Research Article

Evaluation of *Ocimum sanctum* and *Tinospora cordifolia* as Probable HIV-Protease Inhibitors

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ABSTRACT

Ethanol extracts of *Ocimum sanctum* Linn. and *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms. were included for the present *in vitro* study. Pepsin was used as a substitute for HIV-protease to evaluate inhibitory activity of these extracts, as pepsin has close resemblance with HIV-protease in proteolytic activity. Extracts of *O. sanctum* and *T. cordifolia* showed potent inhibitory activity with IC_{50} values of 123.73 and 11.20 µg/ml respectively. In our earlier study, these extracts exerted their anti-HIV activity via multiple mechanisms of action; viz., interference with the gp120 / CD4 interaction and inhibition of HIV-reverse transcriptase. In the present study, they also showed potent inhibitory activity against pepsin enzyme, suggesting that they may be useful as HIV protease inhibitors. The inhibitory activity could be attributed to flavonoids and phenolic content respectively.

Keywords: Anti-HIV, HIV-protease (PR), Pepsin assay, Indian plants, Flavonoids content.

INTRODUCTION

Acquired Immuno Deficiency Syndrome (AIDS), caused by human immunodeficiency virus (HIV), is a serious life-threatening health problem as there is no vaccine, adverse effects of currently approved Anti-HIV drugs, emergence of drug resistance and latent phase of the virus. Hence there is urgent need of safe, effective and economical alternative.[1,2] One of the strategies has been to indentify anti-HIV compounds from natural sources, particularly plants.

HIV protease plays a vital role in viral replication cycle.[3] Blockage of HIV protease leads to formation of immature non-infectious virions.[4] Hence it has become an important target in HIV drug development. Traditional knowledge-driven drug development can follow a reverse pharmacology path and reduce time and cost of development.[5] Several natural products from traditional medicine have been shown to possess HIV-protease inhibitory activity.[6-10] Plants included in the present study were *Ocimum sanctum* Linn. (Tulas) and *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms (Gulvel). The biological activities of *O. sanctum* and *T. cordifolia* have been reported previously.[11,12]

Pepsin has a close resemblance with HIV-protease in proteolytic activity as both of them belong to same aspartate enzyme family.[13] Hence in present study, pepsin was used as a substitute for HIV-protease.[14]

The aims and objectives of present study were to prepare ethanol extracts of selected plants and to evaluate their inhibitory effect on pepsin enzyme.

MATERIALS AND METHODS

Collection of the material

Leaf powder of *O. sanctum* was purchased from Atul medical stores, Mumbai. Stem powder of *T. cordifolia* was procured from Zandu Pharmaceuticals, Mumbai. All the material was identified and authenticated by Dr. J. M. Pathak, Research Director (Pharmacognosy), Zandu Pharmaceuticals, Mumbai.

Preparation of extracts

The plant material was extracted in a Soxhlet apparatus with ethanol directly. All the extracts were made free from solvent and percentage yield of individual extract was calculated which was found to be 8.13% and 5.55% for *O. sanctum* and *T. cordifolia* respectively.

Assessment of pepsin enzyme inhibitory activity

a) Preparation of hemoglobin

Hemoglobin was prepared as stated earlier.[15] Briefly, 2.5 gm hemoglobin (HiMedia) powder was dissolved in 100 ml distilled water. It was blended at maximum speed for 5 min and then filtered through gauze. Eighty ml of filtrate was diluted with 20 ml of 0.3N HCl and stored at 4^0C until further use.

b) Pepsin assay

Pepsin assay was carried out as described by Singh *et al.*[16] Briefly, 50 µg pepsin (HiMedia), 800 µg hemoglobin (HiMedia) and different concentrations of each extract were taken in 500 µl of reaction mixture. The mixture was allowed to incubate at 37^0C for 20 min. After incubation, 700 µl of 5% trichloro acetic acid (TCA) (Himedia) was added to stop the reaction. It was then centrifuged (Rotina 38R) at 14000 rpm for 5 min and the supernatant was collected. Optical density was recorded spectrophotometrically (Cary 50 Bio UV-Visible spectrophotometer) at 280nm. Pepstatin-A (Sigma) was included as a standard. Negative control without extract(s) was set up in parallel. Separate blanks were used for extracts. All the determinations were done in triplicate and the result is expressed as percent inhibition.

Percent Inhibition was calculated as, Inhibition (%) = $(A_{Negative\ control} - A_{Test})/A_{Negative\ control}$ X 100, where A is absorbance. The result is also expressed as IC_{50} value.

Flavonoids content estimation

The method of Oyedemi *et al*[17] was used to estimate total flavonoid content of the extract solutions based on formation of a complex flavonoids-aluminums. Briefly, a volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of each extract solution. After one hour of incubation at room temperature, the absorbance was measured at 420nm using multimode reader (Synergy HT, BioTek). Yellow color indicated presence of flavonoids. Quercetin at various concentrations (20 to 100 µg/ml) was included as a standard. All the determinations were done in triplicate. Mean values of triplicate determinations were used to plot the graph. Total flavonoid content was calculated from the equation (y= 0.008x, R^2= 0.975) obtained from the quercetin standard curve. The result is expressed as Quercetin equivalent in milligrams per gram of dry sample.

Statistical analysis

All the determinations were done in triplicate. Means, standard deviations and IC_{50} values were calculated using a Microsoft Excel program.

RESULTS AND DISCUSSION

Recently considerable attention has been given to screening of various species of medicinal plant extracts for possible anti-HIV activity.[18] Plants have formed the basis of traditional medicine systems and these plant-based systems continue to play an essential role in health care.[19] Ayurveda is a traditional Indian medicinal system being practiced for decades.[20] *O. sanctum* and *T. cordifolia* which are included in present study belong to Indian traditional medicinal system.[21,22]

HIV protease belongs to class of aspartic proteases and has similar structural features and mechanism to aspartic protease enzymes.[23] Aspartic proteases include pepsin, cathepcin D, renin, chymosin and the proteases isolated from numerous fungi.[24] In present study, pepsin was used as a substitute of HIV protease for screening HIV protease inhibitory activity of the selected plants. Similar studies have been carried out by Govindappa *et al*[14] and Singh *et al.*[16] Both the plants showed potent inhibitory activity, wherein, *T. cordifolia* showed the lowest IC_{50} compared to *O. sanctum* (Table 1).

As various previous studies suggested structural and functional similarity between pepsin and HIV protease[25-29], extracts that showed inhibitory activity of pepsin enzyme should also inhibit activity of HIV protease. However, *T. cordifolia* showed no flavonoids content (Table 2). The inhibitory activity of *T. cordifolia* could be attributed to its phenolic content.[30] Group of phenolics includes tannins, simple phenols and phenolic acids, quinones, flavonoids, flavones and flavonols, coumarins to name a few.[31] These phenolics have shown HIV protease inhibitory activity in the past.[32-34] The inhibitory activity of *O. sanctum* could be attributed to flavonoids content (Table 2). Flavonoids have also shown inhibitory activity against HIV protease.[35-37]

Table 1: Effect of extracts on pepsin assay

Plants	Conc. (µg/ml)	% Inhibition (Mean ± SD)	IC_{50} (µg/ml)
O. sanctum	10	31.49 ± 1.2	123.73
	100	43.48 ± 5.0	
	200	71.42 ± 5.5	
	300	74.26 ± 5.7	
	400	82.02 ± 2.6	
T. cordifolia	1	41.43 ± 1.9	11.20
	20	55.48 ± 1.8	
	40	62.98 ± 0.8	
	80	80.28 ± 0.6	
	120	86.97 ± 4.1	
Pepstatin-A (Standard)	-	-	< 0.2

Table 2: Flavonoids content estimation of extracts

Plants	Quercetin equivalent (mg/gm)*
O. sanctum	115
T. cordifolia	No

*Mean of triplicate determinations

In our earlier study, these extracts exerted their anti-HIV activity via dual mechanism of action; viz., interference with the gp120 / CD4 interaction and inhibition of HIV-reverse transcriptase.[38] In the present study, they also showed potent inhibitory activity against pepsin enzyme, suggesting that they may be useful as HIV protease inhibitors. Phytochemical investigation of *O. sanctum* revealed presence of resins, alkaloids, tannins and steroidal terpenes, whereas, *T. cordifolia* showed presence of resins, alkaloids and saponins.[39] These phyto-constituents have shown anti-HIV activity previously.[40-42] Because of such phytochemical diversity, these plants may be involved in the entire process, from virus adsorption to host cell, to formation, growth and activation of virus proteins and budding into mature virions. Similar effects of *Alnus firma* have been reported by Yu *et al.*[43]

CONCLUSION

The present study indicates that *O. sanctum and T. cordifolia* might be of value as sources for novel antiviral compounds. These plants have shown anti-HIV potential by 3 different mechanisms (interference with the gp120 / CD4 interaction, inhibition of HIV-reverse transcriptase and probable inhibition of HIV-protease enzyme). Furthermore, one of the traditional uses of these plants includes immunomodulation. Hence anti-HIV activity could be an added advantage along with the immunomodulatory effect of these plants to fight Acquired Immunodeficiency Syndrome (AIDS).

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