International Journal of Phytopharmacy **Research Article**
ISSN: 2277-2928 (Online)
Journal DOI: https://doi.org/10.7439/ijpp

HIV-1 Reverse transcriptase and Protease assay of different solvent extract of *Elephantopus scaber* L.

Sachin M Hiradeve[*1] and Vinod D. Rangari[2]

[1]*School of Pharmacy, GH Raisoni University, Saikheda, Dhoda-Borgaon, Chhindawara, Madhya Pradesh, India*
[2]*Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur - 495 009 (CG), India*

Abstract

Aim of the study: Anti-HIV activity studies of different solvent extracts of *Elephantopus scaber* L.
Materials and methods*:* petroleum ether, solvent ether, methanolic and aqueous extracts of *E. scaber* was tested for HIV enzyme inhibitory activity against HIV-1 Reverse transcriptase (RT) and HIV-1 Protease (PR). HIV-1 RT assay was performed using non-radioactive HIV-RT colorimetric ELISA kit while the HIV-1 PR assay was performed using a fluorogenic octapeptide substrate, HIV-FRET (fluorescence resonance energy transfer) and a recombinant HIV-1 protease solution (AnaSpec Inc., USA).
Results: The percentage inhibition of controls and *E. scaber* aerial part and root extracts were calculated relative to uninhibited HIV-1 RT in 2% DMSO. The results of the HIV-1 RT assay indicated 25.3% inhibition by aqueous root extract (100µg/ml) and 19.8% inhibition by methanolic extract of root while the aqueous extract of aerial part (100µg/ml) has shown only 11.1 % inhibition as compared to standard Nevarapin (100µg/ml) 27.5 % inhibition. In case of HIV-protease assay, the aqueous extract of root (RAQ) (50 µg/ml) has shown quite high inhibition to the extent of 77% which is very significant.
Conclusion: The results of HIV-1 PR assay indicated that in comparison to standard drugs Ritonavir and Pepstatin, only aqueous extract of root has shown quite high inhibition which is very significant. Hence the study concluded that, as compared the root and aerial part extract of *E. scaber*, the root extract of *E. scaber* have more significant anti-HIV potential as evident from the results.
Keywords: *Elephantopus scaber* , Reverse transcriptase , HIV, Protease assay .

***Correspondence Info:**
Dr. Sachin M. Hiradeve,
School of Pharmacy,
GH Raisoni University, Saikheda, Dhoda-Borgaon,
Chhindawara, Madhya Pradesh, India

***Article History:**
Received: 05/05/2019
Revised: 28/06/2019
Accepted: 28/06/2019
DOI: https://doi.org/10.7439/ijpp.v9i3.5239

QR Code

How to cite: Hiradeve SM and Rangari VD. HIV-1 Reverse transcriptase and Protease assay of different solvent extract of *Elephantopus scaber* L. *International Journal of Phytopharmacy* 2019; 9(3): e5239. Doi: 10.7439/ijpp.v9i3.5239 Available from: https://ssjournals.com/index.php/ijpp/article/view/5239

1. Introduction

HIV is a member of *Retroviridae* family, subfamily *Lentivirirnae* and *Lentivirus* genus in primate lentivirus group. Lentiviruses (*lenti*, Latin for slow) are characterized by slow growth and long asymptomatic incubation period. HIV-1 is the result of zoonotic transmission of SIVcpzin Chimpanzees in West Central Africa. HIV is classified as HIV-1and HIV-2 on the basis of sequence deviation exceeding 50% and on the presence of *vpx* gene in HIV-2. HIV-2 has 40-60% sequence similarity with HIV-1 and was isolated from some AIDS patients of West Africa in 1986. HIV-2 is less predominant than HIV-1, which is found across the world. HIV-2 is mainly concentrated in West African part of the globe, with some cases also identified in America and Western Europe. The clinical manifestation associated with both etiological agents of AIDS is in distinguishable. However, epidemiology studies suggest that the incubation period of disease development is longer for HIV-2 than HIV-1. In addition, HIV-2 maintain slow level of viremia and shows lower rates of transmission and thereby remains geographically localized compared to HIV-1 [1-4].

None of the substances with antiviral activity against HIV are without toxicities and resistance and hence

there is a strong need to improve the current antiretroviral armamentarium. A potential source of novel compounds for HIV is from medicinal plants or other natural products. In order to find such potential anti-HIV agents from medicinal plants, we have screened medicinal plant *E. scaber* commonly used in traditional medicine. In this section, we have reported the in vitro anti-HIV assay activities of different solvent extracts from *E. scaber*. There are tremendous ethnomedical uses of *E. scaber* such as anti-infective, antitumor, antioxidant, wound healing; hepatoprotective etc. and these uses are scientifically validated. This is the first report of *E. scaber* plant utility as an anti-HIV agent.

2. Material and methods

2.1 Collection and authentication of plant material

The whole plant of *E. scaber* was collected in the month of October-November 2012, from the forest of Achanakmar, Chhattisgarh, India. The collected plant was authenticated by the Dr. G. P. Sinha, Scientist D, Ministry of environment and forests, Botanical survey of India, Allahabad, Uttar Pradesh. BSI/CRC/TECH/2014-15/voucher specimen has been preserved in our laboratory for future reference.

2.2 Preparation of plant extracts

The aerial part and root of *E. scaber*has been successively extracted with petroleum ether, solvent ether, methanol, aqueous as described previously [5].

2.3 Sample for Anti-HIV

All the root and aerial part different solvent extract of *E. scaber* has been prepared and the sample selected for cytotoxicity study were as follows

2.3.1 Root extracts of *E. scaber* –

RPE: Pet. ether extract; RDE: Diethyl ether extract; RME: Methanolic extract; RAQ: *A*queous extract. **Aerial part extracts of *E. scaber*** - APE: Pet ether extract; ADE: Diethyl ether extract; AME: Methanolic extract; AAQ: Aqueous extract

2.3.2 Sample preparation

Samples were dissolved in DMSO at stock concentrations of 100 mg/mL. The solvents were used as vehicle controls. Samples were stored at 4 °C.

2.4 Antiviral assay

The HIV-1 p24 Antigen Assay kit (Beckman Coulter, Miami, FL, USA), an enzyme-linked immunosorbant assay (ELISA), was used to detect and quantify HIV-1 p24 core protein. At the end of the7 days incubation, culture supernatant (100 µl) from the HIVinfectedCEM.NKR-CCR5 cultures was transferred to the murine monoclonal-coated 96-well plate for the p24 assay. The protocol was followed as described by the manufacturer, with absorbance measured at 450 nm [6].

2.4.1 HIV-reverse transcriptase assay

The effect of the crude extracts on reverse transcription was tested using a non-radioactive HIV-RT colorimetric ELISA kit from Roche Diagnostics, Germany. The protocol outlined in the kit was followed, under nuclease-free conditions, using 2 ng of enzyme in a well and incubating the reaction for 2 h at 37°C. Negative controls for the assay included HIV-1 RT with only lysis buffer, HIV-1 RT with only solvent (2% DMSO) in lysis buffer, and a blank with just ABTS. The positive control used was nevirapine (kindly donated by Aspen Pharmacare, South Africa), a reverse transcriptase inhibitor used commonly in clinical practice. The HIV-RT inhibition of the plant extracts were measured as a percentage of the inhibition that occurred with HIV-1 RT in the presence of no inhibitor in the same solvent (2% DMSO) as the extracts.

2.4.2 HIV- protease assay

The HIV-1 PR assay was performed using a fluorogenicoctapeptide substrate, HIV-FRET (1) (fluorescence resonance energy transfer) and a recombinant HIV-1 protease solution (AnaSpec Inc., USA). The peptide sequence of HIV- FRET(1) is derived from a natural processing site for HIV-1 PR and has the following structure: 4-(4-dimethylaminophenylazo)- benzoic acid (DABCYL)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-5-[(2-aminoethyl) amino]naphthalene-1 sulfonic acid (EDANS)]. The procedure for the continuous fluorogenic detection of HIV-1 PR was adapted from the method of Matayoshi *et al*. (1990) [7]. The fluorogenic substrate was dissolved in DMSO to 1.3 mM. The stock recombinant HIV-1 protease solution of 200 ng/µl was diluted to a concentration of 75 ng/45 µl with freshly prepared assay buffer (100 mM sodium acetate, 1 M sodium chloride, 1 mg/ml BSA, 1 mM EDTA, 1 mM dithiothreitol, pH 4.7). To the wells of a 96-well black microtiter plate, 45 µl of diluted HIV-1 PR (final concentration was 75ng/well) and 5 µl of extract or control were added and incubated at 37º C for 15 min. During this incubation, the stock substrate was diluted to 16 M by assay buffer and pre-heated to 37°C. The diluted substrate (50 µl) was added, to initiate the reaction of substrate cleavage by HIV-1 PR, and the microplate shaken at 300 rpm for 1 min. The fluorescence intensity was measured kinetically every 30 s over a period of 100 min at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, at a temperature of 37°C, using a Fluoroskan Ascent FL microplate reader (Thermolab systems). The reaction rates were determined by the gradient of the initial linear portions (usually the first 5–10 min) of the plot of RFI (relative fluorescence intensity) as a function of time. Negative controls included were HIV-1 PR with only assay buffer, HIV-1 PR enzyme with DMSO (2%) in assay buffer

and substrate alone. A positive control was pepstatin at a final concentration of 0.2 µM (Bachem, Switzerland). The percentage inhibition of HIV-1 PR was calculated as a percentage of a control with only the solvent (2% DMSO).

3. Result and discussion

3.1 HIV-Reverse transcriptase assay

The percentage inhibition of controls and *E. scaber* aerial part and root extracts were calculated relative to uninhibited HIV-1 RT in 2% DMSO.

The results of the HIV-1 RT assay indicated 25.3% inhibition by aqueous root extract (100µg/ml) and 19.8% inhibition by methanolic extract of root while the aqueous extract of aerial part (100µg/ml) has shown only 11.1 % inhibition as compared to standard Nevarapin (100µg/ml) 27.5 % inhibition. Results of HIV-1 RT inhibition by different solvent extracts of *E. scaber* are given in Table 1 and Fig 1.

Table 1: HIV-1 RT inhibition by different solvent extracts of *E. scaber*

Extracts	Percent inhibition			Average	Activity	Inhibition	SEM
Enzyme	1.1165	1.1385	-	1.128	100	0	0
Nevarapin (100µg/ml)	0.8115	0.8225	-	0.817	72.5	27.5	±0.005
DMSO	1.0365	1.0105	1.0925	1.047	92.8	7.2	±0.024
RPE	1.0235	0.9975	1.0481	1.023	90.7	9.3	±0.014
RDE	1.0595	1.1155	1.1145	1.096	92.2	2.8	±0.018
RME	0.9465	0.9425	0.8225	0.904	80.2	19.8	±0.040
RAQ	0.8035	0.8475	0.8745	0.842	74.7	25.3	±0.020
APL	1.0415	1.0455	1.1145	1.067	94.6	5.4	±0.023
ADE	1.0125	1.0385	0.9735	1.008	89.4	10.6	±0.018
AME	1.0235	0.9975	1.0285	1.017	90.2	9.8	±0.009
AAQ	1.0105	1.0255	0.9715	1.002	88.9	11.1	±0.016

Experiments are repeated three times and values are expressed as ±SEM

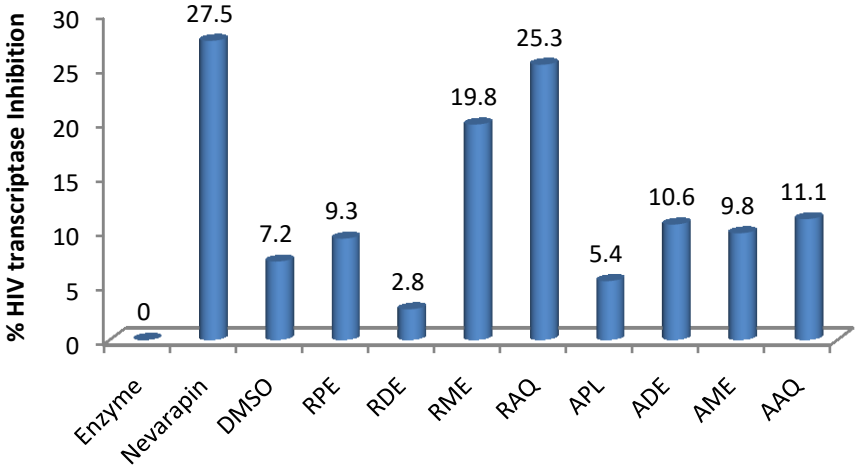


Fig 1: Percentage HIV-1 reverse transcriptase inhibition

The HIV-RT inhibition of the plant extracts were measured as a percentage of the inhibition that occurred with HIV-1 RT in the presence of no inhibitor in the same solvent (2% DMSO) as the extracts. From the results of the HIV-1 RT assay it was clear that solvent ether extract of root (RDE) and petroleum ether extract of aerial part (APL) showed very less HIV-RT inhibition or called ineffective while the RPE, ADE, AME and AAQ extract showed mild to moderate HIV-RT inhibition but the methanolic (RME) and aqueous (RAQ) extract of root showed quite significant HIV-RT inhibition in comparison to standard nevarapine. From results it was concluded that medicinal plant *E. scaber* would be used as a potential anti-HIV agent who provide a safer and more effective platform for newer scaffolds and could lead to better success than routine random screening.

3.2 HIV- Protease assay

Fig 2 (samples) and fig 3 (controls) summarize the EDANS fluorescence kinetic readings over time when the FRET peptide is cleaved by HIV-1 protease.

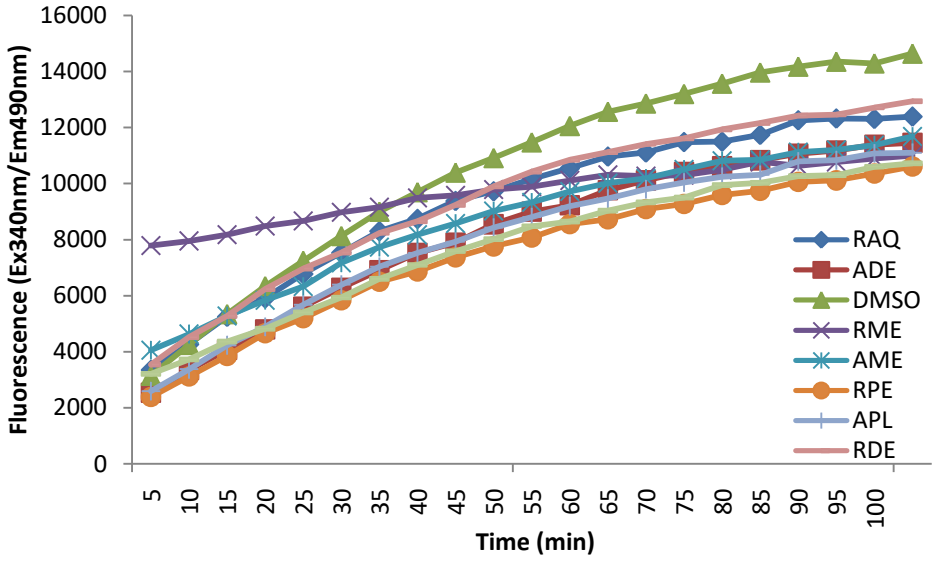


Fig 2: EDANS fluorescence kinetic readings of a FRET peptide upon HIV-1 protease cleavage in the presence of the sample. Readings are the average of duplicate values

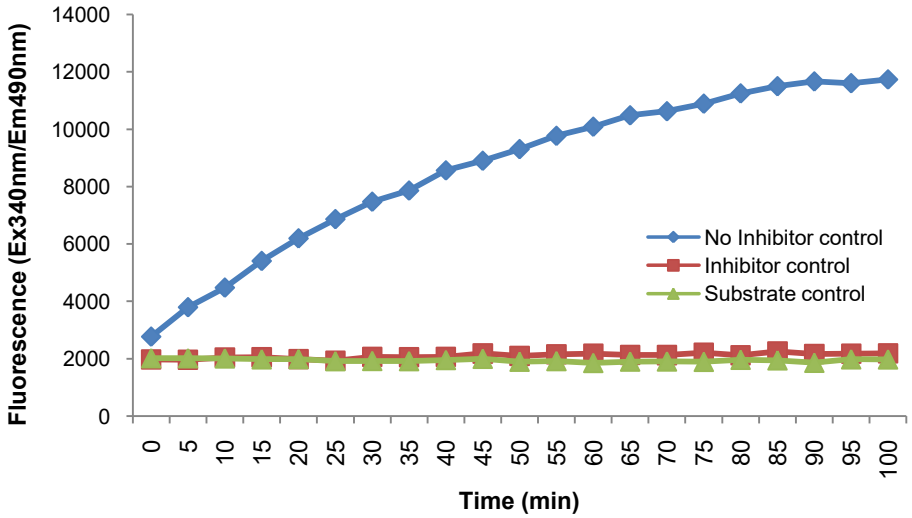


Fig 3: EDANS fluorescence kinetic readings of a FRET peptide upon HIV-1 protease cleavage in the presence/ absence of pepstatin (inhibitor). Readings are the average of duplicate values

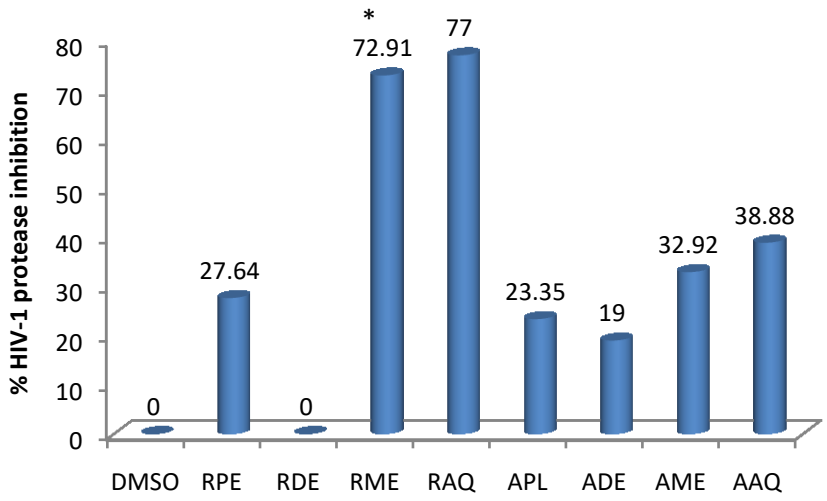
The percentage inhibition of controls and *E. scaber* root and aerial part extracts were calculated relative to uninhibited HIV-1 PR in 2% DMSO.

Table 2: HIV-1 PR inhibition by different solvent extracts of *E. scaber*

Extracts/Standards	Percentage inhibition 50 (µg/ml)			Average % inhibition (Mean)	SEM
Ritonavir	97	96	96	96	±0.33
Pepstatin	96	97	98	97	±0.57
DMSO	0	0	0	0	0
RPE	28.94	26	28	27.64	±0.86
RDE	0	0	0	0	0
RME	74	71	73.75	72.91*	±0.96
RAQ	76	76	78	77	±0.66
APL	23.11	25	21.95	23.35	±0.88
ADE	21	22	16	19	±1.85
AME	31	35	32.75	32.92	±1.15
AAQ	40	39	37.65	38.88	±0.68

Experiment was repeated three times and values are expressed as ±SEM

* Autofluorescence, hence the reading is unreliable.



* Autofluorescence, hence the reading is unreliable.

Fig 4: Percentage HIV-1 protease inhibition

The aqueous extract of root (RAQ) (50 µg/ml) has shown quite high inhibition to the extent of 77% which is very significant. Methanolic extract of root has shown 72.91 % protease inhibition but this inhibition may be because of autofluorescence. Hence the reading was unreliable. Methanolic and aqueous extract of aerial part also shows inhibition but in mild to moderate extent that is 32.92, 38.88 respectively. Results of HIV-1 PR inhibition by different solvent extracts of *E. scaber* are given in Table 2 and Fig 4.

The results of HIV-1 PR assay indicated that in comparison to standard drugs Ritonavir and Pepstatin, only aqueous extract of root has shown quite high inhibition which is very significant. The rest of the extract has shown mild inhibition of HIV-1 PR activity except solvent ether extract of root which has shown zero activity.

4. Conclusion

The study concluded that, as compared the root and aerial part extract of *E. scaber*, the root extract of *E. scaber* have more significant anti-HIV potential as evident from the results. The aqueous extract of root of *E. scaber* has shown significant protease inhibitor activity and reverse transcriptase inhibitor activity. Particularly, methanolic extract of root has shown the significant protease inhibition as well as reverse transcriptase inhibition but because of autofluroscence RT inhibition results was unreliable. Hence the root extract of *E. scaber* indicates the potential of the plant as an anti-HIV agent.

References

[1]. Alizon M, Montagnier L: Genetic variability in human immune deficiency viruses. *Ann N Y Acad Sci.* 1987; 511: 376-384.

[2]. Yokoyama S, Gojobori T. Molecularevolution and phylogeny of the human AIDS viruses LAV, HTLV-III, and ARV. *J. Mol. Evol.* 1987; 24:330- 336.

[3]. Alizon M, Sonigo P, Barre Sinoussi F, Fursika A, Chermann JC, Tiollais P, Montagnier L, Wain Hobson S. Molecular cloning of lymphadenopathy associated virus. *Nature.* 1984; 312:757-760.

[4]. Biberfeld G, Brown F, Esparza J, Essex M, Grifanson F, Horoshoka H, Gallo RC, Montagnier L, Najera R, Robert S, Risser R, Schild G. WHO Working Group on Characterization of HIV Related Retroviruses: criteria for characterization and proposal for a nomenclature system, *AIDS*, 1987;1:189-190.

[5]. Hiradeve SM, Rangari VD. Antibacterial and antifungal activities of *Elephantopus scaber* Linn. *Internat J. Biomed Res* 2015;6:338-45

[6]. Klos M, Van de Venter M, Milne PJ, Traore HN, Meyer D, Oosthuizen V. *In vitro* anti-HIV activity of five selected South African medicinal plant extracts. *J Ethnopharmacol.* 2009; 124:182-188.

[7]. Matayoshi, Ed & T Wang, G &Krafft, Grant & W. Erickson, J. Novel fluorogenic substrate for assaying retroviral proteases by resonance energy transfer. *Science* (New York, N.Y.). 1990; 247:954-8.

[8]. Yang H, Nkeze J, Zhao RY. Effects of HIV-1 protease on cellular functions and their potential applications in antiretroviral therapy. *Cell & Biosciences*. 2012; 2:32.