Development of chemotherapeutic agents from Natural Products

Cancer

Cancer is recognized as the second most dangerous diseases of modern era and spreading continuously with increasing incidences [1-3]. It is caused due to series of intracellular molecular events leading to alteration of the fundamental properties of the cell including regulation of growth and cell cycle progression. These transformed cells develop new morphological and biochemical features and can grow and proliferate in absence of growth signals. These morphological and biochemical changes also promote angiogenesis and metastasis of tumors. It is well established that breast cancer, prostate cancer, lung cancer and colorectal cancer account for approximately 50% of all cancers. In the same way nearly half of the cancer mortality occurs due to these four types of cancers with lung cancer exceeding breast/prostate cancer as the main cause. Prostate cancer is the most commonly occurring cancer in males (about one fourth of new cases) while breast cancer is the most predominant cancer in females (also about one fourth). Other high incident types of cancer include lung, bronchial, colon and rectal cancers which occur equally in men and women. Consequently, the anticancer drug development is the major concern for the research organizations and pharmaceutical industries all over the world. Cancers are classified primarily on the basis of morphological appearance of the tumor, although it has serious limitations. For example tumors with similar histopathological appearance show significantly different clinical outcomes as well as show different responses to therapeutic agents because of significant different in nuclear morphology and molecular markers. Currently the classification of cancer is based on morphological characters, immunophenotyping through antibodies recognizing cell surface, enzyme-based histochemical analyses, and cytogenetic analysis. Recently DNA microarray has provided a powerful tool for cancer classification [4].

Types of cancer

Based on the histological characteristics hundreds of different cancers have been identified, which can be classified into six major groups.

1. **Carcinomas**

Cancers caused due to alteration in epithelial cells covering the surface of skin and internal organs are termed as carcinomas. It is the most common type of cancers, predominantly
occurring in the old age, for example breast, prostate, lung, pancreas, and colon cancers. Carcinomas, account for 80 to 90 percent of all cancer cases. These can be further divided into two major subtypes: (i) Squamous cell carcinoma, which develops in the squamous epithelium and (ii) adenocarcinoma, which develops in an organ or gland.

2. Sarcomas

Cancers arising on different connective tissue including bone, cartilage and nervous system are termed as Sarcomas. These are known to develop from cells originating in mesenchyma outside the bone marrow. Most important examples of sarcoma include fibrosarcoma (fibrous tissue), rhabdomyosarcoma (skeletal muscle), angiosarcoma or hemangioendothelioma (blood vessels), glioma or astrocytoma (neurogenic connective tissue found in the brain) mesothelial sarcoma or mesothelioma (membranous lining of body cavities)

3. Myelomas

These include the cancers arising in the bone marrow plasma cells. These are also called cancers of antibody producing white blood cells

4. Lymphomas

These are developed in the lymph glands and other organs of lymphatic system including tonsils, thymus gland and spleen playing active role in immune system. Lymphomas may also occur in some other organs including breast, stomach and brain. Lymphomas occurring in these organs are known as extranodal lymphomas. These can be further divided into two major subtypes: (i) Hodgkin lymphomas and (ii) non- Hodgkin lymphomas. Both of these subtypes can be differentiated on the basis of the presence of reed-sternberg cells in Hodgkin lymphoma.

5. Leukemias

The cancers occurring in bone marrow are termed as leukemia. It results in the overproduction of immature white blood cells (WBC) rendering the patients immunodeficient and susceptible to subsequent infections. In some cases it also affects red blood cells (RBC) and platelets leading to anemia as well as defective blood clotting. The important examples of leukemia include chronic myelocytic leukemia (CML) occurring in adults, acute myelocytic leukemia (AML) occurring in children, chronic lymphocytic, lymphatic, or lymphoblastic leukemia (CLL) common in adults, acute lymphocytic, lymphatic, or lymphoblastic leukemia (ALL) common in children and adults.
6. Mixed Types

These comprises of more than one component which may be within one category or from different categories. Examples of mixed type include adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma and teratocarcinoma.

Clinical features/symptoms

Different types and stages of cancer can be identified based on potential signs and symptoms:

Appearance of blood in sputum along with shortness of breath, persistent cough and chest pain indicate lung cancer.

Prolonged bleeding in menstrual cycle for more than 10 days or bleeding between two menstrual cycles indicate the presence of uterine cancer.

Presence of a prolonged non-healing ulcer in mouth indicates the oral cancer.

Alternating diarrhea or constipation for a prolonged without any obvious reason followed by blood in stools indicates gastrointestinal cancer.

Appearance of reddish-color in urine with abdominal pain and fever indicate the renal cancer.

Persistent cough and hoarseness of voice could indicate cancer of the throat.

Presence of moles with size more than 7mm in diameter and asymmetrical shape or irregular border as well as multicolor (mosaic) itchy or bleeding moles indicates skin cancer (melanoma).

Presence of rapidly increasing lumps in breast in women above 35 years of age may be cancerous in some cases.

Unexpected continuous weight loss above two months unrelated to dietary changes, stress or exercise also indicate different types of cancer.

These symptoms can be further confirmed based on different tests such as a biopsy or X-ray and used to determine the appropriate treatment.

Tumor cell markers

Cancer cells produce some specific markers which may be present on cell membranes or in cytoplasm. Immunological analysis of these markers in the blood, urine or spinal fluid may be
used in diagnosis of the types of cancer as well as to identify the risk of cancer in individuals. These tumor cell markers are also used to analyze the clinical improvement in cancer patients undergoing cancer therapy as well as to evaluate the efficacy of anticancer treatment.

Tumor cell markers can be classified as antigens (such as carcinoembryonic antigen in adenocarcinomas, alfa fetoprotein in ovarian, testicular and hepatic carcinomas and squamous cell carcinoma antigen in epidermal cancers), enzymes (such as lactate dehydrogenase in leukaemias, acid phosphatase in prostate cancer, alkaline phosphatase in bone cancer, enolase in lung cancer and neuroblastoma and thymidine kinase in leukaemia and lymphomas) and hormones (such as Insulin and gastrin in pancreatic cancer human chorionic gonadotropin in trofoblastic cancers and calcitonin in thyroid and lung cancer).

**Pathophysiology**

Cancer is a genetic disorder in which genetic alterations caused due to unrepaired mutations enable them to divide in the absence of growth signals through increasing the expression of growth factor receptors or through secreting specific growth factors. Activation of telomerase activity also contributes to maintain the length of telomere in cancerous cells resulting in continuous proliferation. Cancerous cells gain growth autonomy through activation of growth promoting oncogenes or inhibition of tumor suppressor genes (growth inhibiting genes), both of which are caused by multiple unrepaired genetic damages due to environmental factors or genetic inheritance. These multiple genetic damages may be subtle or large enough to be detected through the karyotype analysis.

Activation of protooncogenes is known to be caused by one of several processes including point mutation, translocation, gene amplification, enhancer insertion or promoter insertion. It is known to be developed through multistep carcinogenesis process involving three closely associated stages distinguishable from each other.

1. Initiation: it is the first stage of carcinogenesis in which a normal cell converts in to transformed cell or initiated cell in response to exposure of carcinogenic factors. It is a rapid and irreversible process which induces changes in genome of the cells leading to mutation. In addition, chronic inflammation and oxidative stress may also lead to intiation.
2. Promotion: in this stage previously initiated cell undergo a sequence of changes to generate preneoplastic cell. Most of the chemical carcinogens are able to act as initiators and promoters. Deregulation of different cell signaling pathways also lead to carcinogenesis. These signaling pathways include mitogen activated protein kinase (MAPK), serine threonine kinase, nuclear factor kappa B (NF-κB), Akt kinase/protein kinase B (Akt/PKB), Raf/Ras, estrogen receptor, androgen receptor and activator protein 1(AP-1) pathways. These molecular changes ultimately result in inflammation, proliferation and avoidance of apoptosis.

3. Progression: it is the final step of carcinogenesis in which inflammatory changes and proliferation signals lead to excessive production and release of cytokines (angiogenic factors) which induce the preneoplastic cell for proliferation and promotion. Hypoxia is also considered as an important factor in carcinogenesis it is known to induce vascular endothelial growth factor (VEGF) in cancerous cells and induce the expression of matrix metalloproteinase (MMP) in endothelial cells.

**Characteristic features of cancer cells**

There are three stringent properties of cancer cells which make them different from normal cells

(i). Diminished regulation of cell cycle or uncontrolled growth

(ii). Capability of invasion of neighboring tissues and

(iii). Capability of spreading to distant parts of body through metastasis.

After transformation in to cancer cell these cells undergo a variety of morphological and biochemical changes which enable them to proliferate and spread in other locations.

**Morphological changes**

The cancerous cells usually have round shape and show nuclear and cellular pleomorphism, hyperchromatism, altered nuclear: cytoplasm ratio and abundant mitosis. These cells often grow over one another forming multi layers and do not stop replication when come into contact with another cells as well as require low serum level of growth factors.

Cancer cells become able to grow without attachment to surface *in vitro*. An elevated level of telomerase enables these cells to maintain telomere length rendering them to divide continuously as immortal cells. Cancer cells can proliferate in a semisolid or liquid culture media as these do
not require surface attachment and show anchorage-independent growth which result in clonal expansion of malignant cells [5]. Cancer cells undergo karyotypic changes characterized by an increase in the number of chromosomes resulting in increased expression of growth-promoting genes. These cells do not respond to the suboptimal growth conditions and refuse to enter into G0 state. These cells also lose control of cell cycle check points and proceed to replicate the DNA with unrepaired damages leading to accumulation of mutations.

**Biochemical changes**

Cancer cells show enhanced synthesis of Nucleic acids (DNA and RNA) and increased rate of glycolysis in both aerobic and anaerobic conditions. Change in composition of cell membrane results in alteration of permeability and surface charge. Different stages of cancer cells express different cell surface oligosaccharide characteristic to that stage. These surface oligosaccharides have binding specificity for different lectins and thus, can be used as probes to determine the glycoprotein and glycolipid composition as well as the stage of cancer. Change in composition of glycoprotein and glycosphingolipids on cell surface also results in appearance of new surface antigens, glycolipids and proteoglycans as well as altered cell-cell interactions and cell-extracellular microenvironment interactions. Cancer cells express some large glycopeptides containing N-linked oligosaccharides with lead to altered glycosylation patterns significantly different from normal cells [6]. Cancer cell express specific carbohydrate antigens which can be classified into three classes (1) epitopes expressed on glycolipids, epitopes expressed on glycoproteins and epitopes expressed equally on glycolipids and glycoproteins. These specific carbohydrate antigens arise due to incomplete synthesis or processing of carbohydrate chains or due to rearrangement of tumor cell membrane glycolipids or due to glycosyltransferases catalyzed *de novo* synthesis [7]. Cancer cells synthesize altered Proteoglycans which play a crucial role in cellular interactions with extracellular matrix through providing binding sites for components of extracellular matrix as well as act as cell adhesion factors and growth factor reservoir. These cells also secrete mucin with the altered glycosylation pattern which plays an important role in host immune response. The characteristic changes are represented in figure 1.1.

Cancer cells show an increased secretion of certain growth factors into surrounding medium as well as synthesize their own cytokines such as interleukin-6 and interleukin-1. In addition these secrete several lytic enzymes such as proteases, collagenases, and glycosidases which result in
the degradation of extracellular Matrix Components including glycoproteins, proteoglycans, and collagen enabling metastatic cancer cells to invade through tissue barriers, lymphatic system and circulatory system. These cells also express altered integrins, cell surface receptors responsible for cell adhesion to the ECM. These receptor molecules act as a link between the extracellular matrix and intracellular actin cytoskeleton and control the expression of genes involved in cell differentiation and cell adhesion. It functions via a cascade involving ECM-bound integrins intracellular actin fibrils and signal transduction machinery [8].

**Genetics of Cancer**

Cancer is shown to be associated with mutations in three different types of genes proto-oncogenes, tumor suppressor genes and DNA repair genes, these three types of genes work together to maintain integrity of the genome and control the cell cycle. Proto-oncogenes code for different proteins which stimulate cell division through different signaling pathways.

![Figure 1.1 Characteristic features of malignant cell](image-url)

**Figure 1.1** Characteristic features of malignant cell
More than half of the oncogenes act through protein tyrosine kinases. Remaining 50% oncogenes produce various other growth factors including GTP binding protein and DNA binding protein. These growth factors activate a cascade of signaling pathway as shown in figure 1.2. This signaling pathway consists of a receptor molecule with a binding site for the growth factor, some intermediate molecules which transmit the effects produced by growth factor through the cytoplasm, and nuclear transcription factors which induce the gene activation leading to cell division. Followed by unrepaired mutations in genome, proto-oncogenes convert in to oncogenes which permanently activate the signaling pathway leading to an increased production of growth factors. Examples of oncogenic mutations include mutations in ras gene resulting in increased expression of adenilate cyclase and consequently increase in cAMP level leading to severe change in cellular metabolism and uncontrolled cell growth. Mutation in ras has been determined in approximately one third of tumors including thyroid, colon, lung and pancreatic cancers. Another example of oncogenic mutations include, myc encoding the DNA binding protein. Mutated myc gene is identified in seventy percent of cancers.

**Fig. 1.2** Signal transduction pathway: a growth factor binds to a tyrosine kinase receptor on cell surface and activates the membrane protein which in turn activates cytoplasmic kinases ultimately activating the transcription factors which enter the nucleus and stimulate the expression of related genes.
The tumor suppressor genes code for proteins which prevent tumor formation through inhibiting the growth of cell. These proteins may act in different targets present in cytoplasm, nucleus and cell membrane. Mutations in tumor suppressor genes lead to uncontrolled cell growth and proliferation.

The mutations in oncogenes are dominant mutations which results in a gain of function while tumor suppressor gene mutations result in a loss of function thus arise as recessive trait. Examples of cancers caused due to tumors suppressor genes include hereditary retinoblastoma, which occurs in retina in individuals carrying a mutated copy of the RB tumor suppressor gene when the second copy of the gene undergo mutation. Similarly familial adenomatous polyposis of the colon occurs through mutations in both copies of the APC gene. Hereditary breast cancer caused due to mutations in BRCA2 and hereditary breast and ovarian cancer caused due to mutations in BRCA1 also share same mechanism.

Mutations in DNA repair gene avoid the repair of DNA damages allowing the accumulation of further mutations and subsequently increasing the risk of cancer development. Mutation in a DNA repair gene Xeroderma pigmentosum (XP) increase the risk skin cancers caused due to exposure of UV radiation.

Cancer cells are shown to loss the genomic imprinting resulting in expression of both the maternal and paternal alleles leading to double dose of concernrd growth factors. Deletion of genetic material is also frequently observed in several types of cancers leading to loss of heterozygosity, the tendency of the expression of only one allele of a gene. A mutation of a tumor suppressor gene followed by a deletion may results in loss of an important mechanism regulating cell growth.

Mutations in genes encoding proteins involved in histone acetylation, deacetylation and phosphorylation results in alterations in Chromatin structure and function and impaired transcription of DNA leading to malignant transformation. Point mutations of tumor-suppressor gene rb results in deregulation of the histone deacetylation through inhibition of transcription leading to cancer initiation [9]. Several types of cancer also exhibit altered methylation patterns in nuclear DNA it is postulated to be caused due to overexpression of DNA methyltransferase activity [10].
Etiology of cancer

1. Predisposing factors

(a). Age: Age is considered as a significant risk factor for cancer. Incidences of cancer are most common in elders over 55 years of age. After the industrial revolution incidence of cancer has drastically increased due to increased lifespan. Incidence of cancer is very rare in children and youngsters, with leukemia the most frequent. Neuroblastoma is reported to occur in the age of one year comprising 28% of infant cancer. Leukemia is second most common type of cancer in newly born children. Some other cancer types are also known to occur in the early age of life including teratoma, retinoblastoma.

(b). Genetic factor: Certain precancer conditions are inherited from one generation to next generation through inheritance of mutated genes. Susceptibility to childhood retinoblastoma is inherited as autosomal dominant trait and nearly half of these cases are of hereditary origin. Susceptibility to multiple colon poliposis is also inherited as autosomal dominant trait and approximately 40% are known to be caused through inheritance. Similarly Chromosomal DNA instability may be inherited as autosomal recessive trait defect in DNA repair. Xeroderma pigmentosa and skin cancer in UV exposed area are also considered as hereditary cancer.

(c). Environmental factors: 80% human cancers are known to be caused by environmental factors. [11] These include-

Life style

Factors related to modernization of socioculture lifestyle, have increased the risk of cancer. These factors include-

(i) Tobacco consumption and smoking: it is strongly associated with mouth cancer, lung cancer, throat cancer, bladder cancer and pancreatic cancer. Tobacco smoke contributes to as many as half of all deaths caused due to cancer

(ii) Drinking alcohol: it is associated with an increase in oral, esophageal, breast, and liver cancers.

(iii) Excessive energy intake and sedentary lifestyles: in developed countries and most of the developing countries peoples have become habitual of excessive energy intake and sedentary
lifestyles. These habits in association with other factors increase the risk of colon, breast, and other cancers.

(iv) Obesity and increased body mass index: It is known to increase the risk of colorectal, endometrial, thyroid, renal, gallbladder, pancreatic and ovarian cancers. Men are twice as likely as women to have susceptibility to these risk factors.

**Dietary**

A diet low in green vegetable and fruits is also shown to increase the risk of several types of cancer. Further some food products were shown to increase the cancer risk in some individuals for example groundnut infected with fungus like *Aspergillus* produce aflatoxin B1, a well known carcinogen [12]. Further some other lifestyle related and environmental factors are known to increase the risk of cancer these include the application of hormones as therapeutic agents, ultraviolet rays and ionizing radiation, and exposure to chemicals in work places.

**Occupational**

Currently, a large fraction of cancer related mortality in the developed world is known to be caused due to occupational risk factors. It is estimated that at least 200,000 workers involved in different industries die due to cancers related to their workplace all over the world every year. Several studies showed direct association between exposure to respiratory silica particle and lung cancer in workers engaged in ceramic, granite, refractory brick, and diatomaceous earth industries. Dyes, paints, rubber and aromatic amines induce bladder cancer in workers involved in these industries. Exposure to benzene at industrial sites increases the cancer risk in persons involved in these industries. Other examples of occupational carcinogenic agents include asbestos and naphthalamine. Use of organochlorine pesticides in agricultural practices has also been shown to increase the risk of liver and testis cancers [13-15]. Accidental exposure to nuclear radiation in persons working in nuclear reactors or uranium mines is established as risk factor for several types of cancers [16]. It also depends upon ecological and social status of a population for example incidence of liver cancer is very common in China and neighboring countries, probably due to the existence of hepatitis B and aflatoxin in that population. Similarly, incidences of lung cancer occur very frequently in several countries belonging to third world where tobacco smoking is very common.
Iatrogenic

Certain therapeutic drugs may act as carcinogens [17]. Tamoxifen used as an effective drug for the treatment of breast cancer is also recognized as a carcinogenetic agent. It has been reported in a recent study that the women who received tamoxifen in chemotherapy have shown to have increased risk for developing endometrial cancer as compare to normal women [18]. Psoralen ultraviolet A (PUVA) therapy, a combination treatment comprising administration of Psoralen followed by exposing the skin to UV-A (long wave ultraviolet radiation) used for the treatment of severe skin diseases is shown to be associated with an increased risk of skin cancers [19]. Chlornaphazine, used in treatment of polycythemia is shown to be associated with an increased risk of bladder cancer. Similarly, the use of tacrolimus and pimecrolimus as therapeutic agents is shown to increase the risk of Hodgkin's lymphomas and melanomas [20].

(d). Acquired precancerous disorders

Certain clinical conditions are known to increase the risk of developing cancer such as Leukoplakia of oral and genital mucosa can develop in squamous cell carcinomas, some cases of untreated liver cirrhosis can develop in hepatic cancer, hepatoma.

A gastrointestinal disorder known as ulcerative colitis can subsequently produce adenocarcinoma of colon, similarly carcinoma in situ of cervix can produce cervical squamous cell carcinoma. Human papiloma virus which causes cervical cancer and anal cancer is transmitted sexually from one person to other.

2. Carcinogenic agents

Several carcinogenic agents including physical, chemical, and biological have been recognized which can cause cancer. The role of carcinogenic agents is represented in figure 1.3.

(a). Physical agents

Directly effecting DNA

Excessive exposure of high frequency ionizing radiations with enough energy to remove an electron from a molecule, such as X rays, γ rays and ultra violet rays can directly induce mutagenesis through several ways: these can induce single strand or double strand break, formation of pyrimidine dimers in DNA, elimination of Pyrimidine or purine base from DNA or crosslinking between two DNA strands. There is a direct relationship between the effective radiation dose and cancer incidences caused due to these ionizing radiations [21]. Some tissues
such as bone marrow and thyroid glands are highly sensitive and thus more affected by ionizing radiations. Examples of cancers caused due to ionizing radiations include leukemia, thyroid cancer, skin cancer, breast cancer, stomach cancer and lung cancer.

**Indirectly effecting DNA**

Electromagnetic radiations such as X-rays and Y-rays produce free radicals through randomly hitting intracellular macromolecules including proteins, lipids, and nucleic acids leading to molecular damage. These molecular damages in DNA lead to mutations and carcinogenesis. Examples of ultra violet radiation induced cancerous include carcinomas (basal cell and squamous cell cancers) and melanomas of exposed parts of skin prevalent in Newzealand and Australia.

![Figure 1.3](image_url)  
**Figure 1.3** The mechanism of cancer development and role of environmental carcinogenic agents in carcinogenesis.
(b). **Chemical agents**

These are the different chemical compounds which act as procarcinogen and after entering in the body get converted in to ultimate carcinogens

(c). **Biological factors**

Some of the oncogenic viral infections leading to expression of viral oncogenes (v-onc) result in cancer development. These viruses either induce insertion of additional viral oncogenes into the host genome or activate the cellular oncogenes. Normal protooncogenes converted to oncogenes through transduction in to retrovirus or through changes *in situ* leading to alteration in expression and function and subsequently converting them into cellular oncogenes.

**Major classes of virus causing cancer involve-**

**A. DNA viruses:**

Following infection of host cell these viruses form a stable complex with the genome of host cell and transcribed with it. Insertion of the viral DNA near a gene involved in regulation of cell growth and division results in enhanced transcription of that gene and consequently transformation of the cell into cancerous cell. Viral genes transcribed early in the lifecycle of virus produce some specific proteins such as T antigens, E5 and E6 proteins which interfere with cell cycle and growth regulation leading to transformation of the host cell. Examples of cancer causing DNA viruses include-

1. **Herpes virus/ Epstein Barr virus**

   It is the first identified human cancer virus which produces Burkitt lymphoma and nasopharyngeal carcinoma. It is endemic in Northern African and Asian countries and is associated with regular consumption of nitrosamines in diet.

2. **Hepatitis B and Hepatitis C viruses**

   In addition to causing hepatitis these are also shown to produce hepatocellular carcinoma.

3. **Human papiloma virus**

   It induces transformation in host cells through modulating the activity of tumor suppressor genes such as p53 and RB protein rendering the host cell to proliferate and subsequently enabling the replication of viral genome. It is known to cause multiple warts, cervical cancer and anogenital carcinoma [22]. Human papiloma virus is considered as causative factor of genital warts (benign squamous papillomas) shown to be associated with diminished cell mediated immunity. In one third cases, warts may lead to malignant transformation. 99% of cervical cancers and high dose
cervical cancer precursor lesions are caused due to Human papiloma virus infection. Some types of human papiloma virus are associated with increased risk of carcinoma in situ of cervix and cervical squamous cell carcinoma [23].

4. **KSHV/ HHV-8 (Kaposi sarcoma associated herpes virus)** it is known to causes AIDS-related Kaposi sarcoma, a skin cancer, some B cell lymphomas and some other cancers linked with HIV.

**B. RNA viruses:**
All oncogenic viruses are retroviruses which can be divided into two groups (i). Acute transforming retroviruses and (ii). Slow transforming retroviruses.

The acute transforming viruses include in its genome, a transforming sequence known as viral-oncogene (v-onc) which encodes overactive oncogene product leading to transformation of infected cell immediately after infection. Conversely, the slow transforming viruses do not contain transforming sequence and the virus genome is inserted adjacent to a proto-oncogene in the genome of host cell resulting in overexpression of that proto-oncogene subsequently leading to uncontrolled cell division. Since the chance of viral genome insertion near that proto-oncogene is very low, these viruses cause transformation of infected cell slowly as compared to acutely transforming viruses. Examples of cancer causing RNA viruses include

1. Human T cell leukemia virus-1 (HTLV-1) it is the first identified human retrovirus which is associated with human leukemia/lymphoma and endemic in different regions of Japan.

2. Human T cell leukemia virus-2 (HTLV-2)

3. Rouse Sarcoma virus

4. Murine sarcoma virus

5. Murine leukemia virus

6. Avian leukemia and avian sarcoma

Some bacterial infections are also shown to be associated with some types of cancers such as *Helicobacter pylori* increase the risk of gastric cancer, *Opisthorchis viverrini* infection increase risk of cholangiocarcinoma, similarly *Schistosoma haematobium* is shown to be associated with bladder carcinoma.
Incidence of a second cancer in survivors

Cancer survivors have increased risk to develop a new cancer. The usual lifetime probability of developing a primary cancer in cancer survivors is two in nine cases. This increased risk is thought to be mainly due to same risk factors which resulted in the first cancer, for example genetic factor, environmental factors and obesity, and in some cases the therapeutic agents used for the treatment of first cancer.

Role of apoptosis in cancer

Apoptosis is an essential intracellular process of program cell death that plays a crucial role as primary defense mechanism against the occurrence of cancer [24] and thus, loss of apoptosis can interfere with different stages of cancer including initiation, progression and metastasis. Different genes and proteins involved in apoptosis are identified as potential drug targets. The recent advancements in cancer pharmacology and cancer genetics resulted increased understanding of molecular basis of apoptosis and its possible role in development of anticancer agents. It is well documented that some oncogenic mutations results in deregulation of apoptotic events which led to development of cancer. Different studies focused on pathways involving p53 and Bcl-2 postulated the key role of apoptosis in carcinogenesis [25, 26].

p53 play a crucial role as a checkpoint protein recognizing DNA damage and inducing cell-cycle arrest. Physiological conditions such as hypoxia as well as cellular stresses, including DNA damage and mitogenic oncogenes can activate the p53 and promote the apoptosis. Mutations in different components of the p53 pathway have been identified in different types of tumor these mutations are found to be responsible for deregulation of apoptosis. Another pathway involving Ras and PI-3 kinase is also disrupted in different types of cancer. Experimentally induced Bcl-2 overexpression in transgenic mice promoted the carcinogenesis. Similarly, p53 loss produced in transgenic mice promoted the carcinogenesis in several experiments [27]. Furthermore, disruption of p53 downstream components such as bax, caspase-9 and apaf-1 can induce oncogenic transformation and promote tumor progression in experimental animals [28]. Several apoptotic stimuli activate proapoptotic molecules while others act through alleviating the anti-apoptotic signal [29]. p53 induce apoptosis in response to different triggers such as excessive UV exposure, hypoxia or shortened telomeres and thus, loss of p53 function results in survival and clonal expansion of these cells.
Disturbance in apoptotic pathway may also promote metastasis of tumor. To spread in distant locations in the body, metastatic cancer cells should be able to survive in the blood circulation as well as to invade the other tissues. Normally, the epithelial cells are unable to survive in suspension and undergo apoptosis. The process is postulated to be governed by several factors, including focal adhesion kinase, p53 and Bcl-2 as well as different signaling pathways [30]. Thus, loss of apoptosis may contribute to tumor development through affecting different steps including initiation, progression and metastasis.

**Oxidative stress and Cancer**

Oxygen is the responsible factor for evolution of life in the existing form playing the central role in aerobic metabolic processes although about 5% of inhaled oxygen is converted to reactive oxygen species (ROS) such as superoxide or hydroxyl radicals during different steps of metabolic processes. These reactive oxygen species can also be generated through various exogenous sources. The production of these highly reactive molecules is markedly increased during some physiological conditions such as inflammatory response leading to oxidative injuries to cells through damaging the DNA, proteins, lipids, and cell membranes [31]. Superoxide radicals are primarily generated in mitochondria during the production of ATP which require a sequential flow of electrons along different components of the electron transport chain. During this process, some electrons escape and react with molecular oxygen to generate superoxide anions. It is further converted into other more damaging reactive oxygen species such as hydroxyl radical [32]. Some other enzymes such as nitric oxide synthases, NADPH oxidases, and lipoxygenases also produce reactive oxygen species. However, the increased oxidative stress followed by loss of the redox balance in a cellular metabolism results in oxidative damages in cellular membranes as well as in biomolecules through inducing sequential oxidation and fragmentation reactions as well as intermolecular cross-linking between the biomolecules.

Excessive accumulation of reactive oxygen species (ROS) followed by diminished efficiency of host antioxidant defense mechanisms can promote carcinogenesis [33, 34]. Reactive oxygen species may induce genotoxicity through inducing single-strand breaks and mutations in the DNA and are involved in various stages of cancer development [35, 36]. Similarly oxidation of lipid molecules may results in cytotoxicity and genotoxicity [37]. Lipid radical formed due to action of ROS, such as malondialdehyde and 4- hydroxynonenal can further induce DNA damage and mutations [38].
Primary defense against ROS: Catalytic removal of ROS

Under normal conditions these oxidative damages are counteracted by antioxidant defense mechanism composed of different enzymes including superoxide dismutases, catalase, and glutathione peroxidases which reduce the reactive oxygen species and prevent the oxidative modifications of bio molecules and cell membranes. Most common intracellular ROS together with their source and the related antioxidant enzyme system involved in removal of these ROS molecules have been summarized in table 1.1.

Table 1.1 Common intracellular ROS, their source and the related antioxidant enzyme system involved in removal of these ROS molecules

<table>
<thead>
<tr>
<th>ROS Molecule</th>
<th>Source</th>
<th>Antioxidant enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide (O$_2^-$)</td>
<td>Leakage of electron from etc Xanthine oxidase Flavoenzymes Phagocyte activation</td>
<td>Superoxide dismutases (SOD)</td>
</tr>
<tr>
<td>Hydrogen peroxide(H$_2$O$_2$)</td>
<td>Action of SOD on O$_2^-$ Xanthine oxidase Glucose oxidase NADPH oxidase</td>
<td>Glutathione peroxidases (GPx) Catalase (CAT)</td>
</tr>
<tr>
<td>Hydroxyl radical (OH*)</td>
<td>From O$_2^-$ and H$_2$O$_2$ through transition metals (Fe or Cu)</td>
<td>Glutathione (GSH)</td>
</tr>
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Superoxide dismutases (SOD)

These are the first identified ROS metabolizing enzymes which reduce superoxide levels in the cell through catalyzing its conversion to molecular oxygen and hydrogen peroxide [39]. These comprise the first lines of defense against O$_2^-$ produced in mitochondria or other cellular sources. Three isoforms of SOD have been identified in mammalian cells dimeric Cu/Zn SOD present in cytoplasm, tetrameric Mn SOD present in mitochondria, and extracellular SOD.

Catalase (CAT)

These are tetrameric heme containing enzymes primarily located in the peroxisomes and convert H$_2$O$_2$, including those generated through SOD, into oxygen and water. It has the highest turnover rate converting 6 million substrate molecules into products in 1 minute. It efficiently removes the H$_2$O$_2$ produced in peroxisomes through β-oxidation, purine catabolism and photorespiration.
Glutathione peroxidases (GPx)

Glutathione peroxidases can reduce peroxides (hydrogen peroxide as well as organic and lipid hydroperoxides) to water and alcohols. It uses reduced glutathione (GSH) which is converted to glutathione disulphide

\[ \text{ROOH/H}_2\text{O}_2 + \text{GSH} = \text{ROH/H}_2\text{O} + \text{GSSG} \]

Eight isoforms of glutathione peroxidases have been identified which differ in tissue localization and substrate specificity. GPx1 is the most abundant isoform which is responsible for much of the detoxification of \( \text{H}_2\text{O}_2 \) in the cytoplasm [32]. GPx4 is responsible for detoxification of lipid peroxides, such as hydroperoxides of phospholipid and cholesterol esters.

Peroxiredoxins (Prdx)

These are recently discovered class of antioxidants which reduce hydrogen peroxide, peroxynitrite, and different organic hydroperoxides [40]. Six isoforms of Peroxiredoxins have been identified which differ in cellular localization.

Secondary defense against ROS: Free radical scavengers

It comprises small molecules which react with free radicals to produce a less harmful radical. These are called antioxidants. Glutathion is an intracellular antioxidant present in all living aerobic cells in millimolar concentrations. It is essential to maintain the normal reduced environment of the cell and counteracting the oxidative effects of ROS. It is a potential scavenger of reactive oxygen species. Ascorbate, \( \alpha \)-tocopherol and several polyphenolic compounds act as potent antioxidants and can be used to neutralize the effects of reactive oxygen species. Ascorbate quenches reactive oxygen species and inhibits oxidative consequences such as lipid peroxidation [41]. Sufficient dietary intake of ascorbate, Vitamin E and Vitamin A is directly related to decreased cancer risk [42, 43].

Several antioxidant enzymes act through repairing the oxidative damage from biomolecules. Thioredoxins and Methionine sulfoxide reductases can repair the proteins damaged by ROS. However overproduction of reactive oxygen species and impaired defense mechanisms results in oxidative stress which through a sequence of events lead to deregulation of normal cellular
functions giving rise to various pathological conditions including neurodegenerative diseases, cardiovascular disorders, gastrointestinal diseases, cancer and premature aging [44].

Genetic landmarks in cancer research

Major achievements in the field of cancer research have been represented in figure 1.4.

![Figure 1.4 Major landmarks in cancer research](image)

**Diagnosis of cancer**

Most cancers are initially diagnosed on the basis of signs or symptoms or during the diagnosis of other diseases. Both of these provide only intimation to the disease and further require the examination of a tissue sample to determine the type of proliferating cells, genetic abnormalities, its histological grade, and other morphological and biochemical characteristics of the cancer. Some other methods such as blood test, X-rays, CT scans and more recent tests involving Cytogenetics and immunohistochemistry can also be used to diagnose the cancer. Together, these examinations are used to predict the condition of disease and to decide the appropriate treatment.
Cytogenetic and immunological tests are used to predict the molecular changes involved in cancer development including mutations, karyotypic changes and immunological changes leading to prediction of future pathological conditions of the cancer and to select the best treatment.

**Cancer Therapy**

Different types of treatments are suggested for management of different cancer types depending on the type of cancer, its location in the body, and its state of advancement. The major therapeutic approaches involve surgery, chemotherapy radiation therapy, hormonal therapy, immunotherapy and targeted therapy. These different approaches can be applied alone or in combination depending upon type and stage of cancer.

**Surgery**

Surgery is the primary method applied to remove most isolated solid tumors. In localized cancer, surgery typically attempts to remove the entire mass along with the lymph nodes present in the surrounding area. For some types of cancer surgery can be successfully applied to completely eliminate the cancer. It is considered the successful treatment for several early stage cancers and benign tumors. Based on the outcome, various types of surgery are available such as diagnostic surgery- used to isolate a tissue sample for pathological evaluation to determine the type and stage of cancer, curative surgery- used to remove the cancer affected tissue from the specific part of the body, preventive surgery- applied to remove precancer tissue which may consequently develop into a cancer, staging surgery- used to determine the extent of cancer in the whole body. Debulking surgery- applied to removes a portion of a cancer affected tissue when removal of the entire tumor is harmful to the body. It may be followed by chemotherapy and radiation therapy. Palliative Surgery- used to treat advanced stages of cancer in order to minimize the effects of cancer.

Recently some specialized surgeries have been developed for cancer treatment such as cryosurgery in which extremely cold temperatures is used to kill cancer cells in this method, liquid nitrogen is applied directly or through a cryoprobe on the affected tissue. Similarly laser surgery is applied to remove the small tumors without affecting the surrounding tissue using the beams of light energy in place of instruments. Another specialized surgery termed as electrosurgery kill the cancer cells by using electrical current.
Radiation Therapy

Radiation therapy through ionizing radiation is an effective approach to either cure or improve the symptoms of cancer. It kills cancer cells with high-energy radiation directly targeted to the tumor and is applied in nearly 50% of cancer cases. A constant dose of ionizing radiation is applied directly at the affected tissues which act primarily by damaging DNA and preventing its replication leading to the death of rapidly dividing cancer cells. It also kills some rapidly dividing normal cells and the radiation can be from either internal sources in the form of brachytherapy or external sources. Radiation therapy is often used in combination to surgery and or chemotherapy but for certain types of cancer for example early head and neck cancer the radiation therapy may be applied alone. It has been found to be effective in about 70% of cancer patients suffering from painful bone metastasis.

Chemotherapy

Chemotherapy deals with the treatment of cancer with the help of cytotoxic compounds that target rapidly growing cancerous cells. Unlike surgery and radiation therapy which are effective in treatment of cancer at a particular location, chemotherapy effectively kill cancer cells all over the body. Chemotherapy alone or in combination with surgery has proven useful in a number of different cancer types including: breast cancer, ovarian cancer, pancreatic cancer, testicular cancer, colorectal cancer, osteogenic sarcoma, and some lung cancers.

Chemotherapeutic agents primarily act through damaging DNA of the cancer cells, leading to apoptosis or through inhibiting DNA replication and thus, preventing cell division and blocking tumor growth. These agents selectively kill or inhibit rapidly dividing cancer cells. Cell cycle involves 4 phases-

1. G1 phase (Pre-synthetic phase):- It is the first stage in which cells make required preparations for replication of DNA. This phase is also characterized by the synthesis and accumulation of RNA and protein required for cell division.

2. S phase (Synthetic phase):- It involves the replication of nuclear DNA. Histone proteins are also synthesized in this phase, these proteins enter the nucleus and become associated with newly synthesized DNA. At the end of S phase the cell contains two copies of DNA.
3. G2 phase (Post-synthetic phase):- In this phase cell synthesizes several new proteins and complete all required preparations for cell division.

4. M Phase (Mitosis phase):- In this phase nucleus of the parent cell is dissolved, two pairs of chromosomes are pulled to opposite poles and each cell get completely divided into two daughter cells.

Some of these new cells enter the new cell cycle, while the others become non-proliferating cells. Many of the conventional chemotherapeutic agents interfere with the synthesis of DNA precursor molecules, the modulation of the synthesis of these precursor molecules interfere with the cell cycle through preventing the cell to complete the S phase. Several other drugs act through blocking mitosis through preventing the splitting of a cell at the end of mitosis into two daughter cells ultimately preventing the progression of cancer. The anticancer drugs targeting different stages of cell cycle have been shown in figure 1.5.

![Anticancer agent with their specific targets](image)

*Figure 1.5* Types of anticancer drugs targeting different stages of cell cycle with important examples.
Drugs damaging the nuclear DNA

This class of anticancer agents directly damage DNA in the nucleus. These are known to act through directly interrupting the DNA replication in cancerous cells and consequently arresting replication or synthesizing abnormal form of DNA or RNA which are not able to code for useful products. Important drugs of this class include antibiotics - daunorubicin, doxorubicin, cisplatin and etoposide.

Drugs inhibiting the replication of DNA

This type of drugs directly interferes with synthesis of DNA building blocks including nucleotides, folic acid, and nitrogen bases. These drugs interfere with different steps in synthesis of nucleotides or deoxyribonucleotides. This results in decrease in the cellular concentration of these building blocks preventing the synthesis of nucleic acids and ultimately leading to obstruction of replication. Important drugs of this class include methotrexate, mercaptopurine, hydroxyurea and fluorouracil.

Drugs affecting the synthesis and breakdown of the mitotic spindles (Antimitotic agents)

This type of drugs acts by inhibiting the function of microtubules through binding to their subunits and affecting the synthesis and breakdown of the mitotic spindles. Microtubules play central role in equal distribution of chromosome in daughter cells through formation of spindle apparatus during mitosis. Microtubules also play important role in cytokinesis. Thus the compounds targeting the microtubules can be used as potential anti-cancer agents. These compounds are also called antimitotic agents. Three different classes of drugs acting as antimitotic agents have been identified.

1. Taxanes

It is the first identified group of antimitotic drugs which include paclitaxel and docetaxel. These interfere with microtubules through binding to β-tubulin and preventing their disassembly after completion of cell division which results in the mitotic arrest and consequently lead to apoptosis and cell death. Paclitaxel has been isolated from the bark of the *Taxus brevifolia* (Western yew tree) [45]. It is used to treat breast cancer, ovarian cancer, lung cancer and Kaposi's sarcoma. Later on a semisynthetic analog of paclitaxel known as Docetaxel has been synthesized from a compound isolated from *Taxus bacatta* (Himalayan yew tree) [46]. Docetaxel is more potent...
compound and has better toxicity profile as compare to paclitaxel. It is used to treat prostate cancer, breast cancer ovarian cancer and lung cancer.

2. Vinca alkaloids
It is another class of antimitotic drugs which act through binding to the β-tubulin inhibiting it to bind with the α-tubulin thus inhibiting the synthesis of microtubules. These results in cell cycle arrest in metaphase and consequently lead to apoptosis. These include vinblastine, vincristine, vinorelbine, and vindesine isolated from a medicinal plant *Vinca rosea.*
Vinblastine is used in the treatment of bladder cancer, neuroblastoma, testicular cancers, Kaposi’s sarcoma and Hodgkin’s disease. Vincristine is used to treat leukemias and lymphomas of childhood, rhabdomyosarcoma, neuroblastoma and non Hodgkin’s lymphoma. Vinorelbine is used for the treatment of breast cancer and lung cancer. Vindesine is effective in treatment of lung cancer breast cancer and leukemia

3. Colchicine

It is small molecule isolated from Colchicum autumnale which act through binding with β-tubulin and promoting their polymerization eventually inducing apoptosis.

Although the efficiency of chemotherapeutic agents is often limited by its toxic effect on other tissues as these compounds also kill some normal noncancerous cells, particularly those that divide rapidly as part of their physiological roles such as bone marrow cells, germinal epithelium hair follicles and mucosal cells of gastrointestinal tract resulting in common side effects of chemotherapy including low white blood cell count, gastrointestinal disorders, anemia and hair loss. To overcome this problem target based chemotherapeutic agents are developed which selectively target a signaling pathway specific to cancerous cells and thus, showing selective toxicity. Considering on issue of toxicity development of chemotherapeutic agents the search for novel anticancer agents has been extended to compounds derived from natural resources.

On the basis of mechanism of action anticancer drugs are classified in following groups-

Alkylating agents (nitrogen mustards)

These drugs act through producing highly reactive carbonium ion species which can interact with a nucleophile (electron donor species such as hydroxyl, amine and sulphydryl groups) present in biomolecule. The N7 of guanine, N1 and N3 of adenine, and N3 of cytosine are all act as nucleophiles to react with carbonium ion produced by alkylating agents. Most of the drugs of this class have two alkylating groups and may react with two different nucleophilic groups leading to the cross linking of DNA. These drugs act by interfering with transcription as well as replication of DNA causing cytotoxic effect. Examples of alkylating drugs include mechlorethamines, Cyclophosphamide, ifosfamide, chlorambucil, melphalan, ethylenimine and thio-TEPA. Other alkylating agents include busulfan, nitrosoureas, carmustine, lomustine, triazine and dacarbazine.
Antimetabolites

These drugs have structural similarity with DNA constituents or with coenzymes involved in the synthesis of nucleotides. These inhibit the synthesis of purine or pyrimidine or compete with them in synthesis of DNA or RNA. For example methotrexate inhibits the enzyme folate reductase, an enzyme involved in conversion of folic acid to tetrahydrofolic acid. It irreversibly binds with folate reductase, thus inhibiting the synthesis of tetrahydrofolic acid which lead to inhibition of DNA synthesis and subsequently arrest of cell cycle in S phase. Similarly purine and pyrimidine antagonists act through interfering with the synthesis of purines and pyrimidines. Examples of antimetabolites include-

(a) Folate antagonists such as methotrexate
(b) Purine antagonists such as 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine
(c) Pyrimidine antagonists such as 5-Fluorouracil (5-FU) and Cytarabine (cytosine arabinoside)

Plant alkaloids

Vinca alkaloids These are the alkaloids obtained from the plant Vinca rosea. These drugs prevent the spindle formation through binding with tubulin and cause mitotic arrest. Examples: vincristine (Oncovin), vinblastine (Velban) and vindesine.

Taxanes They form stable non-functioning microtubule by promoting polymerization through binding with tubulin, thus resulting in mitotic arrest. Examples- Paclitaxel (taxol), Docetaxel (taxotere).

Podophyllotoxin

Podophyllotoxin and deoxypodophyllotoxin, the active constituents isolated from the roots of Podophyllum peltatum Linnaeus have been found active against different types of cancer, acting through inhibiting the assembly of microtubules and inducing cell cycle arrests in metaphase. Epipodophyllotoxin, an isomer of podophyllotoxin has been shown to be more effective and less toxic against different cancer cell lines. Semi-synthetic analogs of epipodophyllotoxin termed as etoposide and teniposide were found very effective against different human cancers including lymphomas, bronchial cancer and testicular cancer.

Camptothecin analogues

Camptothecin is a cytotoxic alkaloid isolated from, Camptotheca acuminata which is kown to act through inhibiting DNA Topoisomerase I. In order to increase its solubility and improve toxicity profile, certain analogues have been synthesized such as topotecan (hycamtin), irinotecan.
(camptosar), exatecan, rubitecan, lurtotecan and 9-aminocamptothecin (9-AC). Topotecan is found very effective in patients with epithelial ovarian cancer and small cell lung cancer as a second-line treatment. Similarly, irinotecan acts as first- and second-line treatment for metastatic colorectal cancer.

Antibiotics anthracyclines such as doxorubicin (adriamycin), daunorubicin (rubidomycin), actinomycin (dactinomycin) mitoxantrone, bleomycins (blenoxane), mitomycin (mutamycin), mithramycin (plicamycin), dactinomycin (cosmeogen). Different targets of anticancer drugs interfering with the synthesis of nucleic acids have been represented in figure 1.6.

**Drugs inhibiting angiogenesis**

These drugs specifically target the pathways involved in development of vasculature in a tumor preventing blood supply to the tumor. 5,6-dimethylxanthenone-4-acetic acid is postulated to exert anticancer activity through obstructing the development of vascular system in tumors and also showed prominent cancer inhibition when given in combination with other cytotoxic drugs [47]. Angiogenesis inhibitors are known to possess less toxic effect in comparison to other anticancer drugs.
Hormonal agents

These drugs act through restoring the hormonal balance and are useful in treatment of breast cancer, prostate cancer, ovarian cancer and endometrial cancer for example tamoxifen (nolvadex), flutamide (eulexin) and gonadotropin-releasing hormone agonists.

Miscellaneous agents

These include hydroxyurea, L-asparaginase, cisplatin, mitoxantrone (Novantrone), derivatives of retinoic acid and bone marrow growth factors etc.
Enzyme L-asparaginase act through interfering with the metabolism of asparagines. Cancerous cells, particularly lymphatic cancer cells, need continuous supply of asparagines and for this requirement they mainly depend upon dietary asparagines. Recent studies revealed that leukemia cells are unable to synthesize asparagine while normal cells can synthesize it in normal circumstances. This metabolic difference between cancerous and normal cells can be exploited in treatment of leukemia. The enzyme L-asparaginase is recognized as useful agent for the treatment of different types of leukemia. Administration of L-asparaginase degrades the extracellular asparagines through converting into aspartic acid. Cancerous cells become deficient of asparagine and die while normal cells synthesize the required quantity of asparagine internally thus remain unaffected. L-asparaginase was isolated from *E. coli* as well as some rodent animals and was shown to exert significant activity against different types of human leukemias.

**Development of anticancer drugs**

**Historical background**

Most of the conventional anticancer molecules were discovered by serendipity or through random screening of compounds through evaluation of their effect on inhibition of cell division. An important example of coincident discovery of anticancer agent include the findings that nitrogen mustard significantly suppressed the lymphoma and myeloma in the soldiers accidentally exposed to this gas during the World War II. This finding lead to the development of several related alkylating agents, including chlorambucil and cyclophosphamide. In the similar way, the anticancer potential of platinum containing anticancer drugs came into existence coincidently during another study demonstrating the inhibition of bacterial growth after putting them in influence of an electric field. During the experiment, it was observed that multiplication of bacterial cells blocked due to accumulation of the electrolysis product of the platinum electrode which is thought to contaminate the growth medium. This serendipitous discovery has provided valuable anticancer drugs such as cisplatin. Folate antagonists, including aminopterin and amethopterin, came in to existence in 1948. A brief history of development of anticancer drugs is summarized in figure 1.7.
Cancer was recognized as a disease caused due to uncontrolled and excessive cell division. Thus, to develop anticancer agents, attempts were focused in the identification of cytostatic and antiproliferative molecules. Anticancer compounds are initially screened in cell lines derived from different types of cancer possessing characteristic molecular markers and other relevant features related to cell cycle regulation and differentiation. Since an anticancer compound specifically target some molecular pathways, these compounds are evaluated against a panel of different cancer cell lines belonging to different cancer types to determine exact mechanism of action.

Drug found active in *in vitro* screening are evaluated in *in vitro* murine models. Initially developed murine models include L1210 mouse leukemia model and P388 murine leukemia model.
allograft, Murine models of cancer are used for *in vivo* experiments as rapidly growing tumor models provide a more relevant condition in preliminary evaluation of the anticancer potential. Compounds showing significant activity in murine model are further evaluated in other *in vivo* model. *In vivo* study provides more accurate information about activity profile and mechanism of action. In addition to determining the specificity and extent of activity, the *in vivo* studies also provide valuable information on toxicity profile and maximum tolerable doses. Compounds significantly reducing the size of tumor and increasing the life span of the mice are identified as potential agents and recommended for further studies.

National Cancer Institute introduced an advanced *in vivo* model, human tumor xenografts in nude mice. After a detailed analysis and considering all aspects of molecular mechanism related to different types of cancer, NCI recently introduced an *in vitro* system of evaluation of anticancer activity using 60 different tumor cell lines (NCI-60 screen) derived from different types of human cancers including leukemia, breast cancer, prostate cancer, small-cell and non-small-cell lung cancers, and others.

Anticancer drugs development has fundamental difference from other drug development. It need fine selectivity of therapeutic compounds to rapidly dividing cancerous cells without affecting remaining normal cells of whole body. This fact can be easily understood from the fact that out of 600,000 molecules evaluated for anticancer potential, only 38 have reached in clinical trials to be used as drugs.

Development of conventional chemotherapeutic agents is based on the antiproliferative potential of compounds. The screening of molecules is performed to select the compounds with significant cytotoxic or cytostatic effect on different cancer cell lines. The anticancer potential of compounds is further validated through evaluating the effect of these compounds on regression of tumors in murine tumor xenografts or allografts. The anticancer agents were discovered basically through randomly screening the compounds for cytotoxic potential or evaluating their potential to modulatet metabolic pathways related to cell division.

The recent advancements in the field of molecular biology and an understanding of the biochemical basis of cancer have provided new way to develop target-based potent anticancer drugs. After the identification of biochemical pathways and key enzymes regulating these pathways it become easy to develop a therapeutic agent which selectively target a pathway
involved in different stages of the disease including growth, metastasis or angiogenesis. A number of target-based anticancer agents have been developed following these developments.

**Anticancer drugs acting through facilitating the apoptosis**

Different cytotoxic drugs exert their activity through triggering the apoptosis. Most of the currently used anticancer drugs were developed based on evaluation of their potential to selectively kill tumor cells. These can modulate the activity of genes and proteins controlling apoptosis [48, 49]. Many of these are known to act through activating the p53 or inhibiting Bcl-2 activity [50].

Several molecules inhibiting the activity of NF-κB or proteosome or different enzymes involved in kinase/Akt pathway are shown to be potentially inducing cell death in cancerous cells [51]. Molecules modulating the function of Ras and its activator farnysltransferase are also used as potential anticancer agents. The compounds modulating the activity of any of these signaling molecules can restore the apoptosis in cancerous cells or facilitate the other anticancer agents to induce cell death. These drugs induce the damaging effect in the nuclear DNA subsequently resulting in the activation and release of the pro-apoptotic factors from mitochondrial intermembrane space or upregulation of expression of pro-apoptotic receptors (Fas/CD95, TNF-R1 and TRAIL receptors) on cancerous cells, which subsequently interact with pro-apoptotic proteins to initiate apoptotic signaling pathways [52]. Several natural products including flavones and alkaloids have been shown to act through selectively inducing the apoptosis in cancer cells [53, 54]. Similarly, luteolin and apigenin have been shown to induce apoptosis selectively in myeloid leukemia and prostate cancer respectively [55, 56]. Some flavones such as fisetin and wogonin as well as red wine polyphenolic extract have been shown to induce apoptosis mediated by cascade involving caspase 3 [57].

**Anticancer Drug Development based on Molecular targets**

Anticancer agents are developed based on *in vitro* screening of cytotoxic activity using different cancer cell lines followed by *in vivo* screening using animal models. To determining the non toxic dose of these drugs, the preclinical study is followed by early phase of human clinical trials.

Anticancer agents show toxic effect when their concentration exceeds the limiting dose hence it become essential to determine tolerable maximum doses with no detectable toxic effect. In case of anticancer agents based on molecular targets, the possibilities of toxic effect get reduced
considerably as these agents are postulated to be directly targeting a specific pathway. Molecular target based agents usually act through targeting key enzymes of different intracellular and extracellular processes with reduce toxicity and less chance of drug resistance.

Examples of these key enzymes include topoisomerase, farnesyltransferase, receptor tyrosine kinases, dihydrofolate reductase and matrix metalloproteinase.

The development of anticancer agents with molecular targets is an intricate process requiring well understanding of several related intracellular and extracellular processes. Beyond all these obstacles research efforts are focused on development of molecular targets based anticancer agents which involve the identification of a potent target molecule followed by development and validation of a molecular assay specific to that target and the relationship between the molecular target and the cancer type.

Since these anticancer drugs are developed with a pre-determined target and mechanism of action, some modifications are needed in preclinical and clinical studies. Determination of the effects of these drugs on a particular target is not enough to demonstrate therapeutic effect, it should be authenticated through several other studies determining their effects on other related processes.

Furthermore, toxicity profile is the major factor in development of molecularly targeted anticancer drugs. It includes the adverse effects of these drugs on other metabolic pathways and general toxicity. To overcome these effects the compounds should be chemically modified to enhance the activity and consequently to decrease the toxicity.

The combination therapy is another way to overcome these effects as it require comparatively low dose of drugs eventually minimizing the toxic effects. In combination therapy the effects of molecular targets based drugs are enhanced by other chemotherapeutic agents that may act through sensitizing the cancerous cells to these agents.

**Anticancer drugs acting through Inhibition of metastasis**

The spread of the cancer from its origin site to other parts of the body is known as metastasis. It is attributed to the movement of cancer cells to distant locations in the body. The mechanism of Translocation of cancerous cell involves cellular movement together with the extracellular matrix remodeling (ECM remodeling) which facilitates physical entrapment of the cells and
ultimately determines the shape of a tissue at both the site of initial as well as the site of metastasis. Several enzymes including plasminogen activators (PAs), proteases and matrix metalloproteinases (MMPs) have been shown to be associated with the extracellular matrix remodeling facilitating the invasion and propagation of cancerous cells to distant parts of the body. Consequently, the compounds modulating the activity of plasminogen activators, proteases and matrix metalloproteinases may act as potential anticancer agents. Some recent studies postulated that p53 mutations and Bcl-2 overexpression also influence the metastasis [58, 59].

**Anticancer drugs acting through inhibition of angiogenesis**

The development of new blood vessels in cancerous tissues to maintain the blood supply to all the cells in growing tumor is known as angiogenesis. It is an essential step to fulfill the requirement of oxygen and nutrients for the cells present in the center of tumor to grow as well as its spread to other parts of the body. Consequently, the drug candidates preventing the supply of oxygen and nutrients through blocking the angiogenesis may be applied as anticancer agents.

Tumor cells secrete some angiogenic factors which induce the blood vessel cells to divide and develop new blood vessels in tumor. Different studies have shown that some oncogenes such as bcl2 increase the expression of genes encoding different angiogenic factors.

The process of angiogenesis is postulated to be regulated through intricate network of signal transduction cascade and can be divided in three closely associated steps (i) initiation of angiogenesis, (ii) development of blood vessel lining through proliferation and migration of endothelial cells, and (iii) remodeling of the extracellular matrix.

Recent advancement in the understanding of tumor angiogenesis has led to the identification of several novel drug targets including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and receptor tyrosine kinases related to these growth factors. Some other validated targets include matrix metalloproteinases, cell adhesion molecules and plasminogen activators. Compounds modulating the activities of these targets lead to the inhibition of endothelial cell proliferation and subsequent inhibition of angiogenesis. The inhibitory potential may be used in the development of potential anticancer drugs. Taxanes have been postulated to act through disrupting the microvessel mediated by inhibition of VEGF and MMP and enhanced expression of E-cadherin [60, 61].
**Development of antibodies against tumor-specific antigens**

Development of antibodies against tumor-specific and tumor associated antigens is used as powerful strategy to find new anticancer drugs. These antibodies are used to induce antitumor immune responses in cancer patients it is considered to be free of toxic effects. These specific antibodies can be designed to be conjugated with tumor specific drugs, radioisotopes or prodrug specific enzymes and used against different types of tumors. Furthermore different tumor specific antibodies can itself induce tumor regression through stimulation of antibody-dependent cellular toxicity (ADCC) or targeting the complement system. Antibody-dependent cellular toxicity is demonstrated to be mediated through monocytes, granulocytes and natural killer cells. Tumor specific antibodies can also be used to induce the expression of tumor-specific antigens on the antigen-presenting cells/dendritic cells to activate the T-cell immune response against cancer.

**Role of Metabolomics and Pharmacogenetics in treatment of cancer**

These are relatively new techniques came in to existence due to advancement in molecular biology. These used to predict the drug response and toxicity profile in individual patents. Cytotoxic drugs exhibit toxic effect in normal cells at higher doses, considering this fact it become essential to determine the safe dose for a patent. Pharmacogenetics is used to determine the nontoxic dose through monitoring of drug response and toxicity using the genetic constitution of the individual patient to recognize the key factors involved in drug response and toxic effect. It primarily considers the drug-metabolizing enzymes cytochrome P450 (CYP) superfamily several of which are found to be responsible for the inactivation of anticancer drugs.

Variations in drug targets such as thymidylate synthase and epidermal growth factor receptor (EGFR) are also considered. Person to person difference in drug–response can be minimized through it. Quantification of metabolites through metabolite profiling (analyses of all the metabolites in a sample to identify the characteristic metabolite profile of that sample) and target analysis (quantification of metabolites specific to a particular drug or pathway) provide useful information about cellular metabolism. It can be achieved through isotopic labeling of selected metabolites and monitoring their movement through diverse pathways followed by different assays such as NMR, mass spectroscopy, liquid chromatography – tandem MS (LC-MS/MS) and gas chromatography – tandem MS GC-MS/MS). Metabolic profiling can be used as a
marker to determine the disease state and to differentiate the normal tissues from different states of the disease [62].

**Other anticancer agents acting through indirect mechanisms**

Several drugs acting through indirect mechanisms other than cytotoxic and molecularly targeted are used in the cancer treatment. The important examples of these drugs include the drugs reversing the multidrug resistance, drugs modulating immune response, chemoprotective agents, drugs restoring hormonal balance, photosensitizers, and Supportive cancer-care agents.

**Drugs reversing the multidrug resistance**

Multi-drug resistance (MDR) is a major concern in the anticancer drug development. The upregulation of the membrane efflux protein, P-glycoprotein (P-gp) and failure of therapeutic agents to induce apoptosis result in multi-drug resistance. Consequently the inhibitors of the membrane efflux protein may be used to potentiate the activity of anticancer drugs. Several P-glycoprotein inhibitors including zosuquidar, tariquidar, and laniquidar, have showed potential anticancer potential when used in combination with other cytotoxic agents [63].

**Vaccines or immunomodulators**

It is a group of drugs which act through improving the immune response to tumor cells. Tumor-specific antigens are used to generate humoral and/or T-cell mediated immunity. immune-mediated anticancer activity can be attain through a number of approaches such as through potentiating immune interaction by increasing the activity of antigen presenting cells (APC) or directly increasing the immune response by cytokines [64].

**Chemoprotective agents**

These are known to alleviate the toxic effects of anticancer drugs. Nephrotoxicity caused by nitrogen mustard ifosfamide can be neutralized by sodium-2-sulfanylethanesulfonate when administered as combination therapy. Similarly amifostine act as a chemoprotective agent through effectively minimizing the nephrotoxicity caused by anticancer drug cisplatin.

**Drugs restoring hormonal balance**

Another effective non-cytotoxic anticancer therapeutic approach include the hormonal therapy which can be effectively used for the treatment of some particular types of cancer which are
known to be influenced by hormonal imbalance such as breast cancer, prostate cancer, ovarian cancer and endometrial cancer. Steroids such as antiandrogens and antiestrogens are used for the treatment of these cancer types without showing any toxic effect. Recent studies showed that the breast cancers can be treated through removal of the ovaries [65]. Similarly prostate cancer can be treated by removal of testis or by administration of the estrogen [66].

**Photosensitizers**

These are the biologically inactive compounds but become active when irradiated by light. Administration of photosensitizers followed by laser irradiation of cancer affected tissue results in the generation of free radicals in that portion of the body, leading to destruction of the tumor. Precaution should be taken in determination of concentration of the photosensitizer and focused irradiation. 5-Aminolaevulinic acid, a porphyrin precursor is postulated to be effective photosensitizing agent [67].

**Supportive cancer-care agents**

These are the drugs used to reduce the side effects caused by chemotherapeutic agents aiming to enhance their therapeutic efficacy as well as improve the quality of life after chemotherapy. Painkillers such as opiates, anti-emetic drugs such as butyrophenones, and growth factors such as granulocyte colony stimulating factor and monocyte colony stimulating factor are some important drugs of this class [68].

**Process of anticancer drug development**

The development of anticancer drug is primarily based on the identification of cytostatic or cytotoxic compounds which act through reducing the growth of tumor or demolishing the tumor. The process of anticancer drug development has been extended to exploit target specificity of drug candidates to identify the molecular targets as well as to develop molecules selectively acting on these targets. Several other drug types including immunomodulators, chemoprotective agents, Multi-drug resistance-reversing agents, drugs based on hormonal therapy and photosensitizers are in progress with the potential to be used in combination with conventional cytotoxic agents. Moreover the application of recent technologies such as pharmacogenetics and metabolomics is known to improve the process of development of novel anticancer agents. The development of new drug usually consists of following steps:
1. Identification of biologically active compounds

New bioactive compounds can be identified through activity directed fractionation of different extracts from natural resources or by chemical synthesis. This step is supported by several analytical techniques to characterize and purify these compounds as well as to determine their bioavailability and stability under physiological conditions. Different types of chromatography together with spectroscopic techniques are used to purify and characterize the compound. Their physicochemical properties including solubility, melting point, and stability are determined through different techniques. Method is developed and validated to ensure large scale isolation or synthesis of compound as it progresses in the drug development pathway.

2. Screening of biological activity and preclinical pharmacology

The possible drug structure is determined through pharmacophore mapping and docking analysis and comparing their structures to those of existing drugs. Selected compounds are screened using different cancer cell lines to determine the extent and specificity of anticancer activity. Toxicity of these compounds is determined using normal cell lines. These compounds were finally evaluated in animal models to validate their efficacy and toxicity, preclinical study provide sufficient information about efficacy, toxicity and pharmacokinetics of drug candidate.

3. Clinical development

It is the final step of drug development process which encompasses three major steps termed as phase I, phase II and phase III clinical trials. In phase I clinical trials these drugs are evaluated in healthy human volunteers to determine the maximum tolerable dose as well as the toxic effects. The gradually increased doses of the drug candidates are administered to a small group (usually, 20 to 100 persons) of closely supervised healthy volunteers. Subsequently, in phase II clinical trials the drugs are evaluated and their effects are studied in detail in a small group of carefully observed and continuously assessed patients (usually, 100 to 300 patients) suffering from selected types of cancer to determine the efficacy of drug, the method of delivery, effective dosage and the dosing interval as well as to reconfirm the maximum tolerable dosage. To confirm findings of phase I and II trials, the drugs are evaluated in long duration (usually 2 to 10 years) phase III clinical trials in a larger population involving thousands of patients in multiple locations These studies are designed to compare the therapeutic potential of these newly developed drug candidates with that of the existing most potent therapeutic agent as well as to
further confirm the efficacy and maximum tolerable dosage. The drug found successful in Phases I, II, and III clinical trials can be approved by national drug regulatory authority.

**Natural products as source of therapeutic agents**

Natural products are continuously providing new leads in terms of structural novelty as well as novel mechanism of action. Compounds isolated from natural product are shown to have chiral centers, complex ring systems, and in some cases heterocyclic rings, and thus have the potential to exhibit diverse biological activities [69]. Natural products serve as established template for the development of new scaffolds of drugs [70]. An important example of natural product based drug development include the synthesis of the anti-inflammatory drug, acetylsalicylic acid (aspirin), a derivative of natural product, salicin. Similarly, pilocarpine isolated from *Pilocarpus jaborandi* has been used as therapeutic agent for the treatment of several diseases for more than 100 years. Morphine isolated from *Papaver somniferum* L. has provided strong basis for the development of anticancer agents. A representative process for the development of natural product based drug has been illustrated in figure 1.8.

![Strategies for Natural Product based Drug Discovery](image)

**Figure 1.8:** Procedure of the development of therapeutic agents from natural resources
At present time research interest has extremely focused on medicinal plants to find novel bioactive compounds as these could provide relatively simple but promising lead, since it is much easier to identify an active constituent from therapeutically active herbal preparation than to find a new drug from a large group of new synthetic molecules [71]. Furthermore, the molecules obtained from natural resources are relatively more compatible to living system as these are synthesized in living organisms to perform biological functions. These may have biologically relevant structures and can be easily metabolized in living system, hence lesser side effects. Flavonoids and other polyphenolic compounds taken with diet may prevent the development of cancer in alimentary canal. Sufficient intake of green vegetables and fruits rich in these compounds may also reduce the risk of many other types of cancer [72].

Several terrestrial plant species and marine organisms possessing diverse biological activities have been used as source of therapeutic agents. Some important examples include:

Azadirachta indica Juss (Neem)

It is a large tree belonging to the family Meliaceae widely distributed in the tropical countries including India and china and recognized as a potential source of multidirectional therapeutic agent in traditional system of medicine since the Vedic times. All parts of the plants including leaves, fruits, seeds and stem bark have been used as traditional medicine against a variety of human ailments from ancient times. Several researchers have been working in medicinal aspects of Azadirachta indica. Several active constituents have been isolated from this plant out of which azadiractin is the most potent constituent [73-75].

Thymus vulgaris (Ajowan)

It possesses significant antimicrobial potential and it is used in traditional system of medicine for the treatment of some parasitic diseases. Thymol, a phenol compound is the major active constituent present in essential oil of this plant and is found to be responsible for the activity. It exhibit antiseptic and anti diarrheal activities.

Ocimum sanctum Linn (Tulsi)

It is a small erect herbaceous aromatic plant belonging to the family Labiatae commonly known as Tulsi. It is widely distributed in tropical and subtropical regions of India. The leaves of Ocimum sanctum are commonly used to treat a number of diseases including constipation,
postnatal disorders, cough, fever, vomiting, cholera, edema, hydrosarca, septicemia, antiulcer, dog bite and many other ailments [76]. It has also been shown to possess significant anticancer activity in mice model [77]. Leaves are very rich source of essential oils. A wide range of active compounds with diverse biological activities have been isolated from the leaves of *Ocimum sanctum*, out of these compounds eugenol is identified as the major active constituent of the oily fraction of the leaves. Several other components have also been identified including caryophyllene, eugenol methyl ester, terpinene-4-ol, decyldehyde, cervacrol and camphor.

**Garlic**

It is the edible bulb belonging to the family liliaceae. Garlic and other species of the genus like onion, leek, scallion and shallot are used as household remedy. These are termed as allium vegetables. Garlic is a rich source of natural sulfur and iodine and has several therapeutic effects. Consumption of garlic and other allium vegetables in diet provide several enzymes which are capable of neutralizing the effects of some carcinogenic substances. It is traditionally used to treat several diseases including tuberculosis, heart disease, arthritis, hay fever and cancer [78-81]. Several studies showed that different extracts of garlic showed significant antiatherosclerosis and antidyslipidemic potential. Phytochemical investigation of garlic provided several sulfur compounds as well as glutathione precursors, which are known to acts as potential antioxidants and also display anticarcinogenic properties. S-allyl-L-cysteine sulfoxide and diallyldisulfide S-oxide (allicin) isolated from bulbs of garlic have shown antimicrobial property. Allyl sulfur and other several compounds isolated from different extracts of garlic inhibited the growth of cancerous cells. Recent studies have established that different allyl sulfur compounds, naturally occurring in garlic and onions, increase the susceptibility of cells to the stress created by products of cell division.

Natural products have provided the inspiration for most of the existing drugs. 28 % of drugs developed in last two decades were directly based on compounds isolated from natural resources or their semisynthetic derivatives, with another 25% created around a pharmacophore obtained from natural products. Currently, medicinal chemists are involved in synthesis of novel active molecules inspired from natural products [82, 83]. The recent advancement in medicinal chemistry and analytical techniques allowed the medicinal chemists to synthesize the natural
products in lab as well as modify their structure to improve the activity and other qualities such as solubility or stability in the human body [84].

In the field of infectious disease several antibacterial agent have been isolated from natural resources belonging to different classes including β-lactams, tetracyclines, glycopeptides, macrolides and spectinomycins. Similarly a number of antifungal drugs have also been obtained from natural products including nikkomycin Z, amphotericin B and nystatin analogues. Some important drugs in this class including streptogramins have also been produced through genetically modified strains of *Streptomyces*. A potential combination of antibiotic has been prepared by combination of two different water soluble semisynthetic antibiotics namely dalfopristin and quinupristin in ratio of 7:3. It has promising activity against multi drug resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*, and is used for treatment of a variety of microbial Infections. It is known to act through interfering bacterial cell wall synthesis and is effective against a broad range of pathogenic strains. Other potential antibiotics obtained from natural resources or derived from natural products include Ziracin, LY-303, L-743, GR-135402, and GM-237354 [85]. A series of lipopeptide antibiotics has been isolated from *Streptomyces roseosporus* exhibiting potential activity against Gram-positive bacteria. A semisynthetic analog daptomycin prepared by reacylation of the N-terminus with n-decanol was found most effective without any toxicity [86-88]. Different plant-derived flavonoids belonging to different structural groups were evaluated against a wide range of pathogenic bacteria some of these compounds exhibited prominent activity [89]. Several semisynthetic flavones were also showed significant antibacterial activity [90]. *Calophyllum lanigerum* yielded a potent anti HIV molecule Calanolide A which is known to act through inhibiting reverse-transcriptase. Pre clinical studies have showed that Calanolide A is effective against AZT resistant strains HIV- I [91]. A potent antiparasitic drug, ivermectin is isolated from a *Streptomyces* sp. It is a WHO recognized drug used in vector born disease prevalent in several parts of Africa and America. It is also used in treatment and control of parasitic diseases in animals. Considering the neurological disease dopamine receptor agonism remains the focus of most of researchers. Aporphine, a large group of naturally occurring compound isolated from a wide range of plant species is known to exhibit significant activity against neuronal disorders. Apomorphine, a semisynthetic analog with dopamine (DA) receptor agonist activity is used in treatment of Parkinson’s disease [92, 93]. Similarly, varenicline, a semisynthetic molecule obtained through the modification of cytosine is
known to activate nicotinic acetylcholine receptor (nAChR) [94]. Several natural products including pure compounds, plant extracts and other herbal preparations have been explored for their therapeutic potentials in neurodegenerative disorders and some of these have shown considerable neuroprotective potential.
Stem of *Baeckea frutescens* L. is used in traditional Chinese medicine for treatment of rheumatism and snake-bite. Bioactivity directed fractionation of its crude extract yielded a chromone derivative 5-hydroxy-2-isopropyl-7-methoxycromone as active ingredient [95]. In the current era attention has also been given to microbial fermentations leading to the discovery of many clinically useful drugs. A significant number of natural product derived drugs are known to be produced directly by microbes or interactions of microbial with the host plant. In this regard, endophytic fungi have been recognized as a possible alternative source of natural products primarily obtained from plants. Different medicinally active natural products of plant origin including camptothecin, taxol, taxane, hypericin, rohitukine and hypercin have been reported to be produced in significant amount by different species of endophytic fungi obtained from these plants [96-100].

**Bioassay guided drug discovery**

Bioassay-directed fractionation supported by several recent techniques is extensively used to identify the active principles/pure compounds present in crude natural products preparations. This approach can be used systematically to reduce the complexity of the extracts/fractions in order to locate the biological activities of complex materials. It resolves the complex mixtures to more simple and pure form [101, 102]. Considering on therapeutic preparation obtained from crude extracts, only a specific portion of the extract-the active principles/bioactive molecules with specific biological targets is of interest. In view of this fact, a biomolecular interaction step should be included into analytical methods to achieve bio-selectivity and to purify the compounds with specific therapeutic activity.

The technique has been extensively used for very long period. Two different studies have been carried out by Durston and Ames 1974 and Commoner et al. 1974 for detection of metabolic carcinogen intermediates in urine samples [103, 104]. Some other research groups also performed similar studies based on bioassay guided fractionation [105, 106]. Several aspects of activity-guided analysis and the potential role of these analytical techniques in drug development together with different new approaches related to screening of biological activities have been studied in details recently [107-109]. The bioassay-directed fractionation can be divided in to three main steps namely biological assay, fractionation and detection. The biological assay
(pharmacological or biochemical) is the basic and major part, which deals with determination of the active principles or compounds responsible for biological activity.

Biological activities of different plant extracts, fractions and pure compounds isolated from these fractions are determined through several biological assays. The choice of biological assay depends on the type of activity present in plant extracts and compounds. The selected biological assay should be highly sensitive and specific for the activity. It should also be cost effective and easy to handle. Two major types of biological assays are applied- the mechanism-based assays and cell-based assays.

**Mechanism-based assays**

These assays evaluate the effect of the compounds or plant extracts and fractions on a specific target such as enzyme, DNA or receptor involved in a molecular signaling cascade. These include *in vitro* assays performed using several methods. Since *in vitro* conditions significantly differ from normal physiological conditions, it should be standardized as well as supported by additional experiments. *In vitro* assays can accurately determine the biological activity of the compounds or extract at very low concentrations. The major limitation of Mechanism-based assays is that they have significant difference from normal physiological conditions as these assays focus particularly on only target pathway thus overlooking the influence of several other related pathways on that particular pathway. Moreover, several other molecules present in active fractions may show their effects through a different mechanism, modulating the effect of the target molecule on specific signaling pathway. This may result in a misinterpretation of the effect produced by active extract or fractions. In addition, molecules or plant extracts found active on *in vitro* assay may be inactive in normal physiological conditions when evaluated in animal models due to their poor water solubility and less bioavailability.

**Cell-based assays**

These assays involve the assessment of drug-cell interactions with the whole intact cell rather than just isolated systems. These assays are able to determine the activity of wide range of active compounds in small time. Although the exact mode of action of compound cannot be determined through these assays, they can provide the initial information about the possible mechanisms. Compounds that cannot pass through the cell membrane can also be evaluated through this
method. These assays have many advantages as compare to mechanism-based assays since a broad range of compounds can be screened with a much higher hit rate through cell based assays. Although the in vitro study can provide valuable information related to their anticancer potential including mechanism of action but before clinical evaluation, it should be validated through in vivo experiments for example evaluation of anticancer activity in human tumor xenograft in an immunodeficient mouse. Large amount of pure compound is required for in vivo study that can be achieved through re-isolating the compound by repeated collection of the source organism or by additional fermentation if the compound is of terrestrial microbial origin or by production of the compound through total synthesis in a synthetic laboratory. Since these procedures may be quite time consuming, steps can be taken to decide that whether a given lead compound has the potential for further development as an anticancer agent. Recently some rapid in vivo assays such as hollow fiber assay have been developed which requires very less time to predict the activity and also need relatively small quantity of compound. In hollow fiber assay cancer cells are cultured inside fibers which are subsequently implanted in immunodeficient mice [110, 111]. A 60-cell line panel developed by U.S. National Cancer Institute (NCI) is used to test different molecules against different types of human cancers and to determine the mechanism of action of these molecules [112].

The second step is the fractionation step which is performed in order to increase the purity of active ingredients and simultaneously avoiding the presence of undesired compounds. It can be a very simple procedure encompassing partition of the extract into a few fractions through column chromatography or it can be a complex method involving sophisticated techniques like high-performance liquid chromatography [HPLC] and electrophoresis. The third step (detection) comprises different techniques related to structural elucidation, including mass spectrometry (ESIMS, MALDI-TOF), NMR (1H, 13C, COSEY, NOESY, HMBC, HSQC), IR, X Ray crystallography and alimental analysis. Over the time several novel analytical approaches have been introduced which facilitated the field of drug development through increasing the efficiency and sensitivity as well as reducing the time requirement [113-115]. With the help of recent version of liquid chromatography equipped with biochemical detectors different biochemical interactions can be detected online. Complex mixtures can be resolved in relatively simple and more active fractions as well as the active constituents of the complex natural extracts can be purified and characterized with the help of different types of chromatography including
conventional Column chromatography, HPLC, counter current chromatography, UPLC and molecular exclusion chromatography. Although the different organic solvents used as solvents in liquid chromatography may exert their own effects and interfere with actual results of biological assays, these effects can be reduced through evaporation of the fractions to be evaluated. Molecular exclusion chromatography is used for separating the molecules of similar polarity but different molecular weights.

Potential Anticancer Agents obtained from Natural Products

Citrus fruits and green vegetables provide a rich source of vitamins, flavonoids and other polyphenolic compounds, adequate consumption of these plant products reduce the risk of cancer. More than 1,000 non-nutritive plant derived compounds are known to have cancer-preventive activity. More than 400 plant derived compounds with anticancer potential are under investigation. Most of the species of higher plants, microorganisms, arthropods, and marine invertebrates are still not studied and thus these diverse natural resources can be explored to provide novel anticancer agents. Recent studies have identified new species of bacteria, algae, fungi, and vertebrates which are able to provide new anticancer molecules. Some important medicinal plants possessing anticancer potential are listed in table 1.2.

Table 1.2: Some important Medicinal Plants possessing anticancer activity

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Family</th>
<th>Active part</th>
<th>Plant name</th>
<th>Family</th>
<th>Active part</th>
</tr>
</thead>
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<tr>
<td><em>Acacia xanthophloea</em></td>
<td>Leguminosae</td>
<td>Fruit</td>
<td><em>Ipomea batata</em></td>
<td>Convolvulaceae</td>
<td>Rhizome</td>
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<tr>
<td><em>Adenium obesum</em></td>
<td>Apocynaceae</td>
<td>Leaf</td>
<td><em>Juncus acutus</em></td>
<td>Juncaceae</td>
<td>Leaf</td>
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<td>Anacardiaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Aeonium arboretum</em></td>
<td>Crassulaceae</td>
<td>Leaf</td>
<td><em>Lavandula angustifolia</em></td>
<td>Meliaceae</td>
<td>Leaves</td>
</tr>
<tr>
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<td>Meliaceae</td>
<td>Fruit</td>
<td><em>Leptadenia hastate</em></td>
<td>Asclepiadaceae</td>
<td>Bark</td>
</tr>
<tr>
<td><em>Alnus japonica</em></td>
<td>Betulaceae</td>
<td>Wood</td>
<td><em>Ligustrum lucidum</em></td>
<td>Oleaceae</td>
<td>Seed</td>
</tr>
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<td>Scientific Name</td>
<td>Family</td>
<td>Part</td>
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</tr>
<tr>
<td>Aphanamixis polyspachya</td>
<td>Meliaceae</td>
<td>Stem bark</td>
<td>Maytenus canariensis</td>
<td>Celastraceae</td>
<td>Fruit juice</td>
</tr>
<tr>
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<td>Araceae</td>
<td>Root</td>
<td>Maytenus macrocarpa</td>
<td>Celastraceae</td>
<td>Stem bark</td>
</tr>
<tr>
<td>Aster amellus</td>
<td>Compositae</td>
<td>Entire</td>
<td>Maytenus serrata</td>
<td>Celastraceae</td>
<td>Seed</td>
</tr>
<tr>
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<td>Meliaceae</td>
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<td>Monnina obtusifolia</td>
<td>Polygalaceae</td>
<td>Aerial parts</td>
</tr>
<tr>
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<td>Begoniaceae</td>
<td>Entire</td>
<td>Morinda citrifolia</td>
<td>Rubiaceae</td>
<td>Root</td>
</tr>
<tr>
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<td>Meliaceae</td>
<td>Seed oil</td>
<td>Ocotea foetens</td>
<td>Lauraceae</td>
<td>Branchlets</td>
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<td>Root</td>
<td>Pinus parviflora</td>
<td>Pinaceae</td>
<td>Strobilus</td>
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<tr>
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<td>Entire</td>
<td>Piper latifolium</td>
<td>Piperaceae</td>
<td>Leaf</td>
</tr>
<tr>
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<td>Compositae</td>
<td>Entire</td>
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<td>Leaf</td>
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<td>Bark</td>
<td>Pleione bulbocodioides</td>
<td>Orchidaceae</td>
<td>Tuber</td>
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<td>Shoot</td>
<td>Pratia nummularia</td>
<td>Campanulaceae</td>
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<tr>
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<td>Phymatosorus diversifolium</td>
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<tr>
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<td>Rhizome</td>
<td>Phytolacca esculenta</td>
<td>Phytolaccaceae</td>
<td>Root</td>
</tr>
<tr>
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<td>Berberidaceae</td>
<td>Root</td>
<td>Rabdosia rubescens</td>
<td>Labiatae</td>
<td>Leaf</td>
</tr>
<tr>
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<td>Leaf</td>
<td>Ruellia tuberose</td>
<td>Acanthaceae</td>
<td>Bark</td>
</tr>
<tr>
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<td>Euphorbiaceae</td>
<td>Leaf</td>
<td>Salvia chinensis</td>
<td>Labiatae</td>
<td>Entire</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Part(s)</td>
<td>Genus</td>
<td>Family</td>
<td>Part(s)</td>
</tr>
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</tr>
<tr>
<td>Croton lechleri</td>
<td>Euphorbiaceae</td>
<td>Latex</td>
<td>Salvia officinalis</td>
<td>Labiatae</td>
<td>Leaves</td>
</tr>
<tr>
<td>Cynanchum hancoekianum</td>
<td>Asclepiadaceae</td>
<td>Entire</td>
<td>Scirpus holoschoenus</td>
<td>Cyperaceae</td>
<td>Inflorescence</td>
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<tr>
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<td>Fruit</td>
<td>Scutellaria barbata</td>
<td>Labiatae</td>
<td>Entire</td>
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<tr>
<td>Echinops grijisii</td>
<td>Compositae</td>
<td>Root</td>
<td>Scutellaria indica</td>
<td>Labiatae</td>
<td>Root</td>
</tr>
<tr>
<td>Echinops latifolius</td>
<td>Compositae</td>
<td>Root</td>
<td>Sempervivum armenum</td>
<td>Crassulaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Echites vucatanensis</td>
<td>Apocynaceae</td>
<td>Latex</td>
<td>Sempervivum arvense</td>
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<td>Leaf</td>
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<tr>
<td>Epilobium hirsutum</td>
<td>Onagraceae</td>
<td>Entire</td>
<td>Swietenia humilis</td>
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<td>Seed</td>
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<td>Euphorbia ebracteolata</td>
<td>Euphorbiaceae</td>
<td>Aerial parts</td>
<td>Tabebuia impetiginosa</td>
<td>Bignoniaceae</td>
<td>Stem bark and wood</td>
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<td>Euphorbiaceae</td>
<td>Stem</td>
<td>Tabebuia rosea</td>
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<td>Stem bark and wood</td>
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<td>Euphorbia marginata</td>
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<td>Tabebuia serratifolia</td>
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<td>Stem bark and wood</td>
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<tr>
<td>Euphorbia kansui</td>
<td>Euphorbiaceae</td>
<td>Root</td>
<td>Thalictrum fabri</td>
<td>Ranunculaceae</td>
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<td>Euphorbia prolifera</td>
<td>Euphorbiaceae</td>
<td>Latex</td>
<td>Thevetia ahouia</td>
<td>Apocynaceae</td>
<td>Leaf and Stem</td>
</tr>
<tr>
<td>Ficus pretoiae</td>
<td>Moraceae</td>
<td>Sap</td>
<td>Thevetia gaumeri</td>
<td>Apocynaceae</td>
<td>Leaf and Stem</td>
</tr>
<tr>
<td>Hedyotis chrysotricha</td>
<td>Rubiaceae</td>
<td>Entire</td>
<td>Thevetia peruciana</td>
<td>Apocynaceae</td>
<td>Leaf and Stem</td>
</tr>
<tr>
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<td>Elaeagnaceae</td>
<td>Fruit</td>
<td>Uncaria tomentosa</td>
<td>Rubiaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>Hypoxis nyasica</td>
<td>Hypoxiaceae</td>
<td>Rhizome</td>
<td>Virola bicuhyba</td>
<td>Myristicaceae</td>
<td>Seed</td>
</tr>
</tbody>
</table>
Compounds obtained from natural resources tend to be in only one stereoisomeric form since the active sites of enzymes catalyzing the synthesis of these compounds are asymmetric, allowing them to catalyze a stereospecific reaction. This specific feature of natural products allows them to exhibit biological activity which can be exploited in development of therapeutic agents.

The positive and negative effects of secondary metabolites obtained from plants insects and microorganisms were experienced by mankind in ancient time during their quest for food and survival. Secondary metabolites produced in any species have been proposed to facilitate its survival through repelling or attracting other organisms. Various toxic compounds produced by plants, mollusks, insects and reptiles were postulated to be used as defense mechanism by which they avoided being eaten by other animals [116]. Antitumor antibiotics from micro organisms include the anthracyclines (such as doxorubicin), bleomycin, dactinomycin (actinomycin), and mitomycin C. Moreover, four classes of plant derived anticancer drugs are being used for the treatment of different types of cancers these include vinca alkaloids (bisindole alkaloids), taxanes, epipodophyllotoxins and camptothecins. At present time, several potential natural product-derived anticancer agents are in the market and several another are in advanced clinical trials [117, 118]. Compounds with anticancer activity have been discovered from various skeleton types, mainly from plants, but also from other organisms such as fungi and marine organisms [119]. Marine organisms uniquely synthesize halogen atom (mostly) chlorine and bromine containing secondary metabolites, apparently due to their excessive availability in that environment. In addition to plant derived and microorganism derived antineoplastic agents, several natural products of marine origin have also reached in advanced stage of clinical development. Some important anticancer natural products isolated from marine and terrestrial resources have been described bellow and some others are listed in table 1.3.

**Alvaradoin E**

It is an anthracenone C-glycoside which has been isolated from leaves of *Alvaradoa haitiensis* Urb belonging to family picramniaceae through bioactivity-guided fractionation of the extract showing anticancer activity. It showed potent antiproliferative activity against KB, LNCaP, and
Cytotoxic activity of Alvaradoin E has been demonstrated to be mediated through apoptosis. Treatment with Alvaradoin E initiated the chromatin condensation and mitochondrial membrane depolarization leading to apoptosis in LNCaP prostate cancer cell line. Its antiproliferative activity has been further validated through several other studies. It showed potential decrease in cell viability as well as increased the DNA cleavage within 36 hours of exposure [120]. Several other compounds belonging to this series including alvaradoin A and alvaradoin B have also showed significant anticancer potential [121].

**Psammaplin A**

It is a bromotyrosine metabolite isolated from different marine sponges which is shown to possess antiproliferative activity against P388 cells [122]. Since then several other psammaplin compounds have been identified including psammaplins B-L and biprasin [123]. These molecules showed potent antimicrobial activity. Psammaplin A and biprasin were found to have significant DNA methyltransferase as well as histone deacetylase inhibitory activities. In addition, Psammaplin A has been shown to inhibit the aminopeptidase N as well as topoisomerase II activities.

Psammaplin F, showed elective histone deacetylase inhibitory activity while psammaplin G, showed selective DNA methyltransferase inhibitory potential. A synthetic analogue NVP-LAQ824 showed significant *in vitro* anticancer potential with low toxicity and high tolerance in human xenografts. It has been shown to induce apoptosis at smaller concentrations [124].

**Pancratistatin**

It is a phenanthridone alkaloid isolated from bulbs of the *Pancratium littorale* Jacq (Amaryllidaceae) as a major active constituent [125]. Later on, in order to increase the water solubility and bioavailability of parent compound, Pancratistatin 3,4-O-cyclic phosphate sodium salt has been prepared which showed considerable anticancer potential in P388 murine cancer cell line. It also showed significant activity against *in vivo* ovary sarcoma tumor model. Pancratistatin has also been prepared in lab through total synthesis. Recent studies have demonstrated that Pancratistatin induce apoptosis through activation of caspase-3 and flipping of phosphatidyl serine to the outer phase of plasma membrane [126]. It induced the caspase-3 activity in the Jurkat cell line and shown to activate the Fas receptor within lipid rafts in plasma.
membrane. One of its derivative pancratistatin 3, 4-O-cyclic phosphate sodium salt also showed antiproliferative activity in different cancer cell lines.

**Didemnin B**

Didemnin B is a cyclic depsipeptide isolated from *Trididemnum solidum*, a Caribbean tunicate which showed significant anticancer potential and is currently in phase II clinical trials for the treatment of breast cancer, ovarian cancer, lung cancer, myeloma, glioblastoma/astrocytoma, and cervical cancers [127]. It is postulated to act through inhibiting the synthesis of nucleic acids, and proteins as well as through binding to thioesterase [128]. Aplidine a synthetic analog of didemnin B is shown to act as a potent anticancer agent through interfering with the synthesis of DNA and proteins and inducing cell cycle arrest. It also uniquely inhibits the ornithine decarboxylase, an enzyme involved in the growth of tumor and angiogenesis. Antiangiogenic effect is shown to be mediated by decreased secretion of VEGF as well as diminished expression of the VEGF-r1 receptor [129]. Aplidine showed prominent activity against different solid tumor models. It showed low toxicity and high tolerance with the most common adverse events being vomiting, nausea, asthenia, and hypersensitivity. It is currently in phase II clinical trials against a wide range of solid tumors.

**Neopeltolide**

It is a potential anticancer compound of marine origin isolated from a sponge belonging to family Neopeltidae collected from the coastal regions of Jamaica [130]. It has been reported to possess potential cytotoxic activity in the concentration of nM range against various cancer cell lines. It also induced cell cycle arrest at the G1 stage in A549 lung cancer cell lines. Recent studies have showed that it exhibit the anticancer potential through targeting cytochrome bc1 complex.

**Bryostatin 1**

It has been isolated from marine bryozoans through the bioactivity guided fractionation of active extracts. It showed significant anticancer activity mediated through protein kinase C.

**Palmerolide A**

It is an important anticancer compound obtained from marine organism *Synoicum adareanum*, a tunicate distributed in the shallow waters around the Antarctic Peninsula [131]. It was found to
possess anticancer potential against a panel of cell lines. Several follow-up studies revealed that it inhibit different vacuolar-ATPase. Several stereoisomers of palmerolide have been synthesize and evaluated for biological activities [132].

Dolastatin 10

Dolastatin 10 has been isolated through the bioactivity guided fractionation of active extracts of Dolabella auricularia (sea hare). It is a pentapeptide having four structurally unique amino acids including dolaprolie, dolavaline, dolaisoleucine, and dolaphenine. It is thought to be synthesized by cyanobacteria which serve as primary diet of Dolabella auricularia [133]. It has been shown to exhibit potent antiproliferative activity against murine PS leukemia cells. It also inhibited the binding of Vinca alkaloid to tubulin as well as affected the tubulin-dependent hydrolysis of guanosine triphosphate leading to the arrest of cell cycle in metaphase. During the
clinical studies, dolastatin 10 has been shown to develop moderate peripheral neuropathy so it was dropped from clinical trials. It provided the basis for a synthetic analogue Soblidotin (TZT-1027) with potent anticancer activity and reduced toxicity [134]. Administration of TZT-1027 in experimental animals significantly inhibited the growth of P388 leukemia and solid tumor cell lines with better activity profile. It has been shown to exhibit the activity irrespective of the presence of functional p53 gene. Furthermore, it showed significant activity in human xenograft models of lung carcinoma and breast carcinoma. It also interferes with angiogenesis through interacting with VEGF leading to the necrosis of tumor [135].

Pericosine A

It is a cyclohexenoid derivative, isolated from a fungal strain Periconia byssoides found in the gut of Aplysia kurodai [136]. It has been shown to possess selective cytotoxic activity against a wide range of cancer cell lines. It also showed promising anticancer potential in vivo. It is shown to act through inhibiting protein kinase, EGFR and topoisomerase II [137].

Dolastatin 15

Dolastatin 15 was isolated from D. auricularia (sea hare). It is a depsipeptide having seven amino acid or hydroxyl acid residues. It showed potent anticancer activity against P388 lymphocytic leukemia cell line [138]. It directly binds to the Vinca domain of tubulin. In order to enhance the water solubility and therapeutic efficacy synthetic analogues including cemadotin and synthadotin, have been developed based on Dolastatin 15. Cematodin is shown to act through interfering with tubulin polymerization as well as inducing the depolymerization of microtubules leading to mitotic arrest. Synthadotin is shown to be active against non-small cell lung cancer and prostate cancer.

Silvestrol

It has been isolated from ethanolic extract of the fruits and leaves of Aglaia foveolata (Meliaceae) [139], later on it has been isolated from another plant Aglaia leptantha. It is a rocaclaglate derivative containing benzofuran ring. It is shown to exert selective cytotoxicity against different cancerous cell lines. Several studies demonstrated that this class of compounds exerts anticancer activity through modulating the protein synthesis and arresting the cell cycle in G2/M-stage. These are also known to act through inhibiting the activity of NF-κB and tumor
necrosis factor alpha (TNFα) [140]. Furthermore it exhibited potential anticancer activity in different xenograft models with no obvious toxic effect. Mechanistic Studies revealed that it act through modulating different genes involved in apoptosis and cell cycle regulation. It is shown to act through upregulating p21 and p300 and downregulating p53 and MDM2 at transcription and translation levels leading to cell cycle arrest. It also induced cytochrome c release through modulating the mitochondrial membrane potential ultimately leading to apoptosis. Silvestrol was also shown to be effective in combination therapy.

Ecteinascidin-743

It is a tetrahydroisoquinolones alkaloid isolated as major active constituent from aqueous extracts of the *Ecteinascidia turbinata*, a tunicate [141]. It has also been prepared semisynthetically from cyanosafraclin B, an abundant natural product obtained from *Pseudomonas fluorescens*. A recent study has shown that it interferes with nucleotide excision repair during the transcription and induces p53 independent apoptosis. It also interacts with the minor groove of DNA and subsequently induces bending in the DNA helix. Furthermore, it showed significant effect on different types of cancers pretreated with platinum/taxanes in phase I clinical trials [142].

Symplocamide A

It is a depsipeptide with having two unusual amino acid constituents. It was isolated from *Symploca*, a cyanobacterium of marine origin. It was found to be an effective anticancer agent, showing significant antiproliferative activity against different cancer cell lines.

Spiruchostatin A

It is a bicyclic depsipeptide with potential anticancer activity isolated from a *Pseudomonas* extract together with another compound spiruchostatin B. It showed potent antiproliferative activity in different cancer cell lines. It has shown to act through inhibiting histone deacetylase activity leading to increase in acetylated histones and ultimately cell death. It also showed significant activity in different *in vivo* models [143].

Halichondrin B

It has been isolated from *Halichondria okadai*. Its anticancer potential is postulated to be mediated by inhibition of tubulin polymerization leading to breakdown of the mitotic spindle and cell cycle arrest in G2/M-stage [144].
The structural complexity and potential anticancer activity of halichondrins led the development of semisynthetic and total synthetic analogs. Some of these analogs have shown potent anticancer activity. For example E-7389 act through modulating the function of microtubules as well as inducing the phosphorylation of p27 and Bcl2 without involvement of p53 and subsequently leading to apoptosis and cytotoxic effect [145].

**Flavopiridol**

It is a semisynthetic analog of a natural chromone alkaloid that exhibit anticancer potential through inhibiting different cyclin-dependent kinases which regulate the cell cycle [146]. Moreover, it selectively induces apoptosis and exhibit anti-angiogenic properties. During the several recent *in vitro* and *in vivo* studies, it was shown to inhibit the proliferation of a broad range of human cancer cells and is currently under Phase II and Phase III clinical trials, both as a single agent and in combination with other anticancer drugs [147-150].
### Table 1.3: Some important anticancer compounds obtained from Natural resources

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Compound</th>
<th>Organism</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anethole</td>
<td><em>Foeniculum vulgare</em></td>
<td>NF-kB, AP-1, JNK, MAPK</td>
</tr>
<tr>
<td>2</td>
<td>Aaptamine</td>
<td>Sponge</td>
<td>p21, cyclins</td>
</tr>
<tr>
<td>3</td>
<td>Bastadine 6</td>
<td>Sponge</td>
<td>Inhibition of angiogenesis <em>in vitro</em> and <em>in vivo</em> involves apoptosis</td>
</tr>
<tr>
<td>4</td>
<td>Boswellic acids</td>
<td><em>Salai guggul</em></td>
<td>NF-kB, p42 MAPK, p38 MAPK, 5-LOX, 69urviving, cyclin D1, Bcl-2, Bcl-xL, CIAP</td>
</tr>
<tr>
<td>5</td>
<td>Capsaicin</td>
<td><em>Capsicum annum</em></td>
<td>NF-kB, 69urviving, cyclin D1, Bcl-2, Bcl-xL, CIAP, Cdc25, Cdk1, Bcl-2, Bcl-xL</td>
</tr>
<tr>
<td>6</td>
<td>Camptothecin, Topotecan,</td>
<td><em>Camptotheca acuminata</em></td>
<td>topoisomerase I</td>
</tr>
<tr>
<td></td>
<td>Irinotecan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Cephiaostatin</td>
<td>Worm</td>
<td>Apoptosis and increased mitochondrial matrix density</td>
</tr>
<tr>
<td>8</td>
<td>Cortistatin A</td>
<td>Sponge</td>
<td>regulators of angiogenesis</td>
</tr>
<tr>
<td>9</td>
<td>Curcumin, Curcuminoids</td>
<td><em>Curcuma longa</em></td>
<td>Egr1, STAT-1,3,5, PPARγ, EGFR, HER2, TYK2, JNK, PKA, PKC, Bcl-2, IL-6, Bcl-XL, p53, GSH-px, cyclin D1, 5-LOX, COX-2, INOS, MMP-9, TNF, IL-8, IL-12 NF-kB, AP-1, Akt, Src, JAK2</td>
</tr>
<tr>
<td>10</td>
<td>Dibenzoylmethane</td>
<td><em>Glycyrrhiza echinata</em></td>
<td>COX2, LOX, HIF, VEGF</td>
</tr>
<tr>
<td>11</td>
<td>Dictyostatin</td>
<td>Sponge</td>
<td>tubulin polymerization</td>
</tr>
<tr>
<td>12</td>
<td>Ellagic acid</td>
<td><em>Punica granatum</em></td>
<td>NF-kB, COX-2, cyclin D1, MMP-9, PDGF, VEGF, p21/WAF1, p53</td>
</tr>
<tr>
<td>13</td>
<td>Emodin</td>
<td><em>Rheum emodi</em></td>
<td>NF-kB, HER-2/neu, caspase-3, AR, MMP-9, CYP1A1, CYP1B1</td>
</tr>
<tr>
<td>14</td>
<td>Etoposide</td>
<td><em>Podophyllum peltatum</em></td>
<td>topoisomerase II</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>Compound</td>
<td>Source</td>
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<tr>
<td>15</td>
<td></td>
<td>Eugenol, iso Eugenol</td>
<td><em>Eugenia caryophyllus</em></td>
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<tr>
<td>16</td>
<td></td>
<td>Flavonoids, Catechins</td>
<td><em>Camellia sinensis</em></td>
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<tr>
<td>17</td>
<td></td>
<td>Fostrie cin</td>
<td><em>Streptomyces sp.</em></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>Garcinol</td>
<td><em>Garcinia indica</em></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>Genistein</td>
<td><em>Glycine max</em></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>Gingerol, paradol</td>
<td><em>Zingiber officinale</em></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>Guggulsterone</td>
<td><em>Commiphora mukul</em></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>Ingenol</td>
<td><em>Euphorbia ingens</em></td>
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<tr>
<td>23</td>
<td></td>
<td>Laxaphycins A and B</td>
<td>Bacterium</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>Leptosins C and F</td>
<td>Fungus</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>Limonene</td>
<td><em>Elettaria cardamomum</em></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>Linalool, monoterpenes</td>
<td><em>Coriandrum sativum</em></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>Neoamphimedine</td>
<td>Sponge</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>Paclitaxel Docetaxel</td>
<td><em>Taxus brevifolia</em></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td>parthenolides</td>
<td><em>Tanacetum parthenium</em></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>Pentostatin</td>
<td><em>Streptomyces sp.</em></td>
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<tr>
<td>31</td>
<td></td>
<td>Podophyllotoxin Etopophos NK-611</td>
<td><em>Podophyllum peltatum</em></td>
</tr>
</tbody>
</table>
### Review of Literature

<table>
<thead>
<tr>
<th></th>
<th>Medicinal Plant</th>
<th>Biological Effects</th>
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</thead>
<tbody>
<tr>
<td>32</td>
<td>Protopanaxadiol Pana quinquefolium</td>
<td>caspase 3, 8 and 9</td>
</tr>
<tr>
<td>33</td>
<td>Quercetin Citrus sp.</td>
<td>NF-kB, Bax, Bcl-2, cyclin D1, caspase, PARP, Gadd 45</td>
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<tr>
<td>34</td>
<td>Resveratrol Vitis vinifera</td>
<td>COX-2, iNOS, JNK, MEK, AP-1, NF-kB, P21Cip1/WAF1, p53, Bax, caspases, survivin, cyclin D1, cyclin E, Bcl-2, Bcl-xL, CIAP, Egr-1, PKC, PKD, VEGF, IL-1, IL-6, IL-8, AR, Cdc2-tyr15a, endothelin-1</td>
</tr>
<tr>
<td>35</td>
<td>Squalamine (aminosterol antibiotic) shark</td>
<td>Inhibits angiogenesis, prevents growth factor-induced endothelial cell proliferation</td>
</tr>
<tr>
<td>36</td>
<td>Sulforaphane, indole-3-carbinol Brassica sp.</td>
<td>NF-kB, survivin, cyclin D1, Bcl-2, Bcl-xL, CIAP, Cdc25, Cdk1, Bcl-2, Bcl-xL</td>
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<tr>
<td>37</td>
<td>Tecogalan Arthrobacter sp.</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td>38</td>
<td>Taxol Taxus brevifolia</td>
<td>tubulin</td>
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<tr>
<td>39</td>
<td>Variolin B Sponge</td>
<td>cyclin-dependent kinases</td>
</tr>
<tr>
<td>40</td>
<td>Vinblastine Vinca rosea</td>
<td>tubulin</td>
</tr>
<tr>
<td>41</td>
<td>Vincristine Vinca rosea</td>
<td>tubulin</td>
</tr>
<tr>
<td>42</td>
<td>Withonalide Withania somnifera</td>
<td>NF-kB, COX-2, cyclin D1, MMP-9, survivin, cyclin E, Bcl-2, Bcl-xL, CIAP</td>
</tr>
</tbody>
</table>

### Conclusion

Research work done up to date has showed the importance of medicinal plants in the field of different human diseases including cancer, intense efforts and multiple approaches have fruitified in form of number of anticancer drugs in the area but the main problem associated with these drugs is the toxicity, solubility and lack of specificity as healthy cells are also become the target, most of the modern anticancer drugs available are either inspired from natural product or their
analogues, efforts are still required to keep the delicate balance between toxicity, bioavailability and efficacy, drugs available are not good enough to treat cancer at advance stage though may prolong the life span of person, other drawback is the cost of anticancer drugs which must also be taken into account for the countries like India, a lot need to be done and nature has given the direction. More clinical trials are also needed to validate the usefulness of these agents either alone or in combination with existing therapy.
References


