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In-Vitro Screening of Reverse Transcriptase Activityof Selected Indian Medicinal Plants against Human Immunodeficiency Virus Type-1

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Abstract

Human Immunodeficiency Virus type-1 (HIV-1) is that the explanation for Acquired Immune Deficiency Syndrome (AIDS), a serious human viral disease.The high cost of the HAART regimen has impeded its delivery to over 90% of the HIV/AIDS population within the world. The aim of the present study was to evaluate the in vitro anti-HIV activity of selected Indian medicinal plant extracts.Extracts were prepared from dried plant Vitex negundo, Evolvulusalsinoides and Datura metel leaf with Hexane, Methanol, Ethanol, Chloroform, and Petroleum ether. The phytochemical activities and HIV-1 RT inhibition activity (HIV-1 RT colorimetric ELISA kit –Roche Kit method) was carried with the all solvent extracts of Vitex negundo, Evolvulusalsinoides and Datura metelwas determined. The methanolic extracts of Vitex negundo have given the maximum and positive result with 74.76±1.36, which proves that Vitex negundo has a potential antiviral activity against HIV. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal also as physiological activities.The predominant components present altogether the extracts of Vitex negundo are flavonoids, Terpenoids and steroids. Thus this study seems to justify the normal use of plant for the treatment of communicable disease of viral origin.

Keywords: HIV, Vitex negundo, Evolvulusalsinoidesand Datura metelHIV-1 RT, Phytochemical analysis

INTRODUCTION

Human Immunodeficiency Virus (HIV) is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS) that has created a major health care problem in globally[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[3,4].The transmission of Human immunodeficiency virus type -1(HIV-1) infection is unceasing throughout the world with an estimated 35.3 million people are currently living with this virus[5].The current strategy for the

treatment of HIV infection is Highly Active Antiretroviral Therapy (HAART), which is based on combination of inhibitors of reverse transcriptase and protease[1].

A number of anti-HIV drugs used in conventional AIDS therapy are available in the market, unfortunately the administration of these compounds clinically to the AIDS patients exhibited serious side effects[6]. As a result of these difficulties, traditional medicines remain the primary source of medical care to various healthcare needs[7].Traditional medicine has served as a source of alternative medicine, new pharmaceuticals and healthcare products[1]. Natural products are still being explored as potential antiviral agents and more importantly as inhibitors of the various steps of the HIV life cycle[8,9]. The enzymes and the proteins involved in the life cycle of HIV have been inhibited by a variety of natural products. 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[10,11].

Herbal medicine is now globally accepted as a legal, alternative system of therapy for treatment and cure of various diseases and physiological conditions in traditional treatments in the form of pharmaceuticals.The antiviral activity of phytochemicals is attributed to the different mechanisms that plants utilise when fending off plant viral attacks[12] .Currently, there are about 30 anti HIV compounds approved by US FDA for clinical use. This includes the highly active antiretroviral therapy (HAART). HAART is the effective method of choice to treat HIV/AIDS patients[13]**.** HIV that makes HIV/AIDS more of social, emotional problem than mere economical burden1.Screening of potential anti-HIV agents from medicinal plants may be a rapid and effective way for drug discovery.

MATERIAL AND METHODS

Plant collection

Fresh leaves of *Vitex negundo, Evolvulusalsinoides*and *Datura metel*were collected from Kolli Hills, Tamilnadu. The leaves were washed several times with water to remove the dust particles, air dried at room temperature and powder in grinder mixer. The plant authenticated by Botanical Survey of India (BSI-Southern Circle) - Government of India, Coimbatore, Tamil Nadu.

Preparation of extracts

The plant samples *Vitex negundo, Evolvulusalsinoides*and *Datura metel*In fine-grained type were extracted in Hexane, Methanol, Ethanol, Chloroform, and Petroleum ether by soxhlet apparatus and keep at 4°C till they were processed for biological analysis. At 40ºC under pressure the solvent from the extract was removed.The solid was utilized in antiviral assay when dissolving in DiMethyl-SulphOxide (DMSO) taken under consideration that the utmost concentrations of DMSO within the check answer shouldn't exceed common fraction prescribed by Anbalagan*et al.*[14].

Phytochemical screening of active compound

By using the standard method Qualitative phytochemical properties was analyzed to detect the presence of carbohydrates, alkaloids, tannins, flavonoids, proteins, aminoacids,

glycosides, saponinis, phytosterols, phenols and diterpens. The intensity of the coloration determines the abundance of the compound present[15,16].

HIV-1 Reverse Transcriptase Inhibition Assay

HIV-1 Reverse transcriptase activity

The activity of plant extract on RT activity was determined with recombinant HIV-1 enzyme using a non-radioactive HIV-1 RT colorimetric ELISA kit (Roche)[17-19]. A final concentration of 3 mg/mL stock solution was prepared for all plant materials by weighing 3 mg and dissolved in 1 mL DMSO. Ten microliters (10 µL) of stock solution was added to 90 µL of lysis buffer making a final concentration of 0.3 mg/mL. The enzyme was prepared to a stock solution of 0.764 mg/mL and 0.327 µL was added to 1000 µL lysis buffer. In appropriate wells of the microtitre plates, 20 µL of enzyme, 20 µL diluted extract and 20 µL reaction mixture were added together. For positive control; doxorubicin at 100 µg/mL was used; (1) lysis buffer was added with DMSO and (2) lysis buffer was added with no DMSO. Lysis buffer and reaction mixture act as negative control. The plates were incubated for one hour at 37 °C. The microtitre plates were washed five times with 250 µL of the washing buffer. Two hundred microlitres of Anti-Dig-POD working solution was added in each well. The plates were kept for incubation at 37 °C for one hour. The microtitre plates were washed five times with 250 µL washing buffer. The plates were allowed to stand at room temperature for 10 min after adding 250 µL of ABTS substrate solution. The absorbance was read at 405 nm undermicrotitre plate reader. The mean of the duplicate absorbance was analysed using the formula: % Inhibition ={1−(OD SampleOD negative control)}×100.

RESULT AND DISCUSSION

The Reverse Transcriptase Assay Kit method has been used to detect the anti-viral activity of *Vitex negundo*, *Datura metel* and *Evolvulusalsinoides* successfully. The methanol extract of *Vitex negundo* has given a value of 74.76±1.36, 71.96±2.24 by*Datura metel* and 47.09±0.76 by *Evolvulusalsinoides*. The Ethanolic extract of *Vitex negundo* has given the value of 48.36±0.76, 36.79±0.12 by *Datura metel* and 24.08±0.56 by *Evolvulusalsinoides.* The chloroform extracts of *Vitex negundo* showed 38.76±0.19, 29.43±1.42 by *Datura metel* and 20.10±10.12 by *Evolvulusalsinoides*. The petroleum ether extract of *Vitex negundo* gave the value of 41.12±1.45, 23.13±0.18 by *Datura metel* and 18.72±0.36 respectively (Table.1).

Table-1. Inhibition of HIV-RT by plant extracts-reverse transcriptase inhibition (%)

Name of the plant	Methanol extract	Ethanol extract	Chloroform extract	Petroleum ether
Vitex negundo	74.76± 1.36	48.36 ±0.76	38.76±0.19	41.12±1.45
Datura metal	71.96±2.24	36.79±0.12	29.43±1.42	23.13±0.18
Evolvulusalsinoides	47.07±0.76	24.08±0.56	20.10±0.12	18.72±0.36

Note: Inhibition _50% is considered as significant.

The methanolic extracts of *Vitex negundo* have given the maximum and positive result with 74.76±1.36, which proves that *Vitex negundo* has a potential antiviral activity against HIV.The vast reservoir to screen anti-HIV agents was natural products that show

structural diversity with novel structure and antiviral mechanism. Those natural products can inhibit the products and substrates necessary for the maintenance of the growth 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 inhibition[4,10].

The various compounds extracted from the natural products exhibit anti-HIV-1 effect that inhibit HIV at nearly all stages of viral life cycle 20.They include alkaloids, sulphated polysaccharides, saponins, polyphenolics, flavonoids, coumarins, phenolics, tannins, triterpenes, ribosome inactivating proteins, phloroglucinols, lactones, iridoids, O-caffeoyl derivatives, lignans, xanthones, photosensitisers, phospholipids, quinines and peptides [21-24].Antiviral research has been fascinated towards the compounds that interfere in all most all the stages of the viral life-cycle. In Egyptian folk medicine 41 medicinal plant extracts have been screened for their HIV-1 RT inhibitory effects; putranjivain A was identified from *Phyllanthus emblica*Linn as a strong inhibitory substance against HIV-RT[25,26].Tannins have the potential to inhibit HIV-RT. Tannins may interfere in HIV cell interactions or other mechanisms that can inhibit HIV replication in cell culture. From *Castanopsishystrix*tannins likeGalloylquinates and Galloylshikimates (tannins) was isolated. DC, and Caffeoylquinates (tannin) isolated from *Lonicera japonica* Thunb showed HIV-RT inhibition activity[27].Most of the plant species under study had significant anti-viral activity and therefore were all tested for activity against the HIV-1 virus and inhibition of the RT enzyme, which is vital in the lifecycle of HIV and similar study conducted by Moll *et al.*[28].

Methanol extracts of *A. karoo* shows greater activity against the HIV-1 RT, and with similar polarity to the 70% ethanol used [28].Phytochemical analysis of *Vitex negundo*was carried out with solvents Methanol, Ethanol, Petroleum ether and Chloroform. Methanol extractof *Vitex negundo* contains CHO, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids. Ethanol extract of *Vitex negundo*containsalkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids except CHO. Chloroform extract of *Vitex negundo*hasCHO, Alkaloids, flavonoids, terpenoids, glycosides, and steroids; and absence of Tannin and phenolic compound. Petroleum ether extract of *Vitex negundo*hasSteroids, flavonoids, tannin and Sugars.The major compound present in all the above extracts of *Vitex negundo* are flavonoids, Terpenoids and steroids and the results were provided in Table-2.

Table-2. Preliminary Phytochemical screening of various extracts of the leaves of *Vitex negundo*

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	+	+	-	+
Flavonoids	+	+	+	+
Tannin	+	+	+	-
Carbohydrate	-	+	-	+
Terpenoids	+	+	+	+
Glycosides	+	+	-	+
Steroids	+	+	+	+
Phenols	+	+	-	-
Fixed oil	-	-	-	-

Anthroquninone	-	-	-	+
catachol	-	-	-	-

Note: + Present; – Absent

Chitra *et al*.[29] reported that the preliminary phytochemical analysis carried out on the crude ethanol extract indicated the presence of alkaloids, glycosides, lignin, flavonoids and saponins. In *Vitex negundo* chemical constituents like flavonoids, flavone glycosides, volatile oil, triterpenes, tannins and lignin many others were identifiedby Gautam *et al.,* 1999) [30].Phytochemical analysis of *Evolvulusalisnoides* was carried out by the following solvents Methanol, Ethanol, Petroleum ether and Chloroform. In Methanol extractof *Evolvulusalisnoides*contain the components, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. Whereas the Ethanol extract of *Evolvulusalisnoides*contain the constituents, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. In Chloroform extract of *Evolvulusalisnoides*contain Tannin and phenolic compounds. Petroleum ether extract of *Evolvulusalisnoides*contains flavonoids, and Sugars are present. The predominant components present in all the extracts of *Evolvulusalisnoides*are Tannins, Phenols and Flavonoids (Table-3).

Table-3. Preliminary Phytochemical screening of various extracts of the leaves of *Evolvulusalsinoides*

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	+	+	-	-
Flavonoids	+	+	+	-
Tannin	+	+	-	+
Carbohydrate	-	-	+	-
Terpenoids	+	+	-	-
Glycosides	+	+	-	-
Steroids	+	+	-	-
Phenols	+	+	-	+
Fixed oil	-	-	-	-
Anthroquninone	-	-	-	-
Catachol	-	-	-	-

Note: + Present; – Absent

The primary phytochemical screening of *Evolvulusalsinoides*results in the presence of some secondary metabolites like alkaloids, flavonoids, saponins, volatile oil, glycosides, and tannins.In general secondary metabolites present in plants have been reported by Rabe[31], to be responsible for therapeutic activity. Singh & Bhat[32]reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants

CONCLUSION

Thus there's a requirement for the invention of novel therapeutic strategies. One of the strategies has been to spot anti-HIV compounds from natural sources, particularly from plants. Traditional medicine has served as a source of other medicine, new pharmaceuticals

and healthcare products. Plant derived natural products act as an outsized reservoir for the screening of anti-HIV agents. Currently, there are about 30 anti HIV compounds approved by US FDA for clinical use. Many compounds with anti-HIV-1 effect are screened out from natural products and discovered to inhibit HIV at nearly all stages of viral life cycle. Experimental results thus suggested that most of the plant extracts which have been tested in the present study exert their anti-HIV activity and inhibition of viral RT. Thus this study does seem to justify the normal use of plants for the treatment of infectious diseases of viral origin. Proper isolation of the specific compound from the crude extract and the analysis of their mechanism of action will help in the discoveryof anti-HIV drugs.

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