In vitro anti-HIV activity of some Indian medicinal plant extracts

Palshetkar, Aparna, Pathare, Navin, Jadhav, Nutan, Pawar, Megha, Wadhwani, Ashish, Kulkarni, Smita and Singh, Kamalinder

Available at http://clok.uclan.ac.uk/32043/

Palshetkar, Aparna, Pathare, Navin, Jadhav, Nutan, Pawar, Megha, Wadhwani, Ashish, Kulkarni, Smita and Singh, Kamalinder ORCID: 0000-0001-7325-0711 (2020) In vitro anti-HIV activity of some Indian medicinal plant extracts. BMC Complementary Medicine and Therapies, 20 (69). ISSN 2662-7671

It is advisable to refer to the publisher’s version if you intend to cite from the work. http://dx.doi.org/10.1186/s12906-020-2816-x

For more information about UCLan’s research in this area go to http://www.uclan.ac.uk/researchgroups/ and search for <name of research Group>.

For information about Research generally at UCLan please go to http://www.uclan.ac.uk/research/

All outputs in CLoK are protected by Intellectual Property Rights law, including Copyright law. Copyright, IPR and Moral Rights for the works on this site are retained by the individual authors and/or other copyright owners. Terms and conditions for use of this material are defined in the http://clok.uclan.ac.uk/policies/
In vitro anti-HIV activity of some Indian medicinal plant extracts

Aparna Palshetkar¹, Navin Pathare², Nutan Jadhav², Megha Pawar², Ashish Wadhwani², Smita Kulkarni²* and Kamalinder K. Singh¹,3*

Abstract

Background: Human Immunodeficiency Virus (HIV) persists to be a significant public health issue worldwide. The current strategy for the treatment of HIV infection, Highly Active Antiretroviral Therapy (HAART), has reduced deaths from AIDS related disease, but it can be an expensive regime for the underdeveloped and developing countries where the supply of drugs is scarce and often not well tolerated, especially in persons undergoing long term treatment. The present therapy also has limitations of development of multidrug resistance, thus there is a need for the discovery of novel anti-HIV compounds from plants as a potential alternative in combating HIV disease.

Methods: Ten Indian medicinal plants were tested for entry and replication inhibition against laboratory adapted strains HIV-1lmb, HIV-1Adas and primary isolates HIV-1UG070, HIV-1VBS9 in TZM-bl cell lines and primary isolates HIV-1UG070, HIV-1VBS9 in PM1 cell lines. The plant extracts were further evaluated for toxicity in HEC-1A epithelial cell lines by transwell epithelial model.

Results: The methanolic extracts of Achyranthes aspera, Rosa centifolia and aqueous extract of Ficus benghalensis inhibited laboratory adapted HIV-1 strains (IC₈₀ 3.6–118 μg/ml) and primary isolates (IC₈₀ 4.8–156 μg/ml) in TZM-bl cells. Methanolic extract of Strychnos potatorum, aqueous extract of Ficus infectoria and hydroalcoholic extract of Annona squamosa inhibited laboratory adapted HIV-1 strains (IC₈₀ 4.24–125 μg/ml) and primary isolates (IC₈₀ 18–156 μg/ml) in TZM-bl cells. Methanolic extracts of Achyranthes aspera and Rosa centifolia, (IC₈₀ 1–9 μg/ml) further significantly inhibited HIV-1 primary isolates in PM1 cells. Methanolic extracts of Tridax procumbens, Mallotus philippinensis, Annona reticulate, aqueous extract of Ficus benghalensis and hydroalcoholic extract of Albizzia lebbeck did not exhibit anti-HIV activity in all the tested strains. Methanolic extract of Rosa centifolia also demonstrated to be non-toxic to HEC-1A epithelial cells and maintained epithelial integrity (at 500 μg/ml) when tested in transwell dual-chamber.

Conclusion: These active methanolic extracts of Achyranthes aspera and Rosa centifolia, 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 as candidate microbicide.

Keywords: HIV, TZM-b1, PM1, Achyranthes aspera, Rosa centifolia

* Correspondence: kksingh35@hotmail.com; skulkarni@nariindia.org

¹National AIDS Research Institute, 73, 'G'-Block, MIDC, Bhosari, Pune 411 026, India
²School of Pharmacy and Biomedical Sciences, Faculty of Clinical and Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, UK

© The Author(s). 2020 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background

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 [1]. 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 [2]. 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, ten Indian traditional medicinal plants, Albizia lebbeck, Tridax procumbens, Achyranthes aspera, Ficus benghalensis, Mallotus philippinensis, Rosa centifolia, Strychnos potatorum, Ammona reticulate, Ficus infectoria and Ammona squamosa were selected to investigate their 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 [3]. These plants were selected on the basis of detailed patient 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).

Plants such as R. centifolia, S. potatorum, F. infectoria, F. benghalensis and M. philippinensis were selected because other species of the same genera have exhibited anti-HIV activity [56–60]. Its traditional use in gonorrhoea and leukorrhea [61] and suppressive effects on sperm motility [39] further made S. potatorum, a plant of choice for this study. Fruit pulp of A. squamosa has been reported to inhibit HIV replication significantly in H9 lymphocytes [49] therefore the seeds of A. squamosa which have also shown spermicidal property, an additional desirable attribute for a vaginal microbicide [62] was selected for the study. In addition the leaves of other species A. reticulate were also selected for assessing the anti-HIV activity.

Taylor et al., [63, 64] reported methanolic extract of T. procumbens to exhibit in-vitro anti-Herpes Simplex Virus activity in Vero cells; hence it was selected for investigating its anti-HIV activity. Anticipating the potential of spermicide-based vaginal contraceptives in the reproductive health of women such as Nonoxynol (N-9) and Praneem polyherbal (Azadirachta indica leaves, Sapindus mukerossi pericarp of fruit and Mentha citrate oil) [65]; two plant extracts, methanolic leaf extract of A. aspera that has exhibited safety as well as good anti-fertility property [66] and methanolic pod extract of A. lebbeck which has been shown to suppress spermatogenesis and alter the structure and activity of the Sertoli and Leydig cells [4] were considered worthwhile to explore for anti-HIV activity.

Therefore, under the DBT-ICMR sponsored programme (HIV/AIDS and Microbicides, Phase I) developed for screening plant derived HIV microbicidal candidates, we evaluated these 10 plant extracts against 2 CXCR4 (HIV-1HXB HIV-1UUG070) and 2 CCR5 tropic (HIV-1Ada5 HIV-1VIE9) HIV-1 strains.

Methods

Plant materials and extraction

10 plant materials were collected from various parts of India in different seasons. A plant taxonomist at publicly available herbarium, Botanical Survey of India, Pune, India, validated scientific names and classification of these plants. The specimens were also deposited in the herbarium. Table 2 presents ethno-botanical information and solvents used for extraction of the selected plants.

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 was 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.

Preliminary phytochemical investigation

Qualitative tests were carried out to ascertain the presence of various phytochemicals in the plants extract of the selected plants using the methods described by Harbourne [67] (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, flavanoids, tannins, steroids and saponins.

### 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). The HEC-1A (human endometrial adenocarcinoma) cell line was kindly provided by Dr. R. Fichorova (Associate Professor, Brigham and Women's Hospital, Boston, USA) and National Institute of Virology, Pune, respectively. 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.

### Table 1 Ethnomedicinal usages of selected plant materials

| Sr No | Botanical name     | Common name | Family     | Conventional use and published reports                                                                 |
|-------|--------------------|-------------|------------|--------------------------------------------------------------------------------------------------------|
| 1     | Albizzia lebbeck   | Shirisha    | Mimosaceae | Bark: Anti-oxidant, anti-fertility, anti-microbial activity  
Seeds: Anti-inflammatory activity [4, 5].                                                              |
| 2     | Tridax procumbens  | Ghamra      | Asteraceae | Whole plant: Anti-microbial  
Flowers, Leaves: Anti septic, insecticidal, parasiticidal, anti-Cancer Activity  
Aerial parts: Hepatoprotective  
Leaves: Hypotensive, anti-diabetic, immunomodulating activity [6–12].                                      |
| 3     | Achyranthes aspera | Apaamaarga  | Amaranthaceae | Whole plant: Nephroprotective, hypolepidemic activity.  
Roots: anti-oxidant, spermicidal, activity  
Leaves: anti-oxidant, anti-fertility, anti-depressant, anti-cancer, anti-microbial activity  
Aerial parts: Hepatoprotective activity  
Seeds: Anti-microbial activity [13–22].                                                                   |
| 4     | Ficus benghalensis | Vad         | Moraceae   | Whole plant: Anthelmintic, anti-bacterial activity.  
Bark: Anti-inflammatory, anti-bacterial activity.  
Aerial roots: anti-oxidant, anti-diabetic, immunomodulatory activity [23–37].                                |
| 5     | Ficus infectoria   | Pilkhan     |            | Bark and Leaves: anti-oxidant, anti-hyperlipidemic, hypoglycemic activity [28].                          |
| 6     | Mallotus philippinensis | Kamala | Euphorbiaceae | Seeds: Anti-fertility activity.  
Stem bark: anti-oxidant, anti-tumor activity, anti-bacterial.  
Fruits: anti-inflammmatory, immunoregulatory, anti-proliferative activity.  
Leaves: Hepatoprotective activity  
Roots: Anti-leukaemic activity [29–36].                                                                     |
| 7     | Rosa centifolia    | Gulab       | Rosaceae   | Leaves: treating wounds, opthalmia, hepatopathy, hemorrhoids and anti-microbial.  
Flowers: cardio tonic, anti-inflammatory, anti-asthmatic, anti-bronchitic, anti-diarrheal,  
dysmenorrheal, urinary tract infections, anti-tussive activity [37, 38].                                    |
| 8     | Strychnos potatorum | Nirmali    | Loganiaceae | Plant: Anti-diabetic, anti-microbial activity  
Seeds: Contraceptive, diuretic, anti-inflammatory, hepatoprotective, antioxidant, antiarthritic activity [39–45]. |
| 9     | Annona reticulate  | Ramphal     | Annonaceae | Leaves: Anti-oxidant, anti-inflammatory, anti-helmentic activity.  
Seeds: Anti-cancer [46–48].                                                                                   |
| 10    | Annona squamosa    | Sitafal     |            | Bark: Anti-malarial activity  
Seeds: Anti-tumor activity  
Twigs: Anti-ulcer activity  
Leaves: anti-oxidant, hepatoprotective, anti-bacterial activity  
Fruit pulp: Anti-HIV activity [49–55].                                                                   |

Legend: Details of plants selected and their reported conventional use
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, while the Indian isolate HIV-1VB59 (R5, subtype C) was obtained from the National AIDS Research Institute (NARI), Pune. 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 from 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 [68].

**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-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase (Sigma Aldrich, USA) [69]. 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, 5 mg/ml) solution was added and the plates were incubated

**Table 2** Procurement, authentication and solvents used for extraction of plant material

| Sr. No. | Plant Name       | Part of plant | Authentication No. | Solvent for extraction | % yield (± SD) |
|---------|------------------|---------------|--------------------|------------------------|---------------|
| 1       | Albizia lebbeck  | Whole pods    | RR 3794            | Hydroalcohol           | 17.20 (±0.32) |
| 2       | Tridax procumbens| Aerial parts  | KK1                | Methanol               | 7.2 (±1.32)   |
| 3       | Achyranthes aspera| Aerial parts | PADAAP1            | Methanol               | 10.74 (±0.02) |
| 4       | Ficus benghalensis| Leaves       | PADFB1             | Water                  | 7.56 (±0.52)  |
| 5       | Mallotus philippinensis| Leaves | MPADP12           | Methanol               | 5.72 (± 0.09) |
| 6       | Rosa centifolia  | Leaves        | ROAP1              | Methanol               | 9.46 (± 0.50) |
| 7       | Strychnos potatorum| Seeds       | SPAP2              | Methanol               | 15.00 (±0.43) |
| 8       | Annona reticulata| Leaves       | APAR1              | Methanol               | 7.89 (±0.07)  |
| 9       | Ficus infectoria | Leaves       | APF1               | Water                  | 19.08 (±0.02) |
| 10      | Annona squamosa  | Seeds         | SS1/ 2008          | Hydroalcohol           | 10.87 (±0.15) |

Legend: Procurement, authentication no. & extraction details of the plants parts used for the study

**Table 3** Phytochemical screening of selected plant extracts

| Sr. No. | Plant Extracts | Steroids | Saponins | Flavanoids | Alkaloids | Tannins/ Phenolic Compounds |
|---------|----------------|----------|----------|------------|-----------|----------------------------|
| 1       | A. lebbeck     | +++      | +        | +          | +++       | ++                         |
| 2       | T. procumbens  | -        | -        | +          | -         | -                          |
| 3       | A. aspera      | +++      | +        | +          | +++       | +++                       |
| 4       | F. benghalensis| +++      | +        | +          | +++       | +++                       |
| 5       | M. philippinensis| -        | +        | +          | +++       | +++                       |
| 6       | R. centifolia  | -        | +        | +          | +++       | +                         |
| 7       | S. potatorum   | +++      | +        | -          | +++       | +                         |
| 8       | A. reticulata  | +++      | -        | +          | +++       | +++                       |
| 9       | F. infectoria  | ++       | +        | ++         | +++       | +++                       |
| 10      | A. squamosa    | -        | +        | +++        | ++        | ++                        |

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 Steroids: 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 solution 3. Dilute Iodine Solution 4. Lead acetate solution 4. Dilute Potassium permanganate solution

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
The results were expressed as IC_{50} (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 TCID_{50} the viral stock (HIV-1_{UG070} and HIV-1_{VB59}) was pre-treated with sub toxic concentrations of the extracts/fractions, before addition onto the cells (5x10^{4}cells/well). Whereas, in the cell associated assay, the PM1 cells (5x10^{4}cells/well) were pre-infected with 20 TCID_{50} of the viral stock and then exposed to the extracts/fractions [72]. The virus growth was monitored by Vironostika\textsuperscript{TM} 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., [73]. Briefly, HEC-1A cells (1 x 10^{5}/100 \mu l) were cultured for 7 days on the apical chamber of a Laminin coated dual-chamber Transwell\textsuperscript{TM} system (growth area: 0.3cm\textsuperscript{2}, pore size: 3.0 \mu m) (Corning Costar Corp, USA). After 7 days incubation, two-fold serial dilutions of test preparations (100 \mu l) were added on to the HEC-1A cells and incubated for 24 h (37 \degree C, 5% CO_{2}). The test preparations were removed and 100 \mu l of a 1/20 dilution of yellow-green fluorescent microspheres (FluoSpheres\textsuperscript{TM} sulphate microspheres, Molecular Probes Europe NV, Netherlands) were added in the apical chamber. After 24 h, 100 \mu l 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.

Results

Preliminary phytochemical investigation

The preliminary phytochemical evaluation of plant extracts for the presence of steriods, flavanoids, alkaloids,
saponins, tannins and phenolic acids was done for 10 plants extracts from 8 different families. Steroids were not present in T. procumbens, M. philippinensis, R. centifolia and A. squamosa extracts and the Saponins in T. procumbens and A. reticulate extract. Only flavonoids was present in T. procumbens extract while it was not present in S. potatorum extract (Table 3).

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

| Sr. No. | Plant Extract          | CC₅₀ (µg/ml) | IC₅₀ | IC₈₀ | Therapeutic Index |
|---------|------------------------|--------------|------|------|------------------|
|         |                        |              | CF   | CA   | CF   | CA   | CF   | CA   |
|         |                        |              | IIIB | Ada5 | IIIB | Ada5 | IIIB | Ada5 |
| 1       | A. lebbeck             | 203           | No activity |      |      |      |      |      |      |
| 2       | T. procumbens          | 62            | No activity |      |      |      |      |      |      |
| 3       | A. aspera              | 69            | 8.3  | 2.3  | 4.1  | 28.4 | 18   | 21   | 26   | 35   | 14   | 35   | 13   | 3    |
| 4       | F. benghalensis        | 72            | 6    | 6    | 5.2  | 2.25 | 9.6  | 9.6  | 8.32 | 3.6  | 12   | 12   | 14   | 32   |
| 5       | M. philippinensis      | 71            | No activity |      |      |      |      |      |      |
| 6       | R. centifolia          | 132           | 13.6 | 24.8 | 51.9 | 75.4 | 30.4 | 45.2 | 96.1 | 118  | 5    | 6    | 1    | 1    |
| 7       | S. potatorum           | 124           | 4.97 | 36.5 | 18.51| 17.67| 29.17| 35.89| 79.35| 78.43| 10   | 24   | 8    | 7    |
| 8       | A. reticulate         | 11            | No activity |      |      |      |      |      |      |
| 9       | F. infectoria         | 147           | 1.18 | 2.97 | 4.97 | 8.738| 4.24 | 8.6  | 52.49| > 125| 189  | 49   | 27   | 2    |
| 10      | A. squamosa           | 51            | No activity |      |      |      |      |      |      |

CC₅₀, 50% cytotoxic concentration; IC₅₀, 50% inhibitory concentration; IC₈₀, 80% inhibitory concentration; CF- Cell Free, CA- Cell Associated

**Detonation of cytotoxicity in TZM-bl and PM1 cell lines**

Six methanolic extracts, two aqueous extracts and two hydroalcoholic extracts of 10 medicinal plants 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, A. aspera, hydroalcoholic extract of A. squamosa and water extract of F. benghalensis tested were relatively non-toxic to TZM-bl cells at a CC₅₀ value between 51 and 72 µg/ml. The CC₅₀ values of other extracts such as methanolic extract of, R. centifolia, S. potatorum and aqueous extract of F. infectoria were found to be comparatively higher ranging between 118 and 147 µg/ml. However, methanolic extract of A. reticulate was found to be toxic at a very low concentration (CC₅₀ = 11 µg/ml) as compared to the other extracts (Tables 4 and 5).

**Cytotoxicity of plant extracts, A. aspera, F. benghalensis, R. centifolia, S. potatorum, F. infectoria and A. squamosa 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 concentrations ranging from 2.9–46 µg/ml. Aqueous extract of F. benghalensis was toxic at a very low concentration as compared to other extracts (Table 5).**

**Preliminary screening for anti-HIV1 activity against laboratory adapted strains in TZM-bl cell lines**

Plant extracts of A. aspera, F. benghalensis, R. centifolia, S. potatorum, F. infectoria and A. squamosa showed inhibition of HIV-1 IIIB and HIV-1 Ada5 laboratory adapted strains in both cell free and cell associated assays. Aqueous extract of F. infectoria revealed significant activity against the laboratory adapted strains with estimated IC₈₀ in the range of 4.24–125 µg/ml giving TI of 189, 49 and 27 in cell free HIV-1 IIIB, HIV-1 Ada5 and cell associated HIV-1 IIIB respectively. This was followed by methanolic extract of A. aspera which showed activity with preliminary IC₈₀ in the range of 18–35 µg/ml giving TI of 14, 35 and 13 in cell free HIV-1 IIIB, HIV-1 Ada5 and cell associated HIV-1 IIIB respectively. Aqueous extract of F. benghalensis exhibited activity in both laboratory adapted strains with estimated IC₈₀ in the range of 18–35 µg/ml giving TI between12–32. Methanolic extract of S. potatorum showed activity with preliminary IC₈₀ in the range of 29.17–79.35 µg/ml giving estimated TI of 24 in cell free HIV-1Ada5 strain. Methanolic extract of R. centifolia and hydroalcoholic extract of A. squamosa displayed very low activity (estimated TI in the range of 1–6). Hydroalcoholic extract of A. lebbeck, methanolic extract of T. procumbens, M. philippinensis and A. reticulate did not demonstrate any activity against cell free and cell-associated laboratory adapted HIV-1 strains.

**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-1 UG070 and HIV-1 VB59 in TZM-b1
and PM1 cell lines for confirmation of their anti-HIV1 activity.

In TZM-b1 cell lines methanolic extract of A. aspera and hydroalcoholic extract of A. squamosa exhibited a very good activity with lowest estimated IC₈₀ in the range of 4.8–53 µg/ml and 25–27 µg/ml respectively against primary isolates of HIV-1 strains. This was followed by aqueous extract of F. infectoria, methanolic extract of S. potatorum and R. centifolia and aqueous extract of F. benghalensis with preliminary IC₈₀ in the range of 18–73 µg/ml, < 31.25–105 µg/ml, 17– > 125 µg/ml, and < 78–< 156 µg/ml respectively (Table 5).

In PM1 cell lines, methanolic extract of A. aspera and R. centifolia showed activity with estimated IC₈₀ ranging 1–9 µg/ml. Aqueous extract of F. benghalensis exhibited activity at preliminary IC₈₀ of 2.9–3.5 µg/ml in cell free assay. Methanolic extract of S. potatorum exhibited activity at IC₈₀ of 6–29 µg/ml except for HIV-1 VB59 cell free assay. The hydroalcoholic extract of A. squamosa showed very good activity at lower concentration 0.8 µg/ml in cell associated assay. Aqueous extract of F. infectoria showed activity in cell free assay at a concentration ranging 2–29 µg/ml (Table 5). The representative dose response bar graphs for A. aspera and R. centifolia, in cell free and cell associated assays for TZMbl1 and PM1 are given in Additional file 1: (Figures S1 to S5).

Toxicity testing using Transwell epithelial model
Amongst the three extracts which showed highest activity, methanolic extract of R. centifolia exhibited least toxicity both inTZM-b1 and PM1 cell lines, therefore it was further tested for in vitro activity against HEC-1A cells and demonstrated minimal cytotoxicity with CC₅₀ of 1443 µg/ml. Epithelial integrity of HEC-1A in Transwell dual-chamber system was maintained with only 1% relative fluorescence (percent of positive control) detected after treatment with 500 µg/ml of methanolic extract of R. centifolia. At higher concentration of 1000 µg/ml integrity was affected with 24% leaked fluorescence relative to positive control (Fig. 1).

Discussion
Natural products continue to be 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 microbicides as preventive interventions is a promising area in AIDS research [3]. 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 [76].

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 laboratoroy adapted strains HIV-1 IIIB, HIV-1Ada5 and primary isolates HIV-1 UGO70, HIV-1 VB59 in TZM-b1 and primary isolates HIV-1 UGO70, HIV-1 VB59 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 [77].
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 [70, 78]. The results presented here indicate that the methanolic extracts of aerial parts of *A. aspera*, leaves of *R. centifolia* and seeds of *S. potatorum* and aqueous extract of leaves of *F. benghalensis* and *F. infectoria* possess anti-HIV properties of therapeutic interest inhibiting HIV-1 virus at an estimated TI of 3–35, 1–6, 7–24, 12–32, and 2–189 respectively against laboratory adapted strains and at a very low preliminary IC₅₀ ranging from 4.8–26, 17–125, 31–105, 78–156 and 18–73 μg/ml respectively against primary isolates using TZM-b1 assay.

The lead extracts were also confirmed for anti-HIV activity in PM1 cell line which supports persistent HIV-1 infection [72]. 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 [79]. This cell line has been previously used for studying the anti-HIV1 properties of the polyherbal cream Basant [80]. Hence PM-1 was used for confirming the anti-HIV activity of the methanolic extracts of aerial parts of *A. aspera* and leaves of *R. centifolia* which showed anti-HIV activity (IC₅₀) 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 on PBMCs for qualifying the results should also be considered.

Our earlier work has shown that methanolic extract of *R. centifolia* has also shown activity against four strains of *N. gonorrhoeae* [81]. It substantially lacks cytotoxicity even at high concentrations (CC₅₀ greater than 1 mg/ml) when tested in vitro on HEC-1A cell line (endometrial origin) and maintained its epithelial integrity when studied in Transwell model at concentrations up to 500 μg/ml thus showing potential for investigating it further as candidate anti-HIV microbicide.

As per the literature these extracts have not been further analyzed chemically, although the active components such as oleanolic acid and pominic acid isolated from *Rosa wodsi* leaves the other species of *Rosa*, have been reported to inhibit HIV replication in acutely infected H9 cell growth at IC₅₀ of 40 μg/ml [56, 57]. The literature indicates the phytosteroids, polyphenols and saponins present in the methanolic leaf extract of *A. aspera* are responsible for its anti-fertility effect [66] and methanolic root extract possess anti-herpes virus activity at EC₅₀ of 64.4 μg/ml for HSV-1 and 72.8 μg/ml for HSV-2 [82].

The other selected medicinal plants extract showed anti-HIV activity against at least any one of the assay model except for hydroalcoholic extract of whole pods of *A. lebbeck*, methanolic extract of aerial parts of *T. proconcumbens*, methanolic extract of leaves of *M. philippinesis* and methanolic extract of leaves of *A. reticulate*, they were incapable of showing anti-HIV1 activity against cell free and cell associated HIV-1₃HIV, HIV-1₃Ada5 laboratory adapted strains and HIV-1₃UGO70, HIV-1₃VBE primary isolates in TZM-b1 and PM1 cell lines. It’s worth mentioning that these plants were selected on basis of their sub species showing activity against other strains and primary isolates of HIV and the same species having contraceptive and activity related to this infectious

---

**Fig. 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
The inactivity of these plants against our test strains and primary isolates of HIV does not prove that they do not possess anti-HIV1 activity. These plants can be taken further for the activity against other strains and primary isolates of HIV virus using other anti-HIV assays.

Some plants extract such as *F. benghalensis*, *S. potatorum* and *F. infectoria* showed moderate to mild anti-HIV1 activity. These plants extracts had variable activities across the assays presented in this study where the extract exhibited inhibition of one strain of the primary isolates in one assay but did not inhibit the same primary isolates in another assay model. Aqueous extract of leaves of *F. benghalensis* showed anti-HIV1 activity against all HIV-1 laboratory adapted strains and primary isolates using TZM-b1 assay (TI: 12–32, IC₈₀: 78–156 μg/ml) but did not inhibit cell associated primary isolates in PM1 assay. Methanolic extract of seeds of *S. potatorum* showed anti-HIV1 activity against all HIV-1 laboratory adapted strains and primary isolates using TZM-b1 assay (TI: 7–24, IC₈₀: 31.25–105 μg/ml) but was not capable of inhibiting cell free primary isolate HIV-1/Vw95 in PM1 assay. Aqueous extract of leaves of *F. infectoria* showed anti-HIV1 activity against all HIV-1 laboratory adapted strains and primary isolates using TZM-b1 assay (TI: 2–189, IC₈₀: 18–73 μg/ml) but did not inhibit cell associated primary isolates in PM1 assay. Hence these extracts may not altogether be classified as extracts not having anti-HIV1 inhibitory potential.

Hydroalcoholic extract of seeds of *A. squamosa* exhibited activity against all HIV-1 laboratory adapted strains and primary isolates using TZM-b1 assay (TI: 2–4, IC₈₀: 26–27 μg/ml) but was not capable of inhibiting cell free primary isolates in PM1 assay. This plant extract has a some potential to be explored further and may be used supplementary as a replication inhibitor.

**Conclusion**

To conclude the study, out of 10 plants screened for anti-HIV activity using TZM-b1 and PM1 assays, methanolic extracts of aerial parts of *A. aspera* 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 or management of HIV/AIDS. Additionally methanolic extract of leaves of *R. centifolia* have shown good safety and maintained the epithelial integrity on HEC-1A cells. Plant extracts are complex mixtures of many compounds. Some compounds may mask the anti-HIV1 potential of plant extract due to their cytotoxicity. Therefore our next step would be isolating the phyto-constituents and increasing the chances to find active anti HIV1 compounds with low cytotoxicity.

**Acknowledgments**

The authors would like to thank Sh. V. Chelladurai, Research Officer (Botany), S-III, Survey of Medicinal Plants Unit – Siddha, Palayamkottai (Rtd) and S.N.D.T. Women’s University campus for supplying plant materials and specimens for authentication. The authors would also like to thank Botanical Survey of India for carrying out authentication of the plant specimens.

**Authors’ contributions**

AP participated in the design and coordination of the study, carried out the extraction and standardization studies and drafted the manuscript. NP, MP and AW participated and performed the cytotoxicity and anti-viral assays and edited the manuscript. SK designed the research work and participated in cytotoxicity and anti-viral assays and gave final approval for its publication. KKS designed the research work, overviewed the extraction and standardization studies and edited and revised the manuscript critically for important intellectual content and gave final approval for its publication. All authors read and approved the final manuscript.

**Funding**

This study was supported by grant BT/PR965/Med/14/1203/2006 from Department of Biotechnology (DBT, New Delhi) and Indian Council of Medical Research (ICMR, New Delhi).

**Availability of data and materials**

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1. C. U Shah College of Pharmacy, S.N.D.T. Women’s University, Santacruz West, Mumbai 400049, India. 2National AIDS Research Institute, 73, 'G'-Block, MIDC, Bhosari, Pune 411 026, India. 3School of Pharmacy and Biomedical Sciences, Faculty of Clinical and Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, UK.
References

1. United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS data 2019. Available online: https://www.unaids.org/en/resources/fact-sheets/https://www.hiv.gov/hiv-basics/overview/data-and-trends/global-statistics. Accessed Oct 2019.

2. Rege A, Ambaye RY, Deshmukh RA. In-vitro testing of anti-HIV activity of some medicinal plants. Indian J Nat Prod Resour. 2010;1(2):193–9.

3. D'Cruz DJ, Uckun FM. Clinical development of microbicides for the prevention of HIV infection. Curr Pharm Des. 2004;10(3):315–36.

4. Gupta RS, Kachhawa JB, Chaudhary R. Antifertility effects of methanolic pod extract of Albizia lebeck (L.) Benth in male rats. Asian J Androl. 2004;6(1):55–9.

5. Karuppannan K, Subramanian D, Priyadharsini VS, Phytopharmaceutical properties of Albizia species: a review. Int J Pharm Sci. 2013;5(3):70–3.

6. Salihdeen HM, Yermian OK, Alada AR. An effect of aqueous leaf extract of Trixus procumbens on blood pressure and heart rate in rats. Afr J Biomed Res. 2004;7:27–9.

7. Mahato RB, Chaudhary RP. Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. Sci World. 2005;3(3):26–31.

8. Saxena VK, Albert S. b-Sitosterol-3-O-b-D-xylopyranoside from the flowers of Tridax procumbens Linn. J Chem Sci. 2005;117(3):263–6.

9. Wilwanathan R, Shivasangari KS, Devak T. Hepatoprotective activity of Trixus procumbens against d-galactosamine/lipopolysaccharide-induced hepatitis in rats. J Ethnopharmacol. 2005;101:55–60.

10. Bhagwat DA, Kelledar SG, Adnal A. Anti-diabetic activity of leaf extract of Trixus procumbens. Int J Green Pharma. 2008;2:126–8.

11. Oludummore MK, Nutan Modi M, Dezzutti CS, Kulshreshta S, Rawat A, Srivastava S, et al. Immunomodulatory effects of organic solvent extracts of Mallotus philippinensis (Euphorbiaceae). Chem Pharm Bull. 2002;50(12):1560–4.

12. Vishnupriya P, Radhika K, Sivakumar R, Sri Ramchandra M, Prameela Devi V, et al. Antioxidant and anti-inflammatory effects of leaf extract of Mallotus philippinensis (L.) Benth in male rats. Asian J Androl. 2004;6:155–9.

13. Baskar R, Rajeswari V, Sathish KT. Anti-HIV activity of ent-16,17-dihydroxykauran-19-oic acid as an anti-HIV principle and their inhibitory effects on NO production. Molecules. 2013;18:4477–86.

14. Yuan SSF, Hl C, Chen HW, Yeh YT, Kao YH, Lin KH, et al. Annonacin, a mono-terahydrofuran acetogenin, arrests cancer cells at the G1 phase and causes cytotoxicity in a Bax- and caspase-3-related pathway. Life Sci. 2005;72(25):2853–61.

15. Ali M, Shafiq M, Khan MA, Mehmood T, Khan RA. Hexane soluble extract of Mallotus philippinensis (lam). Mueell. Arg, root possesses anti-leukaemic activity. Chem Cent J. 2013;7(1):57–62.

16. Sankaranarayanan R. Evaluation of antitussive activity of Rosa centifolia. IPSR. 2011;60:1473–5.

17. Khan M, Qureshi RA, Hussain MA, Mohammad K, Khan RA. Hexane soluble extract of Mallotus philippinensis (lam). Mueell. Arg, root possesses anti-leukaemic activity. Chem Cent J. 2013;7(1):57–62.

18. Shukla R, Gupta S, Gambhir JK, Prabhu KM, Murthy GS. Antioxidant effect of aqueous extract of the bark of Ficus bengalensis in hypercholesterolaemic rabbits. J Ethnopharmacol. 2004;95:47–51.

19. Taur DJ, Nirmal SA, Patti RY, Kharya MD. Antistress and anti allergic effects of Ficus bengalensis bark in asthma. Nat Prod Res. 2007;14:1256–60.

20. Gupta AK, Dwivedi S, Sharma A, Lodhi GS. Evaluation of antihypertensive, hypoglycemic and antioxidant potential of Ficus religiosa methanolic extract in wistar rats. J Pharmacognosy Phytochemistry. 2013;16:184–201.

21. Dikshanta A, Katsuki S, Wu JB, Kitanaka S. Anti-allergic agents from natural sources (4). Anti-allergic activity of new phloroglucinol derivatives from Mallotus philippinensis (Euphorbiaceae). Chem Pharm Bull. 2002;50(12):1566–70.

22. Thakur SC, Thakur SS, Chaube SK, Singh SP. An ethereal extract of Kamala (Mallotus philippinensis (mull. Arg.) lam.) seed induce adverse effects on reproductive parameters of female rats. Reprod Toxicol. 2005;20(1):149–56.

23. Mooreby K, Srinivasan K, Subramanian C, Mohanasundari C, Palaniyamw M. Phytochemical screening and antibacterial evaluation of stem bark of Mallotus philippinensis var. Tomentosus. Afr J Biotechnol. 2007;6(13):1521–3.

24. Palshetkar MM, Deshmane AA, Deshpande GS, Deshpande WW, Patil RB. Effects of water and methanolic leaves extracts of Buplerum marginatum on reproduction and development of Phthirius pubis in albino rats. Asian J Exp Biol. 2009;46(12):4850–4.

25. Gayathri M, Krishnan K. Antidiabetic and ameliorative potential of Ficus bengalensis bark extract in Streptozotocin induced diabetic rats. Indian J Clin Biochem. 2008;23(4):394–400.

26. Shukla R, Gupta S, Gambhir JK, Prabhu KM, Murthy GS. Antioxidant effect of aqueous extract of the bark of Ficus bengalensis in hypercholesterolaemic rabbits. J Ethnopharmacol. 2004;95:47–51.

27. Yuan SSF, Hl C, Chen HW, Yeh YT, Kao YH, Lin KH, et al. Annonacin, a mono-terahydrofuran acetogenin, arrests cancer cells at the G1 phase and causes cytotoxicity in a Bax- and caspase-3-related pathway. Life Sci. 2005;72(25):2853–61.

28. Dikshanta A, Katsuki S, Wu JB, Kitanaka S. Anti-allergic agents from natural sources (4). Anti-allergic activity of new phloroglucinol derivatives from Mallotus philippinensis (Euphorbiaceae). Chem Pharm Bull. 2002;50(12):1566–70.

29. Thakur SC, Thakur SS, Chaube SK, Singh SP. An ethereal extract of Kamala (Mallotus philippinensis (mull. Arg.) lam.) seed induce adverse effects on reproductive parameters of female rats. Reprod Toxicol. 2005;20(1):149–56.

30. Mooreby K, Srinivasan K, Subramanian C, Mohanasundari C, Palaniyamw M. Phytochemical screening and antibacterial evaluation of stem bark of Mallotus philippinensis var. Tomentosus. Afr J Biotechnol. 2007;6(13):1521–3.

31. Palshetkar MM, Deshmane AA, Deshpande GS, Deshpande WW, Patil RB. Effects of water and methanolic leaves extracts of Buplerum marginatum on reproduction and development of Phthirius pubis in albino rats. Asian J Exp Biol. 2009;46(12):4850–4.

32. Dikshanta A, Katsuki S, Wu JB, Kitanaka S. Anti-allergic agents from natural sources (4). Anti-allergic activity of new phloroglucinol derivatives from Mallotus philippinensis (Euphorbiaceae). Chem Pharm Bull. 2002;50(12):1566–70.

33. Thakur SC, Thakur SS, Chaube SK, Singh SP. An ethereal extract of Kamala (Mallotus philippinensis (mull. Arg.) lam.) seed induce adverse effects on reproductive parameters of female rats. Reprod Toxicol. 2005;20(1):149–56.

34. Mooreby K, Srinivasan K, Subramanian C, Mohanasundari C, Palaniyamw M. Phytochemical screening and antibacterial evaluation of stem bark of Mallotus philippinensis var. Tomentosus. Afr J Biotechnol. 2007;6(13):1521–3.

35. Palshetkar MM, Deshmane AA, Deshpande GS, Deshpande WW, Patil RB. Effects of water and methanolic leaves extracts of Buplerum marginatum on reproduction and development of Phthirius pubis in albino rats. Asian J Exp Biol. 2009;46(12):4850–4.

36. Dikshanta A, Katsuki S, Wu JB, Kitanaka S. Anti-allergic agents from natural sources (4). Anti-allergic activity of new phloroglucinol derivatives from Mallotus philippinensis (Euphorbiaceae). Chem Pharm Bull. 2002;50(12):1566–70.
isolation of the new diterpenoids, annosquamosins a and b from Annona squamosa. J Nat Prod. 1996;59:635–7.
50. Khar A, Pardhasaradhi BV, Reddy M, Ali Mubarak A, KumariLeela A. Antitumour activity of Annona squamosa seed extracts is through the generation of free radicals and induction of apoptosis. Indian J Biochem Biophys. 2004;41:167–71.
51. Mohamed. Hepatoprotective activity of Annona squamosa Linn. on experimental animal model. Int J Appl Res Nat Prod. 2008;1(3):1–7.
52. Jayshree P, Kumar V. Anti-ulcer constituents of Annona squamosa L.: phytochemical analysis and antimicrobial screening. J Pharm Res. 2008;1(1):34–8.
53. Johns. Antimalarial alkaloids isolated from Annona squamosa. Phytopharmacology. 2011;1(3):49–53.
54. Yadav DK, Singh N. Anti-ulcer constituents of Annona squamosa twigs. Fitoterapia. 2011;82(4):666–75.
55. ChandraShekar. Isolation, characterizations and free radical scavenging activity of Annona squamosa leaf. J Pharm Res. 2011;4(3):610–1.
56. Vlietinck AJ, De Bruyne T, Apers S, Pieters LA. Plant-derived leading molecules for the development of new antivirals. Antiviral Res. 1998;36:3–20.
57. Kashiwada Y, Wang HK, Nagao T, Kitanaka S, Yasuda I, Fujioka T, et al. Anti-HIV activity of a fraction of Ophioglossum vulgatum. J Nat Prod. 1998;61(9):853–60.
58. Yu YB, Park JC, Lee JH, Kim GE, Jo SK, Byun MW, et al. Screening of some plant extracts for inhibitory effects on HIV-1 and its essential enzymes. Kor J Pharmacol. 1998;29:338–46.
59. Ramanath B, Premanathan M, Kathiresan K, Nakashima H, Yamamoto N. Studies on some coastal plants for anti-HIV activity. South East Asian Seminar on Herbs and Herbal Medicines, Patna. 165. 1999.
60. Nakane H, Arisawa M, Fujita A, Koshimura S, Ono K. Inhibition of HIV reverse transcriptase activity by some fluroglucinol derivatives. FEBS Lett. 1991;268: 83–5.
61. Yadav KN, Kadam PV, Patel JA, Patil MJ. Strychnos potatorum: phytochemical and pharmacological review. Pharmacogn Res. 2015;13(5):1–9.
62. Singh KK, Parmar S, Tatke PA. Contraceptive efficacy and safety of a polyherbal topical Microbicide candidate inhibits different clades of both CCR5 and CXCR4 tropic, lab-adapted and primary isolates of Human Immunodeficiency Virus in vitro infection. J Virol Antiv Res. 2014;3(4). https://doi.org/10.1016/j.jvirtrres.2014.03.021 Epub 2014 Mar 31.
63. Jadhav N, Kulkarni S, Mane A, Kulkarni R, Palshetker A, Singh K, et al. Antimicrobial activity of plant extracts against sexually transmitted pathogens. Nat Prod Res. 2014;27:1–5.
64. Mukherjee H, Ojhaa D, Baga P, Chandel HS, Bhattacharyya S, Chatterjee TK, et al. Anti-herpes virus activities of Achyranthes aspera: an Indian ethnomedicine, and its triterpene acid. Microbiol Res. 2013;168:238–44.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions