



Original Research Article

***In vitro* screening of anti HBV and anti HIV properties of *Gymnema sylvestre* R.Br leaves from Kolli Hills, Tamilnadu, India**

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ABSTRACT

Keywords

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This study is aimed to determine the activity of ethanolic and methanolic leaf extract of *Gymnema sylvestre* R. Br against HIV-1 Reverse Transcriptase, HBV DNA polymerase and HBsAg. The effect of ethanolic and methanolic leaf extract of *Gymnema sylvestre* R. Br was evaluated using a non radioactive HIV-RT colorimetric kit. The HBsAg binding activity was evaluated by ELISA kit and HBV DNA polymerase activity was studied using Radiometric Kit. Inhibition of 50% (IC₅₀) was taken as positive activity. Methanolic extract was found to possess potent *in vitro* HBsAg binding, HBV DNA polymerase inhibition and HIV-1 RT inhibition activity whereas ethanolic extract is positive for HIV-1 RT inhibition

Introduction

Hepatitis B virus poses a silent epidemic challenge to India. With 3.7% prevalence, over 40 million HBV carriers, India is considered to have an intermediate level of Hepatitis B virus endemicity. Every year one million Indians are at risk for Hepatitis B virus and about 100,000 die from HBV infection (NCDC, 2014). Hepatitis B shows variable clinical manifestation ranging from asymptomatic HBV carrier to fulminant liver failure and it becomes chronic, often progress to chronic hepatic cirrhosis and hepatocellular carcinoma (Wright and Lau, 1993).

Acquired Immunodeficiency Syndrome (AIDS) caused by Human Immunodeficiency Virus (HIV) results in life threatening opportunistic infections and malignancies. With an estimate of 5.7 million HIV infections in India (Steinbrook, 2007), HIV leads to functional impairment of immune system, and destroys the ability of the host to fight infections. Co infection of HIV and HBV is a major emerging problem and treatment requires strategies to combat both (Kourtis *et al.*, 2012). Mutations and emerging resistance of HBV and HIV to current therapeutics and the

expense incurred for the treatment lead to the development of complementary treatment options (Michailidis *et al.*, 2012), (Tang and Shafer, 2012).

Medicinal plants possess innumerable bioactive principles attacking multiple targets, minor side effects, low potentials to cause resistance, low costs. Plant resources are available for exploring the herbs showing antiviral activity as ayurvedha and siddha medicine systems is been in practice in India from time unknown. Hence this study is aimed at exploring the potentials of *Gymnema sylvestre* R.Br possessing anti HBV and HIV activity.

Gymnema sylvestre R. Br. is native to central and western and southern India, tropical Africa and Australia. In Tamil it is called as sirukurinja and is popularly called as gurmar due to its antisaccharine properties. *Gymnema sylvestre* R.Br. belongs to the family Asclepiadaceae, It is a slow growing, perennial, medicinal woody climber. Leaves are opposite, usually elliptic or ovate (1.25–2.0 inch. 0.5–1.25 inch). Flowers are small, yellow, in umbellate cymes. Follicles are terete, lanceolate up to 3 inches in length. It is regarded as one of the plants with potent anti-diabetic properties and being used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammations, family planning and snakebite. In addition, it possesses antimicrobial, anti hypercholesterolemic and hepatoprotective activity. Antiviral inhibition i.e. viral DNA synthesis is reported by Jassim *et al.* (2003).

Gymnema sylvestre R.Br. leaves contain triterpene saponins belonging to oleanane and dammarene classes. The major constituents like gymnemic acids and gymnemasides are dammarane saponins

(Khramov *et al.*, 2008). Besides this, other plant constituents are flavones, anthraquinones, hentri-acontane, pentatriacontane, α and β -chlorophylls, phytin, resins, quercitol, tartaric acid, formic acid, butyric acid, lupeol, β -amyrin related glycosides and stigmasterol.

Materials and Methods

Plant material

The leaves of *Gymnema sylvestre* R.Br. was collected from Kolli hills adjoining downstream areas of Namakkal district of Tamilnadu, India and authenticated (PARC/2011/943) by Dr. Jayaraman, Plant Anatomy Research Centre, National Institute of Herbal Science, Chennai, India. The plant samples were washed, shade dried, powdered and extracted in 95% ethanol, methanol and filtered. The extracts were then concentrated to dryness under reduced pressure and the residue was freshly dissolved in appropriate buffer on each day of experiment for the antiviral assays. The extract that could not dissolve in the buffer was dissolved in DMSO taking into account that the maximum concentration of DMSO in the test solution should not exceed 1 percent.

Antiviral assays

In vitro HbsAg binding study (Thyagarajan *et al.*, 1988)

Equal volume of HBsAg positive plasma and the methanol and ethanol extracts were mixed and incubated at 37° C for 5 days. The mixture was assayed daily using commercial HBsAg ELISA Kit for 5 days. Control tubes containing plasma with solvent alone. The binding effects of the extracts were analyzed every day.

HBV – DNA polymerase inhibition assay

HBV-DNA polymerase inhibition study was performed according to Lofgren *et al.* (1989). Two sets of control reaction were added. Control 1 without plant extract and control 2 Lamivudine 1mg/ml Both the test and control were spotted in Whatman DE 81 filter paper and DNA was precipitated by adding cold 5% trichloroacetic acid and 0.1% pyrophosphate solution. The filter paper was washed thrice with the same solution to precipitate the DNA. It was transferred to scintillation cocktail and radioactivity was measured. The radioactivity was correlated to HBV- DNA polymerase inhibition.

The mean of triplicate counts was used to calculate the percentage of inhibition using the formula

$$\text{Percentage inhibition} = \left(\frac{100 - \text{Mean CPM} \times 100}{\text{Mean CPM negative control}} \right)$$

Plant extract that exhibited an inhibition greater than or equal to 50% were considered as positive.

HIV-1 RT assay

The effect of ethanol and methanol plant extracts on RT activity in vitro was evaluated with recombinant HIV-1 enzyme, using a non radioactive HIV-1 RT colorimetric ELISA kit (Roche) (Harnett *et al.*, 2005). The concentration of the extract used was 200µg/ml. the extracts, which reduced the activity by at least 50% were considered as active. Azidothymidine (AZT) was used as a positive control at 100µg/ml. The control 1 contained only the buffer and the reaction mixture (no enzyme and extracts were added). For control 2, the enzyme and reaction mixture were added for

the reaction to take place. The absorbance was read at 405nm with a reference wavelength of 490nm. The mean of the triplicate absorbance was analysed using the formula

$$\text{Percentage inhibition} = \left(\frac{100 - \text{Mean sample absorbance} \times 100}{\text{Mean control-2 absorbance}} \right)$$

Preliminary phytochemical analysis

Qualitative phytochemical analysis was also carried out to study the phytochemicals present in the ethanolic and methanolic extracts by method of harbore (1998).

Alkaloids by Dragendorff's reagent, anthraquinine by Borntrager's test, Cardiac glycosides by kellar kilianis test, flavanoids by shinoda test, saponin by frothing test, tannins by Braemer test and terpenoids by Liebermann –Burchadt test.

Results and Discussion

Methanol extract of *Gymnema sylvestre* leaves inhibited both HIV-1 RT and HBsAg and HBV DNA polymerase whereas ethanolic extract showed inhibition only for HIV1- RT. This HIV and HBV inhibiting properties are important as they indicate the probability of identifying and extracting some antiretroviral compounds from this plant.

Qualitative analysis of phytochemicals revealed the presence of alkaloids, saponins, quinones and cardiac glycosides in both methanolic and ethanolic fractions and tannins in methanolic fractions.

Gymnema sylvestre is been widely used for its antisaccharine properties. Not much work has been done on its antiviral activity. The hepatoprotective activity as studied by

Srividya *et al.*, 2010 and immunostimulatory effect studied by Malik *et al.*, 2009 shows that this herb could be a potential target especially against hepatitis virus.

Viral enzymes are crucial for disease progression and replication and hence developing inhibitors against them would be a desirable strategy. This study has revealed the effective antiviral activity in ethanolic and methanolic extracts of *Gymnema sylvestre*.

HIV-1 Reverse transcriptase inhibition assay positivity proves the antiviral activity and the RT associated RNase H activity has to

be studied by molecular docking studies. HBV creates a large number of variants through rapid mutagenesis as its reverse transcriptase lacks proof reading function, a phenomenon that is responsible for the low efficacy of the current drugs and the high rate of drug resistance. Therefore it is necessary to develop new anti HBV drugs (Wang *et al.*, 2010). It can be inferred that Methanolic extracts possess considerable HIV1- RT, HBV DNA polymerase activity and HBsAg binding activity. Alkaloids are heterocyclic nitrogen compounds and have proven antiviral activity as studied by Macmohan *et al.* (1995).

Table.1 Antiviral assay of leaves of *Gymnema sylvestre* R.Br

Plant Extract	HBsAg binding activity (150µg/ml)	HBV DNA polymerase inhibition (400µg/ml)	HIV1-RT inhibition(200µg/ml)
Methanol Extract	+	+	+
Ethanol Extract	-	-	+

+ = positive, - = Negative

Table.2 Qualitative phytochemical analysis of leaves of *Gymnema sylvestre* R.Br

Test	Methanol Extract	Ethanol Extract
Alkaloids	+	+
Flavanoids	-	-
Coumarins	-	-
Saponins	++	++
Tannins	-	+
Quinone	+	+
Cardiac glycosides	+	+

Tannins are a group of polymeric substance formed by condensation of flavan derivatives or by polymerization of quinine units. Earlier studies revealed Tannins exhibiting gp41 binding activity as studied by Lu *et al.* (2004). Triterpenoid Saponins besides having strong adjuvant activity also possess substantial antiviral activity as studied Roner *et al.* (2007). Since the plant extract of *Gymnema sylvestre* R.Br. leaves are rich in these phytoconstituents, the antiviral activity of the extracts may be attributed to these compounds either individually or synergistically.

Thus this study justifies the importance of *Gymnema sylvestre* R.Br in targeting HIV-HBV coinfection. Further work on isolation and characterization of active principles from this plant and its pharmacodynamic study would be highly beneficial to this society

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