Available Online through www.ijptonline.com

ANTI-HIV ACTIVITY OF TERMINALIA CHEBULA CRUDE EXTRACTS AND GALLIC ACID

Sravan Kumar Reddy Theepireddy*, Ramakrishna Chinthala1, L. Vaikunta Rao3#, Santosh Vishwakarma2, K.Rathanakar Reddy2, Mallikarjuna Rao Duvvada1

1Department of Environmental Studies, GITAM Institute of Sciences, GITAM University, Visakhapatnam-530045. India,
2Department of Discovery Research, Hetero Research Foundation, Plot No 80 and 81 A.P.I.E, Balanagar, Hyderabad-500018. India,
3#Department of Chemistry, GITAM Institute of Sciences, GITAM University, Visakhapatnam-530045. India.

Email: sravankumar.t@heterodrugs.com

Received on 20-06-2014 Accepted on 08-07-2014

Abstract:

Herbal drug products have special place in the pharmaceutical world. Terminalia chebula is been used in various traditional medicines for treatment of various diseases. The objective of the current research work was to study the anti-HIV I activity of the crude extract of Terminalia chebula and its chemical constituent gallic acid. Terminalia chebula fruit extract and gallic acid showed the IC50 was of 84.81 µg/ml and 71.99 µM respectively for the HIV integrase strand transfer inhibition activity. The crude extract of Terminalia chebula and gallic acid showed dose dependent inhibition on different HIV-1 strains such as NARI-VB 28, NARI-29, NARI-VB 30, NARI-VB 39, NARI-VB 49 and HIV-1 92 HT599. The Terminalia chebula fruit extract and gallic acid did not show the cytotoxicity at the concentration used in the study. Terminalia chebula fruit extract and its constituents can be further exploited for future plant based anti-HIV.

Keywords: Terminalia chebula, Integrase, HIV, Gallic acid.

Introduction:

The greatest challenge in front of global research community is the treatment of HIV infections. HIV is known to be a predominantly sexually transmitted disease, while few cases are also noticed because of either blood transfusion or from an infected mother to infants (1). HIV is a causative agent for AIDS (acquired immunodeficiency syndrome). The main focus in anti-retro viral therapy (ART), given for HIV infection is suppression of viral replication as much as possible for the longest possible period. Though current antiretroviral drugs are improving the quality of life in HIV/AIDS...
patients (2), there is rapid emergence of drug resistance to viral strains. This has led to the thrust for new anti-viral drugs that act by newer mechanism (3). During the process of exploring various methods for treating this deadly infection, researchers have identified HIV-1 protease (PR), reverse transcriptase (RT) and integrase (IN) enzymes as prime targets (4). Raltegravir, Elvitegravir as well as Dolutegravir are now available integrase inhibitors in the market. In continuation of search for new integrase inhibitors, plant derived HIV-1 integrase inhibitors is gaining lot of importance (5).

Use of traditional medicines for fatal diseases like cancer, HIV infection/AIDS, etc., has gained lot of public interest. A detailed review was published by Chinsembu and Hedimbi (6-7) as well as Singh et.al. (5) to identify various traditional plants that are commonly used for treatment of HIV infection. The inhibitory activity of *Terminalia chebula* (Fig. 1) against HIV-1 protease (8), reverse transcriptase (9) as well as integrase inhibition is documented in literature. To our knowledge, till date there is no report on anti-HIV activity using Integrase inhibitory activity of *Terminalia chebula* crude extract and gallic acid, hence we decided to explore its anti-HIV activity against HIV-I viruses.

**Materials and Methods**

**Collection of Plant Material**

Fresh *Terminalia chebula* mature fruits were purchased from Ayurvedic shop, Habsiguda, Hyderabad, India. The fruits were selected according to the uniformity of the shape.

**Figure 1:**

![Fig 1: Leaves & dried fruits of *Terminelonia chebula*](image)
Preparation of *Terminalia chebula* Fruit Extract:
The shade dried fruits of *Terminalia chebula* was powdered in an electrical grinder. The powdered fruits 250 g material was taken in hexane (1000 mL) and stirred for 24 hr at room temperature to remove fatty substances, chlorophylls and the lipids. The hexane layer was decanted, remaining crude residue was extracted with 95% ethanol (1000 mL) for 48 hr at room temperature. The solution was filtered and the residue was extracted twice again with 95% ethanol for 48 hr, the extract was filtered under suction pump. All the three 95% ethanol extracts were combined and concentrated under reduced pressure at 50-55°C to obtained 68 g of residue. The residue was purified by using column chromatography to separate gallic acid.

Isolation of Gallic acid:
The crude 95% ethanol extract was purified with the column chromatographed by using 100-200 mesh silica gel eluted with solvent system methanol in dichloromethane. The fractions were collected each 500 mL with pure dichloromethane, 2.5% methanol in dichloromethane, 5% methanol in dichloromethane, 7.5% methanol in dichloromethane, 10% methanol in dichloromethane, 12.5% methanol in dichloromethane and 15% methanol in dichloromethane. Gallic acid was isolated in the fraction of 12.5% methanol in dichloromethane this was distilled off to obtain gallic acid with 99.6% purity by HPLC, mp. 257-261°C.

Integrase Inhibition activity:
Integrase inhibitor activity of *Terminalia chebula* crude extract and isolated gallic acid was determined (Fig. 2) according to procedure reported earlier (10). In brief, annealed donor DNA was added to streptavidin coated plates. After incubation and wash with buffer, wells were blocked with BSA/sperm DNA. Wash procedure was repeated to remove unbound DNA. Integrase enzyme was added followed by pre-defined concentration of test compound in DMSO (1% final concentration) and target DNA. Incubation continued for next 2 hr and plates were subjected to washing to remove unbound DNA. Secondary antibody coupled with HRP (Anti DIG+ HRP) was added to wells. After further incubation and washing, enzyme substrate TMB was added. Reaction was stopped by adding 0.2N H$_2$SO$_4$ after 5 min incubation and color development was read at λ450. Raltegravir was used as positive control drug.
Figure-2: Effect of *Terminalia chebula* fruit extract and gallic acid on Integrase activity.

*Terminalia chebula* fruit extract (µg/mL); Gallic acid (µM)

The results are IC$_{50}$ ± S.E.M., n = 3 for HIV-I integrase activity.

Cell lines and viruses:

TZM-bl cell line was used as indicator cell for the microtiter syncytia formation assay. The cell lines were cultured in RPMI medium 1640 containing 100 U/ml penicillin, 100 µg/ml streptomycin, 2mM L glutamine, and 10% heat inactivated fetal calf serum. NARI-VB 28, NARI-29, NARI-VB 30, NARI-VB 39, NARI-VB 49 and HIV-1 92 HT599–NIH was prepared and stocked as described previously [11-12]. The cell lines and the virus stock were obtained through National AIDS Research Institute (India) as well as from AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: MT 2 from D. Richman [13, 14]. All the protocols were approved by Institutional Biosafety Committee (IBSC) Department of Biotechnology, India.

**Syncytia formation assay of acute HIV infectivity:**

The effect of *Terminalia chebula* crude extract and gallic acid on acute HIV infectivity was measured by the syncytia formation assay [12, 15]. TZM-bl cells infected NARI-VB 28, NARI-29, NARI-VB 30, NARI-VB 39, NARI-VB 49 and MT-2 cells were infected with HIV-1 92 HT599 plated into microtiter wells at 30,000 cells in 100 µl/well. Cells were treated with 2 µl *Terminalia chebula* crude extract at various concentrations for 1 h. After 4 day, syncytium formation was scored under an inverted microscope.

**p24 assay of HIV replication:**

The effect of *Terminalia chebula* crude extract and gallic acid on NARI-VB 28, NARI-29, NARI-VB 30, NARI-VB 39, NARI-VB 49 and HIV-1 92 HT599 replication in vitro was tested (Table 1) by viral core protein p24 expression using
commercial ELISA kit Advanced Biosciences Laboratories (ABL) as described previously [12, 15]. Briefly, TZM-bl cells were inoculated with a titered stock of NARI-VB 28, NARI-29, NARI-VB 30, NARI-VB 39, NARI-VB 49 and HIV-1 92 HT599–NIH virus at 10 TCID/ well. Cells were incubated at 37°C for 60 min to allow viral absorption. The cells were then washed to remove unbound virus and plated with or without the addition of Termioia chebula crude extract and gallic acid for the duration of the experiment. In this assay, at the multiplicity of infection used, viral production peaks at day 4. Thus, p24 expression was assayed in cell free supernatant sharchested at day 4.

Table 1:

<table>
<thead>
<tr>
<th>Viral Strain</th>
<th>TCE IC₅₀(µg/ml) ± S.E.M.</th>
<th>Gallic acid IC₅₀(µM) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV – I VB 28</td>
<td>6.48 ± 0.16</td>
<td>18.3 ± 0.21</td>
</tr>
<tr>
<td>HIV – I VB 29</td>
<td>4.64 ± 0.19</td>
<td>23.59 ± 0.08</td>
</tr>
<tr>
<td>HIV – I VB 30</td>
<td>13.34 ± 0.04</td>
<td>27.36 ± 1.00</td>
</tr>
<tr>
<td>HIV – I VB 39</td>
<td>9.15 ± 0.07</td>
<td>32.4 ± 1.20</td>
</tr>
<tr>
<td>HIV – I VB 49</td>
<td>4.18 ± 1.00</td>
<td>19.08 ± 1.06</td>
</tr>
<tr>
<td>HIV–I 92 HT599</td>
<td>0.52 ± 0.09</td>
<td>0.49 ± 0.06</td>
</tr>
</tbody>
</table>

The results are IC₅₀ ± S.E.M., n = 3 for HIV-I antiviral activity.

Table-1: Effect of Termioia chebula fruit extract and gallic acid on antiviral activity.

Determination of Termioia chebula crude extract and gallic acid cytotoxicity:

Cytotoxicity of Termioia chebula crude extract and gallic acid was evaluated by the MTT assay. Uninfected cells were grown in the absence or presence of various concentrations of Termioia chebula crude extract and gallic acid for 4 days. The cells were then exposed for 3 h to MTT tetrazolium salt (Cell Titer 96, Promega, Madison, WI) with phenazinemethosulfate as described by the manufacturer. The viability of drug exposed cells is reflected in the activity of mitochondrial hydrogenases of the cells converting MTT into color dense formazan. Optical density was determined in a plate reader set to record the absorbance at 590 nm and compared to absorbance values of the control cells cultured without drugs. The cell inhibition was determined using following formula.

\[
\% \text{ cell inhibition} = 100 - \frac{\text{Abs (Sample)}}{\text{Abs (Control)}} \times 100
\]
Statistics:

For statistical analysis, the values are expressed as a mean± S.E.M. of three determinations. The IC$_{50}$ values were calculated using Graph pad prism 5.02.

Results and Discussion:

*Terminalia chebula* is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. It is used for the treatment of number of diseases like cancer, paralysis, cardiovascular diseases, ulcers, leprosy, arthritis, gout, epilepsy etc. It has been reported as antioxidant (16), antibacterial (17), antiviral (18), antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc. *Terminalia chebula* and its isolated compounds gallic acid chebulagic acid, and chebulinic acid showed moderate *in vitro* cytotoxicity against various cultured human tumor cell (9).

The integration of viral DNA into the host cell chromosome is essential for HIV-1 replication. This process, mediated by integrase involves two chemical reactions. The first reaction is 30 processing in which a specific dinucleotide sequence of the viral DNA 30 ends are cleaved to generate reactive hydroxyl groups. Next the integrase enzyme facilitates the transesterification reaction known as strand transfer, resulting in the successful integration of the viral DNA into the host chromosome (20). Integrase represents an interesting target for therapeutic intervention as it is essential to progression of HIV infection.

The major objective of our study was to understand the anti-HIV activity of *Terminalia chebula* fruit extract and gallic acid. Previous experiments carried out by Mi-Jeong et al., (21) showed that, galloyl moiety play a major role in the expression of the inhibitory activity of HIV-1 integrase. *Terminalia chebula* Retz fruits myrobalanscombreataceae was found to inhibit the 3’- processing activity of HIV-I integrase IC$_{50}$ of 10.3 µg/mL while gallic acid isolated from *Terminalia chebula* exhibited IC$_{50}$ 54.7 µM the isolated gallic acid was characterized using HPLC, NMR and Mass Spectrophotometry (data not shown). Experiment carried out in our laboratory *Terminalia chebula* fruit extract and gallic acid showed the IC$_{50}$ of 84.81µg/ml and 71.99µM (Fig.2) respectively, while the standard integrase inhibitor Raltegravir showed the IC$_{50}$ of 17.8nM as reported in the literature. The IC$_{50}$ for the gallic acid is comparable to the reported value however there are differences in the IC$_{50}$ value of crude extract in HIV-1 integrase inhibition activity from previous report this may be due to habitat of plant, collection time, method and extraction procedure etc. In current...
In this study, we wanted to see the translation of the Integrase inhibition on the antiviral activity. We selected few of the HIV-1 R5 Indian strains obtained from NARI, viz NARI-VB 28, NARI-29, NARI-VB 30, NARI-VB 39, NARI-VB 49 and HIV-1 92 HT599 from NIH AIDS programme to study the anti-HIV activity of the crude extract *Terminalia chebula* and gallic acid. The crude extract and gallic acid showed dose dependent inhibition of HIV-I viruses with the IC$_{50}$ values for each strain represented in the Table 1. Overall the crude extract showed enhanced activity compared to the gallic acid which in-turn supports that there are other constituents in the crude extract which may be playing the role in the anti-HIV activity. No cytotoxicity on uninfected target cells was detected from 0.1 to 1000 $\mu$g/ml to 1000 $\mu$M for crude extract and gallic acid respectively. Thus, *Terminalia chebula* and gallic acid by itself has direct anti-HIV activity in these assays. These results further substantiated the use of Integrase inhibitor for the anti-HIV treatment.

In conclusion, considering the limitations of current treatment options for HIV, it will be important to define whether chemical constituents present in the *Terminalia chebula* can be used as the basic pharmacophore to design a potent Integrase inhibitor with anti-HIV activity.

**Acknowledgements:**

Authors acknowledge the generous support received from Dr. B.P.S. Reddy Chairman & Managing Director Hetero Research Foundation, Hyderabad 500018, India for this research work. Authors also extend their acknowledgements to Dr. Vittal Kurisetty, Dr. Ravikanth, Dr. Jayaprakash Narayana and Mr. Ramesh.

**References:**


Corresponding Author:
Sravan Kumar Reddy Theepireddy*,
Email: sravankumar.t@heterodrugs.com