Chapter 2
Review of Literature
2. LITERATURE REVIEW

2.1. HUMAN IMMUNO DEFICIENCY VIRUS (HIV)

HIV, the aetiological agent of AIDS, belongs to the Lentivirus subgroup of the family Retrovirideae. HIV is an enveloped single stranded RNA virus with a genome consisting of dimer of identical RNA molecules. HIV is the most complex retrovirus studied so far with at least 9 known genes. Long terminal repeats LTRs flank both the sides of these genes. The LTR consists of three regions namely U5, R and U3 and initiates the expression of viral genes in conjunction with RNA polymerase II and auxiliary transcription factors.

2.1.1. STRUCTURE OF HIV

A lipid membrane surrounds the virus. Viral proteins are encoded by three genes that, respectively, encode the capsid protein, the replicative enzymes, and the envelope protein. (Cheesebrough, 1990)

Envelope

It is a lipid bi layer, which is derived from the host cell membrane and is lined by matrix protein layer from the inner side and envelope protein organized into spikes on the outer side. Embedded in the envelope is the viral encoded glycoprotein (gp) gp41. Bound to this is the outer glycoprotein knob gp120. The envelope protein is made as a single large polypeptide and modified by cellular enzymes. The association of the envelope protein with the core occurs as the virus buds from the cell surface.

Capsid

Like other retroviruses, HIV virion contains a virus capsid, which consists of protein p17. The capsid shows icosahedral symmetry. It surrounds the core of the virus.
The capsid proteins are made as a single polypeptide chain and is cleaved late in virus maturation by a viral protease.

Core

It is an elongated dense mass of virus which consists of two identical single strands of viral RNA, structural proteins, the enzyme reverse transcriptase and other enzymes. The main core protein is p25 (p24, p26).

2.1.2. **HIV GENOME**

Fig. 2.1.1: Schematic diagram of an HIV virion

![Schematic diagram of an HIV virion](image)

**Structural genes of HIV**

The genome of HIV consists of a core of genomic RNA. Three genes encode for the structure of HIV

- env - (envelope) gene encodes for glycoprotein gp120 and gp41. Precursor product of the envelope is gp160, which is formed in infected cells.
- **gag** - (group associated antigen) gene which encodes for the proteins that determine the structure of the core and helps in the assembly of the virion in the membrane of host cells. These genes encode p24, p7 and p9 that bind to RNA molecule. p17 is also encoded by this gene and forms the capsid, p55 is the precursor for all these proteins.

- **pol** - (polymerase) gene encodes for viral enzymes Reverse Transcriptase / ribonuclease p61 and p52, protease p10 (p51) and endonuclease p31(p34). p160 is the precursor of these enzymes and is present in intact virus particles.

**Regulatory genes of HIV**

In addition to the three structural genes found in all retroviruses, HIV possesses other genes that regulate the assembly of viral proteins and control infectivity, viral replication, and latency.

- **tat** (trans activator) gene, which is detected mainly in the nucleus of infected cells and is essential for viral replication.

- **rev** (regulatory) gene, involved in regulating the production of viral proteins.

- **vif** (virion infectivity factor) gene, necessary to produce infectious particles.

- **nef** (negative -regulatory factor) gene, slows down the transcription of the viral genome and may therefore be responsible for HIV remaining dormant in infected cells.

- In addition to the above said genes, HIV-1 codes for vpu, which is not present in HIV-2, and HIV-2 codes for vpx not found in HIV-1.
2.1.3. **DYNAMICS OF HIV INFECTION**

Infection of the human immunodeficiency virus type-1, (HIV-1) results in the progressive degeneration of the immune system. Three patterns of virus-host interaction have been identified.

The first phase of cell free viremia is followed by a period of high level of viral antibody and cytotoxic T cell response to the antigen, during which the antigen is undetectable. This state may persist for years. The second phase is that of continuous proliferation of the virus with a decrease in the number of total T cells, this condition persists till death of the individual. (Cheesbrough, 1990)

HIV may infect any cell bearing CD4 antigen receptor. The helper T cells, monocytes and macrophages are the blood cells to be infected first, followed by dendritic macrophages, epithelial cells and renal cells. Infection of replicating CD4 cells results in rapid cell killing, whereas infection of resting T cells results in latent infection. Infection of the monocytes results in low but persistent levels of virus replication, whereas infection of macrophages results in low levels of production of viruses that are not released from the infected cell (Haseltine, 1988)

In another type of cells namely the cells of monocytes – macrophage lineage, the circulating monocytes do not show a significant level of infection as detected by DNA PCR. These macrophages seem to be the major reservoirs of the virus as they are not susceptible to the virus induced cytopathic effects. Their main function is antigen presentation to T cells and in the course they transmit the virus to the activated CD4+ cells.

The major site of viral replication is the peripheral lymphoid organ mainly the lymph nodes and spleen. Some of the viruses produced in the lymph nodes is trapped on specialised cells known as follicular dendritic cells (FDC) which are located in the germinal centres of the secondary lymphoid organs. The FDC binds and presents
been elucidated (Munrova, 1990).

In spite of the enormous amount of work carried out to study the molecular

as co receptors for HIV and SIV enzy(Camills et al., 1998)

addition to the above said receptors, some additional receptors have been shown to serve

the X4 viruses. Viruses which can use both kind of receptors are called R5X4.In

liges, and macrophages. These viruses use only the CCR5 receptors and are called

HI-V I. T helper strains replicate in CD4+ T cells, established CD4+ T cell

receptors (Camills et al., 1998)

vines are classified as R5. All subspecies of HIV-1, HIV-2 and SIV use CCR5 co-

macrophages and CD4+ T cells and use C1 chemoattractant receptors CCR5.These HIV-1

coupled chemokine receptor. Macrophage (M-HoProp) strains of HIV-1 replicate in the

was related to interaction of the virus envelope glycoproteins

HIV infection is initiated by interaction of the virus envelope glycoproteins

CHeXokine receptors and HIV entry

half-life is about 6 hours (Carpenter, 1997).

10 billion HIV particles are produced and destroyed each day and the plasma virus

characterized by a high rate of viral immunity. Current estimates suggest that at least

elements in HIV pathogenesis is the high level of productive infection which is

elements in HIV pathogenesis involving immunological based virus-host interactive mechanisms. The key

The most intriguing area of HIV diseases is the complex picture of HIV

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214. HIV PATHOGENESIS

disrupted leading to increase in concentration of plasma virus (Pirtz et al., 1998)

antigens to B-lymphotropics. In later stages of HIV infection, the FDC network is
Cellular tropism of virus is defined based on the chemokine receptors to which gp120 of the virus binds. Most patient isolates belong to the M-tropic strain which can infect both macrophages and T cells and can use either CCR-5 or CXCR-4 co-receptor, whereas the lab adapted strains grown for many passages on T-cell lines can use only CXCR4 co receptor (Gallo et al., 1999).

The infection of CD4+T cells by HIV-1 results in the presence of virus in the plasma. It is found that T cell tropic X4 HIV-1 isolates can infect CD4+ T cells as soon as they emerge from the thymus; Some of these infected cells after undergoing blast transformation, enter the G0 phase and become the memory cells. These resting cells, which have unintegrated HIV DNA, may act as latent reservoir for the virus. It has a long life span and can carry the provirus, which can be reactivated at a later stage by antigenic activation. The X4 strain can infect all the stages of T cells i.e. from the naïve T cells to the resting cells (Finzi et al., 1998)

The Macrophage tropic (R5) HIV-1 isolates can infect only the activated CD4+ T cells and may not infect the resting cells. These activated CD4+ T cells are highly permissible for replication and produce virus at a high rate. Thus die quickly either due to the cytopathic effects of the virus or due to host cytolytic effector mechanism (Finzi et al., 1998)

** ADSORPTION, PENETRATION, AND UNCOATING**

After binding of virus gp120 to the CD4 antigen present on the cell surface, the core of the virus consisting of RNA, core protein and enzymes enter the host cell (Wang et al., 1999). The single-stranded viral RNA is then converted to double-stranded DNA, called the provirus. This process requires polymerase and the ribonuclease enzymes present within the capsid. The DNA migrates to the nucleus and gag coded integrase helps it to integrate into the host cellular DNA thus ensuring
permanent infection. The provirus can multiply along with the host cell using host machinery.

**REVERSE TRANSCRIPTION.**

Viral genomic RNA acts as template for the synthesis of complementary single stranded DNA copies. This reaction is catalysed by viral encoded reverse transcriptase enzyme using a host transfer RNA as primer.

**POST-TRANSCRIPTIONAL PROCESSING, ASSEMBLY AND RELEASE**

Retroviral mRNAs are translated into precursor polypeptides, which are further cleaved by viral protease and processed to form various structural and functional proteins. The matured virions are then released from the infected cells by a process of budding. (Hirsch, 1985).

**2.1.5. PATTERNS OF HIV INFECTION:**

Depending on the region and the type of individuals infected, four patterns have been described so far (Cheesebrough, 1990).

Pattern I

In North America, Western Europe, Australia and New Zealand, about 80-90% of HIV infections occur in homosexual and bisexual men or in intravenous drug users. Heterosexual spread is rare but increasing. These areas are called Pattern I region.

Pattern II

Heterosexual transmission, with a consequent high level of maternal-child transmission, is the predominant mode of spread in sub-Saharan Africa and parts of the
Caribbean. The ratio of infected males to infected females is approximately equal. This pattern of infection is referred to as Pattern II.

Pattern I/II

A mixture of Pattern I and Pattern II is found in the Caribbean, Central America and South America.

Pattern III

In those countries where there is so far little HIV infection other than that imported by foreigners or brought back from abroad, the pattern is referred to as Pattern III. These areas are characterized epidemiologically by onset of the HIV/AIDS pandemic from mid to late 1980s.

2.1.6. CLINICAL FEATURES OF HIV INFECTION

Centre for disease control (CDC) has proposed the following classification for people with HIV infection:

**Group I:** Seroconversion illness: This is the phase of cell free viremia. Soon after infection person gets glandular fever-like illness with sore throat, skin rash and enlarged lymph glands. Within a few days the illness passes off and the person become asymptomatic.

**Group II:** Asymptomatic: Most people remain asymptomatic after acquiring HIV and seroconverting. They remain well for many years but are infectious. This is the period where level of viral antibody is high with high cytotoxic response to antigen but the antigen as such is undetectable.
Group III: Persistent generalized lymphadenopathy: Many infected people, although otherwise well, develop generalised swelling of their lymph nodes in the neck and under the arm. The lymph nodes are usually 1-2 cm in diameter, mobile and not tender.

Group IV: Symptomatic HIV disease: After a variable period most people in group I to III develop symptoms and signs due to HIV or its consequences and are then classified as belonging to Group IV. In this phase there is continuous proliferation of virus with a decrease in number of total T cells. This condition persists till death.

2.2. ANTIVIRAL CHEMOTHERAPY

In the quest for development of antiviral chemotherapeutic agents against Human Immuno Deficiency virus, the first agent to enter clinical trials and receive approval were Nucleoside analogues, which target reverse transcriptase. In general, reverse transcriptase inhibitors have only moderate clinical efficacy when administered in monotherapy regimens. Their use is further limited by adverse side effects (Ventura, 1999). The second class of reverse transcriptase inhibitors are non-nucleoside reverse transcriptase inhibitors, NNRTIs. A rapid emergence of resistance has limited their clinical efficacy. An alternate target is HIV protease an enzyme required for the terminal maturation of the viral polyprotein into structural and functional proteins (Huff, 1991).

There have been several studies on the viral dynamics and the decay of viral – plasma load during different stages of antiviral chemotherapy. Finzi et al.,(1998) have described three stages in the decay of the viral load in plasma.

- Stage I: In this stage there is an exponential drop in the levels of viral plasma with the decrease being 100 fold within two weeks of chemotherapy. There are two factors which result in decrease in viral load in plasma, the first being that recently infected cells are responsible for majority of plasma virus and both the virus and the naïve cells do not survive long. The second fact being that RT Inhibitors and
Protease inhibitors save the susceptible cells from infection without having any effect on the cells which have already been infected. Thus the initial rapid fall in plasma viral load is due to the prevention of new infection of susceptible cells, the decay of cellular and extra cellular compartments that harbour the virus and the redistribution of CD4+ T cells from the secondary lymphoid organs.

- Stage II: The next phase of viral decay is much slower than the first phase and is observed after 2-3 weeks of chemotherapy. In this stage the viral load falls below the detectable range of current assays (20-500 copies/ml). The anti retroviral drugs may act upon the virions trapped on FDC and also on the virus that infect the macrophages since macrophages have a slower turnover than the CD4+ lymphoblasts.

- Stage III: This phase of HAART therapy deals with the virus, which latently infect resting memory cells. These cells comprise of less than $10^7$ cells and it is possible to postulate that the enhancement of immune response may be able to control the virus originating from these reservoirs.

2.3. **REVERSE TRANSCRIPTASE (RT)**

Reverse Transcriptase (RT) was discovered in 1970 when DNA dependent RNA synthesis in RNA tumour virus was being investigated. It is a DNA polymerase that synthesises double stranded DNA using a single stranded RNA as template. This enzyme is coded by the *pol* gene. It is first produced in the virus infected cells as a *Gag-Pol* fusion polyprotein resulting from a ribosomal frame shift. This polyprotein undergoes a cleavage by viral protease to form a heterodimer of 15,000 and 66,000 dalton molecular weight subunits. It is a multifunctional enzyme possessing:

- RNA directed DNA polymerase (RDDP) activity.
• Ribonuclease H (RNase H), which resides in the larger peptide subunit of 15,000 dalton (p66). RNase H specifically degrades the RNA template of the RNA-DNA hybrid.

• DNA directed DNA polymerase (DDDP) activities.

The synthesis of second DNA strand is also catalysed by viral reverse transcriptase. (Mitsuya, 1990). The inhibition of Reverse transcriptase enzyme arrests the retro transcription of genomic RNA, an essential step for replication of the virus. Reverse transcription generally takes place in the cytoplasm of infected cells, although it has also been demonstrated that retroviruses can initiate reverse transcription prior to infection of target cells. (Ventura, 1999)

The activity of RT may be measured by quantifying one of the multi functions of the enzyme, i.e. RDDP, DDDP or RNase H activity. The majority of assays used for determining enzyme activity measure the incorporation of labelled nucleotides into the newly synthesised DNA, thus quantifying the polymerase activity. (Matthee et al., 1999)

2.3.1 INHIBITORS OF RT

The fundamental role played by reverse Transcriptase (RT) in the replication of retroviruses has made this enzyme a key target in the chemotherapy of HIV infection, especially in the prophylaxis and intervention of AIDS. Antimony tungsten (HPA23) was the first clinically effective HIV-RT inhibitor. Subsequently a number of synthetic analogues have been studied for this purpose, AZT (3’-azido-3’-dideoxy-thymidine), DDC (2’3’-dideoxyctydine) and DDI (2’, 3’-dideoxyinosine) being a few of them. Vanadyl sulfate or polyguanic acid have been found to inhibit RNase considerably but are very toxic. In addition to a number of synthetic analogues, a number of Tannins, flavanoids and alkaloids have been reported as HIV-RT inhibitors (Mekkawy, 1995).
Inhibitors of RT can be divided into 2 groups: Nucleoside analogs (NA) and Non nucleoside reverse Transcriptase inhibitors (NNRTIs). Nucleoside analogues inhibit DNA synthesis by acting as chain terminators once incorporated into DNA. To be active, they must be phosphorylated to their 5'-triphosphate form by the cellular kinases. NNRTIs bind to a hydrophobic pocket near the catalytic site of RT. These two categories of compounds, while different in their way of inhibition must have the ability to reach the replication complex (Ventura et al., 1999).

Nucleoside RT inhibitors (NRTI) were the first anti-HIV agents broadly employed clinically. Currently five NRTIs have been approved by the US food and Drug administration, they are

3TC(Lamivudine),

Zalbactine,

Zidovudine or AZT,

ddI (Dideoxyinosine) and

d4T (Stavudine).

NRTIs are prodrugs and function as competitive inhibitors and chain terminators.

Two non-nucleoside reverse Transcriptase inhibitor NNRTIs have been approved by FDA-Nevirapine (Viramune) and Delvaridine. A natural product Calanolide A, a coumarin NNRTI has been isolated from the antiviral extract of Calophyllum lanigerum and is under the Phase I clinical trial. (Mattee et al., 1999)
2.4. **HIV-1 PROTEASE**

The genome of HIV encodes viral structural proteins and replicative enzymes that are translated as either polyprotein precursor Pr55 Gag or the ribosomal frame shift product Pr160 Gag-Pol. These polyproteins are proteolytically processed by a virus encoded aspartyl protease. This aspartyl protease is a 99 amino acid protein encoded by the 5' portion or retroviral pol, and is translated initially as part of 160,000 molecular weight polyprotein precursor. It auto catalytically processes itself by a poorly understood mechanism. (Winslow, 1995).

HIV-1 protease is a complex enzyme composed of 2 identical halves, or dimers with an active site located at the base of the cleft created by the dimerization process. Large HIV 1 derived polyproteins are inserted into this cleft and undergo a chemical reaction resulting in the cleavage. In 1988, the HIV 1 Protease enzyme was crystallized and its 3-Dimensional structure determined (Deeks, 1997).

Important structural characteristics of the homodimeric form of the enzyme include a substrate binding region, a bridging water molecule extending from the flap region, and a catalytic site aspartic acid residue.

Five non-contagious regions of protease are highly conserved across virtually all clinical and laboratory isolates of HIV 1

- Amino acids 1-9 (amino terminal and S3 sub sites of the substrate binding region),
- 21-32 (sequence around the catalytic site:nd S2 sub site),
- 47-56 (flap region), 78-88 (S1 and S2 sub sites of the substrate binding region) and
- 94 – 99 (carboxyl terminal and dimerization region).
Although HIV-2 and SIV vary by as much as 50% from HIV-1 at the amino acid level, most of the non conservative amino acid replacement occur well outside the catalytic cleft. Three of the 9 cleavage sites catalysed by HIV protease lie between the phenylalanine and proline bonds (Phe-Pro).

2.4.1. **INHIBITORS OF PROTEASE**

The alarming spread of Human Immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS) has initiated an urgent pursuit to comprehend and control this disease. Advances in molecular virology, viral and cell biology have defined numerous targets for potential drug intervention. To date, numerous HIV-PR inhibitors have been reported, but few have been studied in humans because they lack acceptable oral bioavailability. (.Bruce et al., 1994) A series of HIV protease inhibitors possessing a hydroxylamine pentanamide transition state isostere have been developed.

Some of the cytopathic effects of HIV infection seen *in vitro* may be mediated by HIV Protease and can be assayed using a variety of substrates such as synthetic fluorescent labeled peptides or radiolabelled polyproteins. The inhibition of the enzymatic activity by protease inhibitors is expressed as \( k_i \) which is determined as the concentration of the inhibitor giving 50% inhibition of cleavage of either a synthetic peptide or a (35S) – methionine-labeled Gag polyprotein precursor substrate made by *in vitro* translation .Most serious candidate protease inhibitors need \( k_i \) values in the sub nanomolar range. Active inhibitors should not have activity against normal aspartyl proteases such as rennin, pepsin and cathepsin. Majority of inhibitors under *in vitro* study are based on stabilised mimetics of the Phe-Pro bond , as endopeptidase catalysis of hydrolysis of the N- terminus of prolyl residue is not seen in human beings. In contrast to NNRT inhibitors, which are generally not active against HIV-2, most Protease inhibitors have good activity against HIV-2 (Winslow et al., 1995)

The FDA has approved three HIV-1 protease inhibitors for treatment of HIV infections
Saquinavir mesylate (Invirase),

Ritonavir (Norvir) and

Indinavir Sulfate (Crixivan).

The fourth drug, Nelfinavir is available through an expanded access programme.

Although these drugs are clearly effective in short-term studies, their long-term efficacy has not been reported. Moreover, each drug can cause significant adverse effects and result in important, clinically relevant interactions with other common medications. Finally, the inappropriate use of these drugs can lead to the rapid development of viral resistance (Deeks, 1997).

**Saquinavir:**

In its current formulation, it has limited oral bioavailability. In order to achieve adequate absorption, saquinavir must be taken with meals with high fat content. This dietary strategy for improved absorption doesn't overcome the large first pass effect in the liver, where saquinavir undergoes extensive metabolism by hepatic cytochrome P450 3A system. The current FDA approved dose is 1800mg/day. Drugs that induce cytochrome P450 activity such as rifampin and rifabutin reduce the bioavailability of saquinavir and should be avoided. Finally Saquinavir may inhibit the metabolism of terfenadine (Seldane), astemizole (Hismanal) and Cisapride (Proputed) leading to increased plasma concentration of these drugs and potentially serious cardiac arrhythmias. Saquinavir is best tolerated of the compounds, but in its current formulation, has limited bioavailability and the least effect on HIV RNA and CD4+ cell levels.
Ritonavir:

Ritonavir is also a potent protease inhibitor and a high plasma drug concentration can be achieved with oral formulation of 600mg twice daily. It is a potent Inhibitor of P450 3A system and its concurrent use can result in dramatically increased levels of Saquinavir. Ritonavir is potent with sufficient bioavailability, but has greatest rate of intolerance and is associated with a formidable list of drug interactions.

Indinavir:

Indinavir was approved by FDA in 1996. It seems to have a potent effect on CD4+ cell count and HIV RNA levels and metabolized by cytochrome P450 3A enzymes. It inhibits the metabolism of rifabutin; therefore, rifabutin dose should be reduced 50% when the drugs are used concurrently. Ketaconazole interferes with the metabolism of indinavir sulfate, thereby increasing indinavir concentration. The bioavailability of indinavir is reduced with most metals, so it should be taken in fasting state at least 2 hours after or 1 hour before meals. Indinavir has a profound & substantial effect on HIV RNA. Its bioavailability to limit disease progression and prolong life is not yet established.

Nelfinavir:

It is currently available through expanded access programme, and should be administered at a dose upto 750 mg daily with food. Nelfinavir should not be used with terfenadine, astemizole, or Cisapride, rifampin and rifabutin.

All the 4 agents reduce plasma HIV RNA levels and increase CD4+ cell levels. Saquinavir and Ritonavir are effective in delaying disease progression and prolonging life, at least when used in people with moderate to advanced HIV disease.
Table 2.4. HIV-1 protease is known to cleave eight amide bonds in precursor gag and gag-pol proteins as indicated: (Sakurai et al, 1993)

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<tbody>
<tr>
<td>1</td>
<td>P17 * p24</td>
<td>Ser-Gln-Asn-TYR* PRO-Ile-Val-Gln</td>
</tr>
<tr>
<td>2</td>
<td>P24*X</td>
<td>Ala-Arg-Val-LEU*ALA-Glu-Ala-Met</td>
</tr>
<tr>
<td>3</td>
<td>X* p7</td>
<td>Ala-Thr-Ile-MET * MET- Gln-Arg-Gly</td>
</tr>
<tr>
<td>4</td>
<td>P7 * p6</td>
<td>Pro-Gly-Asn-PHE * LEU-Gln-Ser-Arg</td>
</tr>
<tr>
<td>5</td>
<td>*PR</td>
<td>Ser-Phe-Asn-PHE* PRO-Gln-Ile-Thr</td>
</tr>
<tr>
<td>6</td>
<td>PR * RT</td>
<td>Thr-Leu-Asn-PHE * PRO-Ile-Ser-Pro</td>
</tr>
<tr>
<td>7</td>
<td>RT51 * RNase</td>
<td>Ala-Glu-Thr-PHE * TYR-Val-Asp-Gly</td>
</tr>
<tr>
<td>8</td>
<td>RT * IN</td>
<td>Arg-Lys-Ile-LEU * PHE-Leu-Asp-Gly</td>
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2.5 **ANTIRETROVIRAL REGIMEN**

The key element in HIV pathogenesis is the high level of productive infection, characterised by a high rate of virion turnover. Estimate suggests that at least 10 billion HIV particles are produced and destroyed each day with the plasma virus half life being about 6 hours. Studies of HIV DNA and RNA in lymphoid tissue provide direct evidence of moderate levels to high to level replication that is paralleled by detection of virus particles in plasma (Carpenter et al., 1997).

At present antiretroviral therapy is recommended to all patients when:

a) The plasma HIV RNA concentrations greater than 5000 to 10,000 copies / ml regardless of CD4+ cell count.

b) For all subjects with HIV infection and detectable plasma HIV RNA who request it and are committed to lifelong adherence to the necessary treatment.

c) For patients with symptomatic HIV disease or with CD4+ cell counts below $0.50 \times 10^9 / l$ (500 µl), particularly below $0.35 \times 10^9 / l$ (350 µl).
According to the International AIDS Society recommendation the preferred initial regimen is one that is most likely to reduce and maintain plasma HIV RNA levels below the level of detection (below 500 or 400 copies/ml) using the most sensitive assay available.

Table 2.5. Advantages and disadvantages of three-drug regimen

<table>
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<tr>
<th>Regimen</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>NRTI-1 and NRTI-2 and PI</td>
<td>This regimen should be able to achieve plasma HIV RNA levels below limit of detection in large majority of drug adherent patients</td>
<td>Strict adherence to the regimen is crucial; quality of life may be affected; durability of effect, Long-term tolerance, and overall clinical benefits in antiretroviral naïve patients with early disease is not fully defined.</td>
</tr>
<tr>
<td>NRTI-1 and NRTI-2 and NNRTI</td>
<td>Many patients taking this regimen achieve plasma HIV RNA level below limit of detection; it also permits deferral of a PI if this option is chosen</td>
<td>Strict adherence to this regimen is crucial; may not be as potent as a PI containing regimen; it is not recommended for patients with advanced disease (i.e., low CD4+ counts or high plasma viral load); durability of effect and overall clinical benefit not fully defined.</td>
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2.6. **IN - VITRO SUSCEPTIBILITY TESTING METHODS**

In order to test the efficacy of antiretroviral drugs several in-vitro tests have been standardised.

- p24-antigen based assay such as - AIDS Clinical Trials Group (ACTC)/Department of Defence peripheral blood mononuclear cell consensus assay. The antigen test detects the HIV core antigen p24.

- MT 4 cell based assay developed by Intercompany Consortium of AIDS Drug development. The detection of cytopathic effect of T cell tropic virus is the basis of the assay.

- PCR based assay to detect proviral DNA in lymphocytes.

- Indirect immunoflourescence assay: In this assay the HIV infected cells are fixed on special microscopic slides and it is incubated with anti HIV antibody. Anti-human IgG labelled fluorescien isothiocyanate is used to detect the fluorescence pattern.

Other assays include:

- MT 2 cell based, high throughput RNA hybridisation assay.

- HIV yield reduction assay.

- HeLa-CD4 plaque assay should probably not used to evaluate *in vitro* antiviral activity of protease inhibitors since poor penetration of many HIV protease inhibitors into these epithelial cells may result in an underestimate of antiviral activity (Winslow, 1995).

In addition to the above-mentioned assays enormous amount of work is being carried out to develop newer means of detection of the virus and its effect *in vitro*. 
Pauwels et al., (1988) evolved a tetrazolium based colorimetric assay for detection of anti HIV compounds. The MT4 cell line was infected with HTLV-III\textsubscript{B} and incubated with different concentration of compounds dissolved in RPMI media. Five days after infection the viability of mock and infected cells were examined microscopically, or spectrophotometrically by MTT method at 540nm and 690nm. The 50% effective dose i.e. dose achieving 50% protection or ED\textsubscript{50} is determined. This method gave comparable results as shown by trypan blue exclusion method.

Kazero et al., (1992) developed a colorimetric assay for HIV-1 RT detection using a double labelled (biotin and digoxigenin) deoxyuridine triphosphate mixture. H9 cells were infected with HTLV-III B strain, the supernatent was removed on 5\textsuperscript{th} day and incubated with colorimetric mixture containing biotin - digoxigenin-dUTP. Alkaline phosphatase conjugated digoxigenin antibodies were used to detect the presence of RT in the reaction mix by performing ELISA. This method gives results comparable to standard Isotopic RT assay.

Moussazadeh et al., (1998) developed an HIV host infection system for testing various anti retroviral compounds \textit{in vitro}. In this system a non infectious mutant strain of HIV-1 combined with He La cell line was used. This system can be used to express CD4, Tat and Rev regulatory proteins. The cells were infected with HIV-1\textsuperscript{ΔTat/Rev} and grown in MEM containing various dilutions of the test compound. The supernatent was removed at specific time intervals and tested for RT activity. This model provides a safe system for testing of antiretroviral compounds.

In addition to the active principle various external factors play a role in the antiviral activity of a compound which could lead to discrepancy in the activity of the same compound in different laboratories. Hudson et al., (1993) investigated the role of some external factors responsible for the activity of some antiviral compounds. Light has been shown to have a major role in the activity of hypercin, an anthroquinone said to have antiviral activity. In this study hypercin extracted
from aerial parts of *Hypericum perforatum* was pre incubated with the virus in the presence and absence of light and then added to the cell lines to observe viral inhibitory effect of the compound. HIV antigen ELISA was employed to detect the presence of p24 antigen in the culture supernatant. The duration of exposure also had a remarkable influence in the antiviral potential of the compound. When exposed to light for 10 minutes the antiviral activity was not seen. An increase in the time of exposure upto 30 minutes caused a significant antiviral effect leading to total inactivation of the virus after 60 minutes of exposure of the compound to light. The cytopathic effect on the cell line was recorded. The study concluded that in the presence of light more than 75% antiviral activity was seen in 0.1μ g/ml of the compound whereas in the absence of light 0.1μg/ml of the compound could induce no antiviral effect. The study further demonstrated that the compound was non toxic at a concentration of 1μ g/ml irrespective of the presence or absence of light and clearly cytotoxic at a concentration of 10μ g/ml.

Pepstatin has been recognized as a peptide analog inhibitor of HIV-1 Protease, which binds to the enzyme with an affinity in the low micromolar range. Thyagi *et al.*, (1991) report the mechanism of action as being purely competitive with a Ki in the submicromolar range. This transition state analog appears to possess a structure optimal for interaction with active site residues in HIV protease.

### 2.7. DRUG RESISTANCE

The development of resistance and the subsequent loss of drug activity constitute the primary barrier to long-term efficacious use of anti retroviral drugs. Current therapy for HIV infection involves monotherapy or combination with the available HIV inhibitors. Despite modest improvement in survival and delaying clinical disease, progressive therapy with these agents invariably fails, due to selection of viral variants with reduced susceptibility.
HIV is characterised by a high turnover rate and poor Reverse transcriptase fidelity. Both these characters may lead to mutation. These mutations may be fatal to the virus, have no effect, or confer either decreased or increased susceptibility to anti-retroviral agents. In addition, to the above said factors resistance to RT inhibitors may be established even before the start of therapy (Kakuda, 1998).

Studies on the nucleoside and non nucleoside inhibitors show that there exists a temporal relationship between appearance of resistant strains virological and immunological failure. Mutations as the basis of resistance have been shown for all the antiretroviral drugs. The most common mutations underlying AZT resistance include RT M41L, D67N, K70R, and T215Y. Mutations conferring resistance to didanosine (ddI), zidovudine (ddC), thiacytidine (3TC) have also been observed. AZT resistance develops slower than seen for other antiretroviral drugs like nevirapine and the explanation given for this slow emergence of resistance is the fact that some drug resistant mutations may result in impairment of viral replication itself. (Aquila, 1995). A mutation on the RT codon 74 leading to resistance for ddI has been shown to have no risk of progression on ddI therapy.

Mutations within the protease gene conferring resistance have been described for each of the four currently available, protease Inhibitors. Amino acid substitution at codons 48 or 90, or both confers resistance to saquinavir. Mutations at codons 32, 46, 71, 82, 84 confer resistance to indinavir. Ritonavir resistance requires an initial mutation at V82. Nelfinavir resistance requires critical mutation occurring at codon 30. Resistance to Indinavir and ritonavir require multiple mutation., with mutation at codon 82 appearing to be critical with both compounds, but not sufficient enough to develop resistance. Cross-class resistance required a minimum of 4 substitutions, the best characterised involving locations 46, 63, 82 and 84. Viral resistance to indinavir is seen to persist even after withdrawal of therapy (Deeks et al., 1997).

Viral resistance more frequent when HIV is exposed to sub therapeutic levels of PRI, thus allowing for ongoing HIV replication in the presence of selective
pressure. Genotypic analysis of HIV from subjects treated with sub therapeutic doses of indinavir and ritonavir revealed multiple mutations in the gene for protease and subsequently decreased sensitivity of HIV isolates to these agents. In the presence of high concentration of Indinavir and Retinovir the emergence of resistance is dramatically reduced. (Moyle et al., 1996)

Resistance during PI monotherapy develops in some patients even when standard doses of drug are used. When PI is administered in combination with one or 2 nucleoside analogues, development of mutation conferring resistance is reduced (Deeks, 1997).

Mutations generally lie in the pol gene, which encodes for protease enzyme leading to a two amino acid substitution, one in each of the monomers of the dimeric enzyme. These mutations are clustered around the active site of the enzyme (Moyle et al., 1996).

The “gold standard” genotypic assay for HIV-1 antiretroviral resistance is DNA sequencing of relevant HIV gene. In addition to this assay, a selective PCR for detection of mutation to predict clinical progression has been suggested. Other assays to detect resistance mutation are oligonucleotide – specific hybridisation of DNA and RNase A digestion pattern.

The different strategies being adopted to minimize emergence of drug resistance are

1. Combination therapy with more than one type of drug to inhibit different phases of viral life cycle.
2. Keep virus load continuously suppressed by minimizing ongoing dynamism of HIV replication events.
3. Keep changing combination regimens every few months thus not allowing the drug resistant strain from dominating the viral population.
There have also been reports of the clinical strain becoming resistant to chemokine at doses well above those, which could be inhibitory to the laboratory strains. It was observed that viral isolates obtained during the asymptomatic stages generally used only CCRS as a co-receptor and were inhibited by RANTES, MIP-1a and MIP-113, but not by SDF-1. By contrast, the majority of the isolates derived after the progression of the disease were resistant to C-C chemokine, having acquired the ability to use CXCR4, while gradually losing CCRS. Most of these isolates were also insensitive to SDF-1, even when used in combination with RANTES. An early acquisition of CXCR4 usage predicted a poor prognosis. In children who progressed to AIDS without a shift to CXCR4 usage, all the sequential isolates were CCRS-dependent but showed a reduced sensitivity to C-C chemokine. Discrete changes in the V3 domain of gp 120 were associated with the loss of sensitivity to C-C chemokine and the shift in co-receptor usage. These results suggest an adaptive evolution of HIV-1 in vivo, leading to escape from the control of the antiviral C-C chemokine.(Greco et al., 1999)

2.8 DEFINITION AND SCOPE OF IMMUNOMODULATORS

An Immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate the components of the immune system including both innate and adaptive arms of the immune response.(Agarwal, 1999). The first quest for Immunomodulators started as the agent for treatment of cancer in which the discovery of cytokines has been a major stepping stone. Isoprinosine, an immunostimulant, with the ability to control the severity and duration of viral infections was the first one to get approval of the FDA.(Hadden, 1994). Cyclosporin is an immunosuppressent, which has been used, in recent times for prevention of graft rejection (Walsh et al., 1992).

Three classes of immunomodulators have been described so far:

**Immunosdjuvants:** Enhance the efficacy of vaccines and are thus given in conjunction with vaccines e.g Freund’s adjuvant.
**Immunostimulants:**

They support and enhance the defense mechanism of the body. They are non-specific in nature and in healthy individuals they promote the basal level of immune response, but in immunocompromised individuals they act as immunotherapeutic agents (Agarwal et al 1999) This class of immunomodulators has thus gained importance in recent times with the growing pandemic of HIV/AIDS and are administered in conjunction with the chemotherapeutic agents.

**Immunosuppressents:**

They are used to control the pathological effects of immune system as in the case of graft rejection.

**2.8.1. CYTOKINES**

The viral and host factors that under line infection with HIV-1 and subsequent progression to AIDS vary considerably. Accordingly, some individuals, termed long-term nonprogressors (LTNP), remain asymptomatic for >10yrs, whereas others develop AIDS shortly after infection. One host factor that may contribute to the time course of disease progression following infection with HIV-1 is the individuals pattern of cytokine production (Blasdall, 1999).

Infection with HIV-1 of individual CD4+ T cell is influenced by a variety of host factors such as state of cell activation, expression level of viral receptors, and secretion of antiviral proteins. CD8+ T cells are known to produce antiviral proteins such as β-chemokine (RANTES, MIP-1β and MIP-1β) that inhibit the entry of R5 HIV-1 into the cells by down regulating HIV-1 coreceptor, especially CCR5. The entry of X4 HIV-1 through the CXCR4 coreceptor is inhibited by another chemokine, stromal cell derived factor 1 (Maeda et al., 1999).
Maeda et al., (1999) have further shown that β-chemokines act as chemotactic factors for resting and activated lymphocytes and have an independent suppressive role against HIV-1 infection. They showed that the concentration of β-chemokines from the CD4+ T cell rich fraction was about six to eight fold higher than that from the CD8+ T cell rich fraction after 3 days of PHA stimulation whether these fractions were exposed to HIV-1 or not.

Saha et al (1999), have contradictory reports stating that CD4+ T cell clones from non progressor secreted high levels of β-chemokines, whereas AIDS patients produce little or no β-chemokines. Thus, β-chemokines production from CD4+ T cells may play an important role in the clinical progression of disease.

Macrophages and dendritic cells are derived from CD34+ stem cells. Wang et al., (1999) conducted a study on the susceptibility of dendritic cells and their role in HIV transmission to T cells during antigen presentation and also the role-played by β chemokine in the suppression of infection to these cells. The HIV -1 tropic IIIB and Rut strains were propagated in PBMCs in the presence of different cytokines and chemokines. The culture supernatent was used to examine the p24 antigen level by ELISA. This study demonstrated that during the differentiation of CD34+ cells to CD1a+DC and CD1a-CD14+ cells, these cells acquire both macrophage tropic as well as T cell line tropic viruses.

They further reported that in the presence of β chemokines the susceptibility of these cells to macrophage tropic virus (Ba-L) decreased but the infection by T cell tropic viruses namely IIIB and Rut strains of HIV-1 was enhanced. This study revealed that the external intervention might result in a selective pressure resulting in change in viral tropism.

Tumour Necrosis Factor (TNF) is an important factor in HIV disease pathogenesis. It can accelerate HIV replication in vitro and also stimulate the release of
other cytokines. These in turn can accelerate HIV replication in T lymphocytes and macrophages. Lew et al.,(2001) have shown that an increase in serum TNF levels among HIV-1 infected patients is associated with virological relapse and disease progression.

Several recombinant cytokines such as IL-2, IL15, IL12 have been investigated in the treatment of HIV infection. Mitsuyasu, (1999) state that after the reduction in the number of T cells infected by HIV is brought about by HAART there may be chances of stimulation of T cells using exogenous cytokines.

Various cytokines affect every step in the HIV life cycle and cytokine dysregulation may play an important role in HIV disease pathogenesis. In particular, the proinflammatory cytokine Tumour Necrosis factor TNF–α can accelerate HIV replication in vitro and stimulate HIV expression via activation of the cellular transcription factor nuclear factor (NF)-κβ. Enhanced expression and secretion of type 2 cytokines such as interleukine 4 (IL4) may be associated with long term HIV disease progression.

The antiviral effects of IFNs and other cytokines, on HIV replication were studied by Shapiro et al., (1999). The study indicated that IFN α / β inhibit HIV replication in PBL and T cell and monocyte cell lines and a synergistic inhibitory effects was observed between IFNγ and GM-CSF in monocytic cell lines .In addition TNFα reduced the susceptibility of the Human T cell line HUT -78 to HIV infection and a synergistic inhibitory effect was observed in combination with IFN γ.

In apparent contradiction to these results, Hober et al., (1999) suggest that TNFα may enhance replication of HIV invitro. This difference in TNFα susceptibility of HIV replication is probably explainable by varying responses to TNFα in different cell types used in the current studies, and once more emphasizes the complexity of cytokine-virus-cell interactions.
A shift from type 1 towards type 2 cytokine profile has been reported to be associated with long term HIV disease progression. Consistent with this, studies have shown a shift from type 2 to type 1 cytokine profile with successful HAART therapy (Lew et al., 2001). These findings support the important role of cytokines, particularly TNF in HIV infection and suggest that assessing the percentage of T cells spontaneously producing TNF and IL4 might be useful in monitoring responses to HAART.

**Antiviral activity mediated by cytokines**

HIV infection leads to major changes in the CD4 and CD8 cells and the expression of different antigens on these cells, some of the major changes are described in the table 2.8.1. (Mitsuyasu, 1999)

**Table 2.8.1: Changes in CD4 and CD8 cells after HIV infection**

<table>
<thead>
<tr>
<th>CD4 cells</th>
<th>CD8 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A T cell surface molecule present on TCR-positive class II MHC restricted T cells and on macrophages and is a coreceptor for HIV. Most CD4 cells are helper cells and some are expressed on Cytotoxic lymphocytes.)</td>
<td>(A T cell surface molecule present on TCR-positive class I MHC restricted T cells and on macrophages. CD8 binds to class I MHC molecules. Most of the CD8 cells are Cytotoxic Lymphocytes but some are expressed on Helper cells.)</td>
</tr>
<tr>
<td>Decrease in Total CD4 cells</td>
<td>Increase in Total CD8</td>
</tr>
<tr>
<td>Increase in CD45RO+cells</td>
<td>Increase in CD38+</td>
</tr>
<tr>
<td>Increase in CD38+</td>
<td>Increase in HLA DR+</td>
</tr>
<tr>
<td>Increase in HLA-DR+</td>
<td>Increase in CD45RO+/RA- cells</td>
</tr>
<tr>
<td>Decrease in CD45RA+</td>
<td>Increase in CD28- cells</td>
</tr>
<tr>
<td>Decrease in CD62L+</td>
<td>Decrease in CD45RA+</td>
</tr>
<tr>
<td></td>
<td>Decrease in CD45RO</td>
</tr>
<tr>
<td></td>
<td>Decrease in CD38- cells</td>
</tr>
<tr>
<td></td>
<td>Decrease in HLA-DR- cells</td>
</tr>
<tr>
<td></td>
<td>Decrease in Total CD8( In advanced disease)</td>
</tr>
</tbody>
</table>
2.8.1.1 Interleukin 4 (IL-4)

It was originally discovered through its action on B-lymphocytes. Cells of T lymphocyte origin, $T_H$ lymphocytes, probably exclusively produce it (Hannan, 1999). Mosmanns group have delineated 2 kinds of $T_H$ lymphocytes, a suppressor induced (cytotoxic) $T_H1$ subset and a helper induced (non-cytotoxic) $T_H2$ subset. These functionally distinct subsets have been shown to produce different ‘packages’ of cytokines. The production of IL 2, IFN $\gamma$ ($T_H1$) and that of IL4, IL5 ($T_H2$) was found to be mutually exclusive. However, there are difference in opinion as to whether the distinction in terms of cytokine production between $T_H1$ and $T_H2$ are watertight, and certainly in most human CD4+ $T_H$ clones IL2, IL4, IFN$\gamma$ are produced simultaneously. Qualitative and quantitative difference in cytokine production by different $T_H$ cell clones may reflect difference in methods of cultures and stimulation as much as phenotypic distinction.

IL4 has been shown to play a controversial role in the progression of AIDS. It has been reported that at an advanced stage of disease, a predominant T cell response with an elevated level of IL4 was seen in the presence of PHA activated peripheral blood obtained from HIV positive individuals produced higher level of IL4 whereas the presence of IL4 before or at the time of infection had a protective role and could delay disease progression (Husain et al., 1996).

Human IL4 has been cloned and deduced as a sequence shown to be ~50% homologous to murine IL4. The IL4 precursor polypeptide contains 24 amino acid N-terminal signal sequence and a 129 amino acid residue mature IL4 protein sequence. The latter has 3 disulphide bridges and 2 potential N-linked glycosylation sites. Human IL4 is not active in mouse cells and vice versa. The genes coding for IL 4, GM-csf and
Table 2.8.1.2: Different Interleukines with their activity (Jan Klein., 1991)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Synonym</th>
<th>Source</th>
<th>Activity induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL-1</td>
<td>Lymphocyte activating factor, LAF</td>
<td>Monocytes/macrophages</td>
<td>Proliferation or differentiation of B cells, lymphokine release from activated T cells, growth of Fibroblasts, synovial cells and endothelial cells, tissue catabolism, release of prostaglandin E2, collagenase, acute phase protein, fever, NK cell activation, neutrophils, macrophages, lymphocyte chemotaxis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thymocyte activating factor (TAF)</td>
<td>Dendritic cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cell activating factor (BAF)</td>
<td>Natural Killer cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endogenous pyrogen (EP)</td>
<td>B-cell line</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leucocyte endogenous mediator (LEM)</td>
<td>T-cell line</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteolysis inducing factor (PIF)</td>
<td>Endothelial cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catabolin</td>
<td>Epithelial cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mononuclear cell factor (MCF)</td>
<td>Fibroblasts</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Astrocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Keratocytes</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IL-2</td>
<td>T cell derived growth factor (TCGF), T cell- maturation/</td>
<td>Activated T lymphocytes.</td>
<td>Proliferation and differentiation of B cells, Growth of activated T cells and thymocytes, Lymphokine production by T cells, Cytotoxic T cell activity, NK cell activity, Lymphokine activated Killer cell activity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stimulating factor (TMF/TSF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Killer helper factor (KHF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T cell replacing factor (TRF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IL-3</td>
<td>Mast cell growth factor (MCGF)</td>
<td>T-lymphocytes</td>
<td>Survival, growth and differentiation of multipotent stem cells and monocyctic, granulocytic, erythroid and megakaryocytic progenitor cells, growth of mast cells, growth of pre B-cell lines.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-cell stimulating factor (PSF)</td>
<td>Activated T cell clones</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Burst-promoting activity (BPA)</td>
<td>Myelomonocytic cell lines</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemopoietic colony growth factor (HCGF)</td>
<td>Lectin stimulated PBL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erythroid colony stimulating factor (ECSF)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Megakaryocyte colony stimulating factor (Mcc-CSF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eosinophil colony stimulating factor (ECSF)\</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multiple colony stimulating factor (Multi-CSF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>IL-4</td>
<td>IgG1 inducing factor</td>
<td>Activated T lymphocyte</td>
<td>Proliferation of b cells, growth of T cells, foetal thymocytes, mast cells, cytotoxic T cells activity and proliferation of PMA-stimulated thymocytes, formation of giant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cell growth factor (BCGF-1)</td>
<td>Mast cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cell stimulating factor (BSF-P1)</td>
<td>Activated B cell lines</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cell stimulatory factor p1 (BCDFy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B cell differentiation factor (MSF)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|   |   | Macrophage fusion factor (MAF)  
|   |   | Macrophage activating factor (MAF γ)  
|   |   | multinucleated cells, activation of 
|   |   | haemopoietic progenitor cells,  
|   |   | increased Ag presenting ability.  
| 5 | IL-5 | T cell replacing factor (TRF)  
|   |   | B cell growth factor II (BCGF II)  
|   |   | Eosinophil differentiation factor (EDF)  
|   |   | Killer helper factor (KHF)  
|   |   | IgA-enhancing factor (IgA- EF)  
|   |   | Eosinophil colony stimulating factor (Eo-CSF)  
|   |   | T lymphocytes  
|   |   | Proliferation of B cells  
|   |   | Differentiation of Eosinophils  
|   |   | Cytotoxicity of thymocyte (with IL2)  
| 6 | IL-6 | Interferon β2 (IFN β2)  
|   |   | B cell differentiation factor (BCDF)  
|   |   | Hybridoma/plasmacytoma-1 (HP-1)  
|   |   | Plasmacytoma growth factor (PCT-GF)  
|   |   | Hybridoma growth factor (HGF)  
|   |   | B cell stimulatory growth factor-2 (BSF -2)  
|   |   | 26 KDA protein  
|   |   | Hybridoma /plasmacytoma growth factor 9H/PGF  
|   |   | B cell stimulatory factor p2 (BSF-p2)  
|   |   | T lymphocytes  
|   |   | Monocytes  
|   |   | Macrophage lines  
|   |   | Fibroblasts  
|   |   | Stromal cell lines  
|   |   | Certain tumour cells  
|   |   | Growth of plasmacytomas And  
|   |   | hybridomas  
|   |   | Production of acute phase  
|   |   | proteins By hepatoma cells  
|   |   | Proliferation of PMA and IL4  
|   |   | stimulated thymocytes  
|   |   | Increased Class I MHC expression  
|   |   | in fibroblasts  
| 7 | IFNγ | Macrophage activating factor (MAF)  
|   |   | Migration Inhibition factor (MIF)  
|   |   | T cell replacing factor (TRF)  
|   |   | T lymphocytes  
|   |   | killer cells  
|   |   | Decreased viral replication in cells,  
|   |   | decreased cell growth, increased  
|   |   | expression of class II & Fcγ R  
|   |   | molecules on macrophages, increase  
|   |   | class I molecule expression on endo  
|   |   | thelial cells and fibroblasts, Increased  
|   |   | NK cell activity, increased antimicrobial  
|   |   | and tumorocidal activity of  
|   |   | macrophages, enhanced tumour NF  
|   |   | and lymphtoxin activity.  

IFNγ share a similar 4 exon –3 intron organization and IL4 and GM-CSF genes are closely linked on human chromosome 5 (murine chromosome 11).

Receptors for pleiotropic IL4 molecule are widely distributed among lymphoid and myeloid cells such as B cells, T cells, macrophages, mast cells etc. IL4-Receptor has been shown to be totally specific for IL4 ie no other cytokine can bind to it. It has been reported that IFNγ inhibits IL4 mediated B-cell activation and production of IgE, although IFN γ does not interfere with IL4 binding to its receptor. (Meager, 1990)

2.8.1.2 Interferons

In addition to interleukins, Interferons are shown to have a role in HIV prognosis.

3 different types of Interferons have been classified they are:

- **IFNa** - Leucocyte IFN: It represents a group of IFNa subtype, which can inhibit cell multiplication, modulate both cellular and humoral immunity and induce antiviral state. It can suppress and also inhibit HIV replication. (Chehadeh et al., 1999) The mechanism underlying the IFN α / inhibition of HIV replication are not known. By comparison in cells infected with MMLV antiviral action of IFNs appears to be mediated by inhibition of viral assembly.

- **IFNb** - Fibroblast IFN

- **IFNg** - Type II (immune) IFN

**Interferon gamma (IFNg):**

Discovered in late 1960s when it was shown that lymphocytes can produce IFN species, IFNg, in response to stimulation with plant lectin such as PHA or Con A, which act as mitogens. Human IFNg contains 4 exons and 3 introns and is located...
on chromosome 12. Further research showed that IFNγ was mainly the product of T lymphocytes, and could be produced by T lymphocytes in response to their cognate antigens. In the later case, the action of lymphocytes requires binding of Ag presented by accessory cells, eg. Macrophages, in combination with MHC molecules, to the T-cell receptor. There is some evidence that monocytes/macrophages are involved in induction of IFNγ synthesis. Eg. monocyte/macrophages IL1 probably stimulates T cell IL2 synthesis and possibly IL2 in turn is required for IFNγ induction. Human IFNγ contains a high proportion of basic amino acid but no cystein residues, and therefore no interchain disulphide bridge is possible. (Meager, 1990)

The various techniques used to assess changes taking place in the functioning of the various immune cells are

- Skin testing for delayed type hypersensitivity to recall and neo-antigens.
- Assay of T cell function by detecting the ability to proliferate or produce cytokines in response to antigen or mitogens stimulation in vitro.
- Measurement of the phenotype of naïve and memory cells using flow cytometry.
- Study of T cell repertoire using monoclonal antibody and Polymerase Chain reaction.

2.9. IMPORTANCE OF MEDICINAL PLANTS IN HIV

Man since time immemorial, has been using herbs or plant products as medicine for developing immunity or resistance against common ailments. In addition, these herbs have been source for development of anticancer compounds and anti-viral interferons. An IFN stimulator (SN MC) derived from Glycrrhiza glabra has been reported to give protection to patients of sub acute hepatic failure (Gupta, 1998).
A number of research papers have been published in the study of natural products which can act against HIV.

Tan et al. (1991) tested 156 natural products which included Benzophenanthridine alkaloids such as fagaronine chloride, nitidine chloride, columbemine iodide, proberberine alkaloids, isoquinoline alkaloids and iridoid fulvoplumierin indolizidine, pyrrolizidine, quinolizidine and indole. Fagaroi~ine chloride and nitidine chloride, which are known inhibitors of AMV reverse transcriptase, were found to inhibit HIV-RT as well. Columbemine iodide, proberberine alkaloids, isoquinoline alkaloids and iridoid fulvoplumierin were also demonstrated to have HIV-1 RT inhibitory potential whereas Indolizidine, pyrrolizidine, quinolizidine and indole could not inhibit HIV RT. They also isolated fulvoplumierin, an active component from Plumeria rubra, which inhibited HIV RT at a concentration of 45 μ g/ml.

Lau et al. (1993) compared the inhibition of AMV and HIV RT by over 900 strains of lipophilic and hydrophilic extracts of blue green algae. None of the lipophilic extracts exhibited RT inhibition. In contrast, 18 aqueous extracts showed more than 50% inhibition of AMV RT, 7 of the extracts showed more than 75% inhibition which was comparable to AZT which was used as positive control. When tested for HIV RT inhibition the percentage inhibition exhibited by these extracts were seen to be greater than that for AMV RT. Eleven of the extracts showed more than 85% HIV RT inhibition. These extracts could not degrade transcript DNA, template RNA and enzyme and found to have none of these effects.

Extracts of some algae like Nostoc sp, Phormidium sp, Oscillatoria sp; have been shown to have both AMV and HIV RT inhibitory activities and are promising leads for the development of anti HIV compounds. Clavicoronic acid from...
Clavicorona pyxidata have shown some anti HIV potencies. Asterrriquinone isolated from Aspergillus terreus has shown HIV-1RT activity. Several pigments were isolated from Aspergillus terreus which show anti tumour activity. (Knubel et al., 1990)

Sahar et al., (1995) screened the methanolic and aqueous extracts of 41 Egyptian medicinal plants for HIV RT inhibitory potentials. They reported that the methanolic extract of Phyllanthus emblica showed specific inhibition of HIV RT in the presence of Bovine serum albumin. In a graded fractionation of the powdered fruit, the ethanolic as well as ethanol insoluble fraction were found to inhibit RT. They isolated 6 compounds from this fraction all of which showed RT inhibition, the first fraction being the most potent. A tannin like compound from extracts from fruits of Phyllanthus emblica called putrajivani A was shown to have HIV-RT inhibitory activity. Repandusininc acid A was obtained from P.niruri which also had significant RT inhibitory activity but this compound has been reported to have cytotoxic activity.

The extracts from Cestrararia islandica shows HIV-1 RT Inhibition but has a non-specific binding to the enzyme so can not become a potential candidate for drug development (Pengsuparp et al., 1995).

A coumarin secondary metabolite was isolated from Calophyllum inophyllum and is comparable with Calanolide A with respect to its RT Inhibitory potential. (Patel et al., 1996). Eight coumarins were isolated from the fruits and twigs Calophyllum lanigerum, of which Calanolides A and B were found to be most effective in cell based anti HIV assays. Calanolide A fully protected human T lymphoblastic cell from the cytopathogenic effects of HIV-1 at a concentration where no cytotoxicity was observed. Both the Calanoides act as non nucleoside HIV 1 RT inhibitors. Calalnoide A selectively inhibits HIV-1 RT and not HIV-2 RT and thus has potential usefulness as a drug. It is also found to have activity against the resistant strains of HIV (Galins et al., 1996). Dharmaratne et al., (1998) isolated pyranocoumarin derivatives Cordatolide
A and B from *Calophyllum cordata-oblongum* which showed HIV RT inhibition at IC$_{50}$ of 12.3 µM and 19 µM respectively.

Aqueous extracts of red algae *Liagora boergeseni*, *Porteria hornemanni*, *Champia parvula* and brown algae *Sargassum* inhibit replication of HIV. Peyssonol A, the active principle in *Peyssonnelia* sp, diterpenoids dictyodial and hydroxydictodial from brown algae of the genus *Dictyota* inhibited HIV RT. (Matthee *et al.*, 1999)

Although tannins show some HIV inhibition, it is observed that this inhibition is more so due to inhibition of cell adhesion than antiviral activity which is often accompanied by cytotoxicity. Thus the pursuit of RT inhibitors from tannins does not have particular significance (Matthee *et al.*, 1999).

Extracts from *Cephalis ipecacuana* were shown to have a higher activity against HIV-2 as compared to that of HIV-1. The quinoline alkaloid from extracts of flowers, leaves, twigs of *Euodia roxburghiana* was shown to have significant HIV-RT Inhibitory activity and cell based anti-viral activity. Thus the study of quinolone type compounds for HIV inhibitory activity is being carried out with vigour (Tan *et al.*, 1991).

Flavanoids also have been found to have some activities against the HIV-RT. A xanthone glycoside, swertifrancheaside isolated from *Swertia franchetiana* exhibited RT inhibition as well as DNA polymerase inhibition thus as the case of tannins, flavanoids too do not show any promise as potential candidates for drug development. (Wang *et al.*, 1994)

The first marine natural product found to inhibit HIV-RT were a group of quinone derivatives. Quinone derivatives from *Dysidea avara* namely avarol was shown to have significant HIV-1 RT inhibitory activity. They also inhibited RDDP activity of HIV -1 RT to 50 % at a concentration of 140 µM. RNase H activity was inhibited to more than 90% (Nakane *et al.*, 1990). Several compounds from *D. cinerea*
and *Smenospongia* *sp.* inhibited RNase H activity of HIV -1 RT with a high degree of selectivity and had very little effect on α DNA polymerase (Loya *et al.,* 1990). A hydroquinone isolate from marine sponge *Toxiclona toxius* strongly inhibited RDDP function of HIV RT. Certain other compounds derived from *Hippospongia* *sp.*, *Petrosia* *sp.*, *Verongia* *sp.* have shown to have RT Inhibitory potentials (Matthee *et al.*, 1999).

Chen *et al.*, (1995) identified mangostin and γ mangostin from the ethanolic extract of fresh fruit peel of *Garcinia mangostana* which was subjected to column chromatography on silica gel with chloroform/ethyl acetate (3:2). Both these extracts were found to inhibit HIV -1 Protease at IC$_{50}$ of 5.12±0.41 μM and 4.18±0.32 μM respectively. They used Pepstatin A was used as a positive control with an IC$_{50}$ of 76±5.4 nM.

Mahmood *et al.*, (1996) observed that aqueous and methanolic extracts of *Rosa damascena* exhibited moderate anti HIV activity. Out of the nine compounds isolated from the methanolic extract, the tetrahydroxyflavanone (kaempferol) inhibited the viral protease. The pentahydroxyflavanone (quercetin) and two derivatives of kaempferol inhibited HIV infection by inhibiting the binding of gp120 to CD4. 2-phenylethanol-O-(6-O-galloyl)-β-D-glucopyranoside neutralised viral infectivity by reacting with gp120.

Tomoaki *et al.*, (1996) screened some low molecular weight compounds for HIV-1 protease inhibitory activity and found a compound which could inhibit viral maturation as studied on Molt-4 cells at a concentration of 10μM. They also demonstrated that the compound did not decrease the cell viability. The compound acted by formation of a complex with the HIV-1 protease using the halogeno methylketone moiety.

Matsuse *et al.*, (1999) screened 39 Panamanian medicinal plants for the inhibition of HIV induced syncytia, HIV RT Inhibition and Inhibition of HIV protease using HTLV III$_B$ strain of virus and the cell viability was measured by trypan blue.
exclusion method. Out of the 39 plants tested, water extract of *Jatropha curcas* showed inhibition of virus induced cytopathic effect on MT4 cells at a concentration of 25μg/ml, water extract of *Erythroxylum citrifolium*, *Waltheria indica* and methanolic extract of *Xylopia frutescens* showed HIV-1 Protease Inhibition at IC₅₀ of 43.48 and 46 μg/ml respectively. HIV RT Inhibition was exhibited by water extract of *Chamaesye hyssopifolia*, *Cordia spinescens*, *Hyptis lantanifolia* and methanolic extract of *Tetrapiteris macrocarpa* with an IC₅₀ of 8.6, 7 and 8μg/ml respectively. The water extract of *Jatropha curcas* was subjected to fractionation by chromatography which yielded 5,7 dimethoxycoumarin and 6,7 dimethoxycoumarin with moderate inhibition of HIV-1 induced cytopathic effect but was highly toxic. Corilagin and 3-O-β-D-glucopyranoside derived from the extract of *Chamaesye hyssopifolia* showed HIV RT inhibition.

Baba *et al.*, (1988) studied the mechanism of inhibition of two sulfated polysaccharide dextran sulfate and heparin which have been reported as potent antiviral agents. The study indicated that the two compounds neither had an inhibitory activity on Reverse transcriptase nor could they neutralise the virus. This indicates that both these compounds inhibit the adsorption of the virus onto MT4 cells, thus protecting them even at a concentration of 25μg/ml with an IC₅₀ at 9.1μg/ml for dextran sulphate and 7.0μg/ml for heparin.

Nakashima *et al.*, (1992) screened 87 chemically defined tannins and related compounds for their anti HIV activity on MT4 cells using HIV-1 and HTLV IIIIB strains of virus using indirect immunofluorescence and laser flow cytometric analysis. Gemin D, Nobotanin B, Camelliin B and Trapanin B had a 50% inhibition of antigen expression as detected by immunofluorescence staining at 6.0, 1.8, 3.1 and 2.1μg/ml respectively and all the four compounds inhibited virus adsorption at a concentration of 4.0, 0.9, 1.0 and 1.3μg/ml.

Gollapudi *et al.*, (1995) isolated a polysaccharide (MAR 10) from the aqueous extract of *Hyssop officinalis*. The HIV-1 SF infected PBMCs was
incubated with varying concentration of MAR 10 and tested the supernatent for presence of p24 antigen by ELISA. HIV infected HUT 78 cells were incubated with various concentration of MAR 10 and the inhibition of syncytia formation was recorded. They also tested various concentrations of MAR 10 for lymphocyte proliferation of PHA activated PBMC by using FITC conjugated antibodies against CD3,CD4,CD8 and CD20 antigens. MAR 10 inhibited syncytia formation in HUT 78 T cells at the same time did not have any lymphocyte suppression or toxic effect on the lymphocyte subpopulation.

Konoshima et al., (1995) isolated two saponins from Gledistia japonica and (Gledistia saponin) and Gymnocladus chinensis (gymnocladus saponin). Gledistia saponin inhibited HIV-1 replication in H9 cells at EC50 of 1.1μ M and gymnocladus saponin inhibited HIV-1 replication at EC50 of 2.7 μ M. 3,16-Di-O-acetylchinocystic acid was found to be the active component with the EC50 of 2.3μ M.

Wu et al., (1996) isolated two kaurane derivatives from the fruit of Annona squamosa namely annosquamosin A and annosquamosin B. They found the active component to be 17-dihydroxy-ent-kauran-19-oic acid which inhibited HIV replication in H9 lymphocytes with an EC50 of 0.8μ g/ml.

Sulphated polysaccharide of Asparagopsis armata inhibited HIV replication as measured by HIV syncytium formation (Hastin et al.,1995)

Houghton et al.,(1997) isolated the alkaloid fraction of Schumanniophyton magnificium which had anti HIV property as measured by inhibition of viral syncytia formation on C8166 lymphocytes infected with HIV-1 IIIB strain and the measurement of gp120 by ELISA. Out of eight alkaloids extracted schumanni dicine 1 was found to be most active at EC50 of 1.6μg/ml.

Ayehunie et al., (1998) showed that hot water extract of Arthospira platensis inhibited replication of HIV IIIB ,HIVRF and 11G(patient isolate) strains in human T
cell lines, Periphere blood mononuclear cells and Langerhans cells. Antiviral activity was determined by p24 antigen detection in culture supernatent using ELISA. Human Langerhan cells (LC) were used to study the ability of the algae to inhibit the transfer of HIV from infected cells to uninfected ones. They observed that 100\(\mu\)g/ml of algal extract inhibited viral production by 50% and cytotoxicity was negligible at this concentration. The pre incubation of virus and the extract at a concentration \(\geq 48\mu\)g/ml and subsequent addition to CEM-SS cell lines resulted in direct antiviral effect using HIV RF strain. The aqueous extract was precipitated with ethanol and 4 fractions (A-D) were collected. The A and B fractions had potential anti HIV properties.

Lee et al., (1998) demonstrated that Rhamnan sulfate (RS) a sulfated polysaccharide derived from Monostroma latissimum, had antiviral activities against enveloped viruses such as HSV-1 and HIV-1. The IC \(_{50}\) for HIV-1 replication was 1.5 \(\mu\)g/ml when the compound was added at the time of viral infection. It was found to be 4 times more effective if administered at the time of viral infection as compared to administration after infection with the virus. There was inhibition of syncytia formation in Molt-4 cells using HTLV-IIIIB strain of the virus. Syncytia formation was completely inhibited at a concentration of 25\(\mu\)g/ml.

Berge et al., (1999) isolated a water soluble fraction (AF) from a marine diatom Haslea ostrearia. This fraction was incubated with 100 TCIU/ml of HIV-1 on MT4 cell line for a period of 8 days. At a concentration of 1\(\mu\)g/ml and above, syncytial formation was suppressed by the 4\(^{th}\) day but reappeared on the 5\(^{th}\) day. AF was found to be non-toxic till a concentration of 50\(\mu\)g/ml.

Haraguchi et al., (1999) tested 37 metal compounds at a concentration ranging from 5-10 mg/ml for HIV-1 inhibition on human T cell lines MT4, C8166, Molt-4 clone 8 using stocks of HIV-1 prepared from Molt-4/IIIIB and Molt-4 GUNI cells. The antigen expression was detected by indirect immunofluoresence assay, Polymerase chain reaction was used to detect the effect of these metals on HIV-1 DNA synthesis, p24 antigen production was determined using ELISA and Reverse Transcriptase
polymerase chain reaction was employed to study the effect of the metal compounds on transcription of RNA. Zinc acetate, zinc chloride, zinc nitrate, cadmium acetate and mercury chloride inhibited HIV-1 antigen at an IC$_{50}$ of 8µg/ml, 8µg/ml, 13µg/ml, 0.18µg/ml, 0.12 µg/ml respectively. The zinc compounds were cytotoxic only at 1000µ g/ml whereas cadmium acetate and mercuric chloride showed toxicity at 10-100 µg/ml. None of the compounds inhibited reverse transcription while cadmium acetate and mercuric chloride inhibited viral p24 antigen production and synthesis of HIV-1 RNA but could not inhibit of cellular RNA synthesis.

Premanathan et al., (1999) extracted a polysaccharide (RAP) from Rhizophora apiculata and tested for inhibition of HIV-1, HIV-2 and SIV strains using MT4, Molt 4, MAGI-CCR5 and PBMCs. The different tests used in the study were syncytia formation, virion biding to MT4 cells, monoclonal antibody binding assay and competitive RT PCR amplification assay. It was found that RAP showed concentration dependent inhibition of HIV-1 with a 50% effective concentration of 6.5µg/ml, 50% inhibition of syncytia formation at 53.3µ g/ml, complete inhibition of binding of HIV-1 to MT4 cells at a concentration of 100µ g/ml. A reduction in the number of copies of viral mRNA to less than $10^2$ copies was observed compared to the controls which produced a copy number of $10^7$. RAP showed 50% cytotoxicity at a concentration of 1545µ g/ml.

Hayashi et al., (1996) isolated a sulfated polysaccharide calcium spirulan (Ca-SP) from sea algae, Spirulina platensis. This compound demonstrated anti HIV-1 and anti HSV-1 properties comparable to that of dextran sulfate. Anti HIV-1 activity was demonstrated by determination of p24 antigen in the culture supernatent and the reduction of syncytia formation in cells infected with HIV-1.

Jiratchariyakul et al., (2000) isolated MRK 29 from Momordica charantia ripe fruit and seed found in Thailand which had HIV RT inhibition with 50% IR at 18µ g/ml while the crude protein showed 50%IR at the concentration of 120µ g/ml. The salt
cytotoxicity and anti-HIV potentials. Out of the organic, medicinal and aromatic

Apollo et al. (1996) screened 60 species of Callawaya medicinal herbs for

3F, 5F-decachloro(4′)-phthalonitrile from leaves and stem of Eucalyptus gayanoides.

Parkson et al. (1995) isolated an anti-HIV compound, 12- deacetylphthalonitrile

compounds at an EC50 value of 1.1 M.

processsing of 3′ end, strand transfer and disintegration were inhibited by these

substrate with sequence corresponding to 3′ end of HIV LTR. The
dependent inhibition of viral integrase which was studied using oligonucleotide

reproduction, when administered in cells after infection. The compounds showed close

could inhibit HIV-lntegrase in T-lymphocytes and monocytes and also viral

and GAP 31 from Monomorium charitochondrial and Geolomium multiflorum respectively which

Lee et al. (1995) identified, printed and cloned two plant proteins MAP 30

the 28S ribosomal RNA.

8-furylmoic links between Ribose and Adenine of Guanine at A4324 or C4323 of

By introducing the 8-furylmoic activity which is needed for the breaking of

inhibit the polynucleotide elongation process by inactivation of 60S ribosomal subunit

posion and convert supercoiled DNA to topoisomeralen form and also

therapeutic index (TD50/MIC) ranging from 1000-10000. They also as topoisomerase

syngamy formation were used as markers for the study. These agents had a

32 KD) from leaves of Carnation. The P24 antigen production, viral RT and

Suprophosphatase hlinidrols and DAPs 30 and 32 (Dintubins) anti HIV proteins 30 and

Monomorium charitochondrial (Monomorium andHW protein 31 KD) from the seeds of

Harburg et al. (1994) isolated and charaterised MAP30 from Chinese

introduced FMRG at a concentration of 0.175 µM.

preincubated fraction of MRRK29 showed 82% reduction of p24 expression in viral
extracts screened the water extracts were found to have maximum anti HIV properties and were found in plants used to treat lung and liver diseases.

The water soluble polysaccharide from gametic, carposporic and tetrasporic reproductive stages of Mediterranean red alga *Asparagopsis armata* were studied for their HIV –1 inhibitory activity by Hastin *et al.*, (2000). The Gametic and tetrasporic galactans were shown to inhibit HIV replication at 10 and 8 µg/ml respectively as measured by HIV-induced syncytium formation and reverse Transcriptase activity in cell free culture supernatent. The carposporic polysaccharide was ineffective even at 100µg/ml. Maximal antiviral effect was seen when the polysaccharide was present after or during infection and not before infection suggesting that it has an inhibitory activity at an early stage of infection. This polysaccharide has been shown to be non-toxic to the host cells even at a concentration of 500 µg/ml, and does not have direct antiviral action.

Chen *et al.*, (1995) conducted an *in vivo* test by injecting MT4 cells carrying proviral HTLV-1 into BALB/C mouse. AZT, DDC, DDI (nucleoside inhibitors), HEPT (E-EBUdM), TIBO (non nucleoside inhibitors), KNI-272, Ro31-8959, KNI-144 (Protease inhibitors) were administered subcutaneously or orally to these mice. RT activity and p24 antigen were detected in the supernatent of peritoneal wash fluid. Out of the compounds tested AZT, DDC, DDI, HEPT (E-EBUdM), KNI-272 and Ro31-8959 showed good HIV-1 inhibitory activity.

Naphthaquinones derived from *Ancistrocladus korupensis*, a rare tropical rain forest plant from Cameroon, was shown to protect human lymphoblastoid cell lines from HIV-1 and HIV-2. Michellamine B is the most potent and abundant isomer of this compound and is under preclinical stages. Michellamine B has been shown to inhibit cell fusion, syncytia formation and RT Inhibition. It can also inhibit drug resistant HIV-1 and HIV2 strains (Boyd *et al.*,1994).
Ellagitannins, such as oenothein B inhibits HIV replication in vitro. Digallic acid was found to exhibit 90% inhibition of HIV-1 at a concentration of 0.5μg/ml. Tetragalloylquinic acid showed 80-90 % inhibition at 100μM. A hydrolysable tannin shephagenin A and B from Shepherdia argentea has been shown to have activity at 49 and 74nM ,respectively (Matthee et al.,1999).

Nakashima et al (1986) showed that 0.1 μ M AZT showed complete inhibition of Human Immunodeficiency Virus (HIV) replication in vitro in Molt-4/HTLV-III co culture system whereas no inhibition of induction of multinucleated giant cells was seen in presence of 1μ M and 5 μ M AZT. They also demonstrated that in the presence of 1%Neutralising Antibody and 2.5μ M AZT the viability of co cultures was 91% till 10 days of inoculation. The viability was only57% in the absence of neutralizing antibodies and the presence of 1% normal human sera and 2.5μ M AZT.

A lignan identified from Schisandra chinensis was shown to have RT Inhibitory activity against HIV( Furman,1986). These extracts in natural form seem to have some cytotoxicity and attempts have been made to produce synthetic derivatives similar to the natural lignan isolated from the plants. A di-bromated derivative ,compound 1506, a lignan compound 1737 are such promising candidates which have been shown to be more specific and less toxic than their natural counterparts.

The methanolic extract from Tripterygium wilfordii (Spence et al.,1995)showed significant anti HIV activity and the active constituent was found to be a triterpenoid, salspermic acid. This compound could inhibit HIV replication in T lymphocytes at non toxic concentrations. A triterpenoid ,1β-hydroxyaleuritolic acid was isolated from Maprounea africana which showed a significant RT Inhibitory activity but the mode of action seem to be non specific (Litvak et al., 1996). Euphorbia myrsinites has yielded a number of diterpenoid esters which show some weak RT inhibitory activity (Sun et al.,1996).
2.9.2. IMMUNOMODULATORY POTENTIALS OF MEDICINAL PLANTS - CURRENT STATUS

Beuscher et al., (1994) tested plants used in traditional medicine from Africa and Mauritius were tested for invitro induction of IFN \(\alpha,\beta\) using spleen cultures of NMRI mice. Con A at a concentration of 1-10\(\mu\)g/ml was used as positive control. The culture supernatant was tested for the presence of IFN \(\alpha,\beta\) by determination of inhibition of cytopathic effect in murine L929 cells in the presence of vesicular stomatitis virus (VSV) and the methanolic extract of Chironia krebsii at a concentration of 25 \(\mu\)g/ml, ethanolic extract of root bark of Heteromorpha trifoliate at a concentration of 12.5 - 100\(\mu\)g/ml, ethanolic extract of Badulanin sularis at a concentration of 6.3-12.5\(\mu\)g/ml, ethanolic extract of leaves of Xanthocercis zambesiaca and Holarrhena pubescens induced IFN \(\alpha,\beta\) production. The dichloromethane extract of stem and twigs of Jasminum fluminense induced 10U IFN /ml in the absence of Con A. On testing the toxicity of the plants on Vero and He La cells using 4 methylumbelliferyl heptanoate Jasminum fluminense showed no toxic effect on Vero cells whereas high toxicity was observed on GMK cells.

A number of plants have been identified which possesses immunostimulatory, antiviral, antibacterial, anticancer, antiasthmatic, anti inflammatory activities by Chopra et al., (1996). The most promising attributes have been documented for Allium sativum, Aloe vera, Asparagus racemosus, Azadirachta indica, Curcuma longa, Tinospora cordifolia, Withania somnifera, Andrographus paniculata, Nyctanthes arbor-tristis, Ocimum sanctum, Panax ginseng, Panax pseudoginseng, Phyllanthus emblica and Picrorhiza kurroa.

Allium sativum have been shown to inhibit growth of tumors in animals by activating natural killer (NK) cells, stimulation of T lymphocytes and enhanced production of IL2 (Tang et al., 1997).
Aloe vera has been documented to have anti-inflammatory property, and
induction of delayed type hypersensitivity in mice. It improves wound healing and
prevents dermal ischemia by reversing the effects of thromboxane synthetase.
Acemannan, a major carbohydrate fraction of A. vera is known to enhance IL1 and
TNFα production (Peng et al., 1991).

Ethanoilextract of Andrographis paniculata induces stimulation of
antibody and DTH response to sheep red blood cells, stimulate macrophage
migration and in vitro proliferation of splenic lymphocytes. The stimulation was
found to be lower with purified andrographolides than with the ethanolic extracts
indicating the presence of substances other than andrographiloides contributing
towards immunostimulation (Puri et al., 1993).

Azadiracta indica have shown to have non specific immunomodulatory
properties. Spleen cells of neem oil treated animals showed significantly higher
lymphocyte proliferation to concanavalin A. A. indica induces production of IL1, IFN γ
TNF α. In clinical study of patients with psoriasis A. indica leaf extract application
resulted in reduction of erythema, desquamatisation, γ infiltration of psoriatic lesions
which is indicative of immunostimulatory action of A. indica (Upadhyay et al., 1992).
NIM-76, a volatile fraction of A. indica oil was shown to result in increase in
polymorphonuclear (PMN) leukocytes with concomitant decrease in lymphocyte count
in blood. The immunomodulatory activity of NIM 76 was found to be dose dependent
with enhanced macrophage activity, lymphocyte proliferation response while humoral
component unaffected. At higher concentrations of NIM 76 (300mg/Kgbody weight)
there was stimulation of mitogen induced lymphocyte proliferation, while macrophage
activity remain unaffected. The study indicates that NIM 76 primarily acts through cell
mediated mechanisms by activating macrophage lymphocytes. (Sai Ram, 1997)

Jiratchariyakul et al., (2000) isolated MRK 29 from Momordica charantia ripe fruit
and seed which was assayed for TNF α on L929 fibroblastic cells. The percentage
L929 cytolysis of TNF activity revealed a 3 fold increase in activity of the mononuclear cells.

*Phyllanthus emblica* have been shown to have immunomodulatory effect, powdered fruit was found to stimulate natural killer cells (NK cells). *In vitro* studies on organic extracts of *P. emblica* inhibit leukotriene B4 -induced migration of human PMNs (Suresh *et al.*, 1994).

*Picrorhiza Kurroa* is a promising immunomodulatory agent. It has been demonstrated to enhance DTH response. It inhibits ochratoxin induced suppression of chemotactic activity, production of IL1 and TNF α. A 50% ethanolic extract of *P. Kurroa* leaves was found to elicit dose dependent increase in SRBC induced early and delayed hypersensitivity reaction. It augmented responsiveness of murine splenocytes to T cell mitogens viz., phthrohaemagglutinin, Con A, B cell mitogens viz.,LPS. Picroliv is an iridoid derived from plant *P. Kurroa*. Oral administration of Picroliv in mice prior to immunization with SRBC resulted in significant increase in haemagglutinating antibody titre, plaque-forming cells, γ DTH response to SRBC. It also increased macrophage migration, ¹⁴C-glucosamine uptake, phagocytosis of ¹⁴C-leucine labelled *E.coli*, chemileuminescence of peritoneal macrophages, higher uptake of ³H-thymidine in lymphocytes of Picroliv treated mice (Atal *et al.*, 1986)

*Tinospora cordifolia* is a traditional Indian medicinal plant that is ascribed to possess anti bacterial, anti allergic, anti diabetic and diuretic properties. Extracts of *T. cordifolia* was tested for anticancer activity. Maximum invtro mitogenic response is seen in splenic lymphocytes of mice. It was poorly mitogenic to human lymphocytes. Oral administration of T cordifolia extract to mice enhanced humoral immune response to SRBC but T cell response to Con A was suppressed. The active principles, Syringin (TC-4) Cordiol (TC-7) obtained from *T. cordifolia* have been found to possess anti complimentary and immunomodulatory activity. These compounds also give rise to significant increase in IgG antibodies in serum. Humoral and cell mediated immunity were also found to be enhanced in a dose dependent manner. (Agarwal *et al.*, 1999)
Davis et al., (2000) studied the immunomodulatory effect of root extract of *Withania somnifera*. It was found to enhance WBC count and increase phagocytic activity of peritoneal macrophages in Balb/c mice and inhibit delayed hypersensitivity reaction as demonstrated by Mantoux test.

In addition to angiospermic plants, there are few reports of immunomodulators from other sources. An interferon inducer designated 6-MFA, having broad spectrum of antiviral activity and high margin of safety, was isolated from fungus *Aspergillus ochraceus*. This producty was found to be a mixture of polysaccharides and nucleic protein.

### 2.9.3 DOCUMENTED USE OF MEDICINAL PLANTS USED IN THE PRESENT STUDY:

#### 2.9.3.1. *Amaranthus spinosus Linn:*

**Family:** Amaranthaceae

**Vernacular names**


**Distribution:** Throughout India, in waste lands.

**Parts used:** Whole plant

**Documented Use**

Plant is diuretic, laxative, haematinic, appetiser, useful in conditions of hyperdipsia, burning sensation, leucorrhoea, haemorrhoids, abscesses, anaemia, anorexia, agalactia, haemoptysis, haematemesis, leprosy, eczema and bronchitis. (Varier et al., 1994)
2.9.3.2. Aporosa lindleyana

Family: Euphorbiaceae

Vernacular names

Mal: Kotili, Vittil, Vitti., Tam: Kotili, Vittil.,

Parts used: Roots

Documented Use

Decoction of the root is given in jaundice, fever, headache and seminal loss. (Krithikar et al, 1975).

Jayakar et al, (2003) have shown that the aqueous and alcoholic extracts of root of Aporosa lindleyana (100 mg/kg) reduced the blood glucose of normal rat from 80.4\pm2.7 to 69.8\pm2.0 mg% and 82.6\pm1.9 to 70.8\pm3.2 mg%, respectively 3 h after oral administration of the extract and lowered blood glucose level in alloxan induced diabetic rat from 306\pm3.37 to 160\pm2.46 and 328\pm4.15 to 152\pm3.86 mg%, respectively 3 h after oral administration of the extract.

2.9.3.3. Argyreia nervosa (Burm.f.) Boj.

Family: Convolvulaceae

Vernacular names


Distribution: Throughout India, in areas up to 900 m elevation
**Parts used:** Roots

**Documented use:**

They have been documented by Varier *et al.*, (1994) as being useful in conditions of emaciation, wounds, ulcers, anorexia, dyspepsia, flatulence, colic, constipation, cardiac debility, inflammations, cough, bronchitis, strangury, seminal weakness, nervous weakness, cerebral disorders, synovitis, haemorrhoids, obesity, hoarseness, syphilis, anaemia, diabetes, tuberculosis, arthritis, ascites, leucorrhoea and general debility.

**2.9.3.4. *Asparagus racemosus* Willd.**

**Family:** Liliaceae

**Vernacular names**


San: Satavari., Tam: Kilavari, Satavali.

**Distribution:** Throughout India, in areas up to 1,400 m elevation, also cultivated

**Parts used:** tuberous roots

**Documented Use:**

Varier *et al.*, (1994) have documented *Asparagus racemosus* as cited in the Ayurveda literatures as being useful in conditions of *vata* and *pitta*, nervous disorders, dyspepsia, diarrhoea, dysentery, tumours, inflammations, burning sensation, hyperdipsia, ophthalmopathy, nephropathy, hepatopathy, strangury, scalding of urine, throat infections, tuberculosis, cough, bronchitis, gleet, gonorrhoea, leucorrhoea, leprosy, epilepsy, fatigue, hyperacidity, colic, haemorrhoids, cardiac debility, hypertension, abortion, agalactia and general debility.
2.9.3.5. *Baliospermum montanum* (Willd.) Muell-Arg.

**Family:** Euphorbiaceae

**Vernacular names**

Hin: Danti, Mal: Nagandanti, Danti, San: Danti, Tam: Nakatanti

**Distribution:** Throughout India.

**Parts used:** roots, leaves, seeds

**Documented use**

The roots of *Baliospermum montanum* have been documented to be useful in anasarca, dropsy flatulence, constipation, jaundice, haemorrhoids, leprosy, skin diseases, strangury, vesical calculi, wounds, splenomegaly, anaemia, leucoderma, fever and vitiated conditions of *vata*. The leaves are good for asthma and bronchitis. The seeds are drastic purgative, rubefacient, hydragogue and stimulant, and are useful in vitiated conditions of *vata*, inflammations and flatulence (Varier *et al.*, 1994)

Ogura *et al.*, (1978) have conducted work on potential anticancer agents VIII from constituents of *Baliospermum montanum*.

2.9.3.6. *Boerhaavia diffusa* Linn.

**Family:** Nyctaginaceae

**Vernacular names**

Eng: Hogweed, Pigweed, Hin: Sant, Gadahpurna, Mal: Tavilama, Talutama

San: Punarnava, Tam: Mukkurattai, Mukkarattai-kirai

**Distribution:** Throughout India, as a weed in wastelands and road sides.
**Parts used:** whole plant

**Documented Use:**

The plant is bitter, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic, emetic, expectorant, anti inflammatory, febrifuge, laxative and tonic. It is useful in all types of inflammations, strangury, leucorrhoea, ophthalmia, lumbago, myalgia scabies, cardiac disorders, jaundice, anaemia, dyspepsia, constipation, cough, bronchitis and general debility. (Varier et al., 1994)

The roots of *Boerhaavia diffusa* contain the retenoids, boeravinones A1, B1, C2, D, E and F besides the new dihydroisofuorenxanthin and boerhaavine. The roots are credited with anti-convulsant, analgesic, diuretic, laxative and expectorant properties.

It was found to stop intra-uterine-contraceptive-device (IUCD)-induced bleeding. This herb is also known for its anti-inflammatory and analgesic properties, which are comparable to that of ibuprofen. It has also proved useful as a haematinic.

Mehrotra et al., (2002) evaluated the immunomodulatory properties of extract of *Boerhaavia diffusa* on various in vitro tests such as human natural killer (NK) cell cytotoxicity, production of nitric oxide (NO) in mouse macrophage cells, RAW 264.7, interleukin-2 (IL-2), tumour necrosis factor-alpha (TNF-alpha), intracytoplasmic interferon-gamma (IFN-gamma) and expression of various cell surface markers on human peripheral blood mononuclear cells (PBMCs). They demonstrated that ethanolic extracts of *B. diffusa* roots inhibited human NK cell cytotoxicity in vitro, production of NO in mouse macrophage cells, IL-2 and TNF-alpha in human PBMCs. They also demonstrated that Intracytoplasmic IFN-gamma and cell surface markers such as CD16, CD25, and HLA-DR did not get affected on treatment with *B. diffusa* extract.
2.9.3.7. *Eclipta prostrata* (Linn.,)Linn.

Family: Asteraceae

Vernacular names

San: Bhrngarajah, Tekarajah .,Tam:Kayyantakara, kaikesi

Distribution: Throughout India, at all elevations in waste places and on road sides.

Parts Used: Whole plant

Documented Use:

Useful in hepatosplenomegaly, inflammations, gastropathy, skin diseases, fever, jaundice, hypertension, leprosy. (Varier *et al.*,1994)

He J *et al.*, (1992) observed the effects of a Chinese medicine mixture containing *Astragalus membranaceus, Fructus Ligustri Lucidi* and *Eclipta prostrata*, on the immune function. They found that 9g/kg and 20g/kg fed to mice for seven days, these two dosages of AFE could raise the conversion percentage of lymphocytes (P < 0.01) and serum IgG level (41%-47%) of the mice and the weights of the thymus and spleen increased in the two groups compared with those in the normal control and AFE turned out a resistance to the immunosuppressive effect caused by cyclophosphamide.

In India *Eclipta alba* is used in the treatment of liver cirrhosis, infective hepatitis, liver enlargement, jaundice and other ailments of the liver and gall bladder. In scientific studies *Eclipta alba* also shows good antifungal activity. The plant tops are used for skin diseases (inflammation). *Eclipta* has bioactive steroidal alkaloids. They have cytotoxicity against certain cells.
In Suriname’s traditional medicine, *Eclipta* is used against anaemia, dysentery, eye diseases, asthma and liver cirrhosis. The juice of *Eclipta* together with honey is used to treat upper respiratory congestion in children. The herb is used as a tonic and deobstruant in hepatic and splenic enlargements and in skin diseases. The plant juice is administered in combination with aromatics for catarrhal jaundice. The plant possesses antihepatotoxic and anti-inflammatory activities. The fresh plant is considered anodyne and absorbent.

Liu X *et al.*, (2000) studied the regulatory effects of ethyl acetate extract of *Eclipta prostrata* (EAEEP) on immune function of immunosuppressed mice and found that EAEEP can increase the index of spleen, level of haemolysin in serum, Delayed type hypersensitivity in immunosuppressive mice.

2.9.3.8. *Euphorbia ligularia* Roxb.

**Family:** Euphorbiaceae

**Vernacular names**

**Eng:** Common milk hedge, **Hin:** Sehund, Thuhar, **Mal:** Ilakkalli, Kalli

**San:** Snuhi, Sehundah, **Tam:** Ilaiakkalli.

**Distribution:** Throughout India, often grown as hedge plant

**Parts used:** whole plant

**Documented Use:**

The plant is bitter, acrid, thermogenic, laxative, abortifacient, digestive, deobstruant, expectorant, depurative, anti-inflammatory, carminative, febrifuge, stomachic and vermifuge. It is useful in gastropathy, bronchitis, asthma, inflammations, splenomegaly, cutaneous diseases, dropsy, dyspepsia, flatulence,
intermittent fever, jaundice, leprosy, rheumatism and ulcers. The milky juice is acrid, purgative, expectorant and rubefacient and is useful in otalgia and ophthalmia (Varier et al., 1994).

2.9.3.9. *Ficus microcarpa* Linn.f.

**Family:** Moraceae

**Vernacular names**

**Hin:** Kamarup. **Mal:** Itti, Ittiyal. **San:** Plaksah. **Tam:** Kallicci, Icci

**Distribution:** Throughout India, from sea level to about 1,300m.

**Parts used:** Root, bark and leaves.

**Documented Use**
The aerial roots of *Ficus microcarpa* are used to treat dental caries and odontalgia. The bark and leaves are astringent, refrigerant, acrid and stomachic. They are useful in wounds, ulcers, bruises, flatulent colic, hepatopathy, diarrhoea, dysentery, diabetes, hyperdipsia, burning sensation, haemorrhages, erysipelas, dropsy, ulcerative stomatitis, haemoptysis, psychopathy, leucorrhoea and colporrhagia. (Varier et al., 1994)

2.9.3.10. *Gmelina arborea* Roxb.

**Family:** Verbenaceae

**Vernacular names**

**Eng:** Coomb teak. **Hin:** Gamari, Gambhari. **Mal:** Kumilu, Kumpil, Kumil

**San:** Gambhari, Kasmari. **Tam:** Perumkumbil, Kumadi
**Distribution:** Throughout India, in moist deciduous forests

**Parts used:** whole plant

**Documented use:**

The roots are useful in hallucination, fever, dyspepsia, hyperdipsia, haemorrhoids, stomalalgia and burning sensation. Bark is bitter, tonic and stomachic and is useful in fever and dyspepsia. Leaf paste is good for cephalalgia and the leaf juice is a good wash for foul ulcers. The flowers are sweet, refrigerant, bitter, astringent and acrid, and are used in treating leprosy and skin diseases. The fruits are acrid, sour, sweet, bitter, refrigerant, diuretic, astringent, aphrodisiac, trichogenous, alterant and tonic. They are used for promoting the growth of hair and for anaemia, leprosy, ulcers, constipation, strangury, leucorrhoea and colitis. (Varier *et al.*, 1994)

Hosny *et al.*, (1998) isolated 6-O-alpha-L-rhamnopyranosylcatalpol, 6-O-(3'-O-trans-feruloyl)-alpha-L-rhamnopyranosylcatalpol, 6-O-(2'-O-acetyl-3', 4'-O-di-trans-cinnamoyl)-alpha-L-rhamnopyranosylcatalpol, phenylpropanoid glycosides verbascoside (acteoside) and martynoside and 12 acylated iridoid glycosides named gmelinosides A-L from the leaves of *Gmelina arborea*.

2.9.3.11. *Gymnema sylvestre* (Retz.)R.Br.

**Family:** Asclepiadaceae

**Vernacular names**


**Distribution:** Throughout India.

**Parts used:** Whole plant
Documented Use:

In a trial conducted by Gupta et al., (1964) the laxative action was shown to be due to the presence of anthraquinone derivative. Saponins were found to be present in the alcoholic extract of the plant. The somatotropin - induced hyperglycemia was markedly inhibited by the extract in a dose of 200 mg/kg intramuscularly.

*Gymnema sylvestre* is documented to be useful in eye complaints, to cure opacities of lens, cornea, vitreous body. It is cited to be useful in disease of heart, piles, bronchitis, asthma, ulcers leucoderma, inflammations and cure burning sensation. Fruit is bitter, pungent and found to be stomachic, anthelminthic, aletric. Ayurveda literature documents its usefulness in disease of heart, bronchitis, asthma, ulcers. In Bombay and Madras vaids recomend the leaves in treatment of furunculosis and “madhumedha” (glycosuria). The leaves cause hypogycemia by indirect stimulation of insulin secretion by the pancreas. (Krithikar et al., 1975)

Konoshima et al., (1995) isolated two anti-HIV principles Gleditsia saponin C and gymnocladus saponin G from *Gleditsia japonica* and *Gymnocladus chinensis*, Gleditsia saponin C and gymnocladus saponin G demonstrated inhibitory effects against HIV replication in H-9 cells with EC50 values of 1.1 and 2.7 μM respectively.

**2.9.3.12. Hedyotis corymbosa** (Linn.) Lam.

**Family:** Rubiaceae

**Vernacular names:**

**Hin:** Daman pappar, Pitpapra., **Mal:** Parppatakappullu, Parppatakam.,

**San:** Parpatah, Parpatakah., **Tam:** Parpatagam

**Distribution:** Throughout India, both on dry and wetlands as a weed
Parts used: Whole plant.

Documented Use

The plant is useful in fevers, depression, jaundice, heat eruptions, hyperdipsia, giddiness, dyspepsia, flatulence, colic, constipation, helminthiasis, strangury, leprosy, skin diseases, cough, catarrh, bronchitis and hepatopathy. (Varier et al., 1994)

Lin et al. (2002) investigated the anti-inflammatory and hepatoprotective effects of Peh-hue-juwa-chi-cao (PHJCC), a common commercial name for the herbal extract of either *Hedyotis diffusa*, *H. corymbosa* or *Mollugo pentaphylla* in male rats. They found that the extracts of *Hedyotis diffusa*, *H. corymbosa* and *Mollugo pentaphylla* possess anti-inflammatory activity, and the three plant extracts significantly reduced the acute elevation of serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) concentration, and alleviated the degree of liver damage 24 hours after the intraperitoneal administration of hepatotoxins.

2.9.3.13. *Hemidesmus indicus* (Linn)R.Br.

Family: Asclepiadaceae

Vernacular names


Distribution: Throughout India

Parts used: Roots, leaves, stem
Documented Use:

Useful in burning sensation, leucoderma, skin diseases, leucorrhoea, syphilis, fever, leucoderma, inflammations. (Varier et al., 1994)

Prabakan et al., (2000) report that the oral treatment with the ethanol extract of Hemidesmus indicus roots (100 mg/kg, for 15 days) significantly prevented rifampicin and isoniazid-induced hepatotoxicity in rats.

Ravishankara et al., (2002) studied the antioxidant activity of methanolic extract of H. indicus root bark and carried out phytochemical analysis and TLC fingerprint profile of the extract. They studied the radical scavenging activity by DPPH reduction, superoxide radical scavenging activity in riboflavin/light/NBT system, nitric oxide (NO) radical scavenging activity in sodium nitroprusside/Greiss reagent system and inhibition of lipid peroxidation induced by iron-ADP-ascorbate in liver homogenate and phenylhydrazine induced haemolysis using erythrocyte membrane stabilization assay. They found the activity of extracts to have an EC50 = 18.87 and 19.9 microg/ml respectively in scavenging DPPH and superoxide radicals respectively. The extract inhibited lipid peroxidation of liver homogenate (EC50 = 43.8 microg/ml) and the haemolysis induced by phenylhydrazine (EC50 = 9.74 microg/ml) confirming the membrane stabilization activity.

Anoop et al., (2003) evaluated the anti ulcerogenic property of aqueous ethanolic extracts of the roots in animal models.

Sultana et al., (2003) have shown H. indicus to be an effective chemo preventive agent capable of ameliorating hydroperoxide-induced cutaneous oxidative stress and tumor promotion.


Family: Convolvulaceae
Vernacular names


Tam: Palmudamgi, Palmodikkay

Distribution: Throughout India, in deciduous and evergreen forests and coastal tracts.

Parts Used: Tuberous roots

Documented Use

The roots of *Ipomea mauritiana* are useful in conditions of agalactia, emaciation in children, consumption, strangury, leprosy, skin diseases, anorexia, dyspepsia, colic, flatulence, helminthiasis, bronchitis, fever, burning sensation, nausea, vomiting, pharyngodynia, syphilis, gonorrhoea, inflammation, splenopathy, hepatopathy, menorrhagia and general debility (Varier et al., 1994)

2.9.3.15. *Mesua nagassarium* (Burm.f.) Kosterm.

Family: Clusiaceae

Vernacular names


San: Nagapushpah, Nagakesarah., Tam: Nagapppu

Distribution: Throughout India, in evergreen forests upto 1,500m

Parts used: Flowers, oil
Documented Use

The flowers of *Mesua nagassarium* are useful in conditions of asthma, cough, hiccough, halitosis, leprosy, scabies, dermatopathy, pruritus, pharyngodynia, vomiting, dysentery, haemorrhoids, ulcers, burning sensation of the feet, dipsia, impotency, leucorrhoea, haemoptysis, strangury, cephalalgia, fever and cardiac debility. (Varier et al., 1994)

**2.9.3.16.** *Mussaenda frondosa* Linn.

**Family:** Rubiaceae

**Vernacular names**

**Hin:** Bedina, **Mal:** Vellila, Vellilattali, **San:** Sriparnah, Rajatarih, **Tam:** Vellai-ilai, Velli matantai

**Distribution:** Throughout India in forests, also cultivated

**Parts used:** Whole plant

**Documented Use:**

The seed oil is used in skin diseases. It is useful in cough, bronchitis, fever, inflammation, wounds and ulcers, leucoderma, pruritus, ophthalmopathy, jaundice, metropathy and uropathy. (Varier et al., 1994)

A Comparative evaluation of *Butea frondosa* and flurbiprofen for ocular anti-inflammatory activity in rabbits was conducted by Mengi et al., (1995). They found that a gel preparation of *B. frondosa* leaves were superior in reducing elevated intraocular pressure elicited experimentally, when compared to gels prepared from a commercial eye drop flurbiprofen.
Xu et al., (1996) isolated eighteen saponins from *Mussaenda pubescens*, and found that mussaendoside O, the most abundant saponin from this plant could inhibit the secretions of the lachrymal and salivary glands induced by galanthamine and also had immunopromotive and hemolytic activities.

2.9.3.17. *Nyctanthes arbor-tristis* Linn.

Family: Oleaceae

Vernacular names


Distribution: Throughout India upto 1500m

Parts Used: Leaves, flowers, seeds

Documented Use

The leaves of *Nyctanthes arbor-tristis* are bitter, acrid, thermogenic, antibacterial, anodyne, anti-inflammatory, digestive, cholagogue, anthelmintic, depurative, sudorific, febrifuge, expectorant, diuretic, laxative, trichogenous and tonic. They are useful in inflammations, dyspepsia, helminthiasis, pruritus, dermatopathy, chronic fever, bronchitis, asthma, cough, strangury, constipation, hepatopathy, haemorrhoids, greyness of hair and baldness.(Varier et al., 1994)

Puri et al., (1994) observed a strong stimulation of antigen specific and non-specific immunity, in mice fed with 50% ethanolic extract of seeds, flowers and leaves of *Nyctanthes arbor-tristis*. Maximum activity was found in the seeds in which the active principle were associated with lipids. In flowers and leaves, the major activity was found in the aqueous fraction of the 50% ethanol extract.
Paul et al., (1997) studied the effect of the water soluble fraction of the ethanol extract of *Nyctanthes arbor-tristis* (*NAT*) on tumour necrosis factor-alpha (TNF-alpha) level in plasma of arthritic Balb/c mice. They showed a depletion of TNF-alpha from the host plasma. They further demonstrated that the extract reduced plasma interferon-gamma level.

2.9.3.18. *Ocimum kilimandscharicum Guerke*

**Family:** Lamiaceae

**Vernacular Names**

**Eng:** Camphor basil., **Hin:** Kapurtulsi., **Mal:** Karppurattulasi., **San:** Karpuratulasi., **Tam:** Karpuratulasi

**Distribution:** Throughout India, cultivated.

**Parts Used:** Leaves

**Documented use:**

The leaves are acrid, thermogenic, aromatic, anti-bacterial, insecticidal, antiviral, appetising, ophthalmic and deodorant. They are useful in cough, bronchitis, catarrh, halitosis, bacterial and viral infections, foul ulcers and wounds, anorexia, ophthalmopathy. (Varier et al., 1994)

Ayisi et al., (2003) compared the effects of *Ocimum gratissimum, Ficus polita, Clausena anisata, Alchornea cordifolia, Elaeophorbia drupifera* and AZT on in vitro HIV-1 and HIV-2 replication and cytopathicity. They found that all plant extracts inhibited HIV-1 strain HTLVIII B cytopathicity. Even when treatment with extract was done after 2 hours of infection the plant extracts were still very effective against HIV-2 which is not seen with AZT. Similarly in Molt-4 co cultures with
Molt-4/HIV, early cytopathic effect (CPE) of cell fusion was unaffected by AZT but was completely inhibited by all plants at non cytotoxic concentrations. The plant extracts also inhibited HIV-1 reverse transcriptase (RT) activity at EC₅₀ values of <0.01-0.03 mg/ml and HIV-1 proviral DNA copying was completely inhibited by Ocimum gratissimum and Ficus polita at 0.011 and 0.015 mg/ml respectively.

2.9.3.19. Operculina turpethum (Linn.) Silva Manso

Family: Convolvulaceae

Vernacular names


Distribution: Throughout India upto 900m, occasionally cultivated

Parts used: roots

Documented use

The roots of Operculina turpethum are useful in colic, constipation, dropsy, paralysis, myalgia, arthralgia, pectoralgia, bronchitis, obesity, helminthiasis, gastropathy, ascites, inflammations, intermittent fever, leucoderma, pruritus, ulcers, erysipelas, haemorrhoids, tumours, jaundice, and ophthalmia. (Varier et al., 1994) It is an effective laxative. It is used in periodic fevers. In the treatment of anaemia accompanied by splenomegaly. It is also used to relieve flatulence and colic. In the treatment of obesity, it is used to decrease fat (Kirthikar et al., 1975).

2.9.3.20. Oxalis corniculata Linn.

Family: Oxalidaceae
Vernacular names:


Distribution: Throughout India, upto 3,000 m in all moist exposed localities

Parts used: Whole plant

*Oxalis corniculata* is useful in dyspepsia, haemorrhoids, anaemia, tympanitis, fever, dysentery, diarrhoea, scurvy, corns, warts, excrescences of skin, inflamed ulcers, opthalmopathy, toxicity, cardiopathy, haemorrhages, dysmenorrhoea, amenorrhoea, strangury, hepatopathy and burning sensation. (Varier et al., 1994)

2.9.3.21. *Pinus roxburghii* Sarg.

Family: Pinaceae (Coniferae)

Vernacular names


Distribution: Throughout Himalayas, Ooty, Kodaikanal at altitudes of 450-2400m

Parts used: Wood, Oleoresin, Oil

Documented Use

The wood of *Pinus roxburghii* is useful in opthalmopathy, otopathy, pharyngopathy, halitosis, foul ulcers, haemorrhages, haemoptysis, helminthiasis dyspepsia, flatulence, hepatopathy, strangury, bronchitis, inflammations, skin diseases, pruritus and giddiness. The oleoresin is useful in asthma, chronic
bronchitis, otalgia, odontalgia, inflammations, hepatopathy, splenopathy, urethrorrhea, gonorrhoea, scabies, epilepsy, lumbago, haemorrhoids and tuberculous glands. (Varier et al., 1975)

Takayama et al. (1991) demonstrated that PC6, a natural product extracted from cones of *Pinus parviflora* Sieb et Zucc inhibited the expression of all HIV-1 proteins, HIV-1 reverse and forward transcription and LTR-driven gene expression at the transcriptional level in CEM cells. They also demonstrated that PC6 did not affect the posttranslational processing of the HIV-1 proteins.

Eberhardt et al., (1996) isolated a phenylpropanoid (lignin) component from *Pinus nigra*. Arnold (Pinaceae) seed cones which may component having the anti-HIV-1 activity.

2.9.3.22. *Plectranthus amboinicus* (Lour.) Spreng.

Family: Lamiaceae

Vernacular names
Eng: Country borage, Indian borage., Hin: Patta ajavayin, Patharcur.,

Mal: Kannikkurkka, Panikkurkka., San: Karpuravalli, Sugandhavalakam.,

Tam: Karpuravalli

Distribution: Throughout India, cultivated in gardens

Parts Used: Leaves

Documented Use:
The leaves of *Plectranthus amboinicus* are useful in cephalalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea and cholera especially in children, halitosis, convulsions, epilepsy, cough, chronic asthma, hiccup, bronchitis, renal and vesical calculi, strangury, hepatopathy and malarial fever. (Varier et al., 1994)
Franca et al., (1996) have recorded the plants used in the treatment of cutaneous leishmaniasis due to Leishmania (Viannia) braziliensis (L(V)b) among the rural population of Brazil and found that Anacardium occidentale, (Anacardiaceae) is used by 65% of the population, Clidemia hirta, (Melastomataceae) by 39%, Plectranthus amboinicus (Lamiaceae) by 33%, Chenopodium ambrosioides (Chenopodiaceae) by 31%, Solanum americanum (Solanaceae) by 25% and Plantago major (Plantaginaceae) by 2%.

2.9.3.23. Plumbago indica Linn.

Family: Plumbaginaceae

Vernacular names
Eng: Rosy-flowered leadwort, Fire plant, Hin: Lalcitra, Raktacitra,
Mal: Kotuveli, Cettikkotuveli, Cuvannakotuveli, San: Citrakah, Dahanah
Tam: Cenkotiveli, Cittiramulam

Distribution: Throughout India, in moist situations, as well as cultivated

Parts used: Roots

Documented use:

Rojanapo et al., (1990) tested purified extracts of Acanthus ebracteatus Vahl, Plumbago indica Linn, and Rhinacanthus nasuthus Kurz for their mutagenic and antimutagenic potentials and found the petroleum ether, hexane, and chloroform extracts inhibited the mutagenicity of aflatoxin B1 and the activity of rat liver aniline hydroxylase, one of the cytochrome-P450-mediated reactions.

The roots are useful in dyspepsia, colic, inflammations, cough, bronchitis, helminthiasis, haemorrhoids, elephantiasis, chronic and intermittent fever, leprosy,
leucoderma, ring-worm, scabies, hepatosplenomegaly, amenorrhoea, odontalgia, and anaemia. (Varier et al., 1994)

2.9.3.24. **Pterocarpus marsupium** Roxb.

**Family:** Fabaceae

**Vernacular names**

**Eng:** Indian kino tree, Malabar kino tree. **Hin:** Bjasal, Vijayasar. **Mal:** Venna

**San:** Asanah, Bijakah. **Tam:** Vengai

**Distribution:** Throughout India, in deciduous and evergreen forests.

**Parts used:** Heartwood, leaves, gum

**Documented use:**

In the Ayurvedic system of medicine, *Pterocarpus marsupium* whole plant and gum are used as a laxative, anthelmintic and alternative. It has been documented to cure "Vata" and "Kapha" diseases of blood, eruptions on the body, leucoderma, erysipelas, urinary discharge, anal troubles, leprosy, eye trouble and elephantiasis. (Kirthikar et al., 1975)

The flowers are bitter and seen to lead to reduction in the blood sugar level in rabbits. The decoction of bark has significant effect on serum cholesterol in hypercholesterolemic rabbits. Propterols, isolated from the plant, show antibacterial activity against gram-positive bacteria. (Shah, 1967).

William Cook, (1869) described the use of *Pterocarpus marsupium* in combination with chalk or magnesia in cases of excessive mucous discharges not
attended with inflammation, as in sub-acute dysentery, diarrhoea, and leucorrhoea; and in the stages of bowel complaints, In local application, it was documented to dry exhaustive discharges, arrests haemorrhage, used in leucorrhoea and epistaxis; bleeding gums, and in uterine and pulmonary haemorrhage.

Tripathi et al., (1988) isolated two flavanone glycosides, 7-Hydroxy-6, 8-dimethyl flavanone-7-O-alpha-L-arabinopyranoside and 7,8,4'-tri hydroxy-3', 5'-dimethoxy flavanone-4'-O-beta-D-glucopyranoside from the roots of Pterocarpus marsupium.

2.9.3.25. *Pueraria tuberosa* DC

**Family:** Fabaceae

**Vernacular names**

**Eng:** Indian kudzu., **Hin:** Vidarikand, Sural., **Mal:** Mutukku., **San:** Vidari

**Distribution:** Throughout India, upto 1,200 m

**Parts used:** Tuberous roots

**Documented use:**

The roots useful in conditions of arthritis, burning sensation, constipation, agalactia, strangury, emaciation, cardiac debility, intermittent fevers, pharyngitis, leprosy, dyspepsia, tuberculosis, hepatosplenomegaly, cough, spermatorrhoea and general debility. (Varier et al., 1994)

Park et al., (2002) conducted Ames test to test the antimitagenicidity of the methanolic extract of *Pueraria thunbergiana* (Leguminosae) flowers and its fractions and found that saponin fraction called kaikasponin III prevents the metabolic activation of AFB1 and scavenge electrophilic intermediate capable of
mutation and decreased the number of revertants of *S. typhymurium* TA100 by 99% against AFB, and by 75% against N-ethyl-N’-nitro-N-nitrosoguanidine (MNNG).

Lee *et al.*, (2001) extracted six isoflavonoids from the flower of *Pueraria thunbergiana* out of which tectorigenin, glycinein and genistein exhibited cytotoxicity against various human cancer cells. They postulated tectorigenin as a possible therapeutic agent for leukemia since it could cause apoptotic changes of DNA in the cells, inhibit the autophosphorylation of epidermal growth factor receptor by EGF and decrease the expression of Bcl-2 protein.


Family: Rubiaceae

Vernacular names

Eng: Indian madder , Hin: Mamjith, Majith , Mal: Mancatti, Covvallikkoti, Sivolikkoti , San: Manjistha , Tam: Sevelli, Manjitti

Distribution: Throughout India.

Parts used: Roots

Documented Use:

The roots of *Rubia cordifolia* are useful in rheumatoid arthritis, neuralgia, cephalalgia, dyspepsia, flatulence, colic, diarrhoea, dysentery, helminthiasis, leprosy, skin diseases, leucoderma, pruritus, wounds, ulcers, amenorrhoea, dysmenorrhoea, strangury, ophthalmopathy, intermittent fevers, pharyngitis, cough, diabetes, discoloration of the skin, slow healing of broken bones, tubercular conditions of the skin and mucous tissue, otopathy, urethrorrhea, haemorrhoids, jaundice, hepatopathy, splenopathy, arthralgia, leucorrhoea, pectoral diseases and general debility. (Varier *et al.*, 1994).
The traditional therapeutic use of the plant has been in skin disorders. It is found effective in scabies (Menon et al., 1980) and in infection with *Tinea pedis* (Pillai, et al.,1981). Its evaluation as an anticancer compound in the laboratory has been extensive, the extract showing weak activity *in vitro* and *in vivo* against standard tumour cell lines (Itokawa et al.,1984, Zhang,1983, Adwankar et al.,1984, Itokawa et al.,1984). The anti-inflammatory potential has been studied on carrageenin-induced oedema in rat hind paw. The plant water extract showed significant anti-inflammatory activity at a dose of 10 and 20 ml/kg body weight, comparable to that of phenylbutazone (Antarkar et al.,1983). Whereas no such activity was seen with *Rubia linctorum*, the Persian variety of manjistha.

Ho et al., (1996) examined the three naphthohydroquinones namely furomollugin, mollugin, and rubilactone from the roots of *Rubia cordifolia* for antiviral activity. Furomollugin and mollugin strongly suppressed the secretion of hepatitis B surface antigen (HBsAg), both with IC50 = 2.0 µ g/ml, in human hepatoma Hep3B cells while having little effect on the viability of the cells. Evaluation of structurally related derivatives of Furomollugin and mollugin revealed that a 6-hydroxy group and a pyran or furan ring contribute to this suppressive effect.

Kato et al.,(1987) compared the antitumor activity of RA-700, a cyclic hexapeptide isolated from *Rubia Cordifolia* with deoxy-bouvardin and vincristine (VCR). They observed that during the proliferation of L1210 cultured cells, the cytotoxicity of RA-700 was similar to that of VCR but superior to that of deoxy-bouvardin. The IC50 value of RA-700 was 0.05 mcg/ml under our experimental conditions. RA-700 inhibited the incorporation of 14C-leucine at a concentration at which no effects were observed on the incorporation of 3H-thymidine and 3H-uridine in L1210 culture cells in vitro. The antitumor activity of RA-700 was similar to that of deoxy-bouvardin and VCR against P388 leukemia. Daily treatment with RA-700 at an optimal dose resulted in 118% ILS. As with deoxy-bouvardin and
VCR, the therapeutic efficacy of RA-700 depends on the time schedule. RA-700 showed marginal activity against L1210 leukemia (50% ILS), similar to that of deoxy-bouvardin but inferior to that of VCR. RA-700 inhibited Lewis tumor growth in the early stage after tumor implantation, whereas deoxy-bouvardin and VCR did not. As regards toxicity, a slight reduction of peripheral WBC counts was observed with the drug, but no reduction of RBC and platelet counts. BUN, creatinine, GPT and GOT levels in plasma did not change with the administration of the drug.

Gann, (1984) tested an antitumor cyclic hexapeptide RA-V obtained from the roots of *Rubia cordifolia* for activity against cultured tumor cells and found that RA-V and its n-hexylether showed significant effects against human nasopharynx carcinoma (KB), P388 lymphocytic leukemia and MM2 mammary carcinoma cells.

Adwankar et al., (1982) tested RC-18, a pure isolate from *Rubia cordifolia*, on a spectrum of experimental murine tumors, viz. P388, L1210, L5178Y, B16 melanoma, Lewis lung carcinoma and sarcoma-180. They reported that RC-18 exhibited significant increase in life span of ascites leukaemia P388, L1210, L5178Y and a solid tumour B16 melanoma but it failed to show any inhibitory effect on solid tumours, Lewis lung carcinoma and sarcoma 180.

2.9.3.27. *Salacia reticulata* Wight

**Family:** Hippocrateaceae

**Vernacular names:**

**Mal:** Ekanayakam **San:** Vairi **Tam:** Ponkoranti

**Parts used:** Roots
**Documented Use:**

Roots are acrid, bitter, thermogenic, urinary astringent, anodyne, anti-inflammatory, depurative, liver tonic, and stomachic. Used in haemorrhoids, inflammations, leucorrhoea, skin diseases, amenorrhoea, hyperhydrosis, spermatorrhoea. (Varier et al., 1994). Useful in diabetes, leucorrhoea, leprosy, hepatopathy (Kirthikar et al., 1975).

**2.9.3.28. *Sida cordata* (Burm.f.) Borssum**

**Family:** Malvaceae

**Vernacular names**

**Hin:** Bananiyar, Bhyunli, **Mal:** Vallikkuruntotti, **San:** Nagabala,

**Tam:** Palampasi

**Distribution:** Throughout India as a weed on road sides and other waste places

**Parts used:** whole plant

**Documented uses**

The roots are useful in fever, uropathy and arthritis. The bark of the root is used for leucorrhoea, gonorrhoea and hyperdiuresis. The leaves are good for diarrhoea. The flowers and ripe fruits are refrigerant and are useful in relieving burning sensation, hyperdiuresis, pectoral lesions and promoting strength (Varier et al., 1994)

**2.9.3.29. *Sida rhombifolia* Linn.**

**Family:** Malvaceae
Vernacular names

**Hin**: Barela, Lalbarela., **Mal**: Vankuruntotii., **San**: Atibala., **Tam**: Sittamutti, Kuruntotii.

**Distribution**: Throughout India, as a weed of waste places

**Parts used**: Roots, stems

**Documented use**

The stems are used internally in dermatopathy. The roots are useful in conditions of diarrhoea, tuberculosis, leucorrhoea, strangury, burning sensation and dipsia (Varier et al., 1994)

2.9.330. **Sida rhombifolia** Linn. ssp. fetusa (Linn.) Borssum

**Family**: Malvaceae

Vernacular names

**Hin**: Jamglimedhi., **Mal**: Kuruntotti., **San**: Bala., **Tam**: Kuruntotti

**Distribution**: Throughout the warmer parts of India, as a weed of waste places.

**Parts Used**: Roots, leaves

**Documented use**

The roots and leaves are good for rheumatism, flatulence, colic, haemothermia, emaciation, conditions of seminal weakness, arthritis and diarrhoea. (Varier et al., 1994)
2.9.3.31.  *Symlocos cochinchinensis* (Lour.) Moore  

ssp. laurina (Retz.) Nooteboom  

Family:  Symlocaceae  

Vernacular names  


Tam:  Kambli- vetti  

Distribution:  Throughout India, in evergreen forests, ravines.  

Parts used:  Bark  

Documented use  

The bark of this plant is useful in conditions of ophthalmopathy, asthma, bronchitis, dropsy, arthritis, ulcers, tumours, leprosy, skin diseases, fever, ulemorrhagia, haemorrhages, haemoptysis, dyspepsia, flatulence, leucorrhoea, diarrhoea, dysentery and gonorrhoea (Varier *et al.* ,1994).  

Ishida *et al.* , (2001) identified a compound from *Symlocos setchuensis*, which could inhibit HIV replication in H9 lymphocyte cells with an EC_{50} of 0.037 μM and therapeutic index values of 210.  

2.9.3.32.  *Tinospora cordifolia* (Willd) Miers ex Hook.f.& Thoms.  

Family:  Menispermaceae
Vernacular names

Eng: Gulancha tinospora, Tinospora, Hin:Gulanca, Giloy, Amrta,

Mal: Cittamrtu, Amrtu, San:Guduci, Amrta, Tam:Amurutavalli, Cintilikkoti

Distribution: Throughout India in forests

Parts used: Stem

Documented use:

The stem *Tinospora cordifolia* is useful in conditions of burning sensation, hyperdipsia, helminthiasis, dyspepsia, flatulence, stomachalgia, intermittent fevers, chronic fevers, inflammations, gout, vomiting, cardiac debility, skin diseases, leprosy, erysipelas, anaemia, cough, asthma, general debility, jaundice, seminal weakness, uropathy and splenopathy. (Varier et al., 1994)

The hepatoprotective effect of *T. cordifolia* extract has been studied in carbon tetrachloride induced liver damage in rats. While acute damage was enhanced by prior exposure to the drug, it proved effective in the prevention of fibrosis, and in stimulating regeneration in hepatic tissue (Rege, 1984). Clinically, this drug has been tried as a therapeutic modality in rheumatoid arthritis (Kishore, 1980), jaundice (Hemadri, 1984) and diabetes (Saley, 1982). Used in compound formulations for treatment of jaundice, rheumatoid arthritis and diabetes.

2.9.3.33 *Wedelia chinensis* (Osbeck) Merrill

Family: Asteraceae

Vernacular names

Hin: Pilabhamgara, Mal: Mannakkannunni, San: Pitabhrngarajah,

Tam: Manjalkarilamkanni
**Distribution:** Throughout India, in wet place and coastal areas

**Parts used:** Whole plant

**Documented use:**

*Wedelia chinensis* is astringent, bitter, acrid, thermogenic, anti-inflammatory, vulnerary, ophthalmic, cardiotonic, anthelmintic, diuretic, aphrodisiac, sudorific, febrifuge and trichogenous. It is useful in vitiated conditions of inflammation, elephantiasis, otalgia, cephalalgia, wounds, ulcers, nyctalopia, dysopia, hepatosplenomegaly, colic, dyspepsia, helminthiasis, strangury, anaemia, seminal weakness, fever, baldness and greyness of hair. The plant is very specific for ‘viral hepatitis.’ (Varier et al., 1994)

In the light of the above cited reports on natural products, with anti-HIV potentials, and Immunomodulatory potentials, the present study has been planned and conducted to analyse the HIV-enzyme inhibitory and immunomodulatory potentials of certain Indian medicinal plants which have been cited in ancient Ayurveda and Siddha literatures as having blood purifying, Immunomodulating and anti-microbial properties. Attempts have also been made to characterise the bioactive molecules with anti-HIV properties by adopting biology-guided fractionation methodology.