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In Vitro Anti-HIV Activity of *Rosa centifolia*, (Leaves and Roots) Extract on HIV-1UG070 and HIV-1VB59 in TZM-bl and PM1 Cell Lines

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Abstract

Human immunodeficiency virus (HIV) infection is still contributing significantly in morbidities and mortalities in the world today, more especially in developing countries. The drugs normally use to reat the infection is costly, toxic, and less effective due to resistance by HIV. In view of that an assessment of In Vitro Anti-HIV Activity of Rosa centifolia, (Leaves and Roots) Extract on HIV-1UG070 and HIV-1VB59 in TZM-bl and PM1 Cell Lines. Thus, the present therapy also has limitations of development of multidrug resistance, with a need for the discovery of novel anti-HIV compounds from plants as a potential alternative in combating HIV disease. Methods: The medicinal plant was tested for entry and replication inhibition against laboratory adapted strains HIV-1IIIB, HIV-1Ada5 and primary isolates

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HIV-1UG070, HIV-1VB59 in TZM-bl cell lines and primary isolates HIV-1UG070, HIV-1VB59 in PM1 cell lines. The plant extracts were further evaluated for toxicity in HEC-1A epithelial cell lines by trans well epithelial model. Results: The methanolic extracts of Rosa centifolia inhibited laboratory adapted HIV-1 strains (IC_{80} 29.17-78.43µg/ml) and primary isolates (IC_{80} 30.4-118µg/ml) in TZM-bl cells. Conclusion: These active methanolic extracts of Rosa centifolia, (leaves and roots) could be further subjected to chemical analysis to investigate the active moiety responsible for the anti-HIV activity. Methanolic extract of Rosa centifolia was found to be well tolerated maintaining the epithelial integrity of HEC-1A cells in vitro and thus has potential for investigating it further the phytochemical responsible for these activities.

Keywords: In Vitro, Anti-HIV, Rosa centifolia, Leaves, Roots, Extract, HIV-1UG070, HIV-1VB59, TZM-bl, PM1 Cell, Lines

INTRODUCTION

The human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) originated from the simian immunodeficiency viruses (SIVs) of primates. Thus, HIV-1 and HIV-2 each had a zoonotic origin but now spread directly from human to human. HIV-1 was first isolated in 1983 and HIV-2 in 1986 and they represent two different epidemics. The SIV of chimpanzees (SIVcpz) gave rise to HIV-1 in humans, and the SIV of the sooty mangabey monkey (SIVsm) to HIV-2 in humans. [1] It is still uncertain exactly how the transmission of these SIVs to humans occurred, but it may have been during the hunting and preparation of these primates for food, by the indigenous people of these areas in Central and Western Africa, where these primate species live. [2] Studies using molecular clock evolutionary assumptions have suggested that the ancestor virus for HIV-1 appeared in around 1931. [3] and that of HIV-2 in around 1940. [4] After this initial transmission event, it is likely individuals infected with these primate SIVs then transmitted the human form of the viruses (HIV-1, HIV-2) to other people in their communities, from where it spread, world-wide.

The virus is classified into two human immunodeficiency viruses, HIV-1 and HIV-2, they are members of the family of Retroviruses, in the genus of Lentiviruses. Retroviruses have been found in various vertebrate species, associated with a wide variety of diseases, in both animals and humans. In particular, retroviruses have been found to be associated with

malignancies, autoimmune diseases, immunodeficiency syndromes, aplastic and haemolytic anaemias, bone and joint disease and diseases of the nervous system. [1]

The many different strains of HIV-1 have been separated into major (M), new (N) and outlier (O) groups, which may represent three separate zoonotic transfers from chimpanzees. Groups N and O are mainly confined to West and Central Africa (Gabon and Cameroon), though cases of Group O have been found world-wide due to international travel, after contact with infected individuals from these areas. The HIV strains in Group M are the ones mainly responsible for the HIV/AIDS pandemic, and they are so diverse that they have been subclassified into subtypes (or clades) A-K. This huge diversity of HIV-1 is important when diagnostic testing, treatment and monitoring are applied as the results may differ between different subtypes or clades (see HIV Global Genetic Diversity and Epidemiology below). [1] The diversity of HIV-2 is much less, but subtypes A-H have been proposed. [5]

The human immunodeficiency viruses are approximately 100 nm in diameter. It has a lipid envelope, in which are embedded the trimeric transmembrane glycoprotein gp41 to which the surface glycoprotein gp120 is attached (Figure 1). These two viral proteins are responsible for attachment to the host cell and are encoded by the env gene of the viral RNA genome. Beneath the envelope, is the matrix protein p17, the core proteins p24 and p6 and the nucleocapsid protein p7 (bound to the RNA), all encoded by the viral gag gene. Within the viral core, lies 2 copies of the ~10 kilobase (kb) positive-sense, viral RNA genome (i.e. it has a diploid RNA genome), together with the protease, integrase and reverse transcriptase enzymes. These three enzymes are encoded by the viral pol gene. There are several other proteins coded for by both HIV-1 and HIV-2, with various regulatory or immuno-modulatory functions, including vif (viral infectivity protein), vpr (viral protein R), tat (transactivator of transcription), rev (regulator of viral protein expression) and nef (negative regulatory factor). An additional protein found in HIV-1 but not HIV-2 is vpu (viral protein U). Similarly, vpx (viral protein X) is found in HIV-2 and not HIV-1. [6]

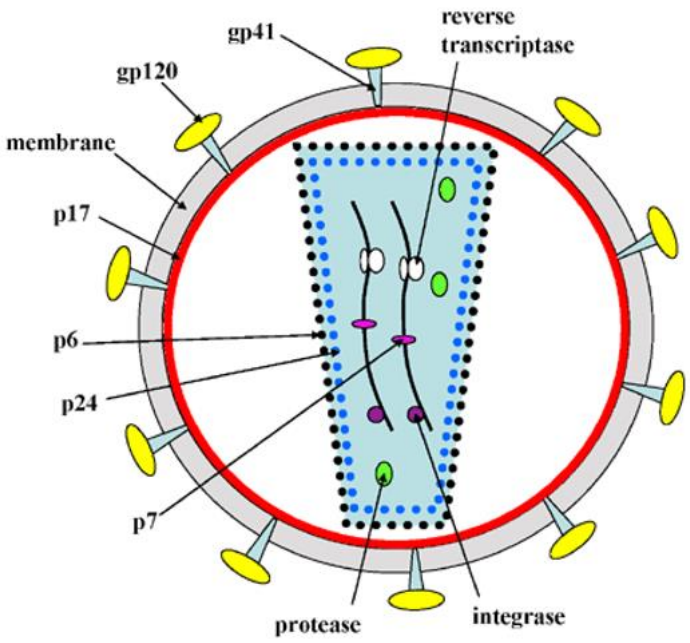


Figure 1: Structure of HIV-1 virus

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 [7]. 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 [8]. 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. For the purpose of this study, In Vitro Anti-HIV Activity of Rosa centifolia, (Leaves and Roots) Extract on HIV-1UG070 and HIV-1VB59 in TZM-bl and PM1 Cell Lines was considered and selected to

investigate its in vitro inhibitory activity against entry inhibition/replication of HIV-1 as first step towards identification of potential anti-HIV microbicide. The microbicides provide protection by directly inactivating HIV or preventing HIV from attaching, entering or replicating in susceptible target cells as well as dissemination from target cells present in semen or the host cells that line the vaginal/rectal wall [9].

The plant was selected on the basis of detailed patent survey and scientific articles on the ethnomedicinal usages of the plant genera directly in HIV/AIDS or for symptoms/conditions closely associated with this disease (Table 1). Plant R. centifolia, S. was selected because other species of the same genera have exhibited anti-HIV activity [10-14]. Its traditional use in gonorrhoea and leukeorrhea [15] and suppressive effects on sperm motility [16].

METHODS

Plant materials and extraction

Rosa centifolia, commonly known as Gulab. Its Rosacea plant, the materials were collected from Michika, the specimens was deposited in the herbarium. Table 2 presents ethno-botanical information and solvents used for extraction of the selected plant part.

The collected plant materials were cleaned, freed of foreign contaminates and washed with water, first air dried and then dried in an electric oven at 40 °C. The dried plant materials were pulverized in an electric mixer. The plant materials were extracted with various solvents individually by hot continuous Soxhlet extraction method for 18–24 h. After extraction, the extract obtained was filtered through 0.2-µm syringe filter and then concentrated on a rotary evaporator by distilling off the solvent under vacuum at 40 °C. The concentrated extracts were finally lyophilized to obtain free flowing powder and stored in airtight bottles in the refrigerator at 4-8 °C. The extractive yields of the individual extracts are recorded in Table 2. Powder was reconstituted in DMSO for final concentration of extract 10 mg/ml and stored at -20 °C until tested for anti-HIV1 activity.

Table 1: Ethnomedicinal usages of selected plant parts

	Botanical name	Plant parts	Common name	Family	Conventional use and published reports
1	Rosa centifolia	Leaves	Gulab	Rosaceae	Leaves: treating wounds, ophthalmia, hepatopathy, hemorrhoids and anti-microbial, Flowers: cardio tonic, anti-inflammatory, anti-asthmatic, anti-bronchitic, anti-diarrheal, [17, 18].
2	Rosa centifolia	Roots	Gulab	Rosaceae	dysmenorrheal, urinary tract infections, anti-tussive activity [17]

Preliminary Phytochemical Investigation

Qualitative tests were carried out to ascertain the presence of various phytochemicals in the plant extract using the methods described by Harbourne [19] (Table 3). It involved the appropriate addition of chemicals and reagents to the concentrated extract of the plant material in a test tube. The changes in the appearance of the colour, as the case may be, confirmed the presence of alkaloids, flavonoids, tannins, steroids and saponins.

Table 2: Procurement, and Solvents Used for Extraction of Plant Material

	Plant Name	Plant parts	Solvent for extraction	Yield (%)
1	Rosa centifolia	Leaves	Methanol	10.74 (±0.02)
2	Rosa centifolia	Roots	Methanol	7.56 (±0.52)

Cells, Viral Strain and Culture Conditions

TZM-bl (recombinant HeLa cells expressing high levels CD4 receptor, CXCR4 and CCR5 co-receptors) and PM1 cells (Clonal derivative of HUT 78) were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (NIH ARRRP), as well as the HEC-1A (human endometrial adenocarcinoma) cell line. The TZM-bl cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, USA) and PM1 and HEC-1A cells in RPMI-1640 (Sigma-Aldrich, USA),

supplemented with 10% heat inactivated fetal bovine serum (FBS, Moregate Biotech, Australia) and standard antibiotic-antimycotic cocktail.

The laboratory adapted HIV-1 strains [HIV-1IIIB (X4, subtype B), HIV-1Ada5 (R5, subtype B)] and the primary isolate HIV-1UG070 (X4, Subtype D) were procured from National Institutes of Health-AIDS Research and Reference Reagent Program, as well as HIV-1VB59 (R5, subtype C) to be used. Phytohemagglutinin-P (5 µg/ml, Sigma Aldrich, USA) activated peripheral blood mononuclear cells (PBMC) derived from healthy donors were used for the growth of all the viral strains. HIV-1 p24 antigen detection kit (Vironostika HIV-1 Antigen, Netherlands) was used to determine the virus production in cell culture supernatants. Samples of viral culture supernatants free form cells were obtained by centrifugation and further filtered and finally stored at -70°C for further use. Spearman Karber formula was used to ascertain the 50% tissue culture infectivity dose (TCID50) of each virus stock in both TZM-bl and PM1 cells [20].

Anti HIV1 Assays

Determination of cytotoxicity in the uninfected TZM-bl and PM1 cell lines

The cytotoxicity of the extracts was determined in uninfected TZM-bl cells using colorimetric assay that measures the reduction of a yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase (Sigma Aldrich, USA) [21]. Briefly, two-fold dilutions of the extracts were prepared, added to 96 well plates pre-seeded with TZM-bl cells (10,000 cells/well) in quadruplicate and incubated for 48 h at 37 °C. The MTT (20 µl, 5mg/ml) solution was added and the plates were incubated further for 4 h. The supernatant was removed, 200 µl of DMSO was added, the plates were incubated for 1 h and the absorbance was read at 550 nm and 630 nm.

The percent viability was calculated by comparing cell viability in the absence of extract using following formula and the results were expressed as CC_{50} (50% cytotoxic concentration).

%Cell Viability = [OD test extract/Average OD control] x 100

The cytotoxicity in uninfected PM1 cells of all the extracts was determined in a similar manner by using a similar dilution scheme and procedure as mentioned above for TZM-bl cells. The cell viability was determined by the trypan blue dye exclusion assay (Sigma Aldrich, USA) and the results were expressed as CC50 [22].

Preliminary Screening for Anti-HIV1 Activity Against Laboratory Adapted Strains in TZM-bl Cell Lines

The anti-HIV1 activity was tested against Cell-free (CF) and Cell-associated (CA) X4 tropic (HIV-1 IIIB) and R5 tropic (HIV-1Ada5) laboratory adapted strains in TZMb1 cell lines.

In cell free assay, the viral stocks (400 TCID50) were pre-treated in duplicate with sub toxic concentrations of the extracts/fractions for 1 h, at 37 °C prior to addition onto the TZM-bl cells (10,000 cells/well). While in cell-associated assay, the cells (10,000 cells/well) were pre-infected with the viral stocks (400 TCID50) for 2 h at 37 °C before exposure to the extracts/fractions [23]. After 48 h, the supernatant was collected and luciferase activity was determined using Britelite plus (Perkin Elmer, USA). Dextran Sulphate (Sigma Aldrich, USA) and Azidothymidine (AZT, CIPLA, Nigerians) were used as positive controls for cell free and cell associated assays respectively. The results were expressed as IC_{50} (50% inhibitory concentration), IC_{80} (80% inhibitory concentration) and Therapeutic Index ($TI=CC_{50}/IC_{50}$).

Confirmation of Anti-HIV1 Activity Against Primary Isolates in TZM-bl and PM1 Cell Lines

The anti-HIV1 activity was tested against Cell-free (CF) and Cell-associated (CA) X4 tropic (HIV-1 UG070) and R5 tropic (HIV-1 VB59) primary isolates in TZM-b1 and PM1 cell lines.

The procedure for anti-HIV1 activity against primary isolates in TZM-bl cell lines followed was same as mentioned above for anti-HIV1 activity against laboratory adapted strains. The results were expressed as percentage inhibition calculated using following equation. The results were expressed as IC_{80} (80% inhibitory concentration).

The anti-HIV activity against primary isolates was also evaluated in PM1 cell lines using 24-well plate (Corning, USA). In the cell free assay, 20 TCID50 the viral stock (HIV-1UG070 and HIV-1VB59) was pre-treated with sub toxic concentrations of the extracts/fractions, before addition onto the cells (5x104cells/well). Whereas, in the cell associated assay, the PM1 cells (5x104cells/well) were pre-infected with 20 TCID50 of the viral stock and then exposed to the extracts/fractions [24].

The virus growth was monitored by Vironostika p24 antigen ELISA (Biomerieux, France). Dextran sulphate and AZT were used as positive controls for cell free and cell associated assays respectively. The percent inhibition was calculated by comparing activity in absence of the extracts/fractions/control drug using the formula mentioned above and the results were expressed as IC_{80}.

Toxicity Testing Using Transwell Epithelial Model Cytotoxicity Assay

The toxicity of selected plant extract was determined in HEC-1A using similar protocol as described for TZM-bl cells, only with a difference of the read-out system, i.e. LDH cytotoxicity detection kit (Roche Diagnostics, Germany).

Determination of Epithelial Integrity in Transwell Dual chamber System

The epithelial integrity was determined as described by Gali et al., [25]. Briefly, HEC-1A cells (1 × 105/100 µl) were cultured for 7 days on the apical chamber of a Laminin coated dual-chamber Transwell system (growth area: 0.3cm2, pore size: 3.0 µm) (Corning Costar Corp, USA). After 7 days incubation, two-fold serial dilutions of test preparations (100 µl) were added on to the HEC-1A cells and incubated for 24 h (37 °C, 5% CO2). The test preparations were removed and 100 µl of a 1/20 dilution of yellow-green fluorescent microspheres (FluoSpheres sulphate microspheres, Molecular Probes Europe NV, Netherlands) were added in the apical chamber. After 24 h, 100 µl of medium was harvested from the basal chamber and the fluorescence was measured using a fluorometer (Perkin Elmer, USA). Untreated HEC-1A cells and 1% Nonoxynol-9 were used as controls for measuring percent transmission.

%Inhibition = 1- [(luminescence in presence of the test extract or fraction

-luminescence of uninfected control cells/luminescence of cells infected with virus

-luminescence of uninfected control cells)] x 100

Table 3: Phytochemical screening of R. centifolia extracts

S/N	Plant Extracts	Steriods	Saponins	Flavanoids	Alkaloids	Tannins/ Phenolic Compounds
1	R. centifolia (Leaves)	-	+	+	++++	++
2	R. centifolia (Roots)	+++	+	-	++++	++

Legend: presence or absence of phytochemical components in plant extracts by different methods: -Absent, ++++: Present in large proportion, +++: Present in good proportion, ++: Present in moderate proportion, +: Present in low proportion: Tests for Steroid: 1. Salkowski Reaction 2. Liebermann- Burchard Reaction 3. Liebermann's Reaction; Tests for Saponins: 1. Foam Test; Tests for Flavanoids: 1. Shinoda Test 2. Lead acetate Test; Test for Alkaloids: 1. Dragendorff's Test 2. Mayer's Test 3. Hager's Test 4. Wagner's Test Test for Tannins and Phenolic compounds: 1. 5% Ferric Chloride 2. Dilute Iodine Solution 3. Lead acetate solution 4. Dilute Potassium permanganate solution.

Table 4: Inhibitory concentrations and therapeutic index of plant extracts against Laboratory adapted HIV-1IIIB and HIV-1Ada5 strains in TZM-bl cell lines

S/N	Plant parts R. centifolia	CC_{50} (µg/ml)	IC_{50}				IC_{80}				Therapeutic Index			
			CF		CA		CF		CA		CF		CA	
			IIIB	Ada5	IIB	Ada5	IIIB	Ada5	IIIB	Ada5	IIB	Ada5	IIIB	Ada5
1	Leaves	132	13.6	24.8	51.9	75.4	30.4	45.2	96.1	118	5	6	1	1
2	Roots	124	4.97	3.65	18.51	17.67	29.17	35.89	79.35	78.43	10	24	8	7

CC_{50}−50% cytotoxic concentration, IC_{50}–50% inhibitory concentration; IC_{80}−80% inhibitory concentration, CF- Cell Free, CA- Cell Associated

Table 5: Inhibitory concentrations of plant extracts against Primary isolates HIV-1UG070 and HIV-1VB59 in TZM-bl and PM1 cell lines

S/N	Plant parts R. centifolia	TZM-bl assay					PM-1 assay				
		IC80(µg/ml)					IC80 (µg/ml)				
		CC_{50} (µg/ml)	CF HIV-1		CA HIV-1		CC_{50} (µg/ml)	CF HIV-1		CA HIV-1	
			UG 070	VB 59	UG 070	VB 59		UG 070	VB 59	UG 070	VB 59
1	Leaves	132	17	33.5	58.9	> 125	20	3.6	3.8	12	18
2	Roots	124	< 31.25	80	60.5	105	46	29.17	4.89	7.35	17.43
Control	DS	5573	7,12	5.3	-	-	4997	20.11	17.13	-	-
	AZT	786	-	-	8.24	18.73	989.78	-	-	8564.29	11,979.32

CC_{50}−50% cytotoxic concentration, IC_{50}–50% inhibitory concentration; IC_{80}−80% inhibitory concentration, CF- Cell Free, CA- Cell Associated.

RESULTS

Preliminary Phytochemical Investigation

The preliminary phytochemical evaluation of plant extracts for the presence of steroids, flavonoids, alkaloids, saponins, tannins and phenolic acids was done for extracts. Steroids were not present in R. centifolia leaves and flavonoid in R. centifolia (Table 3).

Determination of Cytotoxicity In TZM-bl And PM1 Cell Lines

Two methanolic extracts, of the medicinal plant were examined for their ability to inhibit HIV-1 entry and replication. The in vitro toxicity of these extracts to TZM-bl cells was investigated by MTT assay. Methanolic extracts of, R. centifolia roots and the CC_{50} values were found to be comparatively higher ranging between 124 and 4.97µg/ml and, R. centifolia leaves ranging between 132 and 13.6 respectively. However, both the extract of R. centifolia was found not to be toxic at all concentration (Table 4).

Cytotoxicity of plant extracts, for the leaves and roots parts of R. centifolia, showing activity in preliminary anti-HIV-1 assay was carried out in PM1 cells using trypan blue dye exclusion assay. The 50% cytotoxicity was observed at a concentration ranging from 20–46 µg/ml. (Table 5).

Preliminary Screening for Anti-HIV1 Activity Against Laboratory Adapted Strains In TZM-bl Cell Lines

Plant extracts of R. centifolia, (Leaves and Roots) showed inhibition of HIV-1IIIB and HIV-1Ada5 laboratory adapted strains in both cell free and cell associated assays.

The roots extract revealed significant activity against the laboratory adapted strains with estimated IC_{80} in the range of 29.17-78.43µg/ml giving TI of 10, 24, 8, and 7 in cell free HIV-1IIIB, HIV-1 Ada5 and cell associated HIV-1IIIB respectively. This was followed by leaves extract which showed activity with preliminary IC80 in the range of 30.4-118µg/ml giving TI of 5, 6, and 1 in cell free HIV-1IIIB, HIV-1Ada5 and cell associated HIV-1IIIB respectively.

Confirmation of Anti-HIV Activity Against Primary Isolates In TZM-bl And PM1 Cell Lines

The plant extracts showing activity in preliminary screening against laboratory adapted strains were further screened both cell free and cell associated assays against primary isolates HIV-1UG070 and HIV-1VB59 in TZM-b1and PM1 cell lines for confirmation of their anti-HIV1 activity.

In TZM-b1 cell lines methanolic extract of R. centifolia, (Leaves and Roots) exhibited a very good activity with lowest estimated IC80 of 17->175 µg/ml and <31.25–105µg/ml respectively against primary isolates of HIV-1 strains respectively (Table 5).

In PM1 cell lines, methanolic extract of R. centifolia, (Leaves and Roots) showed activity with estimated IC_{80} ranging 12–17.43µg/ml. the extract also exhibited activity at preliminary IC80 of 3.6–29.17µg/ml in cell free for HIV-1VB59 cell free assay. The representative dose for R. centifolia, (Leaves and Roots), in cell free and cell associated assays for TZMb1 and PM1 are given in figure 1.

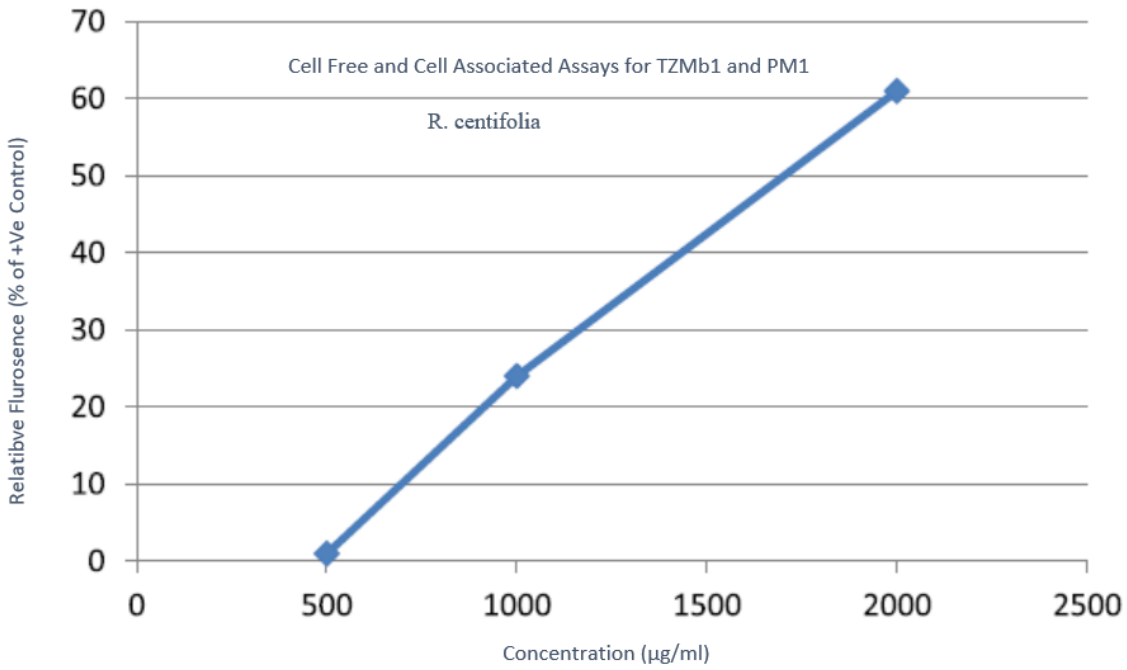


Figure 1: Plot of Relative Fluorescence (%) Vs Concentration (µg/ml) determining epithelial integrity of R. centifolia by measuring permeability to FluoSpheres using the Dual-Transwell Epithelial Model

DISCUSSION

Many researchers have directed their efforts towards the natural product, plants and plant extract has become major sources of innovative therapeutic agents for treatment of infectious diseases, and their exploration has been one of the most successful strategies for the discovery of medicines. The development of new agents against disease and ailments as preventive interventions is a promising area in HIV research [26]. They could be valuable addition in prevention of sexual transmission of HIV-1 and could be an important way to reduce the number of cases of HIV infection globally [74, 75]. Currently available anti-HIV drugs are chemically synthesized and are often limited by side effects and emergence of drug resistance [27].

In order to find such potential anti-HIV in order to find such potential anti-HIV agents from natural sources, ten traditional medicinal plants from India were studied for their inhibitory effects against laboratory adapted strains HIV-1 IIIB, HIV-1Ada5 and primary isolates HIV-1UGO70, HIV-1VB59 in TZM-b1 and primary isolates HIV-1UGO70, HIV-1VB59 in PM1 cell lines. HIV viruses can spread in the body via either a cell-free (virus floating free in plasma) mode or a cell associated (virus particles that remain attached to or within the host cell after replication) mode involving direct cell-cell contact. Hence all the selected plant extracts were evaluated to depict their mechanism of action, whether they will act as an entry inhibitor or at the HIV replication stage [28].

The selected plant extracts were subjected to high throughput (cost-effective, quick and reproducible) TZM-bl assay model which is useful for preliminary screening allowing screening of large number of products against HIV [29, 30]. The results presented here indicate that the methanolic extracts of the roots and leaves of R. centifolia possess anti-HIV properties of therapeutic interest inhibiting HIV-1 virus.

The roots extract revealed significant activity against the laboratory adapted strains with estimated IC_{80} in the range of 29.17-78.43µg/ml giving TI of 10, 24, 8, and 7 in cell free HIV-1IIIB, HIV-1 Ada5 and cell associated HIV-1IIIB respectively. This was followed by leaves extract which showed activity with preliminary IC80 in the range of 30.4-118µg/ml giving TI of 5, 6, and 1 in cell free HIV-1IIIB, HIV-1Ada5 and cell associated HIV-1IIIB respectively.

In TZM-b1 cell lines methanolic extract of R. centifolia, (Leaves and Roots) exhibited a very good activity with lowest estimated IC80 of 17->175 µg/ml and <31.25–105µg/ml respectively against primary isolates of HIV-1 strains respectively (Table 5).

In PM1 cell lines, methanolic extract of R. centifolia, (Leaves and Roots) showed activity with estimated IC80 ranging 12–17.43µg/ml. the extract also exhibited activity at preliminary IC_{80} of 3.6–29.17µg/ml in cell free for HIV-1VB59 cell free assay.

The lead extracts were also confirmed for anti-HIV activity in PM1 cell line which supports persistent HIV-1 infection [31]. The PM1 cell line have been reported to be comparable to peripheral blood mononuclear cells (PBMCs) for culturing of any of the HIV-1 strains and subtypes and thus provide a valuable research tool for studying new anti-HIV therapies [32]. This cell line has been previously used for studying the anti-HIV1 properties of the polyherbal cream Basant [33].

Hence PM-1 was used for confirming the anti-HIV activity of the methanolic extracts of aerial parts of leaves of R. centifolia which showed anti-HIV activity (IC_{80}) ranging between 1 and 8.4 and 2.2–6.8 µg/ml respectively.

These extracts may potentially inhibit the entry and also inhibit HIV-1 replication if the virus enters the vaginal cells. However further work on more replicates and wider concentration range studies are required for confirmation. Future studies should also be considered to isolate pure compound responsible for this activity.

CONCLUSION

To conclude the study, the two plant Parts screened for anti-HIV activity using TZM-b1 and PM1 assays, it was observed that both methanolic extracts of roots and leaves of R. centifolia has prospective anti-HIV1 potential as an entry and replication inhibitors. Hence these experimental moieties may have favourable implications on the prevention and management of HIV/AIDS.

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