Aerva lanata* methanol extract
AZT - Azidothymin

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