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Screening of Natural Products for Anti-HIV Potential: An *In vitro* Approach

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Abstract

Human Immunodeficiency Virus (HIV) is the etiological agent of Acquired Immunodeficiency Syndrome (AIDS). There are 2 types of HIV: HIV-1 and HIV-2. However, worldwide, predominant virus is HIV-1. The development of safe, effective and low cost anti-HIV drugs is among the top global priorities of drug development since the disease is not yet curable, significant toxicity of available anti-HIV drugs, no vaccine and emergence of drug-resistant viruses. One of the strategies has been to identify anti-HIV compounds from natural sources such as plants, herbominerals, marine organisms and microbes. Current review summarizes commonly used *In vitro* assays to screen natural products (crude extracts and/or isolated compounds) for anti-HIV potential. Anti-HIV research should be focused on compounds that interfere with various parts of viral life cycle. Preliminary screening of natural products can be carried out with simple cell-based assays such as MTT or XTT assay and HIV p24 expression using Human T-cell lines. Target specific studies can be achieved through evaluating effect of test substances on HIV gp120/CD interaction, reverse transcriptase, integrase and protease enzymes using sophisticated techniques such as ELISA, Liquid Scintillation Counting, Flourometric, Sprectrophotometric and HPLC-based methods. Nevertheless, further pharmacological and toxicological investigation of active natural product/isolated compound is imperative. To conclude on an optimistic note, natural product derived active compounds serve as important lead molecules as demonstrated in case of development of Calanolide A isolated from *Callophyllum lanigerum* as Non- Nucleoside Reverse Transcriptase Inhibitor (NNRTI). However, more such novel anti-HIV agents are necessary to be discovered.

Keywords: Anti-HIV; ART; MTT assay; XTT assay; HIV-Reverse Transcriptase; HIV-Protease; HIV-Integrase; gp120 ELISA

Abbreviations: HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; SJS: Stevens-Johnson syndrome; TEN: Toxic Epidermal Necrolysis; ARV: Antiretroviral; PMS: Phenazine Methosulfate; ART: Antiretroviral Therapy; CPM: Counts Per Minute; CC50: 50% Cytotoxic Concentration; DABCYL: 4-[4-(dimethylamino)phenyldiazo]benzoic acid; DDDP: DNA Dependant DNA Polymerase; DNA: Deoxyribonucleic acid; EDANS: 5-[(2-aminoethyl)amino]naphthalene-1-sulfonic acid; ELISA: Enzyme Linked Immuno Sorbent Assay; EC50: 50% Effective Concentration; FDA: Food & Drug Administration; FRET: Fluorescence Resonance Energy Transfer; HAART: Highly Active Antiretroviral Treatment; HPLC: High Performance Liquid Chromatography; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NNRTI: Non Nucleoside Reverse Transcriptase Inhibitor; NRTI: Nucleoside Reverse Transcriptase Inhibitor; PBMC: Peripheral Blood Mononuclear Cells; PMS: Phenazine Methosulfate; PR: Protease; RDDP: RNA Dependent DNA Polymease; RNA: Ribonucleic Acid; RT: Reverse Transcriptase; SI: Selectivity Index; SJS: Stevens-Johnson Syndrome; TCAL: Trichloro acetic Acid; TEN: Toxic Epidermal Necrolysis; XTT: sodium 3-[1-(phenylamino)-carbonyl]-3,4-tetrazoliumbis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate

Introduction

Human immunodeficiency virus (HIV), the etiological agent of AIDS is of two types: HIV-1 and HIV-2. Both types are transmitted by sexual contact, through blood, infected needles and syringes and from mother to child, and appear to cause clinically indistinguishable acquired immunodeficiency syndrome (AIDS). AIDS is characterized by extensive immunosuppression that predisposes patients to life-threatening opportunistic infections and unusual forms of neoplasm. Worldwide, the predominant virus is HIV-1. The relatively uncommon HIV-2 type is concentrated in West Africa and is rarely found elsewhere. The strains of HIV-1 can be classified into three groups: the "major" group M, the "Outlier" group O and the "new" group N. These three groups may represent three separate introductions of simian immunodeficiency virus into humans. Group O appears to be restricted to west-central Africa and group N – discovered in 1998 in Cameroon- is extremely rare. More than 90% of HIV-1 infections belong to HIV-1 group M. Within group M there are known to be at least nine genetically distinct subtypes (or clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K. In India subtype C exists [1]. HIV belongs to the family Retroviridae subgroup Lentivirus is a spherical enveloped virus, about 90-120 nm size surrounded by a lipoprotein membrane. The genome of HIV contains 3 structural genes: *gag*, *pol* and *env*. The classical structural scheme of a retroviral genome is: 5' LTR-gag-pol-env-LTR 3'. The LTR (long terminal repeat) regions represent the 2 end parts of the viral genome, that are connected to the cellular DNA of the host cell after integration and do not encode for any viral

proteins [2,3]. HIV-1 is composed of two copies of noncovalently linked, unspliced, positive-sense single-stranded RNA enclosed by a conical capsid composed of the viral protein (p24) and inside the core are three enzymes required for HIV replication called reverse transcriptase, integrase and protease. Also enclosed within the virion particle are Vif, Vpr, Nef. The envelope is formed when the capsid buds from the host cell, taking some of the host-cell membrane with it. The envelope includes the glycoproteins gp120 and gp41 [4-6].

Antiretroviral Therapy (ART)

Currently there are 30 individual or combination antiretroviral drugs licensed and approved by the US Food and Drug Administration (FDA) for use in humans against HIV. The classes of anti-HIV drugs are primarily defined as their sites/targets of action in the HIV life cycle (Figure 1).

HAART

The terminology "Highly active antiretroviral therapy" (HAART) refers to use of combinations of 3 antiretroviral agents for treatment of HIV infection. To date, most clinical experience with use of HAART in treatment-naïve individuals has been based on 3 types of combination regimens: NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitor)-based (1 NNRTI + 2 NRTI), PI (Protease Inhibitor)-based (1-2 PI + 2 NRTI) and triple NRTI (Nucleoside Reverse Transcriptase Inhibitor)-based regimens. Most experience in India is with NNRTI based regimens [7].

Limitations (failure) of HAART

The introduction of HAART has led to a significant reduction in AIDS–related morbidity and mortality. However, there are a number of problems associated with it, including, the difficulties of maintaining long-term adherence, drug-related toxicities and the development of drug resistance, all of which may lead to virological failure, which in turn leads to immunological failure and clinical progression [8].

All FDA-approved NRTIs, NNRTIs and PIs are associated with various adverse effects such as hepatotoxicity (hepatitis, hepatic necrosis & hepatic steatosis), hyperglycemia, hyperlipidemia, lactic acidosis, lipodystrophy, osteonecrosis and osteoporosis to name a few. Furthermore, anti-HIV medications are associated with mild skin rashes as well as serious life-threatening rashes like Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN). Some drugs can even cause bone marrow toxicity. The occurrence of side effects plays a large role in adherence to drug regimens, which in turn can impact the development of drug resistance [8].

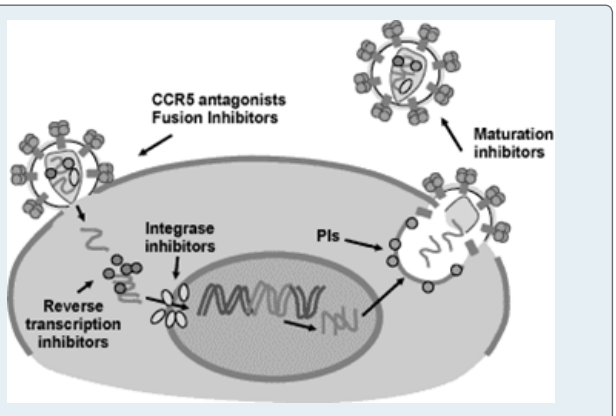

Figure 1: Antiretroviral Targets in HIV Life Cycle.
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http://www.inpracticeafrica.com/~/media/Images/HIV_Pathogenesis_for_Nurses/NSG_HIV_Pathogenesis_Fig3.png

Drug-drug interaction is another aspect responsible for HAART failure. Patients may receive other drugs for supportive care, treatment of opportunistic infections and immunomodulation. Hence, drug interactions are often unavoidable in HIV-infected patients because of the drug classes involved and the number of drugs prescribed. These drug-interactions alter the absorption, transport, distribution, metabolism or excretion of drug that may lead to viral resistance or serious toxic effects resulting in treatment failure [9]. Besides, development of resistance to antiretroviral (ARV) drugs is common cause of ART failure.

Hence there is urgent need for the discovery of novel (safe, effective and cheap) therapeutic alternative as the long-term complications of this disease are multifactorial and can be related to the virus itself or to adverse effects of current antiretroviral therapy [10]. One of the strategies has been to identify anti-HIV compounds from natural products, which can be screened by using various *in vitro* assays.

In Vitro Anti-HIV Assays

The replicative cycle of HIV comprises a number of steps that could be considered adequate targets for chemotherapeutic intervention [11]. Therefore, any effective treatment of HIV-1 infection should target as many aspects of viral life cycle as possible. Natural products with broad structural diversity are good sources for the discovery of anti-HIV agents with low toxicity. Our laboratory has been actively involved in the research of natural products for their potential anti-HIV activity [12-15].

Current paper reviews commonly used *in vitro* assays to evaluate anti-HIV potential of natural products and/or isolated compounds.

HIV-1 replication inhibition assays

Preliminary screening of natural products can be carried out with simple cell-based assays.

MTT or XTT assay: Anti-HIV and cytotoxic effects of natural products or isolated compounds can be evaluated simultaneously with Human T-cell lines such as Jurkat, CEM-SS, MT4, H9 and PBMCs using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) or sodium 3-[1-(phenylamino)-carbonyl]-3,4-tetrazoliumbis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT) assays. The result can be expressed as 50% cytotoxic concentration (CC_{50}), 50% effective concentration (EC_{50}) and the selectivity index (SI) can be calculated as CC_{50}/EC_{50} ratio. Thus, SI reflects both antiviral activity and eventual toxicity of the test material. The high SI value indicates low toxicity of the test compound and high activity against the virus. MTT or XTT assay does not provide detailed information on the mechanism of

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action of anti-HIV compounds. However, it allows an estimation of the *in vitro* therapeutic index for compounds being considered for further preclinical development studies [16].

a). MTT assay: is based on metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide by mitochondrial dehydrogenase of metabolically active cells to insoluble blue formazan that can be measured spectrophotometrically at 540nm. For quantitative estimation of cell viability, formazan must be solubilised prior to colorimetric determination that requires additional centrifugation, pipetting or aspiration steps [17-22].

b). XTT assay: is a slight modification of MTT assay, wherein, sodium 3-[1-(phenylamino)-carbonyl]-3,4-tetrazoliumbis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT), a light yellowish tetrazolium reagent, is metabolically reduced by mitochondrial dehydrogenase in viable cells to a water-soluble, brown coloured formazan product. Phenazine methosulfate (PMS), an electron coupling agent, significantly enhances the production of XTT Formazan. The amount of XTT Formazan produced is correlated with the number of viable cells. Several T-cell lines are sensitive to the lysis caused by HIV replication. Anti-HIV agents protect these cells from lysis. Therefore, XTT plus PMS has been widely used for screening anti-HIV agents [23-25].

HIV-1 p24 Expression assay: The effect of natural products or isolated compounds on HIV-1 replication can be tested by viral core protein p24 expression in cell-free supernatants harvested at day 4 using commercial ELISA kit according to the manufactures' instructions. Briefly, cells are inoculated with viral stock and incubated at 37^0C for 1 hour to allow virus adsorption. The unbound virus is then removed by centrifugation and cells are resuspended in presence or absence of different dilutions of test compound. Cells are harvested on day 4 post-infection, as viral production peaks at day 4 and cell-free supernatants are collected for determination of p24 production by ELISA, wherein, culture supernatant is incubated in microtitre wells pre-coated with anti-p24 antibody and assayed for p24 antigen by biotin-labeled anti-p24 antibody followed by streptavidin-peroxidase conjugate. The amount of captured p24 can be determined by measuring absorbance at 450nm of tetramethylbenzidine substrate [26-29]. This assay can be carried out at different time points; firstly, pre-treatment of virus stock with test compound, secondly, pre-treatment of cells with test compound and delayed addition of test compound to virus-infected cells.

In our study, antiviral activity of the *Jatropha curcas* leaf extracts was assessed using standardized drug susceptibility assays using PBMCs and measured by inhibition of HIV p24 antigen in cell-free supernatants, wherein, it showed effective antiviral and probably entry inhibition activity against potentially drug resistant HIV [resistant to standard anti-retroviral drugs like Zidovudine (AZT), Lamivudine (3TC) and Stavudine (d4T)] [30].

HIV-1 gp120/CD4 interaction inhibition assay

The first step of viral entry into the host is mediated by highly specific interaction between viral envelop glycoprotein gp120 and CD4 molecules [31]. As the binding of gp120 to CD4 is critical to HIV infection, much interest has been focused on agents that block this interaction [32]. Effect of natural products on gp120/CD4 interaction can be analyzed using commercial gp120 capture ELISA kit. In this assay, CD4 molecules have been immobilized on a 96-well microtitre plate. The coated plate is then used to capture free HIV-1 gp120 supplied in the kit. The extent of gp120 binding is assessed by using detector reagent provided in the kit according to the manufacturer's instructions. To determine the mechanism of test compound on binding of CD4 to gp120, the test compound can be pre-incubated with gp120 or it can be pre-incubated with CD4-coated plate or can be added after gp120-CD4 interaction. The result is expressed as percentage inhibition which is calculated as,

$$Inhibition\ (\%) = \left[\frac{\left(A_{control} - A_{Sample}\right)}{A_{control}}\right] \times 100$$

Where, A is Optical Density (OD). Heparin can be included as a standard [31,33,34].

Our study included six natural products, namely, *Ocimum sanctum*, *Tinospora cordifolia*, *Withania somnifera*, *Avicennia officinalis*, *Rhizophora mucronata* and *Shilajit* and their effect on HIV gp120/CD4 interaction was evaluated using gp120 Capture ELISA kit (ImmunoDiagnostics, Inc.). Mangrove plants namely, *A. officinalis* and *R. mucronata* showed effective inhibition of gp120/CD4 interaction by binding to both gp120 and to CD4 ligand and even by displacing gp120 preadsorbed to immobilized CD4, whereas, *O. sanctum, W. somnifera* and *Shilajit* potently inhibited gp120/CD4 interaction by binding to CD4 (but not to gp120) and also competitively inhibited gp120 binding to CD4. *T. cordifolia* inhibited gp120/CD4 interaction by binding to CD4 ligand mainly [35,36].

Besides, enzymes involved in the replication of HIV-1 have been the targets for testing possible anti-HIV substances.

HIV-1 reverse transcriptase (RT) inhibition assay

HIV-1 Reverse transcriptase (RT) is a multifunctional enzyme with 3 enzymatic activities. Firstly, the polymerase domain transcribes viral RNA to viral DNA, a process referred to as RNA-dependent-DNA-polymerase (RDDP) activity. Secondly, in the course of reverse transcription, an intermediary RNA/DNA hybrid is formed. RT through its ribonuclease H (RNase H) domain degrades the RNA component of the hybrid. Thirdly, RT carries out DNA-dependent-DNA-polymerase (DDDP) activity, producing complementary DNA strands. The completion of each of these processes is required for the formation of competent viral DNA capable of integrating into the genome of the infected cell. Hence, RT enzyme is considered as one of the most important targets for antiretroviral substances [37]. Effect of natural products on RT enzyme can be evaluated by using tritium labelled-substrate & poly(rA).p$(dT)_{12\text{-}18}$ as template primer or by using non-radioactive ELISA and/or by ribonuclease H activity.

Using radio-labelled nucleotide: It involves determination of RDDP activity. The inhibition of RDDP activity is measured

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by evaluating the incorporation of methyl-3H-thymidine triphosphate (Methyl [3H]TTP) by RT using polyadenylic acid-oligodeoxythymidilic acid [poly(rA).p(dT)$_{12-18}$] as a template-primer in the presence or absence of test compound. The radioactivity is measured in liquid scintillation counter and is expressed as counts per minute (CPM). From which percentage inhibition can be calculated as,

$$Inhibition\ (\%) = \left[\frac{(CPM\ of\ Negative\ control\ -\ CPM\ of\ Test)}{(CPM\ of\ Negative\ control)}\right] \times 100$$

Azidothymidine (AZT) is included as a standard [37-40].

In our study, *O. sanctum, T. cordifolia, A. officinalis, R. mucronata* and *Shilajit* potently inhibited RDDP function of recombinant HIV-RT (Ambion) and the RT of two clinical isolates. Especially, *Shilajit* showed significant inhibition of both the clinical isolates [35,36,41].

Non-radioactive ELISA method using commercially available kit: In non-radioactive ELISA, instead of radio-labelled nucleotide, digoxigenin and biotin-labeled nucleotides are incorporated into DNA molecule, freshly synthesized by RT. The detection and quantification of synthesized DNA is achieved through a sandwich ELISA protocol. Briefly, biotin-labelled DNA binds to surface of microtitre plate pre-coated with streptavidin. In the next step, antibody to digoxigenin, conjugated to peroxidase, binds to digoxigenin-labelled DNA, followed by addition of substrate. The peroxidase enzyme catalyzes the cleavage of substrate producing coloured reaction product, absorbance of which can be measured at 405nm [42-47].

RNase H activity: The method utilizes radio-labelled RNA/DNA hybrid as a substrate. RNase H activity is evaluated by measuring the degree of degradation of the 3H-labelled RNA strand in a RNA/DNA hybrid by RT in the presence or absence of test substance. Percentage inhibition of RNase H activity is calculated as, [37]

$$\left[\frac{(CPM\ of\ RNA/DNA\ hybrid\ without\ test\ substance) - (CPM\ of\ RNA/DNA\ hybrid\ with\ test\ substance)}{(CPM\ of\ RNA/DNA\ hybrid\ without\ test\ substance)}\right] \times 100$$

HIV-1 integrase inhibition assay

HIV integrase catalyzes the integration of viral DNA into host DNA. Effect of natural products on HIV-1 integrase can be evaluated with recombinant HIV-Integrase by using, **Radiolabelled oligonucleotide substrate** [48,49] or by using **Non-radioactive ELISA** [42,43,47,50-53].

HIV-1 protease (PR) inhibition assay

Another enzyme, protease (PR) is essential for the proper assembly and maturation of fully infectious virus. Blockage of HIV protease leads to formation of immature non-infectious virions [54]. Therefore, PR is another attractive target for the development of anti-HIV agents. Effect of natural products on HIV-protease can be assayed using either of the 2 direct methods.

Fluorometric method: This assay can be carried out using commercially available kit and the recombinant HIV-1 protease. The method is based on quantification of HIV-1 protease activity using a fluorescence resonance energy transfer (FRET) peptide. The phenomenon of FRET occurs when two chromophores, 5-[(2-aminoethyl)amino]naphthalene-1-sulfonic acid (EDANS) and 4-[4-(dimethylamino)phenyldiazo]benzoic acid (DABCYL) interact with each other such that fluorescence emission is modified. This approach has been used to assay bond-cleavage reactions particularly in the proteolysis of peptides [55]. Thus, it involves proteolytic cleavage of EDANS/DABCYL FRET peptide by HIV-1 protease. The sequence of this FRET peptide is derived from the native p17/p24 cleavage site on Pr*gag* for HIV-1 protease. In the FRET peptide, the fluorescence of EDANS is quenched by DABCYL until this peptide is cleaved into two separate fragments by HIV-1 protease at the Tyr-Pro bond. Upon cleavage, the fluorescence of EDANS is recovered, and can be monitored at excitation/emission = 340 nm/490 nm. The assay can be performed in a convenient microplate format [42,43,47,53,56,57].

HPLC-based Method: Here, the recombinant PR and the substrate peptide are incubated in presence or absence of test compound. The reaction is stopped by heating the reaction mixture at 90^{0}C for 1 minute and an aliquot of which, is analyzed by High Performance Liquid Chromatography (HPLC) using RP-18 column. The elution profile is monitored at 280nm. The result is expressed as percentage Inhibition which can be calculated as,

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}}\ X\ 100$$

Where, A is relative peak area of product hydrolysate. Acetyl pepstatin can be included as a positive control [58-62].

Besides, another indirect method can be used to evaluate HIV-1 PR activity using pepsin enzyme as a substitute for HIV-PR. Pepsin has close resemblance with HIV-protease in proteolytic activity as both of them belong to same Aspartate enzyme family [63].

Pepsin assay: In this assay, activity of pepsin enzyme is evaluated by using haemoglobin as a substrate. Briefly, the pepsin enzyme cleaves substrate haemoglobin into smaller soluble peptides after incubation, which is followed by addition of trichloro acetic acid (TCA) to stop the reaction. The undigested precipitated part can be removed by centrifugation and the enzymatic activity is measured spectrophotometrically at 280nm, which is mainly due to presence of tryptophan and tyrosine amino acids (soluble peptides) resulting from digested haemoglobin. Pepstatin-A can be included as a standard [64-66].

Our study showed potent inhibitory activity of *O. sanctum*, *T. cordifolia*, *R. mucronata* and *Shilajit* against pepsin enzyme, suggesting that they may be useful as HIV protease inhibitors [67-69].

In general, six natural products, namely, *Ocimum sanctum*, *Tinospora cordifolia*, *Withania somnifera*, *Avicennia officinalis*, *Rhizophora mucronata* and *Shilajit* were included in our study. Out of these, four (*O. sanctum*, *T. cordifolia*, *R. mucronata* and *Shilajit*) showed anti-HIV potential with 3 different mechanisms

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Table 1: Summary of commonly used *In vitro* Anti-HIV Assays.

Target	Assays
HIV-1 Replication	MTT
	XTT
	p24 expression
gp120/CD Interaction	gp120 ELISA
Reverse Transcriptase Enzyme	Radio-active method
	Non-radioactive ELISA
	RNase H activity
Integrase Enzyme	Radio-active method
	Non-radioactive ELISA
Protease Enzyme	Flourometric method
	HPLC-based method
	Pepsin assay (indirect method)

of action, viz., inhibition of HIV-Reverse Transcriptase enzyme, interference with gp120/CD4 interaction and probable HIV-protease inhibitory activity [14,15] (Table 1).

Successful Candidates

Several review articles have revealed the inhibitory potential of a variety of natural products like plants, microorganisms, marine organisms, minerals to name a few, in the form of crude extracts, as well as isolated compounds against different stages of HIV life cycle [70-76]. Over 60,000 extracts from natural sources have been evaluated against HIV-1, the most important result of which is the class of compounds known as calanolides. Particularly, (+)-calanolide A (NSC 650886), (-)-calanolide B (NSC 661122; costatolide) and (-)-dihydrocalanolide B (NSC 661123; Dihydrocostatolide) extracted from fruits and twigs of *Callophyllum lanigerum.* All three calanolides inhibited the laboratory adapted HIV-1 variants, the clinical viral isolates inclusive of diverse clades (A-F), syncytium inducing and non-syncytium inducing isolates and T-tropic and monocyte-tropic isolates. Furthermore, costatolide exhibited synergy with nucleoside RT, non-nucleoside RT and protease inhibitors. The National Cancer Institute (NCI) has played active and supportive role in the development of calanolide class of compounds including aspects of preclinical development, such as synthesis of analogues, *in vivo* animal assays, formulation, pharmacology and toxicology [77,78]. **Calanolide A**, is found to be a novel, naturally occurring, non-nucleoside reverse transcriptase inhibitor (NNRTI) with potent activity against HIV-1 [79]. It is licensed and evaluated to phase II clinical trials by Sarawak Medichem pharmaceuticals [80].

Likewise, in 2010, phase I human clinical trials of **Prostratin** isolated from *Homalanthus nutans* were carried out by the AIDS ReSearch Alliance in Los Angeles, California [81].

Bevirimat (PA-457), extracted from a Chinese herb *Syzygium claviflorum* is in phase IIb clinical trials by Panacos Pharmaceuticals and is believed to inhibit the final step of HIV Gag protein processing [82].

Besides, modified isomer of pyrocoumarin isolated from *Lomatium suksdorfii*, **3-hydroxymethyl-4-methyl DCK (PA-334B)**, inhibited both clinical and drug-resistant HIV-1 isolates. It is orally bioavailable and preclinical studies revealed minimal toxicities. Panacos Pharmaceuticals has nearly completed the required preclinical studies for IND filing [80].

Conocurvone isolated from *Conospermum incurvum* showed potent anti-HIV activity by a novel mechanism. Conocurvone added 48h after infection, protected T-cells from cytopathogenic effect of HIV-1. It has been under development by the Australian company, AMRAD [80].

These clinical candidates have the potential to come up as drugs for treatment of HIV infection.

Conclusion

Natural products are a proven source of novel anti-HIV compounds and discovery of promising bioactive molecules involves collaborative work of microbiologists, medicinal and synthetic chemists, pharmacologists and toxicologists. However, the recent establishment of Calanolide A as NNRTI gives a boost to invention of many such potential candidates.

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