ABSTRACT

Objective: Anti-HIV activity studies of methanolic extracts of *Adansonia digitata* L. leaves, root bark and fruit pulp.

Methods: Methanolic extracts of *A. digitata* were tested for HIV enzyme inhibitory activity against HIV-1 reverse transcriptase (RT) and HIV-1 protease (PR). HIV-1 RT assay was performed using non-radioactive HIV-RT colorimetric ELISA kit while the HIV-1 PR assay was performed using a fluorogenic octapeptide substrate, HIV-FRET (fluorescence resonance energy transfer) and a recombinant HIV-1 protease solution (AnaSpec Inc., USA).

Results: The percentage inhibition of controls and *A. digitata* leaves, root bark and fruit pulp extracts were calculated relative to uninhibited HIV-1 RT and PR in 2% DMSO. The results of the HIV-1 RT assay indicated 26.5% inhibition by root bark extract (50 µg/ml) and 12.2% inhibition by leaf extract while the fruit pulp extract (100 µg/ml) has shown only 5.9% inhibition as compared to standard Nevirapine (100 nM) 27.5% inhibition.

In the case of HIV-1 protease assay, leaf extract and fruit pulp extract (50 µg/ml) has shown quite high inhibition (≥ 50%) to the extent of 75% and 74% respectively which is very significant. The root bark extract has shown 35% inhibition.

Conclusion: Methanolic extract of the leaves, root bark and fruit pulp of *A. digitata* has shown low anti-HIV-1 RT but significant PR activity. HIV-1 RT activity of root bark extract and HIV-1 PR activity of fruit pulp extract indicates the potential of the plant as an anti-HIV agent.

Keywords: HIV, *Adansonia digitata* L, Reverse transcriptase, Protease

INTRODUCTION

India, with a population of over one billion, is experiencing a rapid and extensive spread of HIV. According to the 2012 report, the estimated number of people living with HIV/AIDS in India was 2.089 million in 2011. The adult (15-49 age-group) HIV prevalence at national level has continued its steady decline from estimated level of 0.41% in 2001 to 0.27% in 2011. But still, India is estimated to have the third-higher number of people living with HIV/AIDS, after South Africa and Nigeria [1].

There are two related but distinct types of HIV: HIV-1 and HIV-2 [2]. HIV-1 is the most pathogenic and causes over 99% of HIV infections [3]. Various studies carried out in different parts of India highlights the fact that HIV-1 subtype C is the most prevalent subtype in India [4]. India initiated HIV-prevention activities in the very early stages of the epidemic and committed to prevention efforts. The National AIDS Control Organization [5] was constituted in 1986 and the National AIDS Control Program launched a year later. To enhance RandD in this area, NACO identifies and promotes Indian academic institutions that conduct epidemiological and operational research for the treatment of HIV/AIDS, including research on indigenous systems of medicine such as Ayurveda and Siddha. Natural products provide a large reservoir for the screening of anti-HIV agents with novel structure and antiviral mechanism because of their structural diversity. A variety of natural products has been found to inhibit unique enzymes and proteins crucial to the life cycle of HIV. The most efficient interventions are at the stages of the reverse transcription process, virus entry, integration of viral DNA into the host genome and protease inhibition [6, 7]. However mechanism of anti-HIV activities of many more natural products is unknown. In India, traditional medicine is used to meet the primary health care need and to treat AIDS patients [8, 9]. Screening of plants worldwide based on ethnopharmacological data to isolate the active constituents from plants for preventing transmission of HIV/AIDS patients increases the potential of finding novel phytoconstituents present in them [10-13].

Many compounds with anti-HIV effect have been screened out from natural products and discovered to inhibit HIV at nearly all stages of viral life cycle [14]. They include alkaloids, sulphated polysaccharides, polyphenolics, flavonoids, coumarins, phenolics, tannins, triterpenes, lectins, phloroglucinol, lactones, iridoids, depsidones, O-cafeoyl derivatives, lignans, and ribosome inactivating proteins, saponins, xanthones, naphthodianthrones, photosensitizers, phospholipids, quinines and peptides [15-18].

*Adansonia digitata* L. family, Bombacaceae, is known by various names such as Baobab and Lemonade tree. It is endemic to Africa. In India, it grows naturally in Mandu region of Madhya Pradesh. It is very common in Andhra Pradesh and also cultivated in Uttar Pradesh, Bihar, Tamil Nadu and Maharashatra [19]. It is one of the largest and reportedly longest living species (6000 y) of the world [20, 21].

A variety of phytochemicals constituents such as terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates, and lipids [21] have been reported from *A. digitata*. The different plant parts are widely used as food, medicine, clothing and shelter. In folk medicine it is used as antipyretic, febrifuge, astringent in diarrhoea and dysentery, also as a substitute for cinchona in various systems of medicine [22]. The pith of the fruit contains high levels of ascorbic acid (vitamin C), tartaric acid, and citric acid and is used in producing a refreshing drink. Seeds are eaten fresh or dried. They can also be ground into a powder and used as a substitute for coffee. The leaves are said to be rich in vitamin C, sugars, potassium tartrate, and calcium. The leaves are freshly cooked as a vegetable or dried and crushed for later use by local people [23, 24]. The leaves are used medicinally against fever by reducing sweating and as an astringent by tightening mucous membranes thus reducing mucous secretions. In West Africa, the leaves and bark are used for treating urinary disorders and diarrhoea. Young roots are cooked and eaten [24-26]. Antibacterial and anti-Trypanosome activities have also been indicated by the plant extracts [27, 28]. Toxicological and pharmacological studies such as anti-inflammatory, analgesic, antipyretic, antibacterial and antiviral activities of the various plant
parts of A. digitata have also been reported [29]. Root bark and leaf extract of A. digitata have shown significant antiviral activity against Herpes simplex, Sindbis and Poliovirus [30]. This has led to the current study to investigate the potential of various plant parts of A. digitata to inhibit HIV-1 enzymes.

MATERIALS AND METHODS

Plant collection and authentication

Fresh leaves, fruits and root bark of A. digitata L. were collected from Mandu, Madhya Pradesh, India, in the month of September 2010. The collected plant was authenticated by Dr. Pramod Patil, Professor, Department of Botany, M. L. B. Girls Post Graduate College, Bhopal, India. Voucher specimen 00919 has been deposited in their laboratory for future reference.

Extraction

The plant material was washed well with water to separate the adhering soil material and dried in the shade. Dried leaves, fruit pulp and bark were comminuted to form a coarse powder. Dried powdered leaves, fruit pulp and bark (500 g each) were extracted with petroleum ether (60-80 °) for 24 h to remove fatty substances. Defatted dried marc was further extracted with methanol. All the extracts obtained were evaporated to dryness under vacuum in a rotary evaporator at 40 °C. All the extracts were subjected to preliminary phytochemical screening to detect various plant constituents.

Analytical studies

Fifty milligrams of the residue extract was dissolved in 5 ml methanol in a volumetric flask, filtered and made up to the volume up to 5 ml for each sample.

HPTLC was carried out on silica gel 60 F254 precoated aluminium plates 0.2 mm thickness, Merck India Limited Mumbai. An Applicator from Camag Linomat-5 (Camag Switzerland: 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used for leaf and fruit pulp extract was toluene: ethyl acetate: diethylamine (7:3:0.5), whereas for root bark extract it was toluene: ethyl acetate (9:1). The HPTLC plates were developed over a distance of 9 cm in a saturated development chamber (Twin through chamber 10X10 cm with SS lid) and visualized under 254 nm, visible light. After derivatization, the plates were again visualized at 254 nm, 366 nm and under visible light. After development, plates were sprayed with 5% methanolic sulphuric acid followed by heating at 105 °C for 5-10 min.

HIV-Reverse transcriptase assay

The effect of the methanolic extracts of the leaves, root bark and fruit pulp of A. digitata was tested by using non-radioactive HIV-RT colorimetric ELISA kit (Roche Diagnostics, Germany). The protocol outlined in the kit was followed, under nuclease free conditions by using 2 ng of the enzyme in each well and incubating the reaction for 2 h at 37 °C. The negative controls for the assay included HIV-1 RT with only lysis buffer, HIV-1 RT with only the reaction for 2 h at 37 °C. The negative controls for the assay conditions by using 2 ng of the enzyme in each well and incubating protocol outlined in the kit was followed, under nuclease free RT colorimetric ELISA kit (Roche Diagnostics, Germany). The microplates were then shaken to 300 rpm for 1 min. and the fluorescence intensity was measured kinetically every 30 sec over a period of 100 min. at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, at 37 °C, using a Fluoroskan Ascent FL microplate reader (Thermolab systems). The reaction rates were determined by the gradient of the initial linear portions (first 5-10 min) of the plot of Relative fluorescence intensity (RFI) as a function of time. Negative controls were HIV-1 PR with only assay buffer, HIV-1 PR enzyme with DMSO (2%) in assay buffer and substrate alone. Positive controls were taken as ritonavir and pepstatin at a final concentration of 0.2 µM (Bachem, Switzerland). The percentage inhibition of HIV-1 PR was calculated as a percentage of control with only solvent (2% DMSO).

RESULTS

Extractive values and preliminary phytochemical screening

The leaves (ASLE), root bark (ASBE), fruit pulp (FPE) of A. digitata afforded methanolic extract in the percent yield of 3%, 2.3% and 1.4% respectively. Preliminary phytochemical screening of all the extracts revealed the presence of flavonoids, steroids, tannins, glycosides and amino acids.

High performance thin layer chromatography

High performance thin layer chromatographic study of the methanolic extract of leaf (ASLE) in the solvent system, toluene: ethyl acetate: diethylamine (7:3:0.5) revealed five spots with Rf values 0.10 (yellow), 0.64 (yellowish green), 0.82 (black), 0.84 (green) and 0.90 (green) when observed under visible light while the fruit pulp extract (FPE) in the same solvent system showed four spots with Rf value 0.34 (light blue), 0.64 (faint) 0.71 (light blue), 0.84 (blue) when observed at 366 nm. Methanolic extract of the bark (ASBE) in the solvent system, toluene: ethyl acetate (9:1) revealed one major and seven minor spots with RF values 0.12 (white), 0.16 (red), 0.26 (sky blue), 0.29 (red), 0.37 (blue), 0.50 (red), 0.60 (blue), 0.66 (red) when observed at 366 nm as reported by Sharma and Rangari [32].

HIV-Reverse transcriptase assay

The percentage inhibition of controls and A. digitata leaves, root bark and fruit pulp extracts were calculated relative to uninhibited HIV-1 RT in 2% DMSO (fig. 1). The results of the HIV-1 RT assay indicated 26.5% inhibition by root bark extract (50 µg/ml) and 12.2% inhibition by leaf extract (50 µg/ml) while the fruit pulp extract (100 µg/ml) has shown only 5.9 % inhibition as compared to standard nevirapine (100 nM) 27.5 % inhibition.

HIV-protease assay

The percentage inhibition of controls and A. digitata leaves, root bark and fruit pulp extracts were calculated relative to uninhibited HIV-1 PR in 2% DMSO (fig. 2). The leaf and fruit pulp extract (50 µg/ml) have shown quite high inhibition (≥ 50%) to the extent of 75% and 74% respectively which is very significant. Since BSA was already present in the protease assay buffer at 0.1%, tannin adsorption would already have taken place.
The results obtained from this experimental work indicated that the flavonoids, proanthocyanins and catechins present in the root bark, leaves and fruit pulp extracts of A. digitata have very strong potential to inhibit HIV-1 RT and PR enzymes. Further phytochemical and anti-HIV activity studies may provide important leads from A. digitata for the development of anti-HIV agent.

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CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. UNAIDS India. Report on the Global AIDS epidemic 2010. Available from: http://www.unaids.org. [Last accessed on 20 Mar 2016].
2. Fletcher CV, Kakuda TN, Collier AC, Talbert RL, Yee GC, Matze GR, et al. Pharmacotherapy-a pathophysiologic approach. 5th edition. Mcgraw-Hill: 2002. Medical Publishing Division, United States of America; 2002. p. 2151-74.
3. Cos P, Maes L, Vanden Berghe D, Hermans N, Pieters L, Vletinck A. Plant substances as anti-HIV agents selected according to their putative mechanism of action. J Nat Prod 2004;67:284-93.
4. Mandal D, Jana S, Bhattacharya SK, Chakrabarti S. HIV type 1 subtypes circulating in eastern and northeastern regions of India. AIDS Res Hum Retroviruses 2002;18:1219-27.
5. National AIDS Control Organization (NACO). National AIDS Control Organisation 2006. Available from http://www.nacoonline.org. [Last accessed on 20 Mar 2016].
6. De Clercq E. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med Res Rev 2000;20:323-49.
7. Cos P, Vanden Berghe D, Bruyne TD, Vletinck A. Plant substances as anti-viral agents: an update (1997-2001). Curr Org Chem 2004;7:1163-80.
8. Harnett SM, Oosthuizen V, van de Venter M. Anti-HIV activities of organic and aqueous extracts of Sutherlandia frutescens and Lobostemon trigonus. J Ethnopharmacol 2005;96:113-9.
9. Zhang K, Wang J, Jiang Y, Xu LZ. Utilization of traditional Chinese medicines in the treatment of 200 people living with HIV/AIDS, China. AIDS STD 2005;11:94-6.
10. Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecoque C, De Clercq E. Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnobotanically selected ethiopian medicinal plants. Phytother Res 2001;15:562-9.
11. Vermanni K, Gorg S. Herbal medicines for sexually transmitted diseases and AIDS. J Ethnopharmacol 2002;80:49-66.
12. Farnsworth NR. Ethnopharmacology and drug development. Ethnobotany, drug development and biodiversity conservation-exploring the linkages. In: Ethnobotany and the Search for New Drugs; Ciba Foundation Symposium. John Wiley and Sons, Chichester; 1995;185:42-59.
13. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001;109:69-75.
14. Wang JH, Tam SC, Huang H, Ouyang DY, Wang YY, Zheng YT. Site-directed PEGylation of trichosanthin retained its anti-HIV activity with reduces potency in vitro. Biochem Biophys Res Commun 2004;317:965-71.
15. Ng TB, Huang B, Fong WP, Yeung HW. Anti-human immunodeficiency virus (anti-HIV) natural products with special emphasis on HIV reverse transcriptase inhibitors. Life Sci 1997;61:933-49.
16. Vletinck AJ, De Bruyne T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. Planta Med 1998;64:97-109.
17. Yang SS, Gragg GM, Newman DJ, Bader JP. Natural product based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics program. J Nat Prod 2001;64:265-77.
18. Rangari VD, Dumbre RK, Dumbre MR. HIV-AIDS and bioactive natural products. Studium Press Llc, Houston, Texas, USA; 2009.
19. Nadkarni KM, Nadkarni AK. Indian Materia Medica- 2. 3rd ed. Popular Prakash, Bombay; 2000. p. 37-9.
20. Ramadan A, Harraz FM, El-Mougy SA. Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of Adansonia digitata. Fitoterapia 1994;LXV:418–22.
21. Shakla YN, Dubey S, Jain SP, Kumar S. Chemistry, biology and uses of Adansonia digitata-a review. J Med Aromat Plant Sci 2001;23:429-34.
22. Ramesh D, Dennis TJ, Shingare MS. Constituents of Adansonia digitata root bark. Fitoterapia 1992;63:278-9.
23. Van wyk BE, Gericke N. People’s Plants: a guide to useful plants of Southern Africa. Pretoria, Briza Publication; 2000. p. 351.
24. Esterhuysen N, Von Breitenbach J, Suhnge H. Remarkable trees of South Africa. Pretoria, Briza Publication; 2001.
25. Wickens GE. The Baobab-Africa’s upside-down tree. Kew Bull 1982;37:173-209.
26. Hudson JB, Anani K, Lee MX, De Souza C, Arnason JT, Gbeassor M. Further investigations on the antiviral activities of medicinal plants of Togo. Pharm Biol 2000;38:46-50.
27. Atawodi SE, Bulus T, Ibrahim S, Amed DA, Nok AI, Mamman M, Galadima M. In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. Afr J Biotech 2003;2:317-21.
28. Kamatou GPP, Vermaak I, Wiljoen AM. An updated review of Adansonia digitata: a commercially important African tree. S Afr J Bot 2011;77:908-19.
29. Matayoshi ED, Wang GT, Kraft GA, Erickson J. Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer. Science 1990;247:954-8.
30. Sharma A, Rangari VD. Antibacterial, the antifungal and antitubercular activity of methanolic extracts of Adansonia digitata. Int J Pharm Biol Sci 2015;10:52-60.
31. Chauhan JS, Chaturvedi R, Kumar S. A new Flavonol glycoside from the roots of Adansonia digitata. Planta Med 1984;50:113.
32. Chauhan JS, Kumar S, Chaturvedi R. A new flavonone glycoside from the roots of Adansonia digitata. Natl Acad Sci Lett 1987;10:177-9.
33. Yasmina A Lim, Ma Chao Mei, Ines T Kusumoto, Miyashiro H, Hattori M. HIV-1 Reverse transcriptase inhibitory principles from Chamaesyce hyssopifolia. J Phytother Res 1997;11:227.
34. Kannan M, Rajendran P, Vedha V, Ashok G, Anushka S, Chandran, et al. HIV-1 reverse transcriptase inhibition by Vitex negundo L. leaf extract and quantification of flavonoids in relation to the anti-HIV activity. J Cell Mol Biol 2012;10:53-9.
35. Shabat AA. Procyanidins from Adansonia digitata. Pharm Biol 2006;44:445-50.
36. Chaomei MA, Nakamura N, Miyashiro H, Hattori M, Shimotohno K. Inhibitory effect of constituents from Cynomorium songaricum and related triterpene derivatives on HIV-1 Protease. Chem Pharm Bull 1999;47:141-5.
37. Vlietinck AJ, Bruyne De T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. Planta Med 1998;64:97-109.

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