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Research Article

Inhibition of Human Immunodeficiency Virus (HIV-1) Reverse Transcriptase by *Acalypha indica* (L.) Plant Leaves Extract

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ABSTRACT

HIV (Human Immunodeficiency Virus) the virus that bases AIDS, is one of the newest zones of medical research nowadays. The objective of the study is to test the HIV-1 reverse transcriptase inhibitory efficiency of *Acalypha indica* leaves extracts. The leaves of *Acalypha indica*, were collected from the selected places of Adilabad District. Leaves were exposed to size reduction to get coarse powder and extracted by sequential maceration method using non-polar to polar solvents. Cell viability was determined in contradiction of PBMC cells by the trypan-blue dye exclusion technique and by MTT assay. The HIV reverse transcriptase enzyme inhibition due to each extract was determined by HIV- 1 RT inhibition assay by using of Retro Sys HIV-1 RT activity kit. At 50 µg/mL n-hexane extract presented the uppermost percentage of HIV-1 RT enzyme inhibition (88.26%) followed by methanol (75%), chloroform (67.3%), ethyl acetate (67.3%) and acetone (63.4%). The weak inhibition was establish at 3.125 µg/mL concentration for all extracts, except n-hexane. At this concentration n-hexane displays more than 50% inhibition. The control drug AZT shows highest inhibition (88.26% and 92.6%) at 25 and 50 µg/mL concentrations. These results achieves that n-hexane crude extract taking more effective activity among the other crude extracts.

1. Introduction

The virus that causes AIDS, known as the HIV (Human Immunodeficiency Virus), is one of the most active research topics in medicine today. According to estimates, 35.3 million persons were HIV-positive worldwide at the end of 2012. (WHO, 2014). After an individual contracts HIV, the virus typically lies latent for a protracted period of time. The virus then starts a cycle of attacking immune system cells by incorporating its genetic material into the cells, using the machinery of the immune cells to make more viruses from the incorporated genetic material, and then rupturing (killing) the cells so that the new viruses can infect more cells. In this way, the immune system is compromised, making the body less able to protect itself from the infections it comes into contact with on a daily basis. Sadly, patients often pass away within a few years of exhibiting AIDS symptoms (i.e., indicators of significantly reduced immunity as a result of the virus's attack on immune system cells).

In India, where there are 1.2 billion people, almost half are adults who are sexually active. Since the discovery of the first AIDS case in India in 1986, reports of HIV infection have been made in all of the country's states and union territories. HIV prevalence is 0.31% among adults (15 years or older) with 2.39 million people living with HIV/AIDS (AVERT, 2011). In India, the spread of HIV has been uneven. India as a whole has a low infection rate, although some areas have been more severely afflicted than others. The extreme north-east and the southern half of the country both have more severe HIV epidemics. Manipur (0.78%) has the highest estimated adult HIV prevalence, followed by Andhra Pradesh (0.76%), Karnataka (0.69%), and Nagaland (0.66%). However, no states reported an HIV prevalence among ANC attendance of 1.0% or more in 2010 for the first time (NACO, 2011).

The Indian plains are home to a weed known as *Acalypha indica* L. It is a typical annual plant found in Indian gardens, backyards, and waste areas all over the country's plains. The root is recommended as a febrifuge, tonic, astringent, and potent purgative Khare (2007). In *E. coli*, the leaf extract reduced mutagenecity, according to Gupta et al (2008). Backwart fever can be treated with an alcohol-based root bark extract. The leaves are emollient and laxative when used externally, and a poultice is applied for chilblains, swelling from bug bites, rheumatism, and facial paralysis (Kirtikar, 2006). The leaves are utilised for external skin eruptions, ringworms, and eczema as well as for jaundice, piles, rheumatism ulcers due to their anti-

periodic and laxative effects. Nadkarni (2009) applies the leaf extract to pustules and bug bites. The roots are utilised for migraine, blood dysentery, joint pain, and chest pain. The blood sugar level was reduced by the root extract by up to 30% (Chopra et al, 2006). Like pulp, both leaves and flowers have purgative properties (Agarwal et al, 2005). To cure cough, use ashes from burned pods mixed with a little honey and a pinch of salt (BenErik et al, 2009). *Acalypha indica* leaf extracts were tested for their capacity to inhibit HIV-1 reverse transcriptase.

2. Materials and Methods

2.1 **Selection of Plant**

Interviews with traditional healers and a review of the literature on the use of traditional medicinal plants in tribal areas of the Jannaram, Kaddam, Utnoor, and Indravelli mandals, Adilabad district, Telangana State, served as the basis for the plant selection for this study. Traditional healers in the Adilabad district used this herb to treat STDs, STIs, and other HIV/AIDS opportunistic infectious infections.

2.2 Collection of Plant Material

In the month of October 2020, *Acalypha indica* leaves were collected from certain locations in the Adilabad District. Taxonomist Prof. Vastsavaya S. Raju, Retired Professor, Department of Botany, Kakatiya University, Warangal assisted in the identification of the plant voucher specimens, which were then deposited at the Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal.

2.3 Preparation of Plant Extracts

Acalypha indica leaves were bulk-collected, cleaned, and shade-dried at room temperature until they were dry. The aforementioned medicinal plant parts were then subjected to size reduction to create coarse powder, and they were extracted using the sequential maceration method using non-polar to polar solvents (hexane, chloroform, ethyl acetate, acetone, and methanol) (Table 1). The extracts were filtered, and it was then dried in a rotating vacuum evaporator under pressure at low room temperature (Thermotech, buchi type model th-012). The yield % was computed and then tested for HIV activity.

Table 1. Extractive values of different extracts of *Acalypha indica* leaves

S.No	Solvent	Initial Weight (gm)	Yield of the extract (in gm)	Percentage yield (%w/w)
1.	Hexane (H)	600	1.910	0.31
2.	Chloroform (C)	600	4.570	0.76
3.	Ethyl Acetate (EA)	600	1.900	0.31
4.	Acetone (A)	600	3.800	0.63
5.	Methanol (M)	600	0.880	0.14

The weight of the residual extract was measured and percent yield was calculated by using below mentioned formula:

Extract yield % = W1/W2 X 100;
Where, W1= Net wt of powder in grams after extraction
W2= total wt of powder in grams taken for Extraction.

2.4 Preparation of Peripheral Blood Mononuclear Cells

PBMC cells are frequently used as the model for the cytotoxicity test in normal cells. Many studies have utilized PBMCs to assess the effects of chemicals or extracts on the proliferation of normal cells (Anazetti et al., 2003; Liu et al., 2004).

At the Kakatiya University health centre, blood samples from healthy participants were drawn via venepuncture and then placed in 15 ml heparin-coated test tubes. It was layered onto HiSep LSM-1077 (HiMedia, Mumbai) media at a volume ratio of 3:1 and centrifuged at 1,000 x g for 30 min after being diluted with PBS in a 1:1 ratio. The centrifugation process separated the PBMCs from erythrocytes and granulocytes by removing them from the plasma and suspending them in the density gradient. After the PBMC layer was removed, PBS was applied twice. The cells were then resuspended in RPMI 1640 media (HiMedia, Mumbai) supplemented with 1 mM L-glutamine, 100 Units/ml penicillin, and 0.1 mg/ml streptomycin, 10% inactivated FCS, and pH 7.2 was adjusted by the addition of 15 mM HEPES after the supernatant had been removed. The MTT assay and the trypan-blue dye exclusion method were used to assess cell viability. In the cytotoxicity investigation, 1 x 10^5 PBMC cells were employed per well of a 96-well tissue culture plate. Dose-response curves between concentrations of the extracts and the percentage of cell viability were created. The curve was plotted, and the IC50 was calculated.

2.5 Cell Viability by MTT assay

The MTT test method was used to measure the vitality of the cells. PBS was used to dissolve MTT (5 mg/ml). After filtering via a 0.2 m filter, the solution was kept between 2 and 8 °C. Prior to being treated with plant extract or AZT (a medication used to treat HIV-1) for 24 hours at 37°C, cells were grown in 96-well plates (3.0 x 104 cells/well) with 100 l medium. Then, each well received 100 l of fresh media containing different quantities of plant extracts or AZT, which was introduced after the previous 48 hours of incubation. Before each experiment, diluted plant extract or AZT solutions were freshly made in DMSO. The (MTT) test was used to measure each well's metabolic activity and compare it to that of untreated cells. After 100 l of the medium had been removed, 15 l of MTT dye solution had been added, and the plates had been incubated at 37 °C for 4 hours. Following that, 100 l of DMSO was added to each well, and the dye crystals were thoroughly mixed. Using a reference wavelength of 630 nm and an ELISA plate reader (Biotek EL 311), the absorbance was calculated. A high intensity of dye colour, or a high number of live cells capable of metabolising MTT ions, was correlated with high optical density readings. The following formula was used to determine the fractional absorbance:

% Cell Survival = Mean absorbance in test wells/
Mean absorbance in control wells x 100

The average cell survival obtained from triplicate determinations at each concentration was plotted as a dose response curve. The 50% inhibition concentration (IC_{50}) of the active substances was determined as the lowest concentration

which reduced cell growth by 50% in treated compared to untreated culture. The IC_{50}s were compared for their activities.

2.6 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).

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).

3. Results and Discussion

3.1 Percentage of Yield Extract

The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 1.910 gm (0.31%), 4.570 gm (0.76%), 1.900 gm (0.31%), 3.800 gm (0.63%), and 0.880 gm (0.14%) respectively (Table-1 and Figure-1). More yield obtained from chloroform crude extracts.

3.2 Cytotoxicity of *Acalypha indica* leave extracts on PBMC cells

After cells were treated extracts of *Acalypha indica* at various concentrations, the cytotoxic effects were investigated using the MTT assay. Cytotoxicity of extracts were determined by an inhibitory concentration at 50% growth (IC_{50}). In the present study cytotoxic activity of solvent extracts 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 extracts including 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL graphically represented in Table-2. MTT assay of extracts shows significant effect on PBMC cell in concentration 3.125µg/mL compared with standard drug AZT.

3.3 Anti-HIV activity of *Acalypha indica* plant leaves extracts

The effect of the five solvent crude extracts of *A. indica* on the HIV-1 RT enzyme by using RT assay were done to attempt to screen for anti-HIV-1 activity. This assay is calorimetric assay where the enzyme activity is determined after treatments in the presence or absence of different concentration of extracts. Inhibition of extracts to the HIV-1-RT enzyme was evaluated based on their percent inhibition compared to a sample that does not contain extracts. The Table-3 showed, the n-hexane extract showed potent inhibitory effect at 50 µg/mL (88.26%) with an IC50 of more than 50 µg/mL compared to RT inhibitor, AZT at 50 µg/mL (92.6%), and the inhibitory activities were dose dependent.

At 50 µg/mL n-hexane extract showed the highest percentage of HIV-1 RT enzyme inhibition (88.26%) followed by methanol (75%), chloroform (67.3%), ethyl acetate (67.3%) and acetone (63.4%). The weak inhibition was found at 3.125 µg/mL concentration for all extracts, except n-hexane. At this concentration n-hexane shows more than 50% inhibition.

4. Conclusion

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. At the concentration of 50 µg/n-hexane extract of *Acalypha indica* shows significant inhibition of HIV-RT. The results obtained in the present investigation indicated that *Acalypha indica* leaves with n-hexane extract shows highest inhibition activity (88% at 50 µg/ml) against HIV-RT while control drug (AZT) shows 92% at 50 µg/ml concentration. These findings suggested that *Acalypha indica* could be a potential source of natural molecules having rich source of secondary metabolites and great importance as therapeutic agent. The *Acalypha indica* leavescan provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections

Table 2. Effect of *Acalypha indica* plant extracts on PBMC cell viability

S.No.	Concentration (µg/mL)	Cell viability (%) of different solvent extracts					AZT
		n-Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	
1	3.125	91.6	72.91	66.66	68.75	64.58	93.5
2	6.25	83.3	64.58	60.41	56.25	62.5	91.6
3	12.5	75	58.33	50	47.91	54.16	83.3
4	25	60.41	43.75	37.5	39.58	54.0	77.56
5	50	43.75	31.25	37.5	27.08	35.41	75
6	100	43	16.6	25	18.75	25	62.5
7	200	25	16.25	18.75	18.33	10.41	25
8	Cell control (0)	100	100	100	100	100	100

Table 3. Inhibition of HIV -1 reverse transcriptase by different concentrations of *A. indica* extracts

Concentration (µg/mL)	HIV-1-RT inhibition of different solvent extracts (%)					AZT -control drug
	n-Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	
3.125	55.76	38.46	32.6	21.15	23.07	59.6
6.25	67.3	40.38	40.38	28.84	26.92	76.92
12.5	76.9	57.69	48.07	57.69	38.46	87.6
25	87.6	63.46	67.3	63.4	55.76	88.26
50	88.26	67.3	67.3	63.4	75	92.6

caused by the organisms. Therefore *Acalypha indica* leaves n-hexane extract was selected for cytotoxic studies and qualitative phytochemical screening.

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Conflicting Interests

The authors have declared that no conflicting interests exist.

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