1.1 Virus
Viruses are ultramicroscopic, acellular, metabolically inert nucleoprotein particles containing bundles of gene strands of either RNA or DNA, with or without a lipid-containing envelope. Unlike free-living bacteria, viruses are obligate intracellular parasites, utilise the host cell machinery to propagate new viruses and can cause ailments as benign as a common wart, as irritating as a cold, or as deadly as the bloody African fever. Hence, the viruses can be termed as the ‘acellular parasites of cellular hosts’, (Chattopadhyay and Khan, 1999). Viruses have numerous invasion strategies and each strain has its own unique configuration of surface molecules (Wagner and Hewlett, 1999; Chattopadhyay and Bhattacharyya, 2008), enabling them to enter into host cells by precisely fitting their surface molecules with the molecules of target cell. For example, the viruses that cause Lassa and Ebola fever and the retrovirus that causes acquired immunodeficiency syndrome (AIDS) spread easily kill swiftly and have no cure or vaccine. Each strain of virus is unique in its surface antigenic structure, its receptors on host cells and its life cycle. As a consequence of genetic variation, variety in mode of transmission, efficient replication and the ability to persist within the host, viruses have adapted to all forms of life and have occupied numerous ecological niches resulting in widespread diseases in humans, livestock and plants (Wagner and Hewlett, 1999; Chattopadhyay and Naik, 2007). Viral diseases, caused by pathogenic virus infections which have high morbidity and mortality rates, are still the leading cause of death in humans worldwide. Although effective vaccines have led or might lead to the eradication of important viral pathogens, such as small pox, polio, and mumps and other viral diseases, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), have proven difficult to combat using the conventional vaccine approach. Moreover, the emergence of viruses resistant to drugs, as well as the serious adverse effects induced by antiviral drugs, has caused serious medical problems, particularly when administered in combination over prolonged treatment periods. Although many new antiviral drugs have been approved in recent years, most of them are used for the treatment of HIV, and these drugs are quite costly, thus limiting their use in developing countries, where infection is most prevalent.

A virus is a unique pathogen which is incapable of replicating without a host cell. It utilizes the host cell environment and cellular factors for its propagation. This unique feature of viruses makes it difficult to design a treatment to attack the virus or its
Antiviral activity

replication directly without any adverse effects on the infected cells. However, viruses share a common stage in their replication cycle, which includes attachment and entry to the host cell, transcription of viral mRNA, replication of viral genome, assembly and budding as progeny virus particles, regardless of different genetic materials (DNA or RNA), or whether has a different invasion strategy of which enveloped with a lipid-containing membrane (enveloped virus) or not (Figure 1.1). Whereas, viruses with an RNA genome, such as HIV, HCV, and influenza, are genetically highly variable, due to the fact that viral reverse transcriptase or RNA-dependent RNA polymerase lack a proofreading mechanism. Accumulated mutations in viral RNA genome have been proven to be associated with the emergence of drug-resistant viruses (Richman, 2006; Yin et. al., 2006; Shafer et. al., 2007). The emergences of drug resistant viruses present a challenge for the design of new drugs. These problems emphasize the need to develop new antiviral drugs targeting different steps in the viral replication cycle.

Figure 1.1: Stages in virus replication and possible targets of action of Antiviral agents

An understanding of the molecular mechanisms of viral invasion and replication enables us to design antiviral drugs targeting the different stages of the viral replication cycle.
Antiviral activity

Although in theory, any viral molecule that is essential for viral replication is a potential drug target, most of the clinically useful antiviral drugs are the molecules that can specifically target a single viral enzyme, which is crucial in viral replication (De Clercq, 2002; Shafer et al., 2007). Targeting virus molecules is likely more specific, and less toxic. However, there is a narrow spectrum of viruses and a higher risk of creating resistant viruses. Whereas drugs which target cellular molecules may possess a broader antiviral activity spectrum and less risk of developing virus resistance, but may be more toxic to the host cell. Ideally, effective therapeutic agents that target multiple stages in the viral replication cycle with combined approaches but with little or no toxicity are desirable.

The discovery of novel anti-viral drugs deserves great efforts. There are several reviews published in recent years stating discovery of new drugs against different viral infections from natural resources. The role of traditional medicine for the development of anti-viral compounds is also been discussed in different reviews. Interestingly, it was found that traditional medicines, like Ayurveda, traditional Chinese medicine (TCM), Chakma medicines, are good sources for potential antiviral drugs (Khan et al., 2005). A wide variety of active phytochemicals, including the flavonoids, terpenoids, organosulfur compounds, limonoids, lignans, sulfides, polyphenolics, coumarins, saponins, chlorophyllins, furyl compounds, alkaloids, polyines, thiophenes, proteins and peptides have been found to have therapeutic applications against different genetically and functionally diverse viruses (Khan et al., 2005; Chattopadhyay, 2006; Naithani et al., 2008). The anti-viral mechanism of these agents may be explained on the basis of their antioxidant activities, scavenging capacities, inhibiting DNA, RNA synthesis, inhibition of the viral entry, or inhibiting the viral reproduction and so on. Large numbers of candidate substances, such as phytochemicals and their synthetic derivatives have been identified by a combination of in vitro and in vivo studies in different biological assays (Naithani et al., 2008; Christopher and Wong, 2006).
1.2 Herbal Medicine

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native people. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

It is common knowledge in many of today’s societies that plants and their extracts continue to provide effective treatment for diseases of all kinds, as they have been for centuries. Ayurvedic medicines in India and traditional Chinese medicine both depend to a large extent on the use for precise plant-based prescriptions for the treatment of specific disorders. Similarly contemporary practices are found in many regions, for example in other parts of Asia, South America, Africa, the Middle East and Russia (Farnsworth et. al., 1985).

1.2.1 Present Status of Herbal Medicine

The wide spread use of herbal medicine is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and German regularly prescribe herbal medicine (Murray and Pizzorno, 2000). The number of patients seeking herbal approaches for therapy is also growing exponentially (Alschuler et al., 1997). With the US Food & Drug Administration (FDA) relaxing guidelines for the sale of herbal supplement (Gottlieb, 2000), the market is booming with herbal products (Brevoort, 1998). As per World Bank reports trade in medicinal plants, botanical drug products and raw material is growing at an annual growth rate between 5 to 15%. The Global pharmaceutical market has risen from US $550 billion in 2004 worth to a close to US$900 billion in the year
Antiviral activity

2012. The herbal industry shares about US$62 billion with good growth potential. In India the value of botanicals related trade is about US$10 billion per annum with annual export of US$1.1 billion while China annual herbal drugs production is worth US$48 billion with export of US$3.6 billion (Kamboj, 2000). Presently the United States is the largest market for Indian botanical products accounting for about 50% of the total exports. Japan, Hong Kong, Korea and Singapore are the major importer of the herbal drugs making 66% share of China botanical drug export. Within the European community botanical medicine represents an important share of the pharmaceutical market. Out of many best 10 popular selling herbal medicines in USA are shown in Table 1.

Table 1.1: Ten Best Selling Herbal Medicine in USA

<table>
<thead>
<tr>
<th>Drug</th>
<th>Botanical Name</th>
<th>Market rank as per sale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinacea</td>
<td>Echinacea species</td>
<td>1</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allium sativum</td>
<td>2</td>
</tr>
<tr>
<td>Goldenseal</td>
<td>Hydratis Canadensis</td>
<td>3</td>
</tr>
<tr>
<td>Inseng</td>
<td>Panax species</td>
<td>4</td>
</tr>
<tr>
<td>Ginko</td>
<td>Ginko biloba</td>
<td>5</td>
</tr>
<tr>
<td>Saw Palmeto</td>
<td>Serenoa repens</td>
<td>6</td>
</tr>
<tr>
<td>Aloe vera</td>
<td>Aloe barbadensis</td>
<td>7</td>
</tr>
<tr>
<td>Ephedra</td>
<td>Ephedra species</td>
<td>8</td>
</tr>
<tr>
<td>Eleuthero</td>
<td>Eleutherococcus senticosus</td>
<td>9</td>
</tr>
<tr>
<td>Cranberry</td>
<td>Vaccinium macrocarpon</td>
<td>10</td>
</tr>
</tbody>
</table>

In the last few decades, a curious thing has happened to botanical medicine. Instead of being killed of by medical science and pharmaceutical chemistry, it has made come back. Herbal medicine has benefited from the objective analysis of the medical science, while fanciful and emotional claims for herbal cures have been thrown out, herbal treatments and plant medicine that works have been acknowledged. And herbal medicine has been found to have some impressive credentials. Developed empirically by trail and error, many herbal treatments were nevertheless remarkably effective (Dwyer and Rattray, 1993). In a recent survey (Cragg et al., 1997) it was estimated that 39% of all 520 new approved drugs in 1983-1994 were natural products or derived from natural products and 60-80% of antibacterial and anticancer drugs were derived from natural products (Harvey, 1999). The penicillin that replaced mercury in the treatment of syphilis has put an end to so many of
Antiviral activity

the deadly epidemics came from plant mold. Belladona still provides the chemical used in ophthalmological preparations and in antiseptics used to treat gastrointestinal disorders. *Rauvolfia serpentina* (The Indian snake root) which has active ingredient, reserpine, was the basic constituent of a variety of tranquilizers first used in the 1950’s to treat certain types of emotional and mental problems. Though reserpine is seldom used today for this purpose, its discovery was a breakthrough in the treatment of mental illness. It is also the principal ingredient in a number of modern pharmaceutical preparations for treating hypertension. But reserpine can have a serious side effect-severe depression. On the other hand tea made of *R. serpentina* has been used in India as a sedative for thousand of years (Dwyer and Rattray, 1993). Examination of the history of medicine and pharmacy reveals a definite pattern. Humankind first utilized materials found in the environment on an empirical basis to cure various ailments. These plant, animal parts and even microorganisms were initially employed in unmodified form, then as concentrated extract to improve their intensity and uniformity of action. Subsequently, pure chemical compounds as prototypes synthetic chemical entities were developed that possessed even greater activity (Robbers et al., 1996). In fact, plant substance remain the basis for a very large proportion of the medications used today for treating heart diseases, hypertension, depression, pain, cancer, asthma, neurological disorders, irritable bowel syndrome, liver diseases and other ailments (Vickers and Zollman, 1999; Carter, 1999; Bensoussan et al., 1998; Schuppan et al., 1999). By 1994, pharmacologist Norman Farnsworth had identified over 119 plant-derived substances that are used globally as drug. Many of the prescription drugs sold in United States are molecules derived from or modeled after naturally occurring molecules in plant. Interest in natural product research has been rekindled by discoveries of novel molecules from marine organisms (such as bryostatin) and potent new chemotherapeutic agents from plants (such as Taxol). Research has been facilitated by new rapid –through put bioassays in which robotic arms and computer controlled cameras test exceedingly small quantities of plant samples for the presence of the compounds active against a multiplicity of disease targets. It is possible to accomplish in a few minutes that once took months to analyze in laboratory. Even with new technology, it appears that one of the best sources for finding plant species to test is still the healer’s pouch, because such plants have often been tested by generations of indigenous people. Yet at this crescendo of enthusiasm for herbal medicine, an increasing number of aged healers are dying with their
knowledge left unrecorded. Too often though forests disappear without any notice. Currently 12.5 percent of all plant species are threatened with immediate extinction. Most botanists regard this estimate by the International Union for the Conservation of Nature (IUCN) as conservative, because it considers only species known to science; numerous undiscovered species pass from the world unrecorded and unmourned (Cox, 2000).

1.2.2 Status of Herbal Medicine in India

India has a rich tradition of herbal medicine as evident from Ayurveda, which could not have flourished for two thousand years without any scientific basis. Ayurveda which literally means knowledge (Veda) of life (Ayur) had its beginning in Atharvaveda (Circa 1500-1000 BC). Charak Samhita and Sushruta Samhita are the two most famous treatises of Ayurveda several other were compiled over the centuries such as Bela Samhita, Kashyap Samhita, Agnivesh Tantra, Vagbhata’s Ashtang hridaya (600), Madhava Nidan (700 AD) (Lele, 1999). Vegetable products dominated Indian Materia Medica which made extensive use of bark, leaves, flower, fruit, root, tubers and juices. The theory of rasa, vipaka, virya and prabhava formed the basis of Ayurveda pharmacology, which made no clear distinction between diet and drug, as both were vital component of treatment (Valiathan, 1998). Charak, Sushruta and Vagbhata described 700 herbal drugs with their properties and clinical effects.

Based on clinical effects, 50 categories of drug have been described – such as appetizers, digestive stimulants, laxatives, anti-diarrhea, anti-haemorrhoid, anti-emetic, anti-pyretic, anti-inflammatory, anti-pruritic, anti-asthmatic, antiepileptic, anti-helminthic, haemoptietic, haemostatic, analgesis, sedative, promoter of life (Rasyana), promoter of strength, complexion, voice, semen and sperm, breast milk secretion, fracture and wound healing, destroyer of kidney stones etc (Lele, 1999).

The advent of western medicine in the eighteen century was a set back to the practice of Ayurveda, which suffered considerable neglect at the hands of the colonial administration. After the first success of reserpine, an enormous amount of characterization of medicinal plants was done in many laboratories and University Departments, but the outcome was discouraging because the effort was disorganized, thin spread and nonfocused (Valiathan, 1998). Molecular pharmacology now provides a new interface between Ayurveda and modern medicine. Using modern techniques, various categories of Ayurvedic drug could
Antiviral activity

provide novel molecular probes. It is now possible to explore the mechanism of action of Ayurvedic drugs in terms of current concept of molecular pharmacology. Some striking example, of Ayurvedic drugs which are understood in terms of today’s molecular pharmacology:

Sarpagandha (*Rauwolfia serpentina*) Reserpine uniquely prevent pre-synaptic neuronal vesicular uptake of biogenic amines (dopamine, serotonin and nor-epinephrine).

Mainmool (*Coleus forskoli Briq*) Forskolin directly stimulates adenylate cyclase and cyclic AMP, with inotropic and Lusitropic effect on heart muscle.

Sallaki (*Boswellia serrata*) Boswellic acid inhibits 5-lipoxygenase and leukotreine B4 resulting in anti-inflammatory and anti-complement effect.

Shirish (*Albizzia lebek*) prevents mast cell degranulation, similar to sodium cromoglycate.

Aturagupta (*Mucona pruriens*) contains L-DOPA

Ashwagandha (*Withania somnifera*) GABA-A receptor agonist.

Katuka (*Picrorhiza kurua*) anti-oxidant action equal to a tocopherol, effect on glutathion metabolism in liver and brain (Lele, 1999). (Sukh Dev, 1997) listed 15 crude Ayurvedic drugs, which have received support for their therapeutic claims. Some of Rasyana dravyas have been shown to increase phagocytosis, activate macrophages and enhance resistance to microbial invasion.

Drugs like *Asparagus racemousus*, *Tinospora codifolia* and *Ocimum sanctum* antagonise the effect of stress (Dhuri et al., 2000).


Use of the herbal medicine in jaundice, presumably viral hepatitis, has been known in India science the Vedic times. About 170 phyto-constituents isolated from 110 plants belonging to 55 families have been reported so far to possess liver protective activities. It is estimated that about 6000 commercial herbal formulations are sold world over as hepatoprotective drugs. Of them about 40 patent polyherbal formulations representing a variety of combinations of 93 Indian herbs from 44 families are available in the Indian market (Bhatt and Bhatt, 1996). However, the following four herbal medicines have been found to be most promising in the treatment of viral hepatitis, (i) Silymarin obtained from

8
the seeds of *Silibum marianum*, (ii) Extracts of *Picrorrhiza kurroa*, popularly known ‘Kutaki’ (iii) Extract of many plant of the genus, Phyllanthus, have been used as hepatoprotective, of them, the most widely used ones have been *Phyllanthus niruri* and *Phyllanthus amarus*, (iv) Glycyrrhizin preparation have been used in the past for peptic ulcer as well as liver diseases with mixed results.

Liv 52, an extract of several plants prepared for Ayurvedic medicine was reported to improve serum biochemistry values in rats with toxic liver damage, and uncontrolled observations in patients with liver disease seemingly gave similar result (Jain and DeFilipps, 1991). Double-blinded and well-designed clinical trials have also been conducted with Argyowardhani in viral hepatitis, *Mucuna pruriens* in Parkinson’s disease, *Phyllanthus amarus* in hepatitis and *Tinospora cordifolia* in obstructive jaundice (Pal, 2002).

1.2.3 Research Approach to Herbal Products

The path of Reverse Pharmacology, arising from observational therapeutics is complementary to other approaches for natural drug development (Fig. 1.2).

**Figure 1.2: R & D path for Natural Products**

The diversity of medical uses of plant is at times daunting for a new entrant to the field. But for a multidisciplinary research and a development network the options of research approach provide deep motivation for identification of new pharmacophores. Besides expanding the herbal therapeutic and preventive armamentarium, new pharmacophores
may help to evolve new targets of drug action as well as a possibility for combinatorial chemistry on the novel pharmacophores. For example, curcumin has been a target molecule for a significant endeavour for a large number of combinatorial compounds. The Council of Scientific and Industrial Research (CSIR), in India has initiated sizeable and meaningful efforts for the development of herbal-based formulations for diabetes, arthritis and hepatitis by a national network programme (Patwardhan et. al., 2004). The industry, the academia and the government research laboratories work in close collaboration. Interesting and novel activities have been detected with the selected plants and some of the active ingredients of therapeutically demonstrable effects e.g. glycaemic control and inhibition of HbA1c (glycosylated haemoglobin) level coupled with a reduction in \textit{in vitro} formation of Amadori products. The diverse approaches to herbal drugs have led to interesting hits and novel activities, which need further in depth drug development efforts, both as herbal as well as new single molecule drugs.