CHAPTER 1

1.1. INTRODUCTION

1.1.1. Cancer

According to World Health Organization (WHO), 'cancer' is defined as a generic term for a group of more than 100 diseases that can affect any part of the body. The other synonyms that are used include malignant tumors and neoplasms. Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading to other locations in the body via lymph or blood. Also known as a multifactor, multifaceted and multi-mechanistic disease enquiring a multidimensional approach for its treatment, control and prevention (Murthy *et al.,* 1990).

It is now widely recognized that cancer results from a series of genetic alterations causing a loss of normal growth controls, resulting in unregulated growth, lack of differentiation, apoptosis, genomic instability, and metastasis. Cancer knows no boundaries and can develop in any tissue of any organ at any age. However, one of the hallmarks of tumor development is a long latent period with no obvious clinical evidence of disease (Baudino, 2004).

The causes of cancer have been divided to two major categories: those with an environmental cause and those with a hereditary genetic cause. Cancer is primarily an environmental disease, though genetics influence the risk of some cancers. Generally, 90-95% of cases attributed to environmental factors and 5-10% due to genetics. Environmental, as used by cancer researchers, means any cause that is not genetic. Common environmental factors that contribute to cancer death include: tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation (both ionizing and non ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants

(Anand *et al.,* 2008). These environmental factors cause or enhance abnormalities in the genetic material of cells.

Roukos, (2009) reported the vast majority of cancers are non-hereditary, also are called 'sporadic cancers'. Hereditary cancers are primarily caused by an inherited genetic defect. Less than 0.3% of the populations are carriers of a genetic mutation which has a large effect on cancer risk. They cause less than 3-10% of all cancer. Some of the syndromes leading to hereditary cancer include:

- Inherited mutations in the genes BRCA1 and BRCA2 have more than 75% risk of breast cancer and ovarian cancer
- Li-Fraumeni syndrome (various tumors such as osteosarcoma, breast cancer, soft tissue sarcoma, brain tumors) due to mutations of p53
- Turcot syndrome (brain tumors and colonic polyposis)
- Familial adenomatous polyposis; an inherited mutation of the APC gene that leads to early onset of colon carcinoma.
- Hereditary nonpolyposis colorectal cancer (HNPCC, also known as Lynch syndrome) can include familial cases of colon cancer, uterine cancer, gastric cancer, and ovarian cancer, without a preponderance of colon polyps.
- Retinoblastoma, when occurring in young children, is due to a hereditary mutation in the retinoblastoma gene.
- Down syndrome patients, who have an extra chromosome 21, are known to develop malignancies such as leukemia and testicular cancer, though the reasons for this difference are not well understood (Roukos, 2009).

Cancer is fundamentally a disease of failure to regulate tissue growth. In order for a normal cell to transform into a cancer cell, the genes, which regulate cell growth and differentiation, must be altered (Croce, 2008). Cell proliferation is an extremely complex process, which is normally regulated by several classes of genes including; 'oncogenes' and 'tumor suppressor genes'. The genes activated during the carcinogenesis process were termed oncogenes because they are capable of transforming certain cells by DNA transfection. Oncogenes may be normal genes, which are expressed at inappropriately

high levels, or altered genes with novel properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells. The genes inactivated or lost in tumor cells were called 'anti-oncogenes' or 'tumor suppressor genes' because the normal unchanged genes were able to suppress tumorigenicity when reintroduced into tumor cells (Fusenig and Boukamp, 1998) (Fig 1.1). Tumor suppressor genes inhibit cell division, survival, or other properties of cancer cells. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in many genes are required to transform a normal cell into a cancer cell (Knudson, 2001).
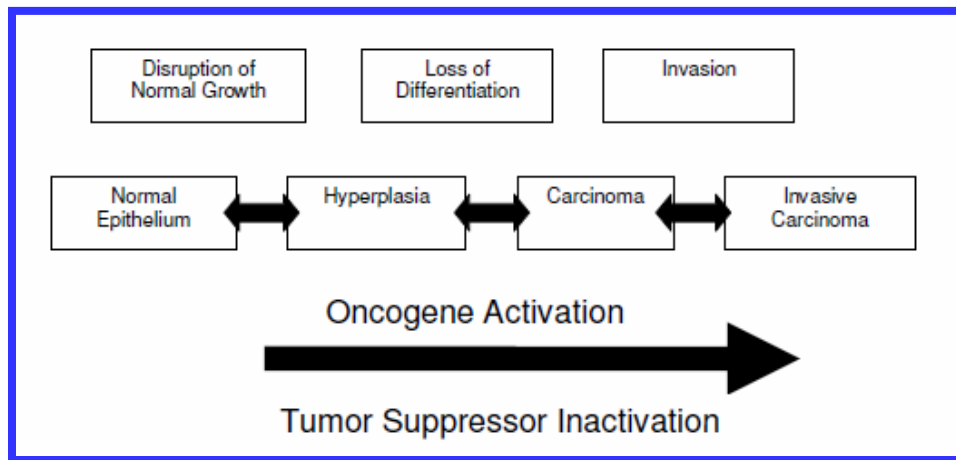

Figure 1.1: Carcinoma development and invasion. The upper row represents disturbances in growth, differentiation and tissue integrity that lead to the phenotypes that characterize the different stages of cancer shown in the lower row (Baudino, 2004).

1.1.1.1.   *Types of cancer*

Cancers are classified by the type of cells that the tumor resembles and is therefore presumed to be the origin of the tumor. The main categories of cancer include:

- Carcinoma; cancer that begins in the skin or in tissues that line or cover internal organs.
- Sarcoma; cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

- Leukemia; cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
- Lymphoma and myeloma; cancers that begin in the cells of the immune system.
- Central nervous system cancers; cancers that begin in the tissues of the brain and spinal cord (National Cancer Institute (NCI, 2010)).

1.1.1.2.   *Cancer stages*

Staging describes the extent or spread of the disease at the time of diagnosis. Proper staging is essential in determining the choice of therapy and in assessing prognosis. A cancer's stage is based on the primary tumor's size and whether it has spread to other areas of the body (Cancer Facts & Figures, 2011). Along development in molecular properties of cancer, prognostic models have been developed for some cancer sites that incorporate biological markers and genetic features in addition to anatomical characteristics. A number of different staging systems are used to classify tumors:

- The TNM (Tumor, Node, Metastasis) staging system assesses tumors in three ways: extent of the primary tumor (T), absence or presence of regional lymph node involvement (N), and absence or presence of distant metastases (M).
- Once the T, N, and M are determined, a stage of I, II, III, or IV is assigned, with stage I being early and stage IV being advanced disease (Cancer Facts & Figures, 2011).
- Different systems of summary staging (in situ, local, regional, and distant) are used for descriptive and statistical analysis of tumor registry data. If cancer cells are present only in the layer of cells where they developed and have not spread, the stage is "in situ". If cancer cells have penetrated the original layer of tissue, the stage is "invasive". If an invasive malignant cancer confined entirely to the organ of origin, the stage is "local". If malignant cancer (1) has extended beyond the limits of the origin directly into surrounding organs or tissues; (2) involves regional lymph nodes by way of lymphatic system; or (3) has both regional extension and involvement of regional lymph nodes; the stage is "regional". If malignant cancer spreads to parts of the body remote from the primary tumor either by direct extension or by discontinuous metastasis to distant organs and tissues, or via the

lymphatic system to distant lymph nodes the stage is "distant" (Bagchi and Preuss, 2005; Cancer Facts & Figures, 2011).

Staging systems are specific for each type of cancer (e.g., breast cancer and lung cancer). Some cancers, however, do not have a staging system. Systems of staging may differ between diseases or specific manifestations of a disease. Cancer staging can be divided into a 'clinical stage' and a 'pathologic stage'. Clinical stage is based on all of the available information obtained before a surgery to remove the tumor. Thus, it may include information about the tumor obtained by physical examination, radiologic examination and endoscopy.

Pathologic stage adds additional information gained by examination of the tumor microscopically by a pathologist (http://en.wikipedia.org/wiki/Cancer_staging). The staging system of the Gleason system according to Bagchi and Preuss, (2005) is as follows:

Stage 0 (Carcinoma in situ): very early cancer. The abnormal cells are found only in the first layer of the primary site and do not invade deeper into the tissue.

Stage I: Cancer involves the primary site but did not spread to nearby tissue.

Stage IA: A very small amount of cancer was found to be visible under the microscope and is found deeper in the tissue.

Stage IB: Here larger numbers of cancer cells were found in the tissue.

Stage II: The cancer has spread to the nearby tissue but is still found inside the primary site.

Stage IIA: Cancer has spread beyond the primary site.

Stage IIB: Cancer has spread to other tissues around the primary site.

Stage III: Cancer has spread throughout the nearby area.

Stage IV: Cancer has spread to other parts of body.

Stage IVA: Cancer has spread to organs close to pelvic area.

Stage IVB: cancer has spread to distant organs, such as the lungs.

- Recurrent: Cancer has recurred at the same location were the original tumor was or at a different location after it has been treated and supposedly eliminated.

1.1.2. Carcinogenesis

'Carcinogenesis' or 'oncogenesis' is literally the creation of cancer. It is a process by which normal cells are transformed into cancer cells. It is characterized by a progression of changes on cellular and genetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thus forming a malignant mass. As a general definition, carcinogenesis is a complex multistage processes including initiation, promotion, and progression steps (Tanaka *et al.,* 2004).

Under normal circumstances, cell division is maintained by regulating normal balance between proliferation and programmed cell death (apoptosis) to ensure the integrity of organs and tissues. Mutations in DNA that lead to cancer (only certain mutations can lead to cancer and the majority of potential mutations will have no bearing) disrupt these orderly processes by disrupting the programming regulating the processes.

Carcinogenesis is caused by mutation of the genetic material (DNA) of normal cells, which upsets both processes of proliferation and death. This results in uncontrolled cell division and the evolution of those cells by natural selection in the body. The uncontrolled and often rapid proliferation of cells can lead to benign tumors; some types may turn into malignant tumors (cancer). Benign tumors do not spread to other parts of the body or invade other tissues, and they are rarely a threat to life unless they compress vital structures or are physiologically active, for instance, producing a hormone. Malignant tumors can invade other organs, spread to distant locations (metastasis) and become life-threatening. More than one mutation is necessary for carcinogenesis. In fact, a series of mutations to certain classes of genes is usually required before a normal cell transforms into a cancer cell (Fearon and Vogelstein, 1990). Only mutations in those certain types of genes which play vital roles in cell

division, apoptosis (cell death), and DNA repair will cause a cell to lose control of its cell proliferation.

1.1.3. *Metastasis*

'Metastasis' is the spread of a disease from one organ to another non-adjacent organ (Chiang, 2008; Klein, 2008). In another word, the process by which cancer spreads from the place at which it first arose as a primary tumor to distant locations in the body is defined as 'metastasis'. Metastasis is the major cause of death by cancer, which depends on the cancer types acquire two separate abilities; increased motility and invasiveness. Metastasis occurs by four routes:

- Spread into body cavities. This occurs by the seeding surface of the peritoneal, pleural, pericardial or subarachnoid spaces. For example, ovarian tumors spread transperitoneally to the surface of the liver. Mesotheliomas can spread through the pleural cavity.
- Invasion of lymphatics. This is followed by the transport of tumor cells to regional nodes and ultimately to other parts of the body; it is common in initial spread of carcinomas.
- Hematogenous spread. This is typical of all sarcomas but it is the favored route in certain carcinomas (e.g. those originating in kidneys). Because of their thinner walls veins are more frequently invaded than arteries and metastasis follows the pattern of the venous flows.
- Transplantation. Mechanical carriage of fragments of tumor cells by surgical instruments during operation or the use of needles during diagnostic procedures (Bacac and Stamenkovic, 2008).

Metastasis is a complex series of steps in which cancer cells leave the original tumor site and migrate to other parts of the body via bloodstream or lymphatic system. Thereafter, malignant cells break away from the primary tumor and are attached to the proteins in surrounding extracellular matrix (ECM). As a result, the tumor is separated from adjoining tissues. By degrading these proteins, cancer cells are able to breach the ECM and escape. When oral cancers metastasize, they commonly travel through the

lymph system to the lymph nodes in the neck. The body resists metastasis by a variety of mechanisms through the actions of a class of proteins known as metastasis suppressors (Yoshida *et al.,* 2000). One of the critical events required for cancer metastasis is the growth of a new network of blood vessels, called 'tumor angiogenesis' (Weidner *et al.,* 1991). It has been found that angiogenesis inhibitors would therefore prevent the growth of metastases (Kumar *et al.,* 2005).

1.1.4. Cell death

Dying cells can engage in a process that is reversible until a first irreversible step or 'point-of-no-return' is trespassed. The Nomenclature Committee on Cell Death (NCCD) suggests that a cell should be considered dead when any of the following molecular or morphological criteria occur: (1) the cell has lost the integrity of the plasma membrane, as defined by vital dyes *in vitro*, (2) the cell including its nucleus has undergone complete fragmentation into discrete bodies (which are frequently referred to as 'apoptotic bodies') and/or (3) its corpse (or its fragments) have been engulfed by an adjacent cell *in vivo*. Thus, 'dead cells' would be different from 'dying cells' that are in the process of cell death, which can occur through a variety of different pathways. Moreover, cells whose cell cycle is arrested (as it occurs in senescence) would be considered as alive and the expression 'replicative cell death' (which alludes to the loss of the clonogenic capacity) should be avoided (Kroemer *et al.,* 2005). A complex cascade of biological processes, also normally part of a cells life (pathways, enzyme systems, functioning of organelles, plasma membrane structure and function, modulation of transcriptional and translational activities etc) was activated in preparation for and during cell death (Darzynkiewicz *et al.,* 1997). It is also vital to understand that every cell is programmed to die subsequent to a suitable stimulus (Trump *et al.,* 1997).

There are several forms of cell death; apoptosis, autophagy, necrosis, oncosis. Cell death is generally defined into two different mechanisms that are mutually exclusive and stand in sharp contrast, apoptosis (programmed cell death) and necrosis (accidental cell death) which are the two fundamental types of cell death (Darzynkiewicz *et al.,* 1997).

1.1.4.1. Apoptosis

The word 'apoptosis' has been coined by Kerr *et al.,* (1972) describing a particular morphological aspect of cell death. Apoptosis has come to be used synonymously with the phrase 'programmed cell death' as it is a cell-intrinsic mechanism for suicide that is regulated by a variety of cellular signaling pathways. For cell death to be classified as apoptotic, rounding-up of the cell, retraction of pseudopodes, reduction of cellular volume (pyknosis), nuclear condensation and fragmentation (karyorhexis), cleavage of chromosomal DNA into internucleosomal fragments and packaging of the deceased cell into apoptotic bodies without plasma membrane breakdown must be observed (Edinger and Thompson, 2004; Kroemer *et al.,* 2005). Apoptotic bodies are recognized and removed by phagocytic cells and thus apoptosis is notable for the absence of inflammation around the dying cell. The morphologic features of apoptosis result from the activation of caspases (cysteine proteases) by either death receptor ligation or the release of apoptotic mediators from the mitochondria. Apoptosis as a tidy regulated cell death requires energy in the form of ATP (Edinger and Thompson, 2004).

Apoptosis is widespread during development and it is often in situation that actually represents a prelethal phase of programmed reaction to injury on a schedule set by various hormonal, nutritional and micro/macro-environmental factors. Apoptosis also occurs following a variety of chemical and microbiologic injuries in many different organ systems (Trump *et al.,* 1997).

1.1.4.2. Necrosis

Necrosis generally defines as nonapoptotic, accidental cell death (Fink and Cookson, 2005). Necrosis is the end result of a bioenergetic catastrophe resulting from ATP depletion to a level incompatible with cell survival and was thought to be initiated mainly by cellular 'accidents' such as toxic insults or physical damage (Edinger and Thompson, 2004). In pathology, necrosis is used to designate the presence of dead tissues or cells and is the sum of changes that have occurred in cells after they have died, regardless of the prelethal processes (Fink and Cookson, 2005). Necrosis is characterized morphologically by vacuolation of the cytoplasm, breakdown of the plasma membrane and an induction of inflammation around the dying cell attributable to the release of

cellular contents and pro-inflammatory molecules. Cells that die by necrosis frequently exhibit changes in nuclear morphology but not the organized chromatin condensation and fragmentation of DNA into 200 bp fragments that is characteristic of apoptotic cell death (Edinger and Thompson, 2004) (Fig 1.2). The most common microscopic settings of necrosis are: 1) cells that died singly displaying the morphological changes of apoptosis, for which we have suggested the term apoptotic necrosis, and 2) groups of cells that died of ischemia, which we can call ischemic necrosis or massive necrosis when the mechanism is not known (Majno and Joris, 1995).

Necrosis, therefore, refers to morphological stigmata seen after a cell has already died and reached equilibrium with its surroundings. Thus, in the absence of phagocytosis, apoptotic bodies may lose their integrity and proceed to secondary or apoptotic necrosis. The term apoptotic necrosis describes dead cells that have reached this state via the apoptotic program. The presence of necrosis tells us that a cell has died but not necessarily, how death occurred (Fink and Cookson, 2005).

1.1.4.3. Oncosis

The term 'oncosis' (from 'onkos', meaning swelling) was coined by von Recklinghausen almost 100 years ago, precisely with the meaning of cell death with swelling. In a monograph on rickets and osteomalacia, published posthumously in 1910, von Recklinghausen described death with swelling primarily in bone cells (Majno and Joris, 1995) (Fig 1.2). The expression 'oncosis' defines a cell death morphology with cytoplasmic swelling, mechanical rupture of the plasma membrane, dilation of cytoplasmic organelles (mitochondria, endoplasmic reticulum and Golgi apparatus), as well as moderate chromatin condensation (Kroemer *et al.,* 2005). The term 'oncosis' has been accepted by many investigators as a counterpoint to apoptosis. The process of 'oncosis' ultimately leads to depletion of cellular energy stores and failure of the ionic pumps in the plasma membrane. Oncosis may result from toxic agents that interfere with ATP generation or processes that cause uncontrolled cellular energy consumption. The changes accompanying oncosis may result from active enzyme-catalyzed biochemical processes (Fink and Cookson, 2005).

In vivo, oncosis typically affects broad areas or zones of cells, e.g., the early changes of cells following total ischemia or the cells in a particular region of the liver or kidney tubule following chemical toxins, such as CCl_4 or $HgCl_2$, respectively. When broad zones of cells are involved followed by death of the cells, a pronounced inflammatory reaction typically occurs at the periphery of the zone, although oncosis is not typical of programmed cell death in development, it has been described in some situations (Trump *et al.,* 1997).

1.1.4.4. Autophagy

Apoptotic bodies and the cellular debris released during lysis of oncotic cells can both be phagocytized and degraded by neighboring viable cells *in vivo*. Another form of cell death, autophagy or type II cell death, features degradation of cellular components within the dying cell in autophagic vacuoles. The morphological characteristics of autophagy include vacuolization, degradation of cytoplasmic contents, and slight chromatin condensation (Fig 1.1). Studies on autophagy suggest that it proceeds through a sequence of morphological changes in a highly regulated process. Briefly, the autophagic pathway begins with the sequestration of cytoplasmic material in double-membrane vesicles known as autophagosomes. The sequestration process is under the control of GTPases and phosphatidylinositol kinases and involves novel ubiquitin-like conjugation systems.

Autophagosomes then fuse with lysosomes in a process depending on microtubules, and the contents are degraded (Fink and Cookson, 2005). It is important to consider that necrosis occurs in cells that are undergoing severe bioenergetic stress, the same conditions that would stimulate autophagy as a mechanism for boosting ATP levels. Thus, it is likely that autophagy and necrosis often occur in parallel, initiated in response to the same stimuli but with completely opposite objectives (Edinger and Thompson, 2004). *In vivo*, cells undergoing autophagy can be phagocytized by neighboring cells (Fink and Cookson, 2005).
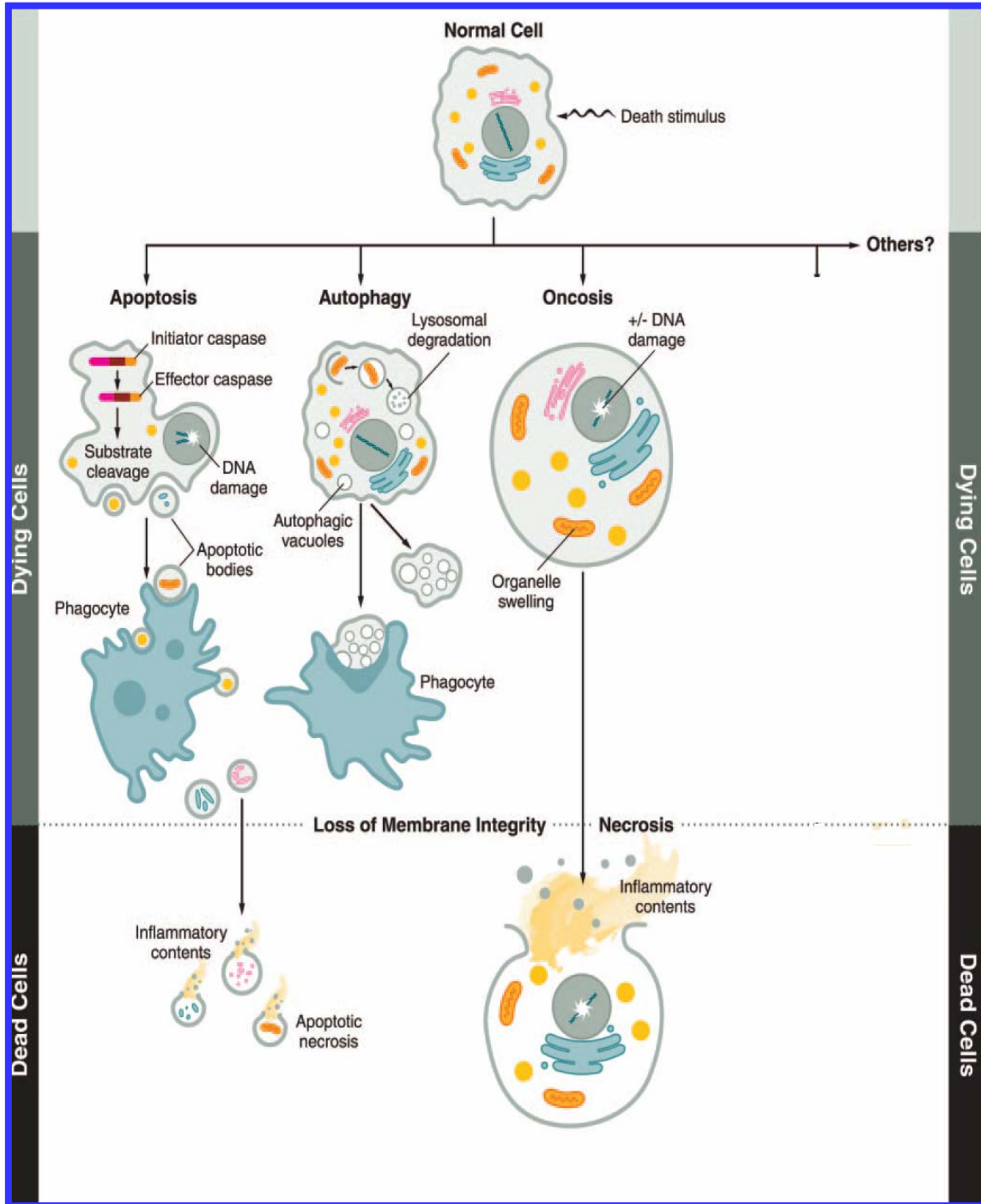
Figure 1.2: Pathways leading to cell death (Fink and Cookson, 2005).

1.1.5. Cancer statistics

The United State (US) National Institutes of Health estimates overall costs of cancer in 2010 to be about $263.8 billion in total of which, $102.8 billion has been spent for direct medical costs (total of all health expenditures); $20.9 billion for indirect morbidity costs (cost of lost productivity due to illness); and $140.1 billion for indirect mortality costs (cost of lost productivity due to premature death) (Cancer Facts & Figures, 2011). In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers) and 7.6 million people died of cancer worldwide (Jemal *et al.,* 2011). Cancer approximately causes 13% of all deaths each year with the most common being: lung cancer (1.3 million deaths), stomach cancer (803,000 deaths), colorectal cancer (639,000 deaths), liver cancer (610,000 deaths), and breast cancer (519,000 deaths) (WHO, 2011). This makes invasive cancer the leading cause of death in the developed world and the second leading cause of death in the developing world. Over half of cases occur in the developing world (Jemal *et al.,* 2011).

Each year, the American Cancer Society estimates the number of new cancer cases and death expected in the United States in the current year, and compiles the most recent data on cancer incidence, mortality, and survival by using incidence data from the National Cancer Institute (NCI) and mortality data from National Cancer for Health Statistics (NCHS). In 2012, 1,638,910 new cancer cases and 577,190 deaths from cancer are reported in the United States (Cancer Facts & Figures, 2012). They estimated 1.44 million new cases of invasive cancer expected among men and women in the United States in 2008 (Jemal *et al.,* 2008).

1.2. LITERATURE REVIEW

1.2.1. Medicinal plants and human health care

Accessibility and affordability of the medicinal herbs have made them as a fundamental part of many people's life all over the world. The selection of medicinal plants is a conscious process, which has led to an enormous number of medicinal plants being

used by plentiful cultures in the world (Heinrich *et al.,* 2004). Surveys conducted in Australia and United State (US) indicates that almost 48.5% and 34% of individuals had used at least one form of unconventional therapy. According to WHO, about 65-80% of the world's population in developing countries, due to poverty and lack of access to modern medicine, depends essentially on plants for their primary health care (Calixto, 2005). Indeed, evaluation of effective plants to cure certain diseases has been recommended by WHO due to the lack of safe modern drugs.

In recent years, efficacy of herbal medicines in diseases like inflammatory bowel disease (Achike and Kwan, 2003; Calixto, 2005; Rahimi *et al.,* 2009; Rezaie *et al.,* 2007), obesity (Hasani-Ranjbar *et al.,* 2009a; Heber, 2003), diabetes (Edzard, 2005; Rahimi *et al.,* 2005), pancreatitis (Ara Tachjan *et al.,* 2010; Mohseni-Salehi-Monfared *et al.,* 2009), cancers (Angelo and Edzard, 2009; Boon and Wong, 2004; Calixto, 2000; Paduch *et al.,* 2007) and inflammatory and oxidant-related diseases (Hasani-Ranjbar *et al.,* 2009b; Rahimi *et al.,* 2010) has been systematically reviewed. Even at dawn of 21th centuray, 11% of 252 drugs considered as basic and essential by WHO was exclusively of flowering plant origin (Rates, 2001). It is estimated that close to 25% of the active compounds in currently prescribed synthetic drugs were first identified in plant resources (Halberstein, 2005) and 20,000 plants have been used for medicinal proposes of which, 4000 have been used commonly and 10% of those are commercial. Out of the 250,000–500,000 plant species on earth, only 1-10% have been studied chemically and pharmacologically for their potential medicinal value (Verpoorte, 2000).

1.2.2. Medicinal plants in South Africa

South Africa, which comprises of less than 1% of the world's land surface, contains 8% of its plant species. This rich plant biodiversity, with over 20,000 different species, is a great source of interest to the scientific community (Fouche *et al.,* 2006). About 70% of these species are endemic to South Africa (SA). It is estimated that there are more than 100,000 practicing traditional healers in the country, with a liable industry worth about R500 million (56,715,000 $) per annum (Mander and Le Breton, 2005). As estimated 27 million indigenous medicine consumers live in South Africa with a large supporting industry (Mander, 1998). Annually up to 700,000 tons of plant materials are consumed,

of which most of them are collected in the wild for local use (Cunningham, 1988) and international trade (Lange, 1997).

Plants harvested from a diverse range of vegetation types, including Valley Thicket, Afromontane Forest, Coastal Forest and Moist Upland Grassland. The Forest Biome was the vegetation type found to be most threatened by over-harvesting (Dold and Cocks, 2002). South Africa is a land with great possibility for discovering novel natural-based products. In an intensive investigation in South Africa, Fouche *et al.,* (2006) reported that among 7,500 randomly selected plant extracts representing 700 taxa, 32 extracts demonstrated potent anticancer activity, represent 24 different plant taxa, which is a hit rate of 3.4% based on the number of taxa screened. The current demand for numerous plant species resulting in intensive harvesting of indigenous plant stocks (this is in association with the lack of main resource management and plant production) has resulted in the rareness of various indigenous medicinal plants.

1.2.3. *Plant-derived anticancer agents*

Plants engaged along history for treatment of cancer. It was estimated that about 67% of pharmaceutical products approved between 1974 and 1994, for human cancer therapy were derived from natural sources (Richard *et al.,* 2005) of which plants have been a main source of several clinically convenient anticancer agents. Approximately five decades longed till several natural and synthetic antineoplastic were discovered. Cragg and Newman, (2005) revealed that there are remarkable numbers of plant-sourced agents in clinical trials for the treatment of cancer. Some are being investigated as direct cytotoxins, whereas others are being studied from the aspect of their potential role as inhibitors of particular cell cycle enzymes, proteins, or pathways. The search for anticancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, 'vinblastine' (VLB) and 'vincristine' (VCR), and the isolation of the cytotoxic 'podophyllotoxins' (Cragg and Newman, 2005). The most important plant derived anticancer agents are depicted in Figure 1.3.

1.2.3.1. Alkaloids and anticancer properties

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. In addition, some synthetic compounds of similar structure are attributed to alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and more rarely other elements such as chlorine, bromine, and phosphorus. Plant alkaloids make up a group of chemotherapy medications used to treat cancer. Alkaloids block cell division by preventing microtubule function which is vital for cell division. Alkaloids are divided into three major subgroups based on the type of plant origin;

- "Vinca alkaloids" bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of the cell cycle). The first agents to advance into clinical use were (VLB) and (VCR), isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae) which was used by various cultures for the treatment of diabetes (Gueritte and Fahy, 2005). Recently, 'vinorelbine' (VRLB) and 'vindesine' (VDS) have been developed as the semi-synthetic analogs of these agents (Lee and Xiao, 2005).

- "Podophyllotoxin" is a plant-derived compound, which help with digestion as well as used to produce two other cytostatic drugs, 'etoposide' and 'teniposide'. These compounds prevent the cell from entering the G1 phase (the start of DNA replication) and the replication of DNA (the S phase). Etoposide and teniposide; semi-synthetic derivatives of the natural product, 'epipodophyllotoxin' (an isomer of podophyllotoxin), could be considered as links to a plant originally used for the treatment of cancer (Lee and Xiao, 2005).
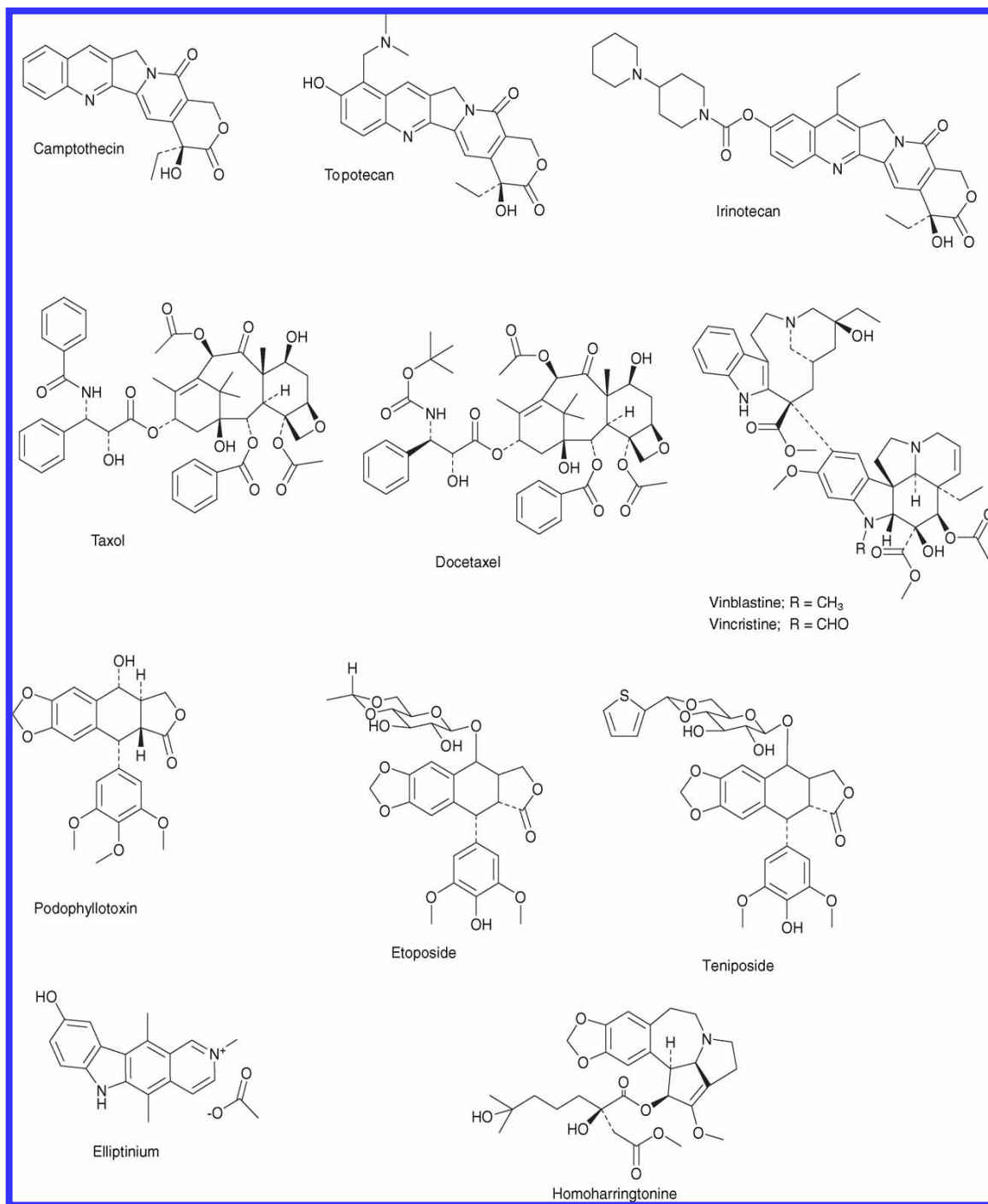
Figure 1.3: Plant-derived anticancer agents in clinical use (Cragg and Newman, 2005).

- "Taxanes" enhance stability of microtubules, preventing the separation of chromosomes during anaphase. The taxane group of plant alkaloids includes paclitaxel, protein-bound paclitaxel and docetaxel. 'Taxanes' and its derivatives (paclitaxel, docetaxel and taxotere) isolated from various Taxus species; active

agents derived from 'camptothecin' (topotecan and irinotecan) isolated from Chinese ornamental tree [*Camptotheca acuminata* Decne (Nyssaceae)] (Rahier *et al.,* 2005); 'homoharringtonine' isolated from the Chinese tree [*Cephalotaxus harringtonia* var. drupacea (Sieb and Zucc.) (Cephalotaxaceae)] (Itokawa *et al.,* 2005); and 'elliptinium' a derivative of ellipticine isolated from species of several genera of the Apocynaceae family etc.

The 'combretastatins' (tubulin polymerization inhibitor) (colchicin related alkaloid), derived from South African bush *Combretum caffrum*, are a family of stilbenes which act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis, and a water-soluble analog, combretastatin A-4 phosphate, has shown promise in clinical trials Phase II (Cragg and Newman, 2005).

1.2.3.2. Coumarins and anticancer properties

Plant coumarins are structurally distinct, non-anticoagulant compounds that have significant medicinal activity (Yarnell and Abascal, 2009). Coumarins, also known as benzopyrones, are present in remarkable amounts in plants, although their presence has also been detected in microorganisms and animal sources (Borges *et al.,* 2005). The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. Coumarins are aromatic compounds with a specific ring structure (Kostova *et al.,* 2006).

The structural diversity found in this family of compounds led to the division into different categories, from simple coumarins to many other kinds of policyclic coumarins, such as furocoumarins and pyranocoumarins (Fig 1.4). Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. The coumarins have been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities (Kostova *et al.,* 2006).

'Coumarin' is a natural substance that has shown anti-tumor activity in vivo, with the effect believed to be due to its metabolites (e.g. 7-hydroxycoumarin). A recent study has

shown that 7-hydroxycoumarin inhibits the release of Cyclin D1, which is overexpressed in many types of cancer. 'Esculetin' (6,7-dihydroxycoumarin) inhibits growth and cell cycle progression by inducing arrest of the G1 phase in HL-60 leukemia cells, resulting from the inhibition of retinoblastoma protein phosphorylation (Aoife and O'Kennedy, 2004).

1.2.3.3. Flavonoids and anticancer properties

Flavonoids (or bioflavonoids) (from the Latin word flavus meaning yellow), are a class of plant secondary metabolites or yellow pigments having a structure similar to that of flavones. Flavonoids are polyphenolic compounds that are ubiquitously in plants. Flavonoids can be classified into: flavonoids, isoflavonoids, neoflavonoids, which are all ketone-containing compounds. Over 5000 naturally occurring flavonoids have been characterized from various plants. They have been shown to possess a variety of biological activities at nontoxic concentrations in organisms (IUPAC Compendium of Chemical Terminology, 1997).

The role of dietary flavonoids in cancer prevention is widely discussed. Compelling data from laboratory studies, epidemiological investigations, and human clinical trials indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy. Many mechanisms of action have been identified, including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these mechanisms (Ren *et al.,* 2003).

Flavonoids were found to be strong topoisomerase inhibitors and induce DNA mutations in the (myeloid/lymphoid or mixed-lineage leukemia) MLL gene, which are common findings in neonatal acute leukemia (Strick *et al.,* 2000). The DNA changes were increased by treatment with flavonoids in cultured blood stem cells (Van Doorn-Khosrovani *et al.,* 2007). A high flavonoid-content diet in mothers is suspected to increase risk particularly of acute myeloid leukemia in neonates (Ross, 1998; Ross, 2000; Spector *et al.,* 2005).
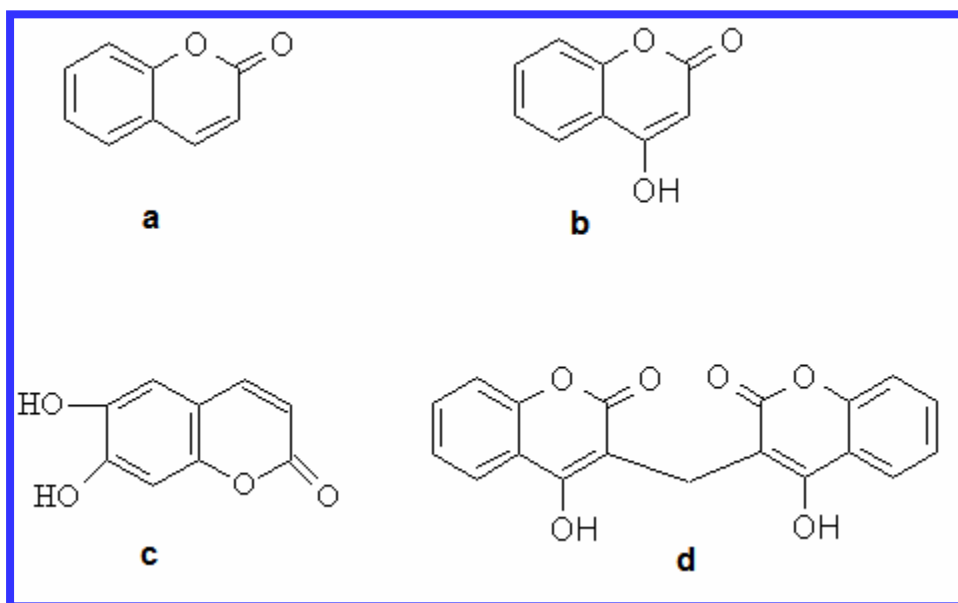
Figure 1.4: Chemical structures of some coumarins: a) coumarin; b) 4-hydroxycoumarin; c) 6,7-hydroxycoumarin and d) bishydroxycoumarin (http://www.people.vcu.edu/~urdesai/cou.htm).

Polyphenols (flavonoids and delphinidin) were found to be strong topoisomerase inhibitors, similar to some chemotherapeutic anticancer drugs including etoposide and doxorubicin. This property may be responsible for both an anticarcinogenic-proapoptotic effect and a carcinogenic, DNA damaging potential of the substances (Bandele *et al.,* 2008; Esselen *et al.,* 2009).

'Flavopiridol' (synthetic polyhydroxylated flavone) (cycline-dependent kinases (CDKs) inhibitor) synthesized by the Indian subsidiary of Hoechst (Aventis) following the isolation and synthesis of the plant-derived natural product, rohitukine, are currently in Phase III clinical trials both as a single agent and in combination with other agents, particularly with paclitaxel and cis-platinum (Cragg and Newman, 2005). Quercetin (3,3',4',5,7-pentahydroxyflavone) passed Phase I clinical trial with antiproliferative activity *in vitro* and is known to inhibit signal transduction targets including tyrosine kinases, protein kinase C, and phosphatidyl inositol-3 kinase (Ferry *et al.,* 1996). 'Genistein' is a well-known isoflavone and is a tyrosine kinase inhibitor. Studies have

indicated that genistein elicits inhibitory effects on cell growth of various carcinoma cell-lines and may be a potential candidate for cancer therapy (Aoife and O'Kennedy, 2004).

1.2.3.4. Saponins and anticancer properties

Saponins are a group of naturally occurring plant glycosides, characterized by their strong foam-forming properties in aqueous solution. The presence of saponins has been reported in more than 100 families of plants out of which at least 150 kinds of natural saponins have been found to possess significant anticancer properties. There are more than 11 distinguished classes of saponins including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes and steroids (Man *et al.,* 2010). Saponins are amphipathic glycosides grouped by the soap-like foaming they produce when shaken in aqueous solutions, and by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. The aglycone (glycoside-free portion) of the saponins are termed 'sapogenins'. The subset of saponins that are steroidal have been termed 'saraponins' (Hostettmann and Marston, 1995).

The pharmaceutical applications of saponins are varied as their origins and chemical structures. Just to mention a few: a number of saponins or saponin-rich mixtures have found to use as anti-inflammatory, antidiuretic, antipyretic, analgesic agent, central nervous system depressants and as treatment for ulcers (Cheeke, 1989). Due to the great variability of their structures, saponins always display anti-tumorigenic effects through varieties of antitumor pathways (Man *et al.,* 2010). The proposed mechanisms of anticarcinogenic properties of saponins include direct cytotoxicity, immune-modulatory effects, bile acid binding, and normalization of carcinogen-induced cell proliferation (Rao and Sung, 1995).

Extracts from *Maytenus diversifolia* have been found to inhibit growth of leukemic lymphocytes *in vivo* (Cheeke, 1989). Rao and Sung, (1995) showed that soybean saponins at the concentration of 150-600 ppm had a dose-dependent growth inhibitory effect on human carcinoma cells (HCT-15). Viability was also significantly reduced. Shibata, (2001) reported that tetracyclic triterpenoid saponins isolated from ginseng, the

root and rhizome of *Panax ginseng* C.A. Meyer (Araliaceae), showed the anti-carcinogenic activity in two-stage anti-cancer-promotion experiments *in vitro* and *in vivo*.

1.2.3.5. Terpenes and anticancer properties

Triterpenoids demonstrated a range of unique and potentially usable biological effects. The history of the use of plants with high saponin/triterpenoid content can be found in the first written herbariums (Dzubak *et al.,* 2006). Terpenes are naturally occurring substances produced by a wide variety of plants and animals. Terpenes are biosynthetically derived from isoprene units with the molecular formula C_5H_8. The basic formula of all terpenes is $(C_5H_8)_n$, where 'n' is the number of linked isoprene units (Gao and Singh, 1998). The most common forms of terpenes are the monoterpenes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) also exist. A terpene containing oxygen is called a terpenoid (Bakkali *et al.,* 2008). The classification and source of terpenoids are presented in Table 1.1 and their chemical structures in Figure 1.5. Terpenes in high concentrations can be toxic and considered as key weapons against herbivores and pathogens. A broad range of the biological properties of terpenoids include; anticancer, cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory and antiparasitic activities (Dzubak *et al.,* 2006; Paduch *et al.,* 2007). Bardon *et al.,* (2002) claimed that various terpenes can act as inhibitors in a dose-dependent manner, to prevent the development of mammary, liver, skin, lung, colon, fore-stomach, prostate and pancreatic carcinomas (Fig 1.6).

Epidemiological studies suggest that dietary monoterpenes may be helpful in the prevention and therapy of cancers, of which among them, D-limonene and perillyl alcohol have been shown to possess chemopreventive and therapeutic properties against many human cancers (Paduch *et al.,* 2007). Chemotherapeutic activities towards human pancreatic cancers have also been shown for other terpenes, such as farnesol and geraniol (Burke *et al.,* 2002). Concerning more than 20000 triterpenoids, known to occur in nature; oleanolic acid (OA), ursolic acid (UA), synthetic oleanane-triterpenoids (SO) and rexinoids are highly effective for the prevention and treatment of cancer in many animal models, but as yet are not definitive agents in clinical practice.

To improve potency, many modifications of the basic triterpenoid structure have been made (Liby *et al.,* 2007).
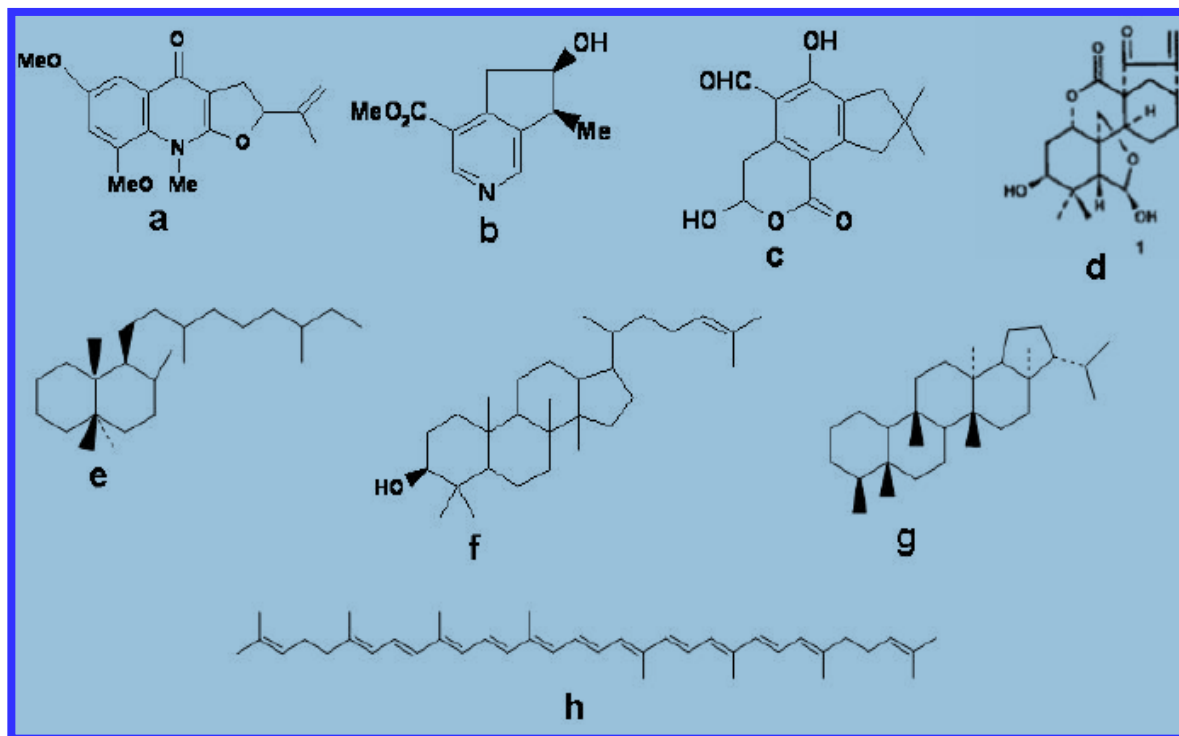

Figure 1.5: Classification of terpenoids: (a) hemiterpenoids (b) monoterpenoids (c) sesquiterpenoids (Manske, 1971); (d) diterpenoids (e) sesterpenoids (f) triterpenoids (g) tetraterpenoids and (h) polyterpenoids (Rahman, 1995).

1.2.3.5.1. Mechanisms of action of terpenes'cytotoxicity

Many attempts have been made to prove the influence of terpenoids on inducing apoptosis as a desired strategy of controlling cancers (Liby *et al.,* 2007; Paduch *et al.,* 2007; Yogeeswari and Sriram, 2005; Zhang *et al.,* 2004). Apoptosis is a process that develops in several phases: 1) an initiation phase, in which the biochemical pathways participating in the process depend on the apoptosis-inducing agent; 2) a decision phase, during which the cell "decides" to commit suicide; and 3) a common degradation phase, which is characterized by the activation of catabolic hydrolases (caspases and nucleases). Although the activation of caspases and nucleases is necessary for the acquisition of the full apoptotic morphology, it appears that the inhibition of such

enzymes does not inhibit cell death induced by a number of different triggers: Bax, Bak, c-Myc, PML), Fas-associated death domain (FADD), glucocorticoid receptor occupancy, tumor necrosis factor, growth factor withdrawal, CXCR4 cross-linking, and chemotherapeutic agents (Costantini *et al.,* 2000).

Table 1.1: Classification of terpenes (Daniel, 2006).

Class	Number of carbon atoms	Number of isoprenes	Sources
Hemiterpenoids	5	1	Volatile oils, esters
Monoterpenoids	10	2	Volatile oils, glycosides, mixed terpenoids
Sesquiterpenoids	15	3	Volatile oils, bitter principles
Diterpenoids	20	4	Resins, chlorophyll
Sesterpenoids	25	5	Rare (mostly in animals)
Triterpenoids	30	6	Resins, waxes, steroids, saponins, cardiac glycosides
Tetraterpenoids	40	8	Carotenoids
Polyterpenoids	α	n	Rubber and gutta

The activation of apoptosis by terpenes occurs via intrinsic cell death pathway, also known as the mitochondrial apoptotic pathway. Yang and Ping Dou, (2010) explained the activation of apoptosis triggered by inhibition of the fas-associated death domain and NF-αB pathways. It has been well documented that suppression of the ubiquitin-proteasome and NF-αB pathways are essential for induction of tumor cell apoptosis.
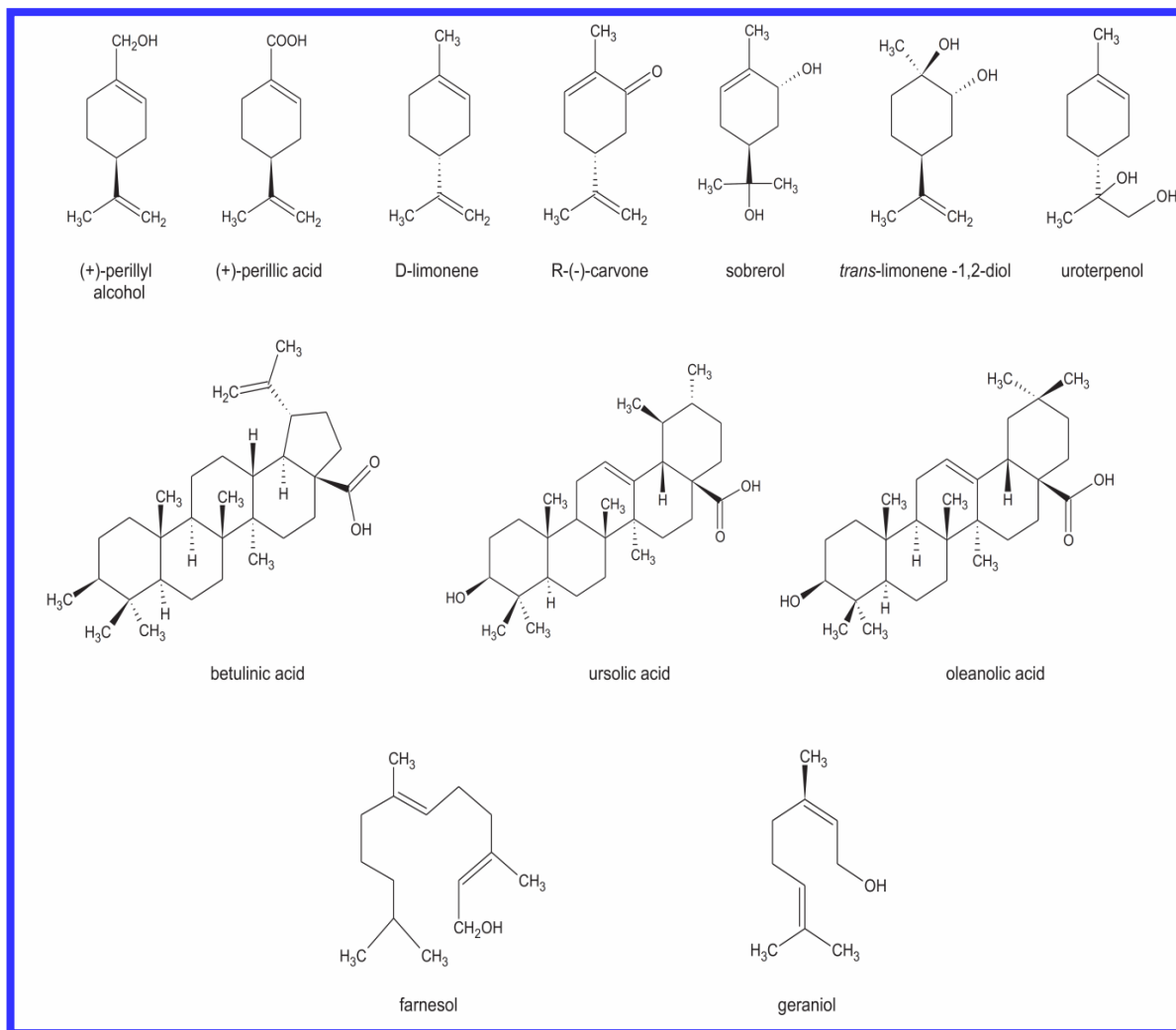
Figure 1.6: Various terpenes with anticancer properties (Paduch *et al.,* 2007).

A proposed mechanism expressed that monoterpenes may activate transforming growth factor (TGF)-β signaling which is produced in a latent form. This activation increases mRNA synthesis encoding (TGF)-β receptors and is closely associated with elevated synthesis of pro-apoptotic proteins (Bax, Bak, and Bad) without influencing p53 or Bcl-2 expression. Moreover, through TGF-β, the cell cycle is down-regulated, influencing the production of cyclin and cyclin-dependent kinases or their reciprocal interactions. In consequence, it leads to G1-phase arrest and cell apoptosis (Ahn *et al.,* 2003). Additional studies revealed that some monoterpenes can influence tumor cells by inhibiting the synthesis of coenzyme Q (CoQ) an important element of mitochondrial

respiratory metabolism. The reduction of CoQ in cell membranes may, therefore, limit cellular signal transduction and metabolism and induce apoptosis of tumor cells (Ahn *et al.,* 2003; Gould, 1995; Paduch *et al.,* 2007).

In another study Ulrich, (2007) showed treatment with ursolic acid (pentacyclic triterpenoid) leads to a significant time- and dose-dependent cell growth inhibition of colorectal cancer cells, coincident with the up regulation of the cell cycle1 regulators cyclin E, p21WAF1/Cip1 and p27Kip1. In addition, ursolic acid significantly induces apoptosis, which is mediated by an increase of BAX/Bcl-2-protein-ratio as well as an up regulation of TRAIL protein which meets in an induction of caspase-3 activity.

1.2.3.5.2. Chemoprevention of terpenes

Cancer chemoprevention, as first defined in 1976 by Sporn, is the use of natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression (Sporn, 1976). According to the National Cancer Institute (NCI), five classes of mainly chemically synthesized chemopreventive agents are characterized of high priority: selective estrogen receptor modulators (SERMs), non-steroidal anti-inflammatory drugs (NSAIDs), calcium compounds, glucocorticoids and retinoids.

In parallel, the NCI identified about 40 edible plants possessing potential chemopreventive compounds, globally known as phytochemicals (National Center for Health Statistics, 2005). For instance, among dietary monoterpenes, D-limonene and perillyl alcohol have been shown to possess chemopreventive and therapeutic properties against many human cancers. There are multiple mechanisms of monoterpene chemopreventive actions. They may act during the initiation phase of carcinogenesis, preventing interaction of carcinogens with DNA, or during the promotion phase, inhibiting cancer cell development and migration. The chemopreventive and therapeutic activities of monoterpenes in later stages of carcinogenesis include induction of cancer cell apoptosis, re-differentiation of tumor cells, and influence on molecular mechanisms regulating their functions. The most important mechanism that monoterpenes influence is post-translational isoprenylation of proteins regulating the growth of cells. Figure 1.7 illustrates further chemoprevention strategies.
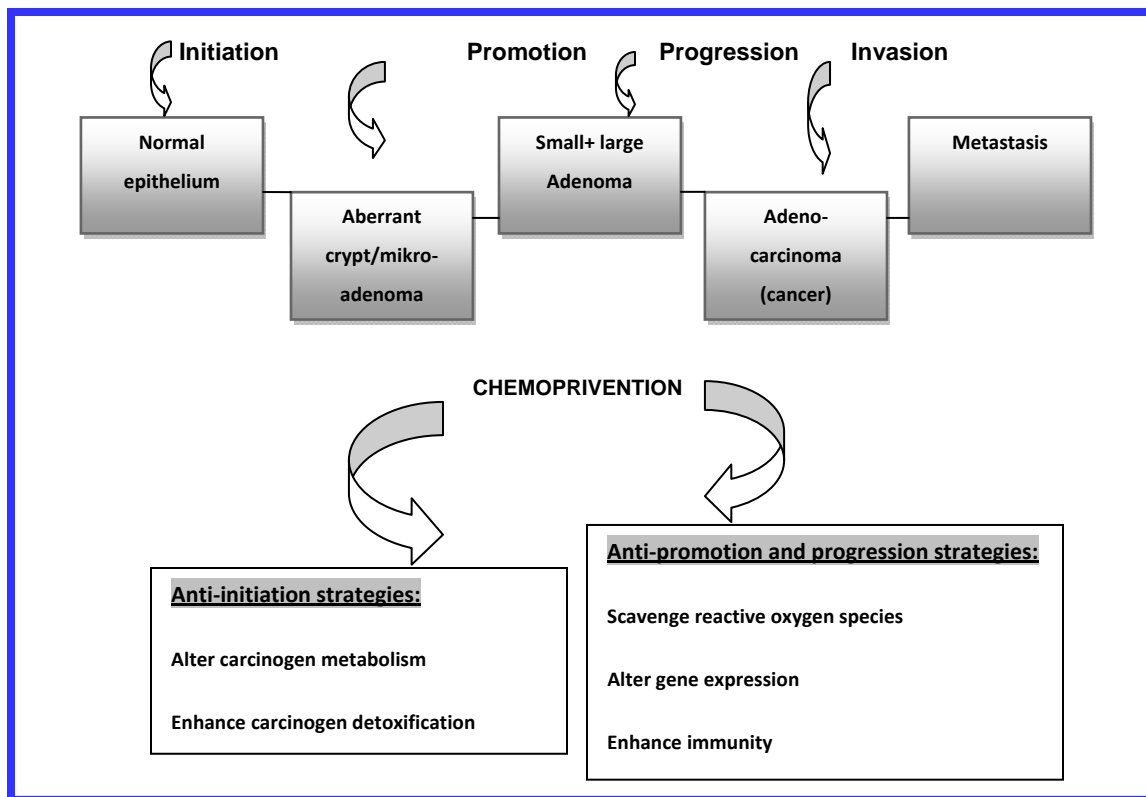
Figure 1.7: Chemoprevention strategies (Ulrich, 2008).

1.3. THE GENUS *Hyaenanche*

The genus *Hyaenanche* belongs to Euphorbiaceae family, which is one of the largest families of plants with about 300 genera and 7500 species, mostly monoecious herbs, shrubs and trees, sometimes succulent and cactus-like, that are further frequently characterized by a milky sap or latex material. Members of Euphorbeaceae family have been investigated as providing potential treatments for cancer, tumors and warts (Donato *et al.,* 2005). The chemistry of the Euphorbiaceae is among the most diverse and interesting of flowering plant families and is comparable to the biological diversity of the family. Because of the presence of unusual secondary metabolites, many species of euphorbiaceous plants are poisonous and have been involved in human and livestock poisoning. Plants of this family have been used in folk medicine, as pesticides or as arrow poisons. Several euphorbias are important food plants, which a number of them are important economically as ornamental plants and as sources of rubber, chemical precursors, lubricants and medicinal compounds (Seigler, 1994). Of all chemical

classes, the most useful for chemotaxonomic study of the Euphorbiaceae appear to be alkaloids, cyanogenic glycosides, diterpenes, glucosinolates, lipids, tannins and triterpenes (Hegnauer, 1963).

1.3.1. Compounds isolated and identified from the genus Hyaenanche

The genus *Hyaenanche* only contains a single species, which is known as *Hyaenanche globosa*. The seeds (possibly also other parts of plant) contain a deadly toxin with a strychnine-like action, which is used as an arrow poison to kill hyenas and other predators (Van Wyk *et al.,* 1997). This plant contains several toxic sesquiterpene lactones, such as; tutin, mellitoxin (also called hyenanchin), urushiol III and isodihydrohyaenanchine. Its main toxin, tutin, is known to cause convulsions, delirium and coma in humans (Van Wyk *et al.,* 1997; Van Wyk *et al.,* 2002).

1.3.2. Hyaenanche globosa

Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Malpighiales*
Family: *Picrodendraceae*
Tribe: *Caletieae*
Subtribe: *Hyaenanchinae*
Genus: *Hyaenanche* Lamb.
Species: *H. globosa* (Gaertn.) Lamb. & Vahl
Synonym: *Jatropha globosa* Gaertn.

Hyaenanche globosa Lamb. is an endemic plant and is restricted to a single flat-topped mountain near Van Rhynsdrop in southern Namaqualand (a province in South Africa). This plant is the single species of *Hyaenanche*. It is a small, rounded tree, with dark green, leathery leaves, characteristically arranged in four along the stems. Male and female flowers are both small and occur on separate trees. The fruits are large rounded capsules with several segments (Fig 1.8). *Hyaenanche* is a Greek word for hyena

poison and was chosen because the fruits were formerly used to poison carcasses in order to destroy hyenas and other vermin (Van Wyk *et al.,* 1997).

Figure 1.8: Aerial parts of *Hyaenanche globosa.*

1.4.   THE GENUS *Maytenus*

The genus *Maytenus* belongs to Celastraceae (or staff vine or bittersweet family; syn. Canotiaceae, Chingithamnaceae, Euonymaceae, Goupiaceae, Lophopyxidaceae, and Siphonodontaceae in Cronquist system), which is indigenous to tropical and subtropical regions of the world, including North Africa, South America, and East Asia. This family consists of about 90-100 genera and 1,300 species of vines, shrubs, small trees, bushes, or lianas, which have resinous stems and leaves (Spivery *et al.,* 2002). The great majority of the genera are tropical, with only *Celastrus* (the staff vines), *Euonymus* (the spindles) and *Maytenus* widespread in temperate climates. The member of genus *Maytenus* are distributed throughout Central and South America, Southeast Asia, Micronesia and Australasia, the Indian Ocean and Africa. They grow in a very wide variety of climates, from tropical to subpolar (http://en.wikipedia.org/wiki/Maytenus).

The variety of bioactivities of the Celastraceae in traditional medicine and agriculture is astonishing, which includes stimulant, appetite suppressive, sedative, emetic, purgative, memory restorative, male contraceptive, antitumor, anti-leukemic, antibacterial,

insecticidal and insect repellent activities (Costa *et al.,* 2008; Souza-Formigonia *et al.,* 1991; Spivery *et al.,* 2002). Cytotoxicity and antitumor activities of *M. ilicifolia, M. ovatus, M. cuzcoina, M. serrate*, *M. diversifolia, M. Molina, M. rigidi* and *M. emarginata* have been reported in literatures (Cargg and Newman, 2005; Hong, 2000; Hui *et al.,* 2009; Lee *et al.,* 1982; Martucciello *et al.,* 2010; Spivery *et al.,* 2002). Anti-microorganism activities of various *Maytenus ssp.* have been stated before (Avilla *et al.,* 2000; Lindsey *et al.,* 2006).

1.4.1. Compounds isolated and identified from the genus Maytenus

Over three past decades, a large number of secondary metabolites displaying a wide range of bioactivity have been extracted from the Celastraceae. Plants of the genus *Maytenus* are extensively investigated for bioactive compounds as they are widely used in folk medicine as an antiseptic, antiasthmatic, fertility-regulating agent, antitumor, as well as for stomach problems (Ghazanfar, 1994). Diverse types of secondary metabolites, including triterpenes (Shirota *et al.,* 1996), oligo-nicotinated sesquiterpenes and sesquiterpene pyridine alkaloids (Corsino *et al.,* 1998), phenolic glucosides (Sannomiya *et al.,* 1998) and agarofurans (Gonzalez *et al.,* 1993), with an interesting spectrum of biological activities have been found in plants belonging to the genus *Maytenus*.

In addition to numerous terpenoids particularly sesquiterpenoids, various bioactive phenylalkylamines, maytansinoids and flavonoids have also been isolated from different species of the Celastraceae. However, the bulk of the bioactive constituents of this family, are terpenoids. All types of terpenoids are found in the extracts of *Maytenus ssp.* (Gonzaleza *et al.,* 2001; Queiroga *et al.,* 2000; Spivery *et al.,* 2002). Two steroids namely; β-sitosterol and β-sitosterol-βD-glucoside were isolated from the leaves of *M. floribunda* (Reiss). In addition, dulcitol, β-sitosterol and β-sitosterol-βD-glucoside; the flavonoids; 4'-O-methyl-(-)-epigallocatechin and proanthocyanidin A; the pentacyclic triterpenes: friedelin, friedelinol and 28-hydroxy-3-oxo-friedelane were isolated from the bark wood of *M. floribunda* (Salazar *et al.,* 1997). Orabi *et al.,* (2001) isolated eight compounds from the ethanol (EtOH) extracts of *M. heterophylla:* (A) dihydroagarofuran alkaloid, (B) 1β-acetoxy-9α-benzoyloxy-2β, 6α-dinicotinoyloxy-β-dihydroagarofuran, β-

amyrin, maytenfolic acid, 3α-hydroxy-2-oxofriedelane-20α-carboxylic acid, lup-20(29)-ene-1β, 3β-diol,(−)-4′-methylepigallocatechin, and (−)-epicatechin. Compounds A and B with moderate antimicrobial activity have been reported.

The aerial parts of *M. undata* yielded different triterpenes such as: 3-oxo-11R-hydroxyolean-12-ene-30-oic acid, 3-oxo-olean-9(11), 12-diene-30-oic acid, 3,11-dioxoolean-12-ene-30-oic acid (3-oxo-18*α*-glycyrrhetinic acid), 3,4-seco-olean-4(23),12-diene-3,29-dioic acid (20-*epi*koetjapic acid), koetjapic acid, and the 12-oleanene artifact 3-oxo-11R-ethoxyolean-12-ene-30-oic acid (Muhammad *et al.,* 2000) (Fig 1.9). 'Pristimerin' (20α-3-hydroxy-2-oxo-24-nor-friedela-1-10,3,5,7-tetraen-carboxylic acid-29-methylester) purified from the ethanol extract of the root bark of *M. ilicifolia*, demonstrated anti-proliferative activity (Costa *et al.,* 2008) (Fig 1.9). Till date, there has been no report on the isolation of compounds from *M. procumbens*.

1.4.2. *Maytenus procumbens*

Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Celastrales*
Family: *Celastraceae*
Genus: *Maytenus*
Species: *M. procumbens* (L.f.) Loes.

Maytenus procumbens (L.f.) Loes. is an indigenous native South African species, also known as Dune Koko (*duinekokoboom* in Afrikaans) tree which characterizes a scrambling shrub or small tree. *M. procumbens* appears as a densely bushy plant with drooping branches that sometimes reach more than 6 meter. Its bark is pale yellowish brown, which sometimes become fissured on old trees. The clusters of white to greenish white flowers may appear in winter, but are sometimes seen up to the end of the following summer. The inconspicuous flowers are followed by spherical fruits that burst open to reveal the bright orange seeds that are loved by birds. *M. procumbens*

occurs along much of the South African south and east coast in dune scrub and wooded areas up to the altitude of about 150 meter (Coates Palgrave, 2002) (Fig 1.10).
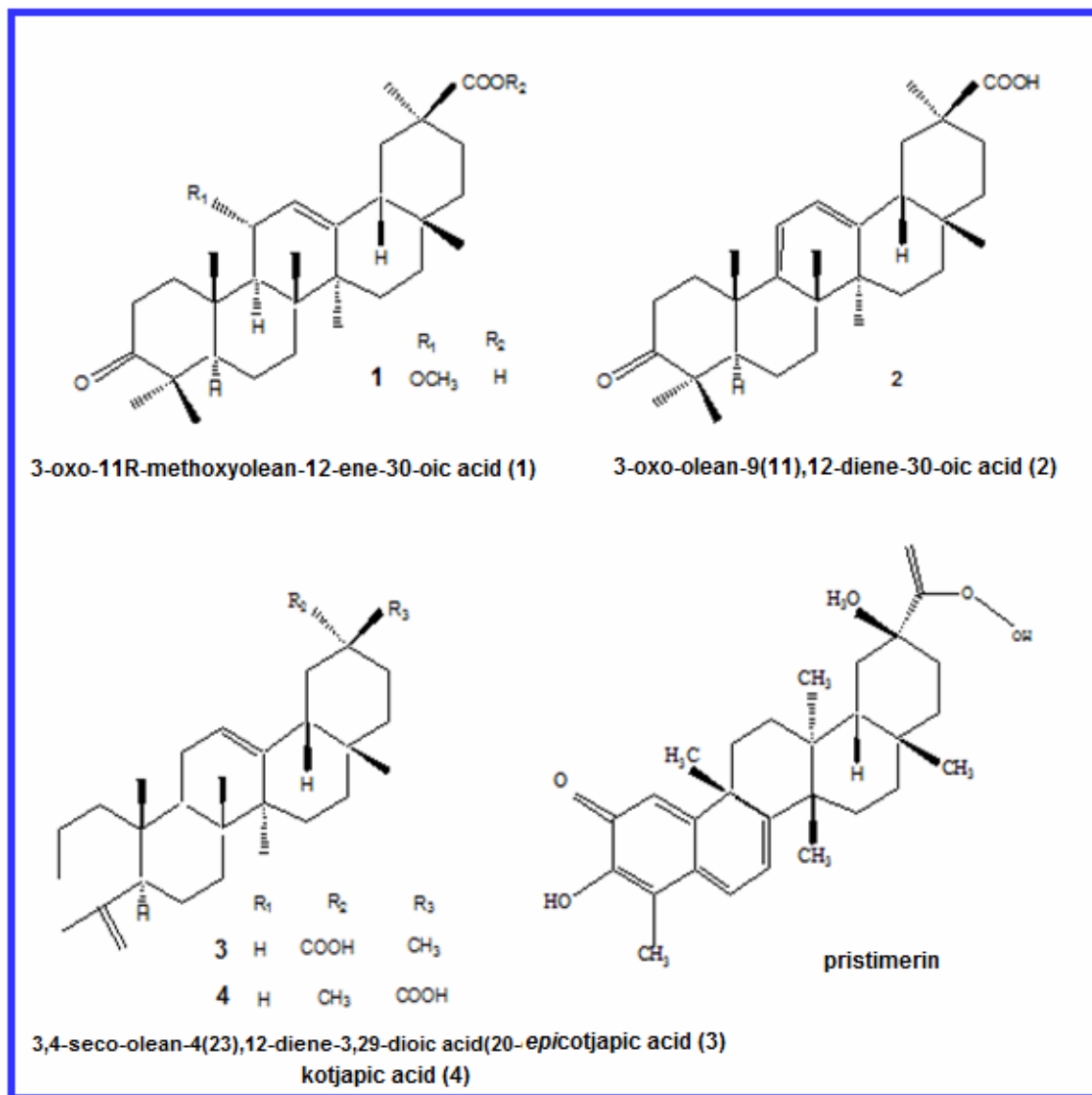

Figure 1.9: 'Pristimerin' isolated from *M. ilicifolia* (Costa *et al.,* 2008); '3-oxo-11R-hydroxyolean-12-ene-30-oic acid (1), 3-oxo-olean-9(11), 12-diene-30-oic acid (2), 3,4-seco-olean-4(23),12-diene-3,29-dioic acid (20-*epi*koetjapic acid) (3), koetjapic acid (4)' purified from *M. undata* (Muhammad *et al.,* 2000).

Figure 1.10: Aerial parts of *Maytenus procumbens.*

1.5. RATIONALE FOR THIS STUDY

Commonly scientific data representing plant-derived agents, display anticancer activity as individuals or in combination with other natural- or chemical-based components. The occurrence of non-selective *in vivo* toxicity and resistance of tumor cells to these drugs still remain problematic. It has been previously proven that different species vary in their biochemical contents resulting in diverse biological activities. In present study, two endemic South African species have been investigated (i) to establish whether these species induce selective cytotoxicity in cancerous cells, (ii) isolate novel compounds with possible cytotoxicity and, (iii) to identify mechanism involved in inducing cytotoxicity?

1.6. AIM AND HYPOTHYSIS

Interest in discovery of herbal-based anticancer agents has increased globally in past decades. Multiple natural agents have been recognized to block carcinogenesis in certain cancer cell lines or animal models. Although much progress has been made in reducing mortality rates, stabilizing incidence rates, and improving survival, cancer still accounts for more deaths all over the world after heart diseases. However, the appearance of drug-resistance tumor cells, non-selectivity of these cancer drugs, and high adverse effects still stay critical. Therefore, acceleration of further progression

regarding to discovery of novel anticancer agents seem to be crucial. Thus, the aim of our study was to characterize the effect of two plant species and their isolated compounds on some human cancer cell models.

1.7. OBJECTIVES

According to defined hypotheses, the objectives of this study were as follows:

a.   To identify the most promising plant extracts by comparing the cytotoxicity of the experimental crude extracts on cancerous and non-tumour cell lines.

b.   Isolation and identification of possible major compounds from the selected extracts.

c.   To determine whether there is a correlation between the antioxidant properties and cytotoxicity of the test compounds.

d.   To determine whether there is a connection concerning the anti-microorganisms activities and cytotoxicity of the selected compounds.

e.   To determine the induced cell death pathway on a cancer cell line with a flow cytometric method.

f.   To investigate whether the selected compounds have an effect on the mitochondrial membrane potentials of cancerous cells or not.

g.   To measure if there is any chance of enhancing mutagenicity in selected cancer cells by test compounds.

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