HIV-1 REVERSE TRANSCRIPTASE INHIBITION BY PHENOLIC COMPOUNDS ISOLATED FROM *ACALYPHA INDICA* (L.) PLANT LEAVES EXTRACT

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Abstract:

HIV (human immunodeficiency virus) the virus that causes AIDS, is one of the hottest areas of medical research today. The objective of the study is to test the HIV-1 reverse transcriptase inhibitory activity of *Acalypha indica* leaves extracts and isolated phenol. The leaves of *Acalypha indica*, were collected from the selected sites of Adilabad District. Leaves were subjected to size reduction to get coarse powder and extracted by sequential maceration method using non-polar to polar solvents. Phenolic compounds were isolated by using TLC, column chromatography and HPLC techniques. Cell viability and cytotoxicity of phenolic compounds were determined against PBMC cells by the trypan-blue dye exclusion method and by MTT assay. The HIV reverse transcriptase enzyme inhibition was determined using HIV-1 RT inhibition assay by using of Retro Sys HIV-1 RT activity kit. At 500 µg/mL concentration of phenolic compound showed the highest percentage of HIV-1 RT enzyme inhibition (88.26%). The weak inhibition was found at 31.25 µg/mL concentration. The control drug AZT shows highest inhibition (92 %) at 500 µg/mL concentrations. These results concludes that the phenolic compounds isolated from *A. indica* having more potent activity.

Keywords: *HIV; Acalypha indica; Reverse Transcriptase, Trypan Blue, MTT assay*.

I. INTRODUCTION

35.3 million people are currently living with Human immunodeficiency virus type 1 (HIV-1) virus and this infection continues to spread throughout the world [1]. Alkaloids, phytosterols, lignans, flavonoids and phenols like phytochemicals from medicinal plants have been found to inhibit unique enzymes and proteins crucial to the life cycle of HIV, including the reverse transcription process, virus entry, the integrase or protease [2,3,4]. Herbal medicines are the globally accepted legal and alternative system of therapy for treatment and cure of various diseases in traditional treatments in the form of pharmaceuticals [5]. Previous investigations established that different medicinal plant extracts inhibit HIV reverse transcriptase in non-specific manner [6,7,8].

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [9]. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities [10]. Therefore, screening of potential anti-HIV agents from medicinal plants may be a rapid and effective way for drug discovery. However, drug toxicity, drug resistance, adverse drug–drug interactions, and accompanying poor patient adherence are still the major factors leading to treatment failure [11]. There is still an acute need for less toxic and more potent HIV drugs and continues be the concern. The present study was to evaluate the HIV reverse transcriptase inhibitory activity of phenolic compound isolated from *Acalypha indica* leaves extracts.

II. MATERIALS AND METHODS

A. Plant collection:

The leaves of *Acalypha indica*, were collected from the selected sites of Adilabad District in the month of July, 2015. The plant voucher specimens identification was done with the help of taxonomist 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.

B. Preparation of plant extract:

After collection of plant material sample was dried at room temperature until they were free from moisture. The dried leaves were powdered and subjected to sequential maceration method used by different solvents (hexane, chloroform, ethyl acetate, acetone, and methanol etc.,) for seven days. The extract was filtered and it was finally dried at low room temperature under pressure in a rotary vacuum evaporator (Thermotech, Buchi type model th-012). The percentage yield calculated and then subjected to anti-HIV activity screening. The weight of the residual extract was measured and percent yield was calculated by using the following 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.

C. Isolation of Phenolic Compound:

Thin layer chromatography (TLC), Column chromatography and HPLC techniques were used for isolation of active compound. Methanol: Chloroform (100:0, 80:20, 60:40, 40: 60, and 20: 80) solvent system was used for TLC analysis.

(i.) Thin layer chromatography (TLC)

Pre-coated silica gel (GF254 on polyester plate) was used for analytical TLC. A strip of the pre-coated silica gel was cut out and a spot of the sample was applied on the aluminium plates about 1.0 cm from the edge in a small chromatographic tank to separate the different fractions based on their relative mobilities in solvent systems and colour reactions. The strips were dried using hot air dryer. The strips were lowered into a small chromatographic jar containing the solvent system. The each jar was covered with a glass lid. The solvent was allowed to ascend until the solvent front was about ¾ of the length of the strip. The strip was removed and dried by a hot air dryer and viewed in iodine chamber to identify spots. The spot was marked and the colour reaction was recorded and the relative Retention factor (Rf) value was calculated.

*(ii.) Column Chromatography***:**

Based on the above TLC results the MC 60:40 solvent system showed good separation, therefore this solvent system is selected for further analysis with column chromatography. The crude extract (methanol) of *A. indica* (2.8 g) was subjected to column chromatography to separate the extract into its component fractions. Silica gel 60G was used as the stationary phase while selected solvent system (MC 60:40) was used as the mobile phase. Total 36 fractions were collected and dried at low room temperature under pressure in a rotary vacuum evaporator. All the fractions were tested for TLC. Based on TLC results, the similar Rf values fractions were pooled. The pooled fractions from 1-4, 5-12, 13-20, 21-25, 26-36 were gives five fractions. These five fractions were tested for HIV-1 RT inhibitory activity.

(iii.) HPLC (High performance Liquid Chromatography):

Pooled fractions which showed HIV-1 RT inhibition was dissolved in HPLC grade Methanol. This was sonicated and then passed through Whatman Nylon Membrane Filter (0.45µm & 47mm diameter) before injecting it into the column. For analytical the Shimadzu liquid chromatograph system (Shimadzu Corp, Kyoto, Japan) equipped with a Shim-Pack VP-ODS C18 column (250 mm × 4.6 mm, 5 µm), coupled with UV detector and sample loop of 20uL capacity. The mobile phase consisted of Methanol/water (60:40) at a flow rate of 0.8 mL/min. Results were acquired and processed by the Shimadzu Lab Solution software (Shimadzu Corp).

D. HIV-1 Reverse Transcriptase Inhibition Assay:

The HIV reverse transcriptase enzyme inhibition due to each extract and isolated compound 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 (Gronowitz et al., 1992). 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. All fractions were selected for anti-HIV activity. 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).

2.5 Phytochemical screening of isolated active compound:

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins, were carried out in extracts and in isolated compound by using standard procedure [12].

III. RESULTS

A. Percentage of Yield

The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol crude extracts are 5.5 gm (1.5%), 8.070 gm (1.70%), 2.430 gm (0.84%), 3.650 gm (0.7%), 12.120 gm (3.1%), respectively (Table-1). More yield obtained from chloroform crude extracts.

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

S.No	Solvent	Color of extract	Yield of the extract (in gm)	Percentage yield(% w/w)
1	Hexane	White	5.5	1.5%
2	Chloroform	Light brown	4.0780	1.70%
3	Ethyl acetate	Light brown	2.430	0.84%
4	Acetone	Light brown	3.650	0.7%
5	Methanol	Dark brown	12.120	3.1%

B. HIV-1 RT activity of the isolated compound

The effect of the five different fractions 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 figure-1 showed, the fraction-4 showed potent inhibitory effect at 500 µg/mL (88.26%) with an IC_{50} of 32 µg/mL compared to RT inhibitor, AZT at 500 µg/mL (92.6%), and the inhibitory activities were dose dependent ($p<0.05$). Therefore Fraction-4 was selected to check the purity in HPLC.

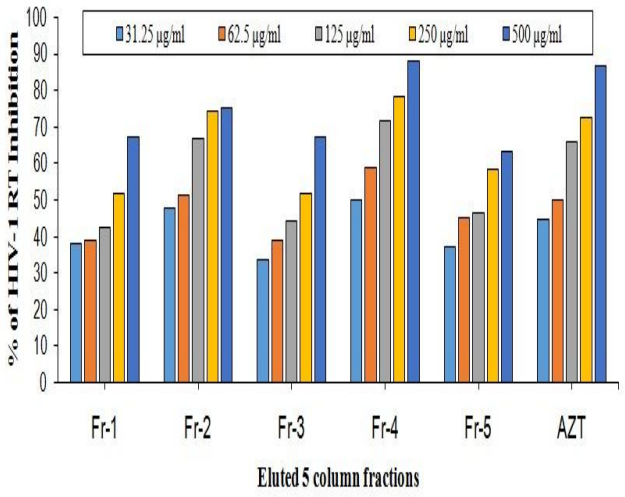

Fig-1. HIV-1 RT inhibition of fractions collected from column chromatography

At 500 µg/mL fraction-4 showed the highest percentage of HIV-1 RT enzyme inhibition (88.26%) followed by fraction-2 (75%), fraction-1 (67.3%), fraction-3 (67.2%) and fraction-5 (63.4%). The weak inhibition was found at 3.125 µg/mL concentration for all five fractions, except fraction-4. Standard drug IC_{50} value is <31.25 µg/ml. The least inhibition among the 5 fractions studied is Fraction-3 whose IC_{50} value is 251 µg/ml. Fraction-1, 2 and 3 have shown moderate inhibition with IC_{50} values 252, 64 and 251 µg/ml respectively. These results concludes that fraction-4 having more potent activity among the other fractions. From the five fractions obtained from column chromatography, and based on the

activity the Fraction-4 was selected to check the purity in HPLC.

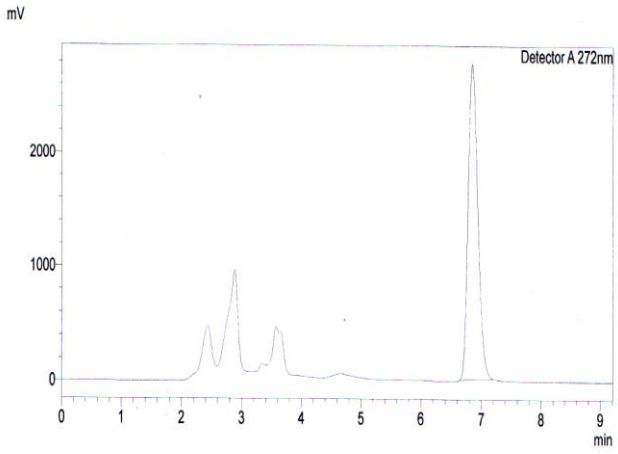

Fig-2. HPLC chromatogram of isolated compound (Fraction-4).

C. HPLC analysis

The fraction was detected at 272 nm and chromatogram was recorded. The peaks were recorded at retention times of 2.2 (1st peak), 3.1 (2nd peak), 4.3 (3rd peak) and 6.8 (4th peak) minutes. The dominant signal occurred at 6.8 minutes (Figure-2). Peaks 1,2,3 were base-line resolved and 4th peak was confirmed the isolated pure compound and tested for phytochemical analysis.

D. Phytochemical analysis

The isolated active fraction was checked for the determination of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols and flavonoids. The phytochemical results revealed that the compound belongs to phenol.

IV. DISCUSSION

Acalypha indica L. belongs to Euphorbiaceae family and is a weed, widely distributed throughout the plains of India. This plant has been reported in treating pneumoniae, sexual transmitted infections, asthma, severe fever, rheumatism and several other ailments [13]. The dried leaves of *Acalypha indica* was made into a poultice to treat wounds and the juice of *Acalypha indica* is added to lime and used to treat a variety of skin disorders and acute infections. The leaves of *Acalypha grandis* have also been reported to possess contraceptive activity [14] and several chemical investigations have been carried out on this plant. Although, anti-HIV-1 activity has been reported previously from different crude extracts of *A. indica* [15], no such activity has been shown from the isolated compounds from this plant. Therefore, an attempt has been made to evaluate the isolated phenols of *A. indica* for activity against HIV-1.

In an attempt, to explore the inhibition of HIV-1 reverse transcriptase enzyme by the crude extracts and isolated fractions of *A. indica*, the *invitro* assay was performed. The crude extracts and also separated five fractions exhibited a dose-dependent inhibition of HIV-1 Reverse transcriptase at concentrations ranging from 31.25 to 500 ug/ml. Among five crude extracts the methanolic extract showed more potent activity and this extract was selected to isolate the active compound. In different investigations by several other groups, n-hexane crude extract have been reported to inhibit HIV-1 RT [16]. The TLC, column chromatography and HPLC were used for isolation of the active compound. Total 36 fractions were collected from column and pooled these fractions based on the TLC results. Finally after pooling, total five fractions were obtained and tested for HIV-1 RT inhibition.

This study showed that *A. indica* plant contained compounds of medicinal importance. This phytochemical screening and analysis revealed that the phenols are mainly present in the active fraction. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The phenols and other phytochemicals produce a wide spectrum of biological activities in animals and humans and are particularly considered an efficacious anti-lipidemic agent [17]. In one of the study phenolic acids such as gallic acid, caffeic cacid and syringic acid from methanolic extracts were obtained with using RP-HPLC analysis [18] and showed potent biological activities. The results of the some experiments shows that gallic acid was the major phenolic acid present in *A. indica* [19]. In previous studies, the isolated phenolic compounds were shown anti-bacterial, anti-oxidant and anti-inflammatory activities [19].

The anti-HIV activity of plant phenols has been reviewed by several scientist and Potential role of

phenols both in the etiology as well as in the prevention of immunological diseases were done and also experiments in animals and cell cultures indicated that plant phenols and glucosides may exert biological activities [19]. 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. The results obtained in the present investigation indicated that *Acalypha indica* leaves with methanolic crude extract shows highest inhibition activity against HIV-RT when compared to control drug (AZT).

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. Therefore the leaves of this plant can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics and antiviral compounds for the treatment of infections caused by the organisms. So the active compound contains the phenols needed by the pharamaceutical companies as well as in food supplements. The qualitative analysis of these phytocompounds will be an interesting area for further purification the advanced spectroscopic studies are required for the structural elucidation, identification and characterization of the active compound would be our priority in future studies.

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