1.0 INTRODUCTION

Since ancient times the parts of plants and other natural sources with medicinal properties have been in use. The early man used these medicinal sources in raw form. The medicinal and therapeutic usefulness of various plants has been recognized by almost all early civilized nations such as Greece, Egypt, China, India and Arabian countries. In India AYURVEDA—the science of treatment of diseases, has been existing since 3000 B.C. Charakasamhita and Susrutasamhita are the two Ayurvedic treatises describing the methods of preparation of medicines from plants including other sources and methods of treatment. In early 14th century the knowledge of preparation of enriched medicaments from plants and other natural sources as galenicals, tinctures, extracts etc was developed by few Apothecarists.

Developments in Science and Technology during the last two centuries revolutionized the preparation of bio-pharmaceuticals (drugs) from natural sources. The phytochemical techniques such as thin layer chromatography and column chromatography resulted in the isolation of large number of pure compounds. The advanced spectroscopic techniques of $^1$HNMR (Proton Nuclear Magnetic Resonance) and $^{13}$CNMR (Carbon Nuclear Magnetic Resonance) made the structure elucidation task easier and faster. Simultaneously, there was also tremendous development in the
standardization and evaluation of the bio-pharmaceuticals for various pharmacological activities.

Modern drug discovery is a long research process of multidisciplinary nature and encompasses a host of various experiments starting from the synthesis/isolation of active principles (compounds) in pure form till the establishment of therapeutic efficacy. Among these various research experimentations, the evaluation of a substance/ material whether natural or synthetic for its potential biological activity is the key step.

1.1. Introduction to Evaluation:

Historically Pharmacological evaluations are being carried out on various experimental animals such as frogs, mice, rats, rabbits, guinea pigs. These animal experimentation models have been well established during the past 50-100 years (Gerhard Vogel H, 2002). This is the classical approach of drug evaluation. Animal experimentation models for drug evaluation are called the in vivo tests. The in vivo tests are developed for evaluation of various pharmacological actions on different organ systems in the body. Few such important in vivo pharmacological tests include tests for action on Cardiovascular, Respiratory, Renal, Central Nervous and Autonomic Nervous Systems, Gastrointestinal tract, Liver, Analgesic, Antipyretic, Anti-inflammatory, Antidiabetic, Anti-lipidemic and Immunomodulatory activities.
Till 1970s, the drug evaluation was totally dependent on *in vivo* tests. The discovery of new drugs has taken momentum all over the world and many pharmaceutical companies started the pharmacological evaluation of large number of synthetic/natural products by using the animal models. The availability and handling of animals has become problem and at the same time the expertise and skill required to carryout experiments on these models is limited. In view of this large scale use of animals in drug discovery, many environmental/social organizations and individuals have raised objections for use of animals. Therefore, Governments of many countries have brought up Rules and Acts to restrict the use of animals for experimentation. In India, Committee for Purpose of Control and Supervision of Experimental Animals (CPCSEA)- a statutory body was formed in 1998 under the rules of Prevention of Cruelty to Animals Act-1960, to restrict and control the large scale use of animals in Pharmacological experiments and also suggested the use of alternatives.

1.2 Introduction to *in vitro* evaluation

The knowledge and understanding of structure and function at the cellular level has been increasingly available during the past 30 years. There has been a rapid progress in Cell Biology and Biotechnology since then. As an alternative to the animals use in initial pharmacological evaluation, *in vitro* (without the use of whole animal) models have been developed
(Ramakrishna Seethala, 2001). The principle of chemical change as a result of biological response is being exploited in the development and design of *in vitro* tests. In addition to the early citation of the biological activity of any compound, the *in vitro* tests offer to the understanding of the mechanism of action also. As there is no involvement of live animals in these tests, the conduct of these experiments is very convenient and easy.

As a result of increased Research and Development in synthesis or isolation of new molecules it has become necessary to screen all these compounds on animal experimentation models. However, it is impossible to carry out *in vivo* evaluation at a pace on par with the speed of synthesis of compounds. Eventually, the discovery process will suffer in meeting its goal. Hence the in vitro tests have become a boon to the discovery process, which has made possible to screen thousands of compounds within no time. The *in vitro* tests are developed for evaluation of various pharmacological or biological activities based on various concepts. The *in vitro* assays are broadly classified into *in vitro* Biochemical Assays and *in vitro* cell based assays.

### 1.2.1 *In vitro* Biochemical Assays

These are cell free assays to screen the compounds for their activity on targets such as enzymes, receptors, antigen-antibody interactions and protein–protein interactions. The *in vitro* biochemical assays are classified
into Homogeneous assays and Heterogeneous assays. The classification is shown in Figure 1.1.

**Figure 1.1 Classification of *in vitro* biochemical assays**

The *in vitro* biochemical assays have several advantages viz:

a. Easy accessibility of the compound to the target
b. Target identification without ambiguity
c. A well-defined mechanism of action
d. Application of new detection techniques
e. Miniaturization of experiments as per laboratory conditions
1.2.2 *In vitro* cell based assays

In these assays the targets screened involved signal transduction pathways. The cell-based assays are very close the environment of a living cell. Generally these assays are conducted for the targets where biochemical assays are not possible. So these cell based assays are also classified into Homogeneous assays and Heterogeneous assays. They are further sub-divided based on the nature of the cell and detection technique. The classification is shown in Figure 1.2

**Figure. 1.2 Classification of cell based assays**
With the advent of various techniques of detection, these cell based assays have become simpler and faster. The cell-based assay techniques are also used as primary screening methods.

The cell based assays have the following advantages:

a. The interaction of the compound with the target can be known.

b. The sequence of the cellular interactions can be traced at different time points

Therefore the cell based assays provide preliminary information on the mechanism of action of the compound at the cellular level.

**Homogeneous assays** are one pot assays without any further steps of transfer or washings. All the reagents are added at a time or in series and the signal is read in a multi well plate reader. **Heterogeneous assays** are multi step assays involving the series of steps such as incubations, washings, transfers, filtrations etc. in these assays also the signal is read in a multi well plate reader

In both of these *in vitro* assays the end point is measured by colorimetry, fluorescence, luminescence and radiometric detection techniques. These assays are carried out in 96 or 384 well micro titer plates. Recently a number of non-radioactive detection techniques have been developed. The fluorescence based techniques have become popular and widely used in cell based *in vitro* assays. The non-radioactive tests are:
i. Absorbance assays: In these assays the absorbance difference of the substrate and substrate enzyme complex are measured and quantified.

ii. Fluorescence assays: A number of fluorescence techniques have been developed and these techniques are 100-200 times sensitive than Colorimetric and Spectrophotometric techniques. The various fluorescence based assays are:
   a. Fluorescence intensity assays
   b. Fluorescence polarization
   c. Fluorescence Resonance Energy Transfer (FRET) assays
   d. Homogeneous Time-Resolved Fluorescence (HTRF).
   e. Fluorescence Correlation Spectroscopy
   f. Fluorescence Life time Assays
   g. Amplified Luminiscence Proximity Homogeneous Assays (ALPHA)
   h. Fluorescence Microvolume Assay Technology
   i. Microvolume Fluorimetry
   j. Laser- Scanning Imaging
   k. Microvolume Two Photon excitation
   l. Electro Chemiluminescence
   m. Magnetic Bead based Screening
   n. Flowcytometry
The anticancer *in-vitro* tests have become popular and important. In the anticancer research field almost 90% of compounds are first tested *in vitro*. In these anticancer tests various cancer cell-lines are used. The *in vitro* cell based evaluations for cancer are carried out by using different human cancer cell lines such as A549 (lung), H1299 (NSCLC), MCF7 (breast), PaCa2 (pancreatic), SH-SY5Y (neuroblastoma), HeLa60 (liver), K562 (AML, CML), HCT116 (colorectal), PC3, LNCaP (prostate), HepG2 (hepatoma) (Masters JRW, 2000).

Once the *in vitro* tests show a promising result, such compounds will be further subjected to other screening tests including the animal experimentation models. The *in vitro* models are best suited for the early detection of pharmacological and biological activity. Therefore, these tests are well accepted and widely used in initial screening methods, particularly in the field of cancer research.

**1.2.3 Flowcytometry:**

Flowcytometry is a technique of measuring the particles or cells, which was developed for the first time by Mack Fulwyer in 1965. Commercial instruments were made available from 1974 onwards. This technique is based on the principle of light scattering and emission of fluorescence by the particles/cells. A laser beam of light is directed on to the hydro dynamically focused fluid stream containing particles or cells in a suspension. The particles or cells may themselves have the fluorescence or
otherwise fluorochromes (fluorescence emitting reagents) are attached to the cells. The intensity of light scattered and fluorescence emitted by the particles is proportional to the number of cells and is detected by various detectors placed at different angles to the direction of stream of particle flow.

1.2.4 Fluorescence-Activated Cell Sorting:

Fluorescence-Activated Cell Sorting (FACS) is a particular flowcytometry technique by which heterogeneously mixed biological cells are sorted out in two or more containers. This sorting of one cell each time is based on the particular cell’s light scattering and fluorescence pattern. Thus in a pool of cells, various biologically/chemically distinct type of cells can be quantified. The data obtained from flowcytometer is plotted in a single dimension as histograms or in two dimensions as dot plots or even in three dimensions. The regions of these plots are further divided into subsets called gates which denote qualitatively and quantitatively different cell types. Because of this technical advantage, the FACS- flowcytometry technique has become indispensable equipment in molecular biology, pathology, immunology, marine biology and medicine. FACS- flowcytometry has wide applications in measurement of cell characteristics. A few such important applications are:

1. Morphological complexity and volume of cells.
2. Total DNA content (cell kinetics, analysis of cell cycle, proliferation etc.)

3. Apoptosis (measurement of DNA degradation, quantification changes in permeability, mitochondrial membrane potential and caspase activity.)


5. Intracellular antigens (cytokines, secondary mediators etc.)


7. Protein expression and Localization.

8. Analysis of Chromosomes and sorting.

**1.2.5 Western Blot (Protein Immunoblot):**

It is a technique to detect the specific type of proteins expressed or present in tissue /cell homogenate. Basically this is gel electrophoresis method specifically employing the Sodium Dodecyl Sulphate–Poly Acrylamide Gel Electrophoresis (SDS-PAGE) technique. The proteins separated on the gel are transferred to a membrane onto which specific antibodies are being adsorbed. The membrane is probed for the specific protein of interest with a modified antibody which is linked to reporter enzyme. Then the membrane is exposed to an appropriate substrate resulting in a color reaction or fluorescence or chemiluminescence. These are detected either visually or with the help of spectrophotometer or fluorescence detection equipment.
1.3 BIOPHARMACEUTICALS FROM NATURAL SOURCES:

The word Biopharmaceuticals is understood with a broad meaning of medicaments obtained from natural sources. So, these biopharmaceuticals include plant, animal and mineral based products which are used as such as medicines, pharmaceutical aids, lead compounds and pure natural product drugs. The plant based biopharmaceuticals include various products obtained or prepared from medicinal plants. These can be mixtures as well as pure compounds. A number of biopharmaceuticals have been evaluated for various pharmacological activities. A few such important biopharmaceuticals possessing the pharmacological activity are shown in Table 1.1.
Table 1.1 Biopharmaceuticals from Natural sources

<table>
<thead>
<tr>
<th>NATURAL SOURCE</th>
<th>COMPOUND/PRODUCT</th>
<th>USE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cannabis sativa</em></td>
<td>Cannabis</td>
<td>Hallucinogenic</td>
</tr>
<tr>
<td><em>Rauwolfia serpentine</em></td>
<td>Reserpine</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td><em>Anamrita cocculus</em></td>
<td>Picrotoxin</td>
<td>Analeptic</td>
</tr>
<tr>
<td><em>Claviceps purpurea</em></td>
<td>Ergotamine</td>
<td>Vascular constrictor</td>
</tr>
<tr>
<td><em>Papaver somniferum</em></td>
<td>Papaverine</td>
<td>Smooth muscle relaxant</td>
</tr>
<tr>
<td><em>Cassia anguistifolia</em></td>
<td>Sennosides</td>
<td>Mild purgative</td>
</tr>
<tr>
<td><em>Cephaelis ipecacunha</em></td>
<td>Emetine</td>
<td>Emetic, Amoeabicide</td>
</tr>
<tr>
<td><em>Digitalis pupurea</em></td>
<td>Digoxin</td>
<td>Cardiac stimulant</td>
</tr>
<tr>
<td><em>Ginkgo biloba</em></td>
<td>Ginkgolides</td>
<td>Antagonist of platelet activation</td>
</tr>
<tr>
<td><em>Colchicum autumnale</em></td>
<td>Colchicine</td>
<td>Treatment of Gout</td>
</tr>
<tr>
<td><em>Eugenia caryophyllus</em></td>
<td>Clove oil</td>
<td>Dental Analgesic</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>Withanolides</td>
<td>Potent Immunomodulator</td>
</tr>
<tr>
<td>Animal</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Apis mellifera</em></td>
<td>Honey</td>
<td>Nutritive</td>
</tr>
<tr>
<td><em>Gadus morrhua</em></td>
<td>Cod liver oil</td>
<td>Source of Vit-D</td>
</tr>
<tr>
<td>Mineral</td>
<td></td>
<td></td>
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<tr>
<td>Native talc</td>
<td>Talc</td>
<td>Pharmaceutical aid</td>
</tr>
</tbody>
</table>

1.3.1 Medicinal Plants and Traditional Systems of Medicine

Plants have been one of the important sources of medicines since the beginning of the human civilization. In spite of tremendous developments in the synthesis of organic compounds as drug molecules, plants still continue
to be the major source of drugs in modern as well as traditional medicine throughout the world. Approximately one third of all pharmaceuticals are of plant origin. (Singh VK et al, 2003)

Herbal medicine was used from ancient times and it was the only health care system in vogue at that time. It was found that herbs were used by all the civilizations and continues to be so, even today. The early man too appreciated the existence of plants available for their diverse utility. The plants not only provided food and shelter but also medicine and clothing. The usage of plants as medicine seems to have evolved by observing wild animals’ behavior and also through trials. Eventually, every tribe contributed to the knowledge of curing efficacy of herbs. The methodical collection of herbal information has led to development of detailed Herbal Pharmacopeia. Till 2000 AD, a major portion of official scientific medicines was resulted from herbal medicine. (http://www.herbalpalace.com)

Human beings by nature, palate and practice used parts of plant for curing which were hither to not consumed in their regular food. Neanderthal entombment site revealed in 1960 is the proof of herbal medicine that existed approximately 60,000 years. (http://www.herbalsupplement.com)

Herbs produce various chemical constituents which act on human beings. Herb is a biosynthetic laboratory and contains different types of
chemical compounds like glycosides, alkaloids, flavonoids, phenolics, terpenoids, steroids etc. These compounds are secondary metabolites and are responsible for medicinal property of the drug.

India is known as hub of herbal medicine as there are well documented medicinal plants, extensive traditional practice knowledge, second largest producer, with 6000 herbs in usage, catering to 75% of the world’s requirement and having nearly 7000 industries processing and formulating herbal medicines with or without standardization. (Dubey et al, 2004). Except few commercially important medicinal herbs all others are found in India. The herbal preparations of Aloe barbadensis, Panax and Allium sativum species which are extensively used World over are available in India.

In Ayurveda, Unani and Siddha several herbs are used in various forms for various ailments. The indigenous system of medicine is based mainly on the use of plants. Charaka Samhita (1000 BC – 100 AD) records the use of 2000 herbal medicines. In 1908 the publication of “The Indian Materia Medica” took place. There are nearly 3,000 herbal medicine monographs mentioned in it.

In China, Huang DI, the legendary yellow emperor is credited with writing The Yellow Emperor’ s Classic Internal Medicine (Huang Di Nei Jing) which lists 12 herbal prescriptions. The authorship of China’s first materia medica (Shen Nong Ben Cao Jing) is credited to the Mythical Shen
Nong (Divine Father), the yellow emperor’s predecessor. (Benky et al, 1993; www.ncam.nih.gov)

The Egyptians are also known for the use of herbs and official schools for herbalists existed in Egypt as early as 3000 B.C. The Ebers Papyrus was written around 1500 B.C. and discovered in 1862. Many of the founders of the ancient Greek schools of medicine owed their learning to the Egyptians. Hippocrates was tutored by Egyptian priest Doctors and his writings mention over 250 medicinal plants. The Greco-Roman knowledge of herbs was elaborated by Arabs which was lost in Europe in the dark ages.

Many Civilizations recognized that the Indian population was skilled at using the native plants as medicines and they began to incorporate them into their list of remedies. (http://www.holisticonline.com) In Britain, professional herbalists survived through establishment of National Institute of Medical Herbalists in 1864, which is flourishing till today. It has the oldest register of practicing medical herbalists in the world.

The WHO estimated that 4 billion people i.e. 80% of the world population presently use herbal medicine for their primary health care. WHO identified 119 plant derived pharmaceuticals used in modern medicine in ways that correlated directly with their traditional usage as plant medicines by natives of various cultures. Major pharmaceutical companies are conducting extensive research on plant materials gathered from the rain
forests and other places for their potential medicinal value. (WHO report 2003).

Medicinal plants have received renewed attention of scientists and farmers in recent years owing to their increased demand by pharmaceutical industries of Indian system of medicines. Medicinal herbs are forging ahead into main stream usage, as large number of people are seeking remedies and health approaches free from side effects that are caused by synthetic chemicals.

Considerable attention is being paid for utilization of eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. Considering the adverse effects of synthetic drugs, the western population is looking towards natural remedies, which are safe and effective. (WHO, World wide review, 1998).

1.4 Introduction to Cancer:

Cancer has become more prevalent in recent times throughout the World second to diabetes. The cell division is a natural phenomenon in all the living organisms including human beings. The older cells die and the new cells will be forming at a particular rate and in a controlled way. DNA is present in all cells and it is responsible for cell division and all its functions. Sometimes in a particular tissue/ tissues, the cell division continuously takes place without any control. When any damage occurs to DNA, the cell repairs it or otherwise the cell dies. If the cell doesn’t repair the damaged
DNA and doesn’t die, then it continues to divide and form numerous new
cells in uncontrolled manner, containing the same type of DNA. These cells
are not necessary to the body. The reasons for DNA damage are mistakes
during cell division, which is due to a number of factors of which a few are
environmental, diet/food, genetic etc. Many times no clear cause is found.
This uncontrolled cell division leading to extra mass in tissue/tissues is
called cancer. However, all the extra masses formed are not cancerous
growths. These extra masses are called tumors. Tumors are of two types.

(A) **Benign Tumors:**

- Benign tumors are not cancers.
- They can be removed.
- They don’t come again
- They don’t spread to other parts of the body.
- They are rarely a threat to life.

(B) **Malignant Tumours:**

- Malignant tumors are cancers
- Cells divide abnormally without any control.
- Spread to nearby tissues and organs and cause damage.
- The cells can break from a malignant tumor and enter into
  blood stream and lymphatic system.
- Cancer invasion is called as metastasis.
These cancers are named based on the cells in which they occur. For example skin cancer called as melanoma as it starts in melanocytes of the skin. When cancer spreads to other parts of the body such as liver, spleen, kidney, brain etc. from its origin, then the cancer is said to metastasize and the abnormal cells of the new cancer are similar to the primary cancer cells. Therefore, this new cancer is named as the metastatic cancer of the initial organ. For example, if lung cancer spreads to brain it is called as metastatic lung cancer and not as brain cancer. (www.medicine world.org)

1.4.1 Multistage carcinogenesis:

Models of rodent skin were used primarily in the development of multistage carcinogenesis (Rous et al., 1941; Mottram 1944). The data relating to epidemiology also suggest that the progression of cancer is through a multistage route. Evidence from experiments revealed that at least three distinctive stages are involved in carcinogenesis.

(a) Initiation:
This is the initial step wherein a heritable mutation which is not fatal, occurs in one of the somatic cells. The growth benefit required in second phase of promotion is met by the initial mutation. These mutated cells circumvent the normal mechanism of regulation of cell which does not occur in normal cells in neighbourhood.
(b) Promotion:
In this stage, in the mutated cell, there occurs a phenotypical clonal growth, after exposure to tumour promoter. These promoters act as external or internal stimuli for growth of the initiated cells leading to the promotion of carcinogenesis (Cerutii et al., 1985; 1991;1994). The direct or indirect effects of the tumour promoters provide the signal for clonal expansion of the neighbouring cells. The first and second stages in the carcinogenesis jointly turn out comparatively benign growths.

(c) Malignant conversion:
After promotion stage, the initiated tumour cell will be converted into malignant growth. At this stage, sensitivity of the tumour cells to the dietetic compounds will disappear. The tumours will become more independent and drastic intrusions are required to control the growth of cells at this stage (Guyton et al., 1993). This stage is at a low pace and is prolonged. The growth interfering agents such as vitamins, folic acid, retinoic acid, hormones, calcium etc., can affect this stage to a greater extent.

1.4.2 General Signs & Symptoms of Cancer:
There are many symptoms for cancer, but these symptoms at an early stage are common to many other general ailments. Only at later/advanced stage these symptoms will become characteristic of cancer. Therefore at
initial stage it is difficult to identify the cancer based on the symptoms (http://www.cancer.about.com).

(A) Weight Loss:
When a person loses weight without any known reason, it is called as unexplained weight loss. An unexplained weight loss of 10 pounds per month is considered as the first sign of cancer. This is generally found in cancers of stomach, esophagus, spleen and lung.

(B) Fever:
Fever is very common in cancer and is more often when cancer spreads to other parts. Fever is also common when the cancer treatment affects the immune system.

(C) Fatigue:
As the cancer spreads, fatigue will become more prominent symptom. It is an early symptom in case of blood cancers as they cause blood loss which leads to fatigue.

(D) Pain:
In bone and testicular cancers, pain is an early symptom. Cancer of colon, rectum and ovary causes back pain. Headache is severe in brain tumor.

(E) Changes In Skin:
Not only the skin cancers but also some other cancers cause skin changes such as hyper pigmentation (darkening of skin), jaundice (yellowish skin), erythema (reddened skin), pruritis (itching) and excessive hair growth.
1.4.3 Risk Factors:

A number of scientists are doing research on various aspects of cancer. It is observed that many factors are associated with the chance of cancer occurrence (IARC 1987, 2002; US Report on carcinogens, 2004; American Cancer Society, 2005). These risk factors are:

(a) **Growing Older:**
This is the most important risk factor and the people above 65 years are more prone to attack by cancer. However there are incidences of cancer in children also.

(b) **Tobacco:**
Every year thousands of people die because of the cancer caused by tobacco use in one or the other form. Smokers are more prone to develop different types of cancers like lung, larynx, mouth, esophagus, bladder, throat, kidney, pancreas and acute myeloid leukemia.

(c) **Sun Light:**
UV radiation through sunlight can cause early aging of the skin by damaging the skin cells which ultimately leads to skin cancer.

(d) **Ionizing Radiation:**
Radioactive fallout generated during accidents at nuclear power plants or during the production and testing of atomic weapons leads to leukemia and thyroid, lung, stomach and breast cancers.
(e) Certain Chemicals & Other substances:
Certain professionals like painters, construction workers and workers in chemical industries may develop different types of cancers. Exposure to Asbestos, Benzene, Benzedrine, Cadmium, Nickel, Vinyl chloride etc. increases the risk of development of cancers.

(f) Some Viruses & Bacteria:
Human Papilloma virus cause cervical cancer, Hepatitis B and C viruses can develop liver cancer. People with HIV infection are at great risk of cancer. Epstein-Barr virus increases the risk of lymphoma and Helicobacter pylori can develop stomach cancer and lymphoma of stomach lining.

(g) Certain hormones:
Generally doctors prescribe hormones to control the problems in menopause and these hormones i.e. estrogen and progestin increase the risk of breast cancer.

(h) Family History of Cancer:
Certain types of cancers like melanoma, breast, ovarian, prostrate and colon cancers run in some families. This may be due to the mutations in the genes which are caused by a person’s life style or environment. These genetic changes pass from parents to children and are present in all cells at the time of birth.
(i) Alcohol:
The risk of cancer gradually increases with increase in alcohol quantity. The person who consumes alcohol more than two times a day for many years will be at the risk of developing cancers of mouth, throat, larynx, esophagus, stomach and liver. The risk increases if the person uses tobacco products also.

(j) Poor Diet, lack of Physical Activity & Over Weight:
The people who take diet with high fat content are at risk of colon, uterus and prostrate cancers. Lack of physical activity, being overweight leads to cancers of breast, colon, esophagus, kidney and uterus.

1.4.4 Conventional Cancer Treatments and Their Side Effects:

(a) Surgery:
Surgery is one of the oldest forms of cancer treatments which are used to remove only the tumor or the entire organ. This method of treatment is used when cancer has not spread to other parts of the body. Surgery though successful in many cases, causes one or all of the following side effects (Weiger W A et al, 2002; Werneke U et al 2004; Cassileth B R et al, 2004; www.cancer.gov)

- Risk of wound infection
- Damage may occur to internal organs and blood vessels during surgery.
• After surgery blood clots may form in deep veins of the legs, in the cases in which the person remains in bed for a long time.

• Reactions may occur to anesthesia or to other medicines which may become serious and can lead to low blood pressure.

• Problems may occur in other organs such as lungs, kidneys or heart and can be life-threatening.

(b) Radiotherapy:

Radiation therapy is used to kill cancer cells and shrink tumors. Radiation can be administered externally (external beam radiotherapy) or internally (brachy therapy). The side effects caused by radiation therapy are as follows:

• Fatigue is the common side effect of radiation therapy.

• Severe blood changes including drop in production of new cells.

• Nausea, vomiting, anemia.

• Irritation and burning of the skin.

• Infertility, exposure of testicles to radiation can cause permanent loss of sperm production.

• Immune system will become weak

• Brain disorders such as changes in brain function which may lead to memory loss, lower sexual desire etc.

• Skin becomes tender or sensitive, dry and itchy. Peeling of skin may also occur.

• The radiation itself can cause second cancers (metastasis)
(c) **Chemotherapy:**

Chemotherapy is a type of systemic treatment which not only affects cancer cells but also the healthy cells. This effect depends on the dosage regimen of the drugs. The anti-cancer drugs arrests cell division at various phases of cell division. Along with the effects on cancerous cells, the white blood cells are also affected to a greater extent. Therefore, a sudden considerable drop in WBC cell count is a serious side effect which is to be monitored carefully and the situation can also lead the patients to succumb to various infections. The other important side effects are anemia, hair loss, intestinal disorders, lesions in the mouth etc.

(d) **Hormone therapy:**

The side effect of hormonal therapy depends on the type of treatment used and the medicines prescribed. For example, Tamoxifen, deprives the effect of estrogen on cancer cells and also causes hot flushes, irritation or vaginal discharge, nausea and irregular menstruation.
1.5 Development of Bio-Pharmaceuticals from Plants for Treatment of Cancer:

Plants have been used in treatment of cancer since long. There are about 3000 plant species that were reported to possess anticancer properties. (Li Q et al, 2002; Newman D J et al, 2002, 2003; Cassady et al, 2004; Cichewitz et al 2004). 60% of the currently used anticancer drugs are directly or indirectly originating from natural sources which include plants as well as marine organisms and micro-organisms. The molecules derived from the above natural sources are playing a key role in the discovery of lead compounds for the development of potential drugs for the treatment of cancer.

The search for anti-cancer principles in plants was started in early 1950’s. The extract of *Vinca rosea* caused significant life extension in mice having a transplantable lymphocytic leukemia and led to the discovery of most widely used anti-cancer drugs from *Vinca rosea* or *Catharanthus roseus* (Apocyanaceae) *i.e.* vinca alkaloids – vincristine (VCR) and vinblastin (VLB). VLB is used in treatment of leukemia, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi’s sarcoma. VCR is effective against acute lymphocytic leukemia in children. Recent semi synthetic analogues of these are Vinorelbine (VRLB) and Vindesine (VDS). These are used along with other chemotherapeutic agents. VRLB is effective against non-small-cell lung cancer (NSCLC) and advanced breast cancer.
In 1960, United States National Cancer Institute (USNCI) initiated extensive plant collection and discovery of some taxanes and camptothecins having cytotoxic properties. But, it took 30 years for their development into clinically active agents.

The Podophyllum species, *P. peltatum* and *P. emodii* have a good history of medicinal use for the treatment of skin cancers. During 1960s and 1970s, there was an extensive research in Sandoz Laboratories in Switzerland which led to the development of Etoposide and Teniposide which were effective in the treatment of lymphomas, bronchial and testicular cancers.

A more recent plant derived chemotherapeutic class of molecules is Taxanes. The leaves of *Taxus baccata* are used in Indian Ayurvedic medicine for the treatment of cancer. Paclitaxel (Taxol) was isolated from the leaves of *T. brevifolia* and is used in the treatment of breast, ovarian and non-small-cell lung cancer (NSCLC) and also shown efficacy against Kaposi sarcoma. Docetaxel, analog of paclitaxel is primarily used in treatment of breast cancer and NSCLC.

Another important anticancer plant is *Camptothecin acuminata*, a chinese ornamental tree from which camptothecin was discovered. But, it was later dropped because of its bladder toxicity. The derivatives of camptothecin are Topotecan (Hycamtin) and Irinotecan (Camptosar). Topotecan is used for the treatment of small-cell- lung cancers and
Irinotecan is used for the treatment of colorectal cancers. Many camptothecin derivatives are in preclinical development.

Homoharringtonine (HHT) is another plant derived drug in clinical use which is isolated from *Cephalotaxus harringtonia* (Cephalotaxaceae). Elliptinium is a derivative of ellipticin isolated from several genera of Apocyanceae family, which shows potential anticancer properties and is used for the treatment of breast cancer. A racemic mixture of harringtonine and homoharringtonine is used for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Purified HHT is effective against the leukemias those are resistant to standard treatment and found that it produces complete hematologic remission (CHR) in patients with late chronic phase myelogenous leukemia (CML).

There are many plant derived drugs under clinical development for the treatment of cancer. They include Flavopiridol analogue of Rohotukine (*Dysoxylum binectariferum*), combrestatins (*Combretum caffrum*), Roscovitine derived from Olomucine (*Raphanus sativus*) etc.

A list of Plants, their botanical name and the part of the plant mentioned to be useful in cancer is mentioned in Table 1.2.
**Table 1.2 Medical Plants with Anticancer Properties:**

<table>
<thead>
<tr>
<th>NAME OF THE PLANT</th>
<th>PLANT PART USED</th>
<th>ACTIVE CONSTITUENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acanthus ilicifolius</em></td>
<td>Whole plant</td>
<td>derivatives of benzoxazoline</td>
</tr>
<tr>
<td><em>Adhathoda zeylanica</em></td>
<td>Whole plant</td>
<td>Pyrroloquinazoline alkaloid: vasicine, vesicinone, vesicinolone.</td>
</tr>
<tr>
<td><em>Ajuga bracteosa</em></td>
<td>Whole plant</td>
<td>Glycoside, tannins, sitosterol, cerotic and palmitic acids.</td>
</tr>
<tr>
<td><em>Aloe vera</em></td>
<td>Leaf</td>
<td>Anthracene glycosides</td>
</tr>
<tr>
<td><em>Alstonia macrophylla</em></td>
<td>Whole plant</td>
<td>Polyphenol, tannin, and choline.</td>
</tr>
<tr>
<td><em>Annona purpurea</em></td>
<td>Stem and leaf.</td>
<td>Alkaloid.</td>
</tr>
<tr>
<td><em>Apium graveolens</em></td>
<td>Whole plant</td>
<td>Isoquercitin, choline, eudesmol, £,β- sedanolide, limoline.</td>
</tr>
<tr>
<td>NAME OF THE PLANT</td>
<td>PLANT PART USED</td>
<td>ACTIVE CONSTITUENTS</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Avena sativa</em> Poaceae</td>
<td>Whole grain</td>
<td>Alkaloid, saponin, flavonoid, vitamin and protein.</td>
</tr>
<tr>
<td><em>Bacopa monnieri</em> Scrophulariaceae</td>
<td>Whole plant</td>
<td>Saponin glycoside: triterpenoid type, flavonoid and β sitosterol</td>
</tr>
<tr>
<td><em>Bauhinia variegata</em> Leguminosae</td>
<td>Whole plant.</td>
<td>β Sitosterol, kaempferol-3-glucoside</td>
</tr>
<tr>
<td><em>Bruguiera gymnorrhiza</em> Rhizophoraceae</td>
<td>Whole plant.</td>
<td>β sitosterol, lupeol, oleanolic acid.</td>
</tr>
<tr>
<td><em>Butea monosperma</em> Fabaceae</td>
<td>Whole plant</td>
<td>Vitamin, protein and mineral.</td>
</tr>
<tr>
<td><em>Cardiospermum halicacabum</em> Sapindaceae</td>
<td>Whole plant</td>
<td>Alkloids, β sitosterol. L-triacontanol, n-pentacosane, and n-triacontane.</td>
</tr>
<tr>
<td>NAME OF THE PLANT</td>
<td>PLANT PART USED</td>
<td>ACTIVE CONSTITUENTS</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Cleome viscosa</em></td>
<td>Whole plant</td>
<td>Macrocyclic diterpene, bicyclic Diterpene cleomeolide and coumarinolignan.</td>
</tr>
<tr>
<td>Cappardiaceae</td>
<td>Root</td>
<td>Sterol, glycosides, campesterol, sitosterol and clerodin.</td>
</tr>
<tr>
<td><em>Clerodendrum infortunatum</em></td>
<td>Whole plant</td>
<td>Eugenol and dianchinenosides A,B,C,D.</td>
</tr>
<tr>
<td>Verbenaceae</td>
<td>Tuber</td>
<td>Alkaloid: Tabernaemontanine</td>
</tr>
<tr>
<td><em>Dianthus chinensis</em></td>
<td>Whole plant</td>
<td>Diterpene and linoleic acid.</td>
</tr>
<tr>
<td>Caryophyllaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eulophia nuda</em></td>
<td>Tuber</td>
<td>Coumarin, isoflavone, triterpenoids, saponin, glycyrrhizin, glabranin.</td>
</tr>
<tr>
<td>Orchidaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Excoecaria agallocha</em></td>
<td>Whole plant</td>
<td>Kosins, kosotoxin and protokosin.</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glycyrrhiza glabra</em></td>
<td>Whole plant</td>
<td></td>
</tr>
<tr>
<td>Leguminosae</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hagenia abyssinica</em></td>
<td>Whole plant</td>
<td></td>
</tr>
<tr>
<td>NAME OF THE PLANT</td>
<td>PLANT PART USED</td>
<td>ACTIVE CONSTITUENTS</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td><em>Kaempferia rotunda</em> Zingiberaceae</td>
<td>Tuber</td>
<td>Essential oils.</td>
</tr>
<tr>
<td><em>Nicotiana tabaccum</em> Solanaceae</td>
<td>Leaf</td>
<td>Nicotine, piperidine, N-methylpyrrolidine and N-methyl-l-anabatine.</td>
</tr>
<tr>
<td><em>Ophirrhiza mungos</em> Rubiaceae</td>
<td>Root</td>
<td>β-sitosterol and amorphous alkaloid.</td>
</tr>
<tr>
<td><em>Oxallis acetosella</em> Oxalidaceae</td>
<td>Whole plant</td>
<td>Oxalic acid, potassium oxalatae, and vitamin C.</td>
</tr>
<tr>
<td><em>Rubia cordifolia</em> Rubiaceae</td>
<td>Root</td>
<td>Purpurin, pseudopurpurin, alizarin and xanthopurpurin.</td>
</tr>
<tr>
<td><em>Rumex acetosa</em> Polygonaceae</td>
<td>Leaf</td>
<td>Hyperoside, oxymethylanthraquinone, oxalic and tartaric acid.</td>
</tr>
<tr>
<td>NAME OF THE PLANT</td>
<td>PLANT PART USED</td>
<td>ACTIVE CONSTITUENTS</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><em>Salvadora persica</em> Salvadoraceae</td>
<td>Leaf</td>
<td>Alkaloid: trimethylamine and β sitosterol.</td>
</tr>
<tr>
<td><em>Salvia officinalis</em> Labiatae</td>
<td>Whole plant</td>
<td>Diterpene, phenolic acid, flavonoid and tannin.</td>
</tr>
<tr>
<td><em>Stephania hernandiifolia</em> Menispermaceae</td>
<td>Root, rhizome and plant.</td>
<td>d- and dl- Tetrandrine, fangchinoline, d-iso chondodendrine alkaloids.</td>
</tr>
<tr>
<td><em>Syzygium cornocarpum</em> Myrtaceae</td>
<td>Whole plant</td>
<td>Oleanolic acid and eugenol.</td>
</tr>
<tr>
<td><em>Terminali catappa</em> Combretaceae</td>
<td>Whole plant</td>
<td>Oleic, palmitic, and linoleic acids</td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em> Menispermaceae</td>
<td>Stem</td>
<td>Berberine, tinosporine, giloin and gilonin</td>
</tr>
<tr>
<td><em>Tylophora indica</em> Asclepiadaceae</td>
<td>Root and leaf</td>
<td>Alkaloid, £- amyrin and quercetin.</td>
</tr>
<tr>
<td>NAME OF THE PLANT</td>
<td>PLANT PART USED</td>
<td>ACTIVE CONSTITUENTS</td>
</tr>
<tr>
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<td>-------------------</td>
</tr>
<tr>
<td>Urgenia indica</td>
<td>Bulb</td>
<td>Cardiac glycosides: Scillaren-A, and Scillaren-B</td>
</tr>
<tr>
<td>Liliaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscum album</td>
<td>Whole plant.</td>
<td>Acetyl choline, propionyl choline, lupeol, viscotoxin, flavonoid and sterol-A.</td>
</tr>
<tr>
<td>Loranthaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitex negundo</td>
<td>Leaf</td>
<td>Essential oil and nishindine alkaloid.</td>
</tr>
<tr>
<td>Verbenaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitex trifolia</td>
<td>Leaf</td>
<td>Camphene, diterpene and flavonoid.</td>
</tr>
<tr>
<td>Verbenaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthium strumarium</td>
<td>Root and whole plant</td>
<td>Sesquiterpene lactones: xanthin, xanthininim and xanthatin.</td>
</tr>
<tr>
<td>Asteraceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yucca aloifolia</td>
<td>Flower.</td>
<td>Agafoline</td>
</tr>
<tr>
<td>Agavaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zea mays</td>
<td>Whole plant</td>
<td>βCarotene, and vitamins C,E,K</td>
</tr>
<tr>
<td>Graminae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zosima orientalis</td>
<td>Root and fruit</td>
<td>coumarin</td>
</tr>
<tr>
<td>Apiaceae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.6 Apoptosis:

The living system has to continuously shed the cells that are damaged or superfluous in an automated and regulated manner. This process of automated cell death is known as Apoptosis. This process is based on cell genetic program and is an essential requirement for the growth and functioning of every living creature. The initiation of Apoptosis takes place either by extrinsic pathway at the surface of the cell or by intrinsic pathway inside the cell. The cells die through cleavage of cellular substrates which is achieved by the release of caspases in the cell by either of the pathways. The extrinsic pathway results through the death receptors on the cell surface. This occurs by employing ligand activated adopter proteins and resulting in activation of caspase-8. Apoptosis by intrinsic pathway, is by releasing proapoptotic factors like cytochrome C from mitochondria into the cell cytoplasm, which triggers the caspase cascade. This pathway is mainly regulated by Bcl-2 proteins. The Apoptosis pathways are shown at Fig 1.1 (www.sabiosciences.com),
Figure 1.3  The apoptosis pathway
1.7 Role of Free Radicals in Cancer Mechanism:

Growth of cancer is characterized by the sequential multi-faceted events that take place at molecular and cellular levels. It is known that the free radicals including active oxygen species interfere with cellular changes and cause mutagenesis. Further, confirmation is available for the involvement of free radicals as intermediaries in pheno type and geno type changes at cellular level leading mutation to neoplasia. Though free radical production generally occurs in all respiring creatures, their levels are found to rise during stress, exposure to carcinogens and disease progress. It is evident experimentally and also endorsed by epidemiological and clinical studies that the beneficial effects observed by the use of antioxidants in the cancer treatment provide proof of the free radicals involvement in cancers (Tamer Fouad, 2002). The free radicals were also found to be involved in oxidative stress in chronic inflammatory situations leading to increased probability of carcinogenesis. It was also believed that several toxic chemicals produce free radicals or their metabolites which lead to carcinogenesis.

1.7.1 DNA Damage - Role of Reactive Oxygen Species:

Chromatin in the cell nucleus offer defence mechanism to certain extent to prevent the base mutation of the cell by DNA based oxidation which is an unending process. Nevertheless, enhanced levels of either exogenous or endogenous free radicals overcome this defence mechanism
and produce mutations. Prolonged presences of these free radicals pass oxidative stress to the cells to stimulate further phases of carcinogenesis (Guyton et al., 1993).

It was found that the free radicals’ mutagenicity is because of direct involvement of the hydroxyl groups at DNA rather than the free radicals as such. The experimental evidence was obtained from electron paramagnetic spectroscopy technique which detected damage to DNA induced by hydroxyl radical. *In vitro* experiments revealed that super oxide and hydrogen peroxide did not interact with DNA to cause any lesions in the cell (Breimer et al., 1990). The free radicals when interact with transition metals like iron and copper, produce the hydroxyl radicals which provided evidence that the oxidative stress caused by the free radicals is via the production of hydroxyl radical.

Further evidence for the interaction of hydroxyl radicals with DNA was elicited by the inhibition of mutation and malignant conversion, by metal chelators which chemically block the hydroxyl radicals (Aruoma et al. 1989).

Reasons for the cause of direct DNA damaged genetic lesions by hydroxyl radical are that it is highly reactive and unstable. Hydroxyl radical also produce indirect effects through increase in intra-cellular calcium levels which cause strand breaks in DNA leading to the formation of degradation products. Reactive oxygen species bring about many changes in the cell such as production of base free sites, base modifications, cross links
between DNA and proteins, strand breaks, deleterious frame shifts and re-arrangements in chromosomes (Halliwell et al. 1991).

**1.7.2 Role of Oxidative DNA damage in initiation:**

The beginning of cancer in initial cells occurs by passing on permanently altered genetic material to the progeny by the cancer cells. In the initial cancer cells, modified DNA must be resistant to the repairing process and at the same time should not be so powerful to create death of the cell. The oxidants induced genetic damages will be lethal. Nevertheless, genetic material deregulation happens in the form of incapacitation, delete or rearrange the promoter or enhancer areas. Thus, the DNA damage caused by the free radicals resulting in the loss of tumour suppressor gene or its total inactivation lead to further stages of cancer. Even the single base pair modification would cause incapacitation of tumour suppressor gene. Analysis of point mutations caused by oxidant exposure in *in vitro* experiments conducted by Halliwell and Dizdaroglu proved that, G and C alterations in base pairs were predominant (Halliwell et al., 1991).

**1.7.3 Role of transition Metals in Oxidative DNA Damage:**

It was observed that exogenous addition of transition metals like copper and iron to the DNA solution cause impairment. This is due to the oxygen species that are generated impairing the DNA bases. Copper was
found to produce higher mutations than the iron (Guyton et al., 1993). This was explained by high affinity of DNA to bind to the copper than to iron. The copper bound with DNA also brings about several changes in physiology of the cell which include stabilizing framework of DNA during its synthesis and transcriptional phases of metaphase in cell division. It even behaves as link amidst DNA and related nuclear proteins.

Copper-DNA direct interaction occurs through specific binding at G-C paired sites. Though the bondage of copper to stabilize DNA is essential, it also gives an opportunity by providing a site for reactive oxygen species to exhibit their damaging effects. Particularly, when copper is present, it promotes the formation of extremely reactive hydroxyl radical from hydrogen peroxide and superoxide radicals that are not so reactive otherwise.

1.7.4 Role of Free Radicals in Tumour Promotion:

The oxidative stress leads to later phases of carcinogenesis (Cerutti, 1985), which is evidently proved to be indirect. It was observed that many biochemical processes promoting carcinogenesis were found to decrease in presence of antioxidants and oxidant detoxifiers. Further, evidence revealed that the oxidative stress situation was created by tumour promoters. This stress environment is also associated with a sustained decrease in natural anti-oxidant defence mechanism existing in the cell such as glutathione peroxidase, catalase, superoxide dismutase activities.
Tumour promotion is accompanied by a series of complex changes at cellular level which stimulate the initiated cell population through clonal expansion while the normal cells are left out. This may be due to direct growth stimulating effect of tumour promoter on initiated cell or indirect consequential effect on normal cells. The normal cell proliferation occurs through a selective terminal differentiation while in the initiated cell the proliferation takes place to fill the gaps left by the exclusion of the counter parts. The direct and indirect stimulatory effects of phorbol ester promoters on initiated cell have been experimentally proved. Oxidative stress also can be expected to cause similar dual signal. The initiation process is associated with one or few phenotype changes.

1.7.5 Role of Free Radicals in Malignant Conversion:

The tumor promoter produce clonally derived benign growths by selective modifications of the gene in initiated cells. Further damage to DNA converts benign growths to fast growing malignant neoplasms. It was experimentally proved that papilloma cells when treated with free radical-generating chemicals like benzoyl peroxide or any other initiator, cause cancer from benign growths. The process may cause further change in DNA or genetic material. This results in formation of a heritable genetic lesion in benign tumor cells which transform to irreversible growing autonomous cancer (Guyton et al, 1993).
1.8 CHEMOPREVENTION OF CANCER:

Now a days, cancer is the largest death causing disease subsequent to cardiovascular diseases (Olivera et al, 1997). Cancer is biochemical status of substantial alterations in cell causing several physiological changes resulting in unrepressed malignant growth (Hanahan et al, 2000). Cancer is a multifarious progression encompassing a number of intricate factors (Nowell, 1986). Many biochemical and systematic accrual of genetic changes occur during carcinogenesis (Pitot et al, 1994). Increased information and knowledge about the cellular and molecular processes in carcinogenesis led to the study of chemo prevention of cancer. Chemo prevention of cancer is explained as a medical approach aimed to arrest or reverse the cancerous cell processes by using nutritional supplement and/or medicinal compounds, particularly by using anti-oxidants during the intervening stages of initial and progressing tumor cells (Kelloff et al, 1994). Cancer is a multiphase progression usually occurring in several years providing ample opportunity for intervening with novel methods to arrest the cancer process (Kelloff et al, 1996; Green Wald, 2001). The advance studies at molecular and cellular have helped in detecting the genetic lesions and cell regions responsible to initiate and progress the malignancy. These detections help in targeting the use of chemoprevention.

Antioxidants were found to be particularly useful as chemo preventive natural substances. In recent times there is an increased awareness about the beneficial effects of antioxidants in chemoprevention. This fact can
better be utilized for reducing the incidence of cancer occurrence and also reducing mortality. Many oncologists have recognized the efficacy of chemopreventive agents even in the treatment of advanced cancers. Since carcinogens cause cancers, the chemoprevention may neutralize or block their effects and inhibit the cancer cell growth. Several compounds from natural sources i.e biopharmaceuticals have been intensively used in the chemoprevention of cancer essentially for their beneficial effects of non-toxic and insignificant chemo resistant properties. These biopharmaceuticals used in cancer treatment, act distinctly by initiating apoptosis in carcinogenic cells in addition to inducing detoxifying enzymes that are non-toxic to healthy cells.

1.8.1 Role of Antioxidants in Chemoprevention of Cancer:

Antioxidants as the name indicates scavenge various free radicals and neutralize ions, which are associated with cancer and/or other disorders. Therefore, the antioxidants have become popular as adjuvants in cancer or other disease treatment or prevention. Many of the herbal neutraceutical formulations are containing one or few antioxidants. The herbal antioxidant formulations are also being sold as OTC drugs. About 25% of cancer patients take antioxidants with or without the recommendation by their doctors. The antioxidants at proper dosage at proper time reversed the cancer in early stages and lowered the cancer metastasis (http://www.drlam.com). The antioxidants must meet some
prerequisites, if they are to be used as adjuvants in anticancer treatments (Roller et al., 1998). They are:

- The antioxidants should have scientific justification for their antioxidant property.
- Mechanism of action of antioxidants must be known.
- They should not cause any toxicity in human body.
- They should not interfere with chemotherapy or radiotherapy.

Recent studies indicate that antioxidants used at appropriate doses improve the tumor response towards chemotherapy and radiation therapy. It was also shown that antioxidants selectively suppress the growth of cancer cells without affecting the normal cells (Blot, 1993). This is a widely accepted hypothesis by the physicians who are nutrition minded. The most commonly used antioxidants in cancer treatment include vitamins, minerals, enzymes and other nutrients. These constituents are considered as a part of antioxidant regimen along with chemotherapy. Different antioxidants work at different levels in the cell.

The selection of the antioxidants/nutrients is based on the type of cancer, chemotherapy and the condition of the patient (Simone et al 2007). Various antioxidants used in cancer therapy are Beta carotene, Vitamin-C, Vitamin- E, Lipoic acid, Poly MVA, Bioflavonoids and Selenium. (Omen et al., 1994)
(a) Beta carotene:

Beta carotene (vitamin-A) is a strong immune booster and activates the immune cells against the cancer cells. Beta carotene stops or lowers the initiation of cancer and the attack of free radicals onto DNA and hence decreases the risk of cancer. High doses of beta carotene even for longer periods do not cause any toxicity.

A low intake of carotenoids from poor diet and/or lacking vitamin supplementation could be associated with an increased risk of breast cancer and may have community health relevance. It has been shown that higher levels of beta carotene in blood can have a protective effect against prostrate, lung and bladder cancers. Beta carotene plays a key role in directing the immune system to kill the cancer cells (Toniolo et al., 2001).

(b) Vitamin-C:

Vitamin C is one of the best antioxidants and it scavenges superoxide, hydroxyl and peroxide radicals generated during the metabolism. Research was extensively carried out to find out the role of vitamin C in cancer therapy and the studies proved that it improved the effect of chemotherapeutic agents. Administration of vitamin C along with Doxorubicin decreased the toxicity of Doxorubicin and improved the effectiveness of Doxorubicin, Paclitaxel and Cisplatin in in vitro studies.
(c) Vitamin-E:

Vitamin E is a very important nutrient which is necessary for strong immune response. It has been proved that vitamin E prevents the cancer. Natural succinate form of vitamin E can be best absorbed. In fibrocystic breast cancer vitamin E is highly beneficial. In vitamin E treated fibrocystic patients, the blood levels of estriol (E₃) and progesterone were raised. Estriol is a natural hormone present in the body and progesterone shows an opposing action to estrogen dominance, which is a causative factor for the cancer of breast, ovary and uterus. Tamoxifen shows better action along with vitamin E rather than alone in breast cancer. vitamin E protects the normal cells against the effect of radiation.

(d) Selenium:

Selenium is a powerful antioxidant and protects the cells from oxygen free radicals. The study reveals that selenium combines with glutathione peroxidase and decreases the damage caused by free radicals and protects the cell membrane. Selenium also reduces the side effects such as nausea, vomiting, headache caused by chemotherapy.

(e) Lipoic Acid:

Lipoic acid is called as universal antioxidant as it is soluble in water and fat and improves the efficiency of other antioxidants. The most important character of lipoic acid is that it can cross blood-brain barrier whereas no other antioxidant has this ability. One more important property of lipoic
acid is that it can regenerate other antioxidants such as vitamin C, vitamin E, co-enzyme-Q10 and glutathione.

(f) Poly MVA:
Poly MVA is an alpha lipoic acid complexed with palladium and a non-toxic poly nucleotide reductase. MVA indicates Minerals, Vitamins and Amino acids. It can cross the cell membranes and blood brain barrier. It protects cellular DNA, donates electron and generates the water and because of these characters it is beneficial in cancer treatment. Cancer cells are having less respiration and hence less water, more sugar, very less oxygen, thereby, does not have oxygen radical pathways. When poly MVA enters into the cancer cell, it damages the proteins which results in inhibition of cancer. Brain tumors have shown best response to Poly MVA and others include breast, ovarian, prostrate and lung cancers.

(g) Bio-Flavonoids:
These are natural compounds found in many plants. Most widely used flavonoids in cancer are green tea and quercetin. Green tea improves the efficacy of chemotherapeutic agents. Quercetin damages cancer cells and protects normal cells. Quercetin shows synergistic action with tamoxifen and cisplatin and also decreases the metastatic potential of the cancer cells. Quercetin increases the apoptosis of cancer cells.
1.9 SCOPE AND SIGNIFICANCE OF IN VITRO EVALUATION OF GYMNEMA SYLVESTRE:

The search for new chemical entities or lead compounds is a continuous and long process leading to the drug discovery. The research towards discovery of new drug or different pharmacological or biological activity for an existing chemical entity is a continuous ongoing activity in many pharmaceutical R&D and academic institutions.

In spite of the intense research and development activities for new drugs, there is dearth of therapeutic & safe drugs for some of the diseases or disorders. For example, cancer is the major disease for which there is no drug without exhibiting serious side effects. The therapeutic efficiency of the existing drugs is also questionable in many cases. Therefore, the search for new anticancer agents is occupying the center place in pharmaceutical research. Among the anticancer drugs discovered till now, majority are plant based (natural products) like vinca alkaloids- vincristine, vinblastine; Etoposide, taxol- paclitaxel, docetaxel; camphothecin, harringtonine etc.

The recent research findings reveal that free radicals are associated with a number of diseases. In some instances there is direct correlation with increased incidence of free radicals with progression of the disease. Literature review on free radicals and their involvement in cancer disease reveal that free radicals cause a number of mutagenic changes in the cell. Particularly, the reactive oxygen species-hydrogen peroxide, super oxide anion, hydroxyl radicals are associated with cancer initiation, progression
and conversion to malignancy. The addition of Antioxidants as an adjuvant therapy in cancer treatment was found to give beneficial effects in suppression of tumor growth. With this current knowledge of involvement of free radicals in cancer, it is prudent to investigate the plants for antioxidant activity in addition to anticancer activity.

In this present investigation, we have aimed to investigate a few plant species and biopharmaceuticals obtained from natural sources for anticancer activity. Initially literature of the few medicinal plants and their natural products i.e. *Gymnema sylvestre, Picrorhiza kurroa, and Tinospora cordifolia* was reviewed. *Picrorhiza kurroa and Tinospora cordifolia* have been investigated for anticancer activity. whereas *Gymnema sylvestre* has been exhaustively studied for anti-diabetic activity. However, *Gymnema sylvestre* plant has not been tested for anticancer activity to any greater extent. There is only one report comparing the anticancer activity of Gymnemagenol from *Gymnema sylvestre* and Dayscyphin C from *Eclipta prostrata* on HeLa cells by MTT assay only. However, analysis of the major constituents of *Gymnema sylvestre* extracts and the effect of these compounds on cell lines has not been studied in detail.

Therefore, we have selected *Gymnema sylvestre* for thorough *in-vitro* evaluation with the following objectives:

i. Screening of Gymnema Sylvestre plant extracts for *invitro* antioxidant and anticancer activity.

ii. To isolate and confirm the chemical structures of major compounds from the extracts which show the anticancer activity.
iii. To test these isolated compounds for anticancer activity.

iv. To study the mechanism of action of these active compounds at molecular level

These objectives have been addressed using the standard techniques of extraction from plant, cell culture methods, fractionation and analysis for the molecular mechanism of chemoprevention

1.9.1 Plan of work:

I. Obtain Gymnema sylvestre plant material, authentication & extraction

II. Phytochemical screening (chemical tests & TLC)

III. Isolation of biopharmaceuticals

IV. In vitro evaluation

a) Antioxidant activity

b) Anticancer activity

i. MTT Assay

ii. Apoptosis mechanism and

iii. Cell cycle analysis