Keywords: *Jatropha curcas* Linn., Leaf extract; Anti-HIV activity; Drug-resistant HIV; Secondary metabolites

Introduction

People living with HIV/AIDS often choose traditional or complementary alternative medicine to complement or replace conventional treatment. The presence of multi-drug or even multi-class resistance in HIV also warrants the need to explore additional means to combat HIV and provide further justifications for the need of alternative and complementary medicines in the treatment of HIV/AIDS.

Most of the traditional systems of medicine in India include some form of ‘medicinal plant’, herbs or natural plant products. It is therefore not surprising that the activity of these traditional medicines against HIV can be scientifically analysed to deduce the role of natural plant products in their anti-HIV activities. A number of medicinal plants have been reported to have anti-HIV properties [1]. Over the past two decades, substantial progress has been made in research on the natural products possessing anti-HIV activity. A variety of secondary metabolites obtained from natural origin showed moderate to good anti-HIV activity [2].

*Jatropha curcas* Linn., (colloquially known as errand/danti/katri in Marathi) is a multipurpose, drought resistant, perennial plant that belongs to Euphorbiaceae family, widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia. The genus name ‘*Jatropha*’ derives from the Greek word ‘*jatros*’ (doctor) and ‘*trophi*’ (food), which implies medicinal uses [3]. Practically all parts of *Jatropha* have some use including green manure, soil erosion control, soap production from oils, leaves as animal feed, biocidal activity from natural products, leaves and/or stems for sericulture and vermiculture and the more famously known biodiesel production from oil apart from its numerous medicinal uses from various parts [4,5]. All parts of *Jatropha* (seeds, leaves and bark) have also been used in traditional medicine and for veterinary purposes for several centuries [6]. Some of the known medicinal properties of *Jatropha curcas* Linn. include antitumor activities, molluscicidal, insecticidal and fungicidal properties. The seed oil (Jamalgota) can be applied to treat eczema and skin diseases and to soothe rheumatic pain [6]. The latex from branches have been found to be strong inhibitors to watermelon mosaic virus and the leaves and latex are used in healing of wounds, refractory ulcers, and septic gums and as a styptic in cuts and bruises [7]. Topical application of *Jatropha* root powder in paste form is a common ethnobotanical practice for the treatment of inflammation being followed by Bhil tribes from Rajasthan area in India and the methanol extract of these roots exhibited systemic and anti-HIV activity [1].

Abstract

**Objectives:** The presence of drug-resistant HIV is a major global concern and warrants the development of novel anti-virals as alternative and inexpensive therapy. In the current study, potentially drug-resistant HIV was isolated and previously unreported anti-viral activity of *Jatropha curcas* Linn. leaf extracts was assessed.

**Methods:** HIV isolation was done using *in vitro* micro co-culture methods followed by drug susceptibility assays to determine resistance to Zidovudine (AZT), Lamivudine (3TC) and Stavudine (d4T). Soxhlet apparatus was used for extraction of metabolites from leaves of *Jatropha curcas* Linn. and Methanolic and Aqueous Extracts were chosen for further study. Secondary metabolites were detected by High-Performance Thin Layer Chromatography and *in vitro* cytotoxicity established by MTT assay. The extracts were then used in post- and pre-infection studies by measuring inhibition of HIV replication to determine anti-viral activity.

**Results:** Seven HIV isolates were obtained (isolation rate: 23.33%) with drug IC<sub>50</sub> values ranging from 0.001418-82.73 µM AZT, 2.645-15.35 µM 3TC and 18.55-66.23 µM d4T. Tannins, Flavonoids, Saponins were detected in Aqueous Extract and Flavonoids, Saponins in Methanolic Extract. The CC<sub>50</sub> values were 32.07 mg/mL and 35.5 mg/mL for Aqueous and Methanolic Extracts respectively. Anti-viral activity was evaluated by inhibition of HIV replication as determined by HIV p24 antigen ELISA. Post-infection (4 isolates) interaction studies showed IC<sub>50</sub> values ranging from 0.0255-0.4137 mg/mL and 0.00073-0.1278 mg/mL for Aqueous and Methanolic Extracts respectively and pre-infection (1 isolate) interaction studies showed 100% inhibition by Methanolic and 97.19% inhibition by Aqueous Extract at 25 mg/mL each.

**Conclusions:** HIV isolates potentially resistant to AZT/3TC/d4T were obtained and *Jatropha curcas* Linn. leaf extracts showed effective anti-viral and probable entry inhibition activity against potentially drug-resistant HIV, which has not been reported earlier. The study indicates that *Jatropha curcas* Linn. is a good candidate for anti-HIV therapy with further research.
significant anti-inflammatory activity in acute carrageenan-induced paw oedema in albino mice [8]. Four antitumor compounds, including jatropham and jatrophone, are reported from other species of Jatropha [8]. The plant has also been homoeopathically used for cold sweats, colic, collapse, cramps, cyanosis, diarrhoea, leg cramps [7,8]. Some other potential antimicrobial activity of Jatropha curcas Linn. have also been found [9,10].

Although several studies have shown the antimicrobial activity, there are a very limited number of previous reports on the specific anti-HIV activity of Jatropha curcas Linn. One such study showed that Jatropha curcas Linn. had specific in vitro anti-RT enzyme activity and that the water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity [9]. Several unknown active components of plants belonging to the family Euphorbiaceae (Jatropha curcas, J. multifida, Spirostachys africana and Trigonostemma xyphophyllodes) were found to possess anti-HIV activities by inhibiting HIV-1 cell entry [11-13]. Another study that looked at traditional medicines in the management of HIV/AIDS in Tanzania found that Jatropha curcas Linn. leaves were being used for treatment in HIV related conditions such as skin rash and oral candidiasis [14].

With this background, the current study was designed to study drug-resistant HIV-1 in the patient cohort in Mumbai. A small sample size was chosen to evaluate the presence of drug-resistant HIV-1 by phenotypic drug resistance assays. Alternative strategies such extracts from the leaves of medicinal plant Jatropha curcas Linn. were evaluated for the potential antiviral and virucidal and/or entry inhibitory activity of these plant extracts.

Materials and Methods

Study population

A total of 30 HIV-seropositive patients (both on Antiretroviral therapy (ART) and drug naïve) attending the ART centre at AIDS Research and Control Centre (ARCON), Sir J J Hospital Campus, were included in the study. Institutional Ethics Committee approval and written informed consent was acquired prior to blood collection.

HIV isolation

Isolation of HIV from blood samples was done using a PBMC micro-co-culture assay [15]. Briefly, healthy, HIV-seronegative donor PBMCs were stimulated with the mitogen phytohemagglutinin-P (PHA-P; at a final concentration of 5.0 μg/mL; Sigma-Aldrich, St Louis, USA) for 24-72 hours before use to promote blast transformation and that the water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity [9]. Several unknown active components of plants belonging to the family Euphorbiaceae (Jatropha curcas Linn. J. multifida, Spirostachys africana and Trigonostemma xyphophyllodes) were found to possess anti-HIV activities by inhibiting HIV-1 cell entry [11-13]. Another study that looked at traditional medicines in the management of HIV/AIDS in Tanzania found that Jatropha curcas Linn. leaves were being used for treatment in HIV related conditions such as skin rash and oral candidiasis [14].

With this background, the current study was designed to study drug-resistant HIV-1 in the patient cohort in Mumbai. A small sample size was chosen to evaluate the presence of drug-resistant HIV-1 by phenotypic drug resistance assays. Alternative strategies such extracts from the leaves of medicinal plant Jatropha curcas Linn. were evaluated for the potential antiviral and virucidal and/or entry inhibitory activity of these plant extracts.

Materials and Methods

Study population

A total of 30 HIV-seropositive patients (both on Antiretroviral therapy (ART) and drug naïve) attending the ART centre at AIDS Research and Control Centre (ARCON), Sir J J Hospital Campus, were included in the study. Institutional Ethics Committee approval and written informed consent was acquired prior to blood collection.

HIV isolation

Isolation of HIV from blood samples was done using a PBMC micro-co-culture assay [15]. Briefly, healthy, HIV-seronegative donor PBMCs were stimulated with the mitogen phytohemagglutinin-P (PHA-P; at a final concentration of 5.0 μg/mL; Sigma-Aldrich Corporation, Bengaluru, India), in the presence of human interleukin-2 (IL-2; Sigma-Aldrich, St Louis, USA), for 24-72 hours before use to promote blast transformation and that the water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity [9]. Several unknown active components of plants belonging to the family Euphorbiaceae (Jatropha curcas, J. multifida, Spirostachys africana and Trigonostemma xyphophyllodes) were found to possess anti-HIV activities by inhibiting HIV-1 cell entry [11-13]. Another study that looked at traditional medicines in the management of HIV/AIDS in Tanzania found that Jatropha curcas Linn. leaves were being used for treatment in HIV related conditions such as skin rash and oral candidiasis [14].

With this background, the current study was designed to study drug-resistant HIV-1 in the patient cohort in Mumbai. A small sample size was chosen to evaluate the presence of drug-resistant HIV-1 by phenotypic drug resistance assays. Alternative strategies such extracts from the leaves of medicinal plant Jatropha curcas Linn. were evaluated for the potential antiviral and virucidal and/or entry inhibitory activity of these plant extracts.

Materials and Methods

Study population

A total of 30 HIV-seropositive patients (both on Antiretroviral therapy (ART) and drug naïve) attending the ART centre at AIDS Research and Control Centre (ARCON), Sir J J Hospital Campus, were included in the study. Institutional Ethics Committee approval and written informed consent was acquired prior to blood collection.

HIV isolation

Isolation of HIV from blood samples was done using a PBMC micro-co-culture assay [15]. Briefly, healthy, HIV-seronegative donor PBMCs were stimulated with the mitogen phytohemagglutinin-P (PHA-P; at a final concentration of 5.0 μg/mL; Sigma-Aldrich Corporation, Bengaluru, India), in the presence of human interleukin-2 (IL-2; Sigma-Aldrich) for 24-72 hours before use to promote blast transformation and that the water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity [9]. Several unknown active components of plants belonging to the family Euphorbiaceae (Jatropha curcas, J. multifida, Spirostachys africana and Trigonostemma xyphophyllodes) were found to possess anti-HIV activities by inhibiting HIV-1 cell entry [11-13]. Another study that looked at traditional medicines in the management of HIV/AIDS in Tanzania found that Jatropha curcas Linn. leaves were being used for treatment in HIV related conditions such as skin rash and oral candidiasis [14].

With this background, the current study was designed to study drug-resistant HIV-1 in the patient cohort in Mumbai. A small sample size was chosen to evaluate the presence of drug-resistant HIV-1 by phenotypic drug resistance assays. Alternative strategies such extracts from the leaves of medicinal plant Jatropha curcas Linn. were evaluated for the potential antiviral and virucidal and/or entry inhibitory activity of these plant extracts.

Materials and Methods

Study population

A total of 30 HIV-seropositive patients (both on Antiretroviral therapy (ART) and drug naïve) attending the ART centre at AIDS Research and Control Centre (ARCON), Sir J J Hospital Campus, were included in the study. Institutional Ethics Committee approval and written informed consent was acquired prior to blood collection.

HIV isolation

Isolation of HIV from blood samples was done using a PBMC micro-co-culture assay [15]. Briefly, healthy, HIV-seronegative donor PBMCs were stimulated with the mitogen phytohemagglutinin-P (PHA-P; at a final concentration of 5.0 μg/mL; Sigma-Aldrich Corporation, Bengaluru, India), in the presence of human interleukin-2 (IL-2; Sigma-Aldrich) for 24-72 hours before use to promote blast transformation and that the water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity [9]. Several unknown active components of plants belonging to the family Euphorbiaceae (Jatropha curcas, J. multifida, Spirostachys africana and Trigonostemma xyphophyllodes) were found to possess anti-HIV activities by inhibiting HIV-1 cell entry [11-13]. Another study that looked at traditional medicines in the management of HIV/AIDS in Tanzania found that Jatropha curcas Linn. leaves were being used for treatment in HIV related conditions such as skin rash and oral candidiasis [14].

With this background, the current study was designed to study drug-resistant HIV-1 in the patient cohort in Mumbai. A small sample size was chosen to evaluate the presence of drug-resistant HIV-1 by phenotypic drug resistance assays. Alternative strategies such extracts from the leaves of medicinal plant Jatropha curcas Linn. were evaluated for the potential antiviral and virucidal and/or entry inhibitory activity of these plant extracts.
The HIV isolate/s were first allowed to infect PBMCs and the infected cells were plated into wells of 96-well tissue culture plate. Next, washed carefully to remove any uninfected HIV and 100.0 μL of viral isolates afterwards. Briefly, a volume of 100.0 μL of viral isolates were individually added to 1.0 mL of PHA-stimulated PBMCs in a 1.5 mL microcentrifuge tube and incubated for 1 hr in a CO₂ incubator to allow the virus to infect the cells. After incubation, the cells were washed carefully to remove any uninfected HIV and 100.0 μL of infected cells were plated into wells of 96-well tissue culture plate. Next, 100.0 μL of 2X concentrations of different plant extracts were added. The plates were then incubated for 7 days at 37°C in the CO₂ incubator. The contents of each well was transferred to microfuge tubes, cells were pelleted and the supernatants used for determining the p24 antigen levels. The results obtained were then analysed to calculate p24 antigen inhibition.

**Results**

**HIV isolation by PBMC co-cultivation and determination of phenotypic drug resistance**

Thirty samples were processed for Peripheral Blood Mononuclear Cells (PBMC) co-cultivation from which seven HIV isolates were obtained giving an isolation rate of 23.33%. The phenotypic drug resistance was determined by calculating the fold increase in IC₅₀ values of the drugs against the HIV isolates compared with reported IC₅₀ values against a standard strain of HIV (HIV-1IIIb) [19]. The fold increases in IC₅₀ values are given in Table 1.

**Solvent extraction and detection of secondary metabolites**

Solvent extraction using Soxhlet apparatus yielded in 2.2 g of Hexane extract, 1.5 g of Dichloromethane (DCM) extract, 1.3 g of Methanolic extract and 1.8 g of Aqueous extract from leaves of Jatropha curcas Linn. Since the Hexane and DCM extracts were extremely sticky and insoluble in water or RPMI-1640 medium, only Methanolic (ME) and Aqueous extracts (AE) were used for all the further assays.

Secondary metabolites of Jatropha curcas Linn. leaf extracts were detected by HPTLC analysis. The plant extracts showed the presence of Tannins, Flavonoids and Saponins in the ME and Flavonoids and Saponins in the ME (Table 2). Figure 1 shows the HPTLC fingerprint of the plant extracts.

**In vitro cytotoxicity of plant extracts**

The in vitro cytotoxicity was carried out using MTT assay and CC₅₀ calculated. The CC₅₀ values of Jatropha curcas Linn. plant extracts were 35.49 mg/mL for Methanolic and 32.07 mg/mL for Aqueous extract as shown in Figure 2.

**Anti-HIV activity of Jatropha curcas Linn. methanolic and aqueous extracts**

Anti-HIV activity of Methanolic and Aqueous extracts of Jatropha curcas Linn. was studied using the in vitro drug susceptibility assay with an endpoint determination of p24 antigen. Percent inhibition of p24...

Antigen was determined against 4 isolates, and it was observed that the IC50 values of the Methanolic leaf extracts ranged from 0.00073-0.1278 mg/mL (Figure 3; Selective index = 19095.34 ± 17709.64) while those of the Aqueous leaf extracts ranged from 0.0255-0.4137 mg/mL (Figure 4; Selective index = 705.86 ± 438.33) as shown in Table 3. Since this was a preliminary study, the pre-exposure interaction to assess the potential entry inhibitory activity was carried out only against 1 isolate (15C), and percent p24 antigen inhibition was determined. It was seen that the methanolic extract showed 100.0% inhibition while the aqueous extract showed 97.19% inhibition.

Discussion

The need for novel antivirals for managing HIV/AIDS is indisputable. The nature of the virus to evolve faster than the antivirals being made available has made it imperative that newer approaches to combating the virus are considered.

Our laboratory has been actively involved in the research of novel experimental moieties for their potential anti-HIV activity. Previous studies carried out in our laboratory with Mangrove plants such as Ocimum sanctum, Withania somnifera, Tinospora cordifolia, Avicennia officinalis, Rhizophora mucronata and ‘Shilajit’, a herbo-mineral [20] and other medicinal herbs such as Phyllanthus amarus [21] have shown tremendous potential in their anti-HIV activities. The leaf extracts of medicinal plant Jatropha curcas Linn. have not been studied extensively in the past. A preliminary study carried out by our laboratory for assessing the anti-HIV activity of Jatropha curcas Linn. extracts showed promising results [22]; hence, in this study an attempt was made to determine the potential anti-HIV activity using potentially drug-resistant clinical isolates of HIV-1.

<table>
<thead>
<tr>
<th>HIV Isolate</th>
<th>Methanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 (mg/mL)</td>
<td>CC50 (mg/mL)</td>
</tr>
<tr>
<td>HI/15C</td>
<td>0.0007333</td>
<td>35.5</td>
</tr>
<tr>
<td>HI/23C</td>
<td>0.1288</td>
<td>275.62</td>
</tr>
<tr>
<td>HI/35C</td>
<td>0.0001361</td>
<td>26083.76</td>
</tr>
</tbody>
</table>

Table 3: Selectivity indices of Jatropha curcas Linn. Extracts.
The plant extracts showed effective antiviral activity as compared to standard drug AZT, 3TC and d4T (Figure 5) in HIV p24 antigen inhibition assays. The aqueous leaf extracts showed IC50 values ranging from 0.0255-0.4137 mg/mL and the methanolic leaf extracts showed IC50 values ranging from 0.00073-0.1278 mg/mL; while in the pre-infection studies, the aqueous leaf extracts showed 97.19% p24 inhibition and the methanolic leaf extract showed 100.0% p24 inhibition at 25 mg/mL each indicating potential denaturation of virus (virucidal activity) or entry inhibitory activity.

These findings are quite significant since the extracts seem to work in both pre- and post-infection regimes and hence suggest that the effect may be mediated by a direct action on the virus particle. Perhaps it blocks binding or uptake to cells or causes immediate particle lysis. While we have planned experiments to elucidate a preliminary mode of action to evaluate the future potential of the extracts, it would be unreasonable to expect a precise mechanism at this stage.

Previous studies using Jatropha curcas Linn. stem bark extracts have shown inhibition of HIV-induced cytopathic effects [9,23]. Recently, the anti-HIV effect of 12-deoxyphorbol-13-phenylacetate, a compound synthesised from Jatropha phorbal esters, has been demonstrated; it inhibits HIV entry into target cells [24,25]. The anti-HIV activity of Jatropha curcas Linn. may be attributed to the secondary metabolites present in the leaf extracts. The aqueous leaf extract showed the presence of Tannins, Flavonoids, and Saponins as secondary metabolites present in the leaf extracts. The aqueous leaf extracts were equally effective against isolates that were resistant to standard anti-retroviral drugs AZT, 3TC and d4T.

Conclusion
To conclude, the study has evaluated that Jatropha curcas Linn. has prospective antiviral activity against potentially drug-resistant HIV-1 and that these experimental moieties have favourable implications on the prevention or management of HIV/AIDS. The plant extracts may be used in the formulation of microbicides or surface disinfectants. It can be therefore deduced from that study that Jatropha curcas Linn. is a good candidate for anti-HIV therapy with further research.

Acknowledgements
The authors would like to thank the staff of ARCON, Mumbai for their assistance in collection of blood samples.

Author Disclosure Statement
The authors declare that there is no conflict of interests regarding the publication of this article.

References


