1. INTRODUCTION & REVIEW OF LITERATURE
Cancer has been known since before the first humans walked the Earth. Fossilized dinosaur bones show evidence of tumors, and archaeologists have ascertained a 2,700-year-old human skeleton with evidence of cancer. The Greek medico Hippocrates named the disease after the Greek world for ‘crab’, possibly because branching web of tumor blood vessels reminded him of the multilegged fauna [Barbara, 2012].

There is a manifesto that cancer was found in antediluvian human remains in the medical literature since ancient times, from the time of the pharaohs in ancient Egypt and the ancient world. Although, it is difficult to interpret the diagnosis of doctors, who lived many centuries ago, we can feign that many of their descriptions related to the cases of cancer. The recent detonation of biochemical noesis of the molecular basis of human cancer has radically changed our understanding of the malignant state and laid the cornerstone for developing new strategies for prevention, diagnosis and treatment of cancer. With an excellent opportunity to surpass heart disease as the number one killer of humans, cancer has become a more unnerving populace foe than ever before [Tandon, 2004].

Cancer is a huge global health challenge, touching every region and socio-economic level. Today, cancer accounts for one out of every eight deaths worldwide (more than HIV/AIDS, tuberculosis and malaria combined). In 2008, there were 12.7 million cases of cancer diagnosed and 7.6 million cancer deaths worldwide. More than 60 percent of all cancer deaths occur in low and middle income countries, many of which lack the medical resources and health systems to support the burden of the disease. In addition, the global burden of cancer is increasing at an alarming rate, in 2030, about 21.4 million new cancer cases and 13.2 million cancer deaths are expected to occur simply due to the growth and aging of the population. In India, about 5, 55,000 people died from cancer in 2010, according to estimates published in 'The Lancet today' (March 28, 2012). About 1,63,8910 new cancer cases are expected to be diagnosed in 2012. In 2012, about 5, 77,190 Americans are expected to die of cancer, more than 1,500 people a day (Figure 1.1). The future of the burden may be increased by the adoption of ill behaviors and lifestyles associated with economic
development and urbanization (e.g. smoking, poor diet, lack of physical activity and reproductive models) in low-and middle-income countries [American Cancer Society, 2012].

![Figure 1.1 Ten leading cancer types, estimated new cancer cases and deaths by sex in United States, 2012.](image)

Cancer development is now commonly recognized as a micro evolutionary process that requires the cumulative action of multiple events. All cancers are multifactorial in origin and include genetic, hormonal, metabolic, physical, chemical and environmental factors. Most human cancers are spontaneously produced without a particular cause which could be pinpointed. The events may occur in a single cell clone and can be explained by a simplified three stage model. These stages include (a) the induction of DNA mutation in a somatic cell known as initiation (b) stimulation of the initiated cell and its clonal expression referred as promotion and (c) malignant conversion of benign tumor into cancer termed as progression [Athar, 2002].
To understand cancer and a rational means of treating it, the broad knowledge and understanding of cell working and their social interactions in the body tissues is required. Thus, the efforts of cancer research are deeply benefit a much wider field of medical knowledge than from cancer alone. "Cancer" refers to more than 100 types of the disease and almost every tissue in the body may appear malignant tumors, and some even yield several types of it. So, cancer is not one disease but a group of diseases in common. Normal cells reproduce only when instructed to do so by other cells in their vicinity. This continued cooperation will ensure that each tissue retains its size and architecture to meet the needs of the organism. Cancer cells breaks this pattern, they become deaf to the normal control of cell division/proliferation and to follow its own internal agenda for proliferation (hyperplasia). In addition, they have an even more insidious property, the ability to migrate from the site, where they invade the surrounding tissue and form masses in distant parts of the body (metastasis). Tumors are composed of these malignant cells is becoming more and more aggressive over time, and they become deadly when they destroy the tissues and organs necessary for survival of organisms in general [Weinberg, 1996]. The two classes of genes, which together constitute a small fraction of the full set of genetic control of cell division and proliferation, play an important role in triggering cancer. Proto-oncogenes promote cell division and expansion, whereas, tumor suppressor genes inhibit it. The activation of oncogenes or inactivation of tumor suppressor genes (due to a mutation) can cause excessive proliferation of normal cells, which can lead to the cancer [Kumar, 2005].

1.1. Cancer Chemoprevention

There are several ways to treat cancer in modern medicine, which include chemotherapy, radiotherapy and surgery. Chemoprevention, by definition, is a tool in the fight against cancer, in which the occurrence of the disease can be completely prevented, slowed or canceled by the administration of one or more chemical compounds. The general public frustration at the lack of effective strategies for prevention and treatment of cancer is
becoming more apparent. The side effects of conventional anti-cancer modalities, whether through the process of radiation or chemical treatment, is a general weakening of the body's immune system resulting in immunosuppression that can significantly increase the risk of infection for the patient [Chorawala et al., 2012]. Thus, people tend to move in the direction of traditional medicine and alternative medicine in recent years. The concept of chemoprevention is gaining attention due to their safety, low toxicity, cost, and general acceptance.

According to the conventional classification proposed by Lee Wattenberg, chemopreventive agents are divided into two main classes- blocking agents and suppressing agents. Blocking agents prevent carcinogens from reaching the target sites, from undergoing metabolic activation or from subsequently interacting with crucial cellular macromolecules (for example, DNA, RNA and proteins). Suppressing agents, on the other hand, inhibit the malignant transformation of initiated cells, in either the promotion or the progression stage. Chemopreventive phytochemicals can block or reverse the premalignant stage (initiation and promotion) of multistep carcinogenesis. They can also halt or at least retard the development and progression of precancerous cells into malignant ones [Wattenberg, 1985].

The participation of several factors and stages of development and our deeper understanding of cancer at the epigenetic, genetic, molecular and cellular levels has enormous potential to interrupt and reverse the initiation/progression of the disease [Manson, 2003]. Therefore, the ability of any single chemopreventive phytochemical to prevent tumor development should be recognized as the outcome of the combination of several distinct sets of intracellular effects, rather than a single biological response.

Recent studies of tumor-inhibiting compounds of plant origin bring an impressive field of new structures. Natural products and their isolated components play an important role in the treatment of cancer today, a large number of anticancer drugs used now days are natural or from natural products from various sources such as plants, animals and marine organisms. The effect of natural products in the apoptosis signaling pathways and/or different targets in
cancer means that they can be a useful starting point in the design and development of new cancer prevention tools.

In the past few years, a natural product based drugs is increasing through new technologies such as combinatorial synthesis and high throughput screening, and their related approaches. Some of the more promising and more effective drugs come from plant sources, such as taxol, camptothecin, combrestatin, epipodophyllotoxin and vinca alkaloids (vincristine and vinblastine), whereas, actinomycin D, bleomycin, doxorubicin, L-asparaginase, and mitomycin C are the drugs that are coming from the microbial sources and citarabine is the first drug derived from marine sources.

To date, the new generation taxane, antracyclines, vinca alkaloids, camptothecines, as well as a new class of epoophilones have been developed. Some of them are used in clinical practice, others in clinical trials. All these drugs are characterized by different mechanisms of action, like interaction with microtubules, inhibition of topoisomerase I or II, alkylation of DNA, and interference with tumor transmission. In addition, many other natural compounds that are in clinical trials of cancer are tabulated in accordance with their leading source of compounds: plants, marine and organisms. Over the past 50 years, more than 1 million compounds have been tested, but only seven herbal anticancer drugs have received Food and Drug Administration (FDA) authorization to clinical application [Ma and Wang, 2009].

1.2. History of natural cancer therapeutics

In recent times, some evidence of the most encouraging clinical value of herbs in cancer treatment allows us to reconstruct the history of these plants and their potential use in these cases. The past 50 years have seen an explosion in our understanding of this disease the most fundamental, and new discoveries occur on an almost weekly basis. For this reason, it is possible to find evidence of the relationship between plants and cancer in recent times. Some botanical compounds, which have been shown to have positive effects in cancer treatment, have a long history behind them. For example, it was recently shown that green tea
antioxidant EGCG (epigallocatechin-3-gallate) significantly slowed the growth of breast cancer in female mice: its use is attested in ancient Japanese texts [Thangapazham et al., 2007].

The use of fruit juice, peel and oil of Punica granatum was also shown to possess anticancer activity, including interference with tumor cell proliferation, cell cycle, invasion and angiogenesis [Lansky and Newman, 2007]. Promising and selective anti-cancer effects were observed with saffron (stigmas of Crocus sativus L.) in vitro and in vivo announced but not yet in clinical trials [Abdullaev, 2005; Schmidt et al., 2007]. Modern scientific research has revealed that a variety of food and medicinal functions of garlic can be attributed to sulfur compounds present in or generated from garlic, which can be effective for cancer treatment/prevention [Ariga and Seki, 2006]. The search for anticancer lead compounds has been the mainstream of marine chemistry. Consequently, a number of marine natural products with unique mechanisms of action have been identified and recently entered clinical trials [Tasdemir et al., 2002; Marshall et al., 2003].

Myrrh is derived from the dried resin of trees of the desert, Commiphora Myrrha and other species. In biblical terms, it was chosen, with incense and gold, as a gift the Magi to the newborn Christ. Hailed for its anti-inflammatory and disinfectant properties, myrrh has historically been used for foods as diverse as stomach pain, indigestion, poor circulation, wound healing, certain skin diseases and menstrual cycle’s irregularities. What makes myrrh such an exciting player as an anti-cancer agent is not only how it kills cancer cells in general, but how it kills those who are resistant to other anticancer drugs. Although, myrrh does not seem to be as powerful as other anti-cancer agents like, vincristine, vinblastine and paclitaxel, its advantage seems to lay in the fact that it can damage the cancer cells without harming healthy cells, something these other drugs do not [Kinghorn, 2003].

One of the most important plant compounds in the fight against cancer was discovered in the bark, and at low levels in the needles, the relatively rare Pacific Yew, Taxus brevifolia. In the 1970s, the NCI (National Cancer Institute) tested plants in a number of collections,
including an extract from Pacific Yew collected by the United States Department of Agriculture in 1962. They discovered taxol, now called paclitaxel, which has become one of the most effective drugs against breast and ovarian cancer and has been approved worldwide for the clinical treatment of cancer patients [Song and Dumais 1991]. The first clinical trials with paclitaxel have been carried out in 1983 and by 1988 preliminary data have shown impressive results in patients with ovarian cancer. In earlier studies of patients with progressive disease, the removal of more than 30% of cancers experienced at least half of them had a response that lasted over a year. This led to the development of broad and successful clinical trials in ovarian and breast cancer. Later, focus of interest shifted to the development of improved analogues of this drug [Adams et al., 1993].

The discovery of the first anti-tumor antibiotics was made by Selman Waksman and H. Boyd Woodruff, in 1940; actinomycin D isolated from Actinomyces antibioticus [Waksman and Woodruff, 1941]. This compound, a chromosomal oligopeptide that acts as an inhibitor of RNA polymerase, is a member of a group of antibiotics (actinomycins) prevalently characterized by antibacterial activity and all discovered by the same research group at the Rutgers University. The clinical antitumor activity of actinomycin D in a number of childhood tumors including metastatic Wilms' tumors is also described [Farber et al., 1956; Keidan, 1966].

The study of anthracycline glycosides and their aglycones isolated from various Streptomyces species began in the 1905s and scored extremely relevant when, in the 1960s, a compound called daunomycin (also known as daunorubicin) with antileukemic properties was isolated from a strain of Streptomyces peucetius var. caesius by Di Marco and colleagues [Di Marco et al., 1964]. The 14-hydroxy derivative of daunorebicin (i.e. Adriamycin), also produced by Streptomyces peucetius and related strains, was isolated in 1969 by Arcamone et al., [1969] at the Farmitalia Research Laboratories. The name of this compound (now known as doxorubicin) comes from the Adriatic Sea, near which the original strain of daunorubicin was collected.
Compared with the study of terrestrial natural products, the study of marine natural products is still in its infancy. Although, the oceans have attracted the attention of researchers since the 1950s with the discovery of the Caribbean sponge, *Cryptotheca crypta*, nucleoside derivative spongouridine spongouridine [Bergmann and Feeney, 1950]. The technical difficulties of collecting marine organisms as well as poor knowledge of this habitat posed a relevant obstacle. This discovery provided the pharmacophore used in the synthesis of a close analogue of spongouridine, known as cytosine arabinoside, marketed by Upjohn (now Pharmacia) as Ara-C (cytarabine), pyrimidine nucleoside analogue, in 1969 to treat leukemia and lymphoma [Newman and Cragg, 2004; Carte, 1996]. The use of cytarabine has now been rejuvenated by the introduction into the clinic of a liposomal formulation indicated for the intrathecal treatment of lymphomatous meningitis [Kripp and Hofheinz, 2008]. Recently, advances in offshore technology and collection of aquatic cultivation give the large number of compounds derived from marine organisms that enter preclinical and early clinical evaluation as potential anticancer agent. A large number of marine compounds (over 16,000), but only few of them have crossed the preclinical and clinical evaluation [Rawat et al., 2006].

Bryostatins in the same years, a group of macrolide lactones were isolates of the bryozoan species *Bugula neritina*. In 1970, dolastatin 10 was isolated from the mollusk *Dolabella auricularia* lives in the Indian Ocean, it has failed to demonstrate the antitumor efficacy, but provided a basis for studies of structure-activity (for example the synthesis of TZT -1027, soblidotin) [Simmons et al., 2005]. Therefore, a variety of other marine anticancer compounds or their semi-synthetic or synthetic derivarives underwent clinical investigation. These compounds include halichondrins and didemmins isolated in the 1980s and characterized by the classical mechanism of cytotoxic action, as well as compounds isolated from the late 1990s and characterized by the most intriguing mechanisms of action (e.g. salinosporamide that inhibits the proteasome or neovastat that blocks vascular endothelial growth factor (VEGF) binding to its receptor) [Singh et al., 2008].
1.3. Current plant based therapies

1.3.1. Tubulin-binding agents

Microtubules are essential components of cell cytoskeleton and are involved in a number of cellular functions. They are critical to the movement of organelles during interphase and during mitosis; they form the mitotic spindle that carries the daughter chromosomes to separate poles of the dividing cell. Drugs that interfere with the main function of microtubules to the misalignment of chromosomes and their bipolar spindle attachment, this effect leads to mitotic arrest in the metaphase/anaphase transition, followed by apoptosis [Jordan and Wilson, 2004]. This has been suggested as the primary anti-neoplastic action mechanism of tubulin-binding drugs but has also been postulated that at least part of anti-tumor effect of these agents is related to their effect on microtubules in interphase cells. Vinca alkaloids and taxanes represent the major classes of tubulin-binding agents.

1.3.1.1. Microtubule destabilizing agents

1.3.1.1.1. Vinca alkaloids

Vinca alkaloids are isolated from Madagascar periwinkle, Catharanthus roseus, also known as Vinca rosea possess many therapeutic effects including anti-tumor activity [Noble, 1990]. Overall 70 alkaloids in periwinkle have been identified with various uses. Vincristine (1) and vinblastine (2) are the first Vinca alkaloids with anti-tumoral activity to be identified. Vindesine (3) (Eldisine® and Fildesin®) and vinorelbine (4) (Navelbine®) are the two semi-synthetic vinca alkaloids to emerge from structural modification studies. Vinflunine (5), a bis-fluorinated derivative of vinorelbine, exhibits a superior anti-tumor activity compared to other Vinca alkaloids [Fahy, 2001]. Due to its favourable preclinical anti-tumoral activities, including microtubule dynamics disruption, antiangiogenesis and prolonged multidrug
resistance development, vinflunine is now being widely studied in phase I-III clinical trials [Yun-San Yip et al., 2008].

Vinca alkaloids disrupt mitotic spindle assembly through interaction with tubulin. In particular, bind specifically to β-tubulin and blocking its activity to polymerize with α-tubulin in microtubules. This leads to the death of actively dividing cells by inhibiting the progression through mitosis. However, the new vinca alkaloids, vinorelbine and vinflunine, have proven to be binding weak in contrast with the binding of vincristine and intermediate level of vinblastine. Evidence suggests that vinorelbine and vinflunine affect microtubule dynamics different from vinblastine [Ngan, 2001].

Classical vinca alkaloids are largely used in the treatment of haematological and lymphatic neoplasm [especially vincristine (Oncovin®)] as well as in several solid tumors (e.g. vinblastine (marketed as velban) in breast, testicular cancer, choriocarcinoma; vindesine in non-small cell lung cancer (NSCLC), breast cancer, etc.). The newer drugs are used primarily in solid tumors, such as lung, breast and ovarian cancers. Common side effects of these drugs are myelosuppression and neurotoxicity. Vinorelbine is used for the treatment of non-small cell lung cancer and metastatic breast cancer.
The main toxic effect of vinorelbine is granulocytopenia with only modest thrombocytopenia and less neurotoxicity than other vinca alkaloids [Aapro et al., 2007].

Vinflunine has been used in treatment of bladder, non-small cell lung and breast cancers; its main side effects are myelosupression and constipation which apparently are more manageable compared to the other vinca alkaloids [Yun-San Yip et al., 2008].
The most widely recognized mechanism of resistance to vinca alkaloids is due to the multidrug resistance-associated P-glycoprotein (P-gp) [Nobili et al., 2006] and the multidrug resistance protein (MAP) [Lautier et al., 1996]. The overexpression of these two proteins belonging to the ATP-binding cassette (ABC) transporter family has been associated with reduced intracellular accumulation of vinca alkaloids and corresponding reduction of cytotoxicity.

Vinca alkaloids are most commonly administered weekly by short intravenous injection (1-15 min), more rarely by continuous infusion. Vinorelbine is the only alkaloid available orally and is administered as a single weekly dose [Leveque and Jehl, 2007].

1.3.1.1.2. Other microtubule destabilizing agents

Cryptophycins are potent macrolide antimitotic cytotoxins produced by cyanobacteria of the genus Nostoc. They work by attacking the tubulin protein found in eukaryotic cells and thereby preventing cell division and reproduction. They are extremely potent suppressors of microtubule dynamics by slowing it in a concentration-dependent manner and depolymerizing microtubules in an irreversible way probably due to covalent drug-target interaction. The presence of several amide and ester linkages within the cryptophycin core provides access to very convergent total synthetic approach. However, the in vivo hydrolytic instability of the compound was a key obstacle to finding a clinical candidate. This problem has been somewhat ameliorated in the totally synthesized cryptophycin-52 (6) [Eggen and Georg, 2002]. Despite the initial enthusiasm deriving from the possibility that cryptophycins would be able to overcome multidrug drug resistance in experimental systems, this occurrence has not been confirmed in clinical trials. In addition, cryptophycin-52 showed only modest activity in patients with platinum-resistant advanced ovarian cancer [D’Agostino et al., 2006].
Halichondrin B (7) is a naturally occurring compound originally isolated from the Japanese sponge *Halichondria okadai*. They are potent tubulin inhibitors that non-competitively bind to vinca binding site [Bai et al., 1991]. Eribulin (8) (E7389, ER-086526, NSC-707389), a synthetic macrocyclic ketone derivative of halichondrin B, was approved by the U.S. Food and Drug Administration, to treat patients with metastatic breast cancer who have received at least two prior chemotherapy regimens for late stage disease, including both anthracycline- and taxane-based chemotherapies [http://www.clinicaltrials.gov].
Dolastatins are peptides, originally isolated from Dolabella auricularia marine mollusks. The pentapeptide dolastatin-10 (9) was the most promising natural dolastatin agent. A synthetic derivative of dolastatin-10, TZT-1027 (10), seems to possess a good safety profile and some anti-tumour activity as reported in a phase I trial [Tamura et al., 2007].

1.3.1.2. Microtubule stabilizing agents

1.3.1.2.1. Taxanes

Paclitaxel (11) (Taxol®), initially extracted from the bark of *Taxus brevifolia*, is now obtained by semisynthesis from 10-deacetylbbaccatin III, which is extracted from the needles of the English yew tree, *Taxus baccata*. Docetaxel (12) (Taxotere®), a semisynthetic taxane
with anticancer activity was directly obtained from 10-deacetylbaccatin III [Dorr, 1997]. With the aim of ameliorating the tolerability of taxanes and reducing clinical resistance, many efforts have been made to find new taxane formulations (e.g. albumin, nanoparticles, emulsions, liposomes, and polyglutamates) or new taxane analogues and prodrugs including orally bioavailable compounds [Ten Tije et al., 2003; Hennenfent and Govindan, 2006]. Compounds such as abraxane, U1-2103, docosahexenoic acid (DHA)-paclitaxel, are examples of new taxanes that have shown higher activity than paclitaxel in taxane-resistant cancers, as well as in tumors that have been unresponsive to paclitaxel. In addition, compared to the prototype, they have a safer toxicological profile and their administration does not require pre-medication for hypersensitivity reactions [Rowinsky and Calvo, 2006].

Due to the hydrophobicity of these drugs, they are administrated in formulations including two different polyoxyethylated surfactants. Since both solvents are biologically and pharmacologically active, they lead to adverse effects such as hypersensitivity reactions [Weiss et al., 1990], peripheral neuropathies [Onetto et al., 1993] or pharmacokinetic alterations especially for paclitaxel [Winer et al., 2004]. A new Taxol derivative, MAC-321 (13) is now being widely studied in phase I-II clinical trials for colorectal, metastatic breast,
and non-small cell lung cancer and is the sole taxane that can be given orally [http://www.clinicaltrials.gov].

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Taxanes exhibit cytotoxic activity by stabilizing microtubules rather than destabilizing them as vinca alkaloids do. In particular, they promote the assembly of microtubules and prevent their depolymerization, thus interfering with a number of normal cellular functions that depend on the physiological balance between tubulin and microtubules [Gueritte-Voegelien et al., 1991; Horwitz et al., 1993]. Paclitaxel and docetaxel have a different effect on cell cycle. Paclitaxel inhibits the cell-cycle traverse at the G2-M phase junction [Dorr, 1997] while docetaxel produces its maximum cell-killing effect against cells in the S phase [Hennequin et al., 1995].

The mechanism of resistance to taxanes are likely to be multifactorial, including the overexpression of the membrane efflux pump P-gp [Nobili et al., 2006], the presence of α and β tubulin mutations, increased microtubule dynamics association with altered MAP expression. Moreover, functional aberrations in multiple molecular pathways, such as cell cycle control, growth promotion and apoptosis can all contribute to taxane resistance [McGrogan et al., 2008].
Paclitaxel and docetaxel are very active in the spectrum of solid tumors (ovarian, breast, lung, head and neck, gastro-oesophageal, bladder, testis, endometrium neoplasm) and in some haematological and paediatric malignancies. Both drugs are active as single agents and in combination chemotherapy [Mekhail and Markman, 2002; Ramaswamy and Puhalla, 2006]. Clinical efficacy of taxanes is accompanied by significant side effects such as neutropenia, mucositis, and hypersensitivity reactions neuropathy. Peripheral neuropathy is less common and less severe for docetaxel than paclitaxel [Scripture et al., 2006].

1.3.1.2.2. Other microtubule stabilizing agents

The epothilones (14) are a new class of cancer drugs originally identified as metabolites produced by the soil-dwelling myxobacterium Sorangium cellulosum. Like taxanes, they prevent cancer cells from dividing by interfering with tubulin, but in early trials epothilones have better efficacy and milder adverse effects than taxanes [Steven, 2005]. Various epothilones (A to F) have been identified and characterized [Spreitzer, 2008]. Due to their better water solubility, cremophors (solubilizing agents used for paclitaxel which can affect cardiac function and cause severe hypersensitivity) are not needed [Julien and Shah, 2002]. Endotoxin-like properties known from paclitaxel, like activation of macrophages synthesizing inflammatory cytokines and nitric oxide, are not observed for epothilone B [Muhlradt and Sasse, 1997].

Several epothilone analogs are currently undergoing clinical development for treatment of various cancers. One analog, ixabepilone (15), was approved by the U.S. Food and Drug Administration for use in the treatment of aggressive metastatic or locally advanced breast cancer no longer responding to currently available chemotherapies [Puhalla and Bruksy, 2008]. Ixabepilone, in combination with capecitabine, has demonstrated effectiveness in the treatment of metastatic or locally advanced breast cancer in patients after failure of an anthracycline and a taxane [Thomas et al., 2007]. It has been investigated for use in treatment of Non-Hodgkin's lymphoma [Aghajanian, 2007]. In pancreatic cancer phase II trial it
showed some promising results (used alone) and combination therapy trials are ongoing [Vulfovich and Rocha-Lima, 2008]. Epothilone B and its various analogues are currently undergoing various clinical phases (patupilone (EPO906) and sagopilone (SH-Y03757A, ZK-EPO) are in phase II trials; BMS-310705 and BMS-247550 in phase I trials).

(14) Epothilones A (R = H) and B (R = CH₃) (15)

(+)-Discodermolide (16) is a recently discovered polyketide natural product isolated from the Caribbean marine sponge Discodermia dissoluta. It is found to be a potent inhibitor of tumor cell growth [Gunasekera et al., 1990; Gunasekera et al., 2002]. Discodermolide has been shown to inhibit the proliferation of human cells by arresting the cell cycle in G2- and M-phase. It hyper-stabilizes microtubules, especially prevalent during cell division. Hyper-stabilization of the mitotic spindle causes cell cycle arrest and cell death by apoptosis [Ter Haar et al., 1996].

Eleutherobin (17) is a diterpene glycoside initially isolated from the soft coral Eleutherobia sp. from Western Australia. Eleutherobin was found to stabilize microtubules by competing for the paclitaxel binding site. Very recently, eleutherobin, along with new derivatives, was found by the Andersen group (University of British Columbia) in
_Erythropodium caribaeorum_, an encrusting coral found in South Florida and the Caribbean. While this finding provides small amounts of eleutherobin for further development as an anticancer agent, there is not a sufficient supply of eleutherobin in nature. However, this observation does provide an exciting opportunity to complete a detailed biosynthetic study of eleutherobin.


![Chemical Structures](image)

Laulimalide (18) and Isolaulimalide (19) are natural products of marine sponge _Cacospongia mycofijiensis_. They act by stabilizing microtubules and produce synergistic action with taxanes [Mooberry _et al._, 1999]. These agents possess either low level or no substrate affinity for P-gp and other ABC transporters, and retain various degrees of activity against taxane-resistant cells _in vitro_, but the clinical implications of these characteristics are not clear [Rowinsky and Calvo, 2006].
1.3.2. Topoisomerase inhibitor

The DNA topoisomerase are nucleol enzymes that reduce torsional stress in supercoiled DNA, allowing selected regions of DNA to become sufficiently untangled and relaxed to permit its replication, recombination, repair and transcription. Inhibitors of topoisomerase I and II are anticancer drugs active in variety of haematological and solid tumors. They exhibit different pharmacological and toxicological profiles [Hartmann and Lipp, 2006; Cortes-Funes and Coronado, 2007].

1.3.2.1. Topoisomerase I inhibitors

1.3.2.1.1. Camptothecins

In the 1950s, an extensive screening programme of the NCI led to the isolation of an extract of the Chinese tree *Camptotheca acuminata* characterized by cytotoxic activity against a variety of leukemias and solid tumors. In 1966 camptothecin (20) was identified as the active constituent of the extract [Wall *et al.*, 1966]. Despite promising preclinical and clinical anti-tumor activity the use of the first camptothecin formulation was hindered by severe and unpredictable toxicity. In 1996 two semisynthetic camptothecin analogues,
irinotecan (21) and topotecan (22) entered the clinics for the treatment of colorectal and ovarian cancer, respectively [Malonne and Atassi, 1997].

Topotecan is a semisynthetic derivative of camptothecin with a basis \( N, N \)-dimethylaminomethyl functional group at C-9 that confers water solubility to the molecule. Irinotecan is a water-soluble prodrug designed to facilitate parental administration of the potent \( \gamma \)-ethyl-19-hydroxy analogue of camptothecin (SN-38). During the catalytic cycle, topoisomerase I binds covalently to double-stranded DNA through a reversible trans-esterification reaction. The trans-esterification reaction leads to the formation of covalent binding between topoisomerase I and DNA (cleavable complex) [Pommier, 2006]. Camptothecins cause DNA damage by stabilizing the covalent topoisomerase I-DNA complex, thus preventing relegation [Hsiang et al., 1985].

![Chemical structures of irinotecan (21), topotecan (22), and camptothecin (20)](image)

The most widely recognized mechanisms of resistance to camptothecins are ATP transporters such as P-gp, MRP and especially BCRP, which are responsible for the cellular efflux of topotecan and irinotecan from tumor cells. Prolongation in the duration of the cell cycle has been associated with the resistance to camptothecins, presumably by reducing the proportion of cells in S phase at any given time [Xu and Villanova-Calero, 2002].

Topotecan is found clinically effective in patients with epithelial ovarian cancer and small cell lung cancer as a second line treatment. The dose-limiting toxicity for topotecan is
neutropenia, with or without thrombocytopenia [Creemers et al., 1996]. Irinotecan acts as first- and second-line treatment for metastatic colorectal cancer [Board and Valle, 2007]. Encouraging results have also been reported in other types of solid tumors (e.g. small cell and non-small cell lung cancer, cervical, ovarian cancers). The dose-limiting toxicities are delayed diarrhea and neutropenia [Hartmann and Lipp, 2006]. A cholinergic syndrome resulting from inhibition of acetyl-cholinesterase activity by irinotecan also frequently occurs within the first 24h after dosing [Hyatt et al., 2005].

Today several synthetic camptothecin analogues are in various stages of clinical evaluation (e.g. iurotecan, exatecan, mesylate, karenitecin, gimatecan). They present some advantages compared to classical semisynthetic camptotecins. In particular, some of these are not a substrate for P-gp (gimatecan, exatecan) [Hartmann and Lipp, 2006; Teicher, 2008], and for the breast cancer resistance protein (BCPR) (gimatecan) [Teicher, 2008]; karenirecin is a very lipophilic compound that might show potential clinical advantages by virtue of its increased lactone stability and enhanced oral bioavailability [Garcia-Carbonero and Supko, 2002]. Exatecan seems to have better aqueous solubility, tumor efficacy and lesser toxic effects compared to captothecin and other derivatives and these agents are currently in phase I-II trials.

1.3.2.2. Topoisomerase II inhibitors
1.3.2.2.1. Podophyllotoxins

Podophyllotoxins are extracted from the roots of podophyllum species, namely, podophyllum peltatum Linnaeus and podophyllum emodi Wallich. Podophyllotoxin was isolated in 1880s, and their structure was elucidated in 1950s. Epipodophyllotoxin is an isomer of podophyllotoxin. Two clinically active semi-synthetic analogs generated from epipodophyllotoxin are etoposide (23) and teniposide (24) [McLeod, 2005].
Etoposide and teniposide are similar in their action and in their spectrum of human tumor activity. Etoposide and teniposide form a ternary complex with topoisomerase II and DNA and prevent resealing of the DNA break. During the presence of epipodophyllotoxins, the topoisomerase II-DNA intermediate cannot be reversed, resulting in DNA double strand-breaks leading to cell death [Watt and Hickson, 1994; Lowe et al., 1993].

Mechanism of resistance to both epipodophyllotoxins is associated with membrane efflux pumps, including P-gp. Other mechanisms of resistance due to mutations or decreased expression of topoisomerase II have been described in epipodophyllotoxin-resistant cells [McLeod, 2005].

Etoposide is indicated for the treatment of lung cancer, choriocarcinoma, ovarian and testicular cancers, lymphomas and acute myeloid leukaemia. Teniposide is approved for central nervous system tumors, malignant lymphoma, and bladder cancer. Myelosuppression represents the total negative impact etoposide; leucopenia is a dose-limiting toxicity while thrombocytopenia occurs less often and is usually not serious. The gastrointestinal toxicities (nausea, vomiting, stomatitis, and mucositis) occur in about 15% of patients with IV etoposide and in about 55% were treated with oral etoposide. Hair loss is a common, but
reversible and hypersensitivity, in response to both drugs has been met [Hartmann and Lipp, 2006].

1.3.2.2.2. Anthracyclines

The anthracyclines are among the most used antitumor antibiotics in the clinic and exert antitumor activity mainly by inhibiting topoisomerase II [Binasci et al., 2000]. Daunorubicin or daunomycin (25) (daunomycin cerubidine) is chemotherapeutic of the anthracycline family that is given as a treatment for some types of cancer. It was initially isolated from Streptomyces peucetius. It is most commonly used to treat specific types of leukaemia [acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL)]. A liposomal formulation of daunorubicin is marketed in the United States as DaunoXome. In addition to its major use in treating AML, daunorubicin is also used to treat neuroblastoma. Daunorubicin has been used with other chemotherapeutic agents to treat the blastic phase of chronic myelogenous leukemia [Jermias et al., 1998].

Doxorubicin or hydroxydaunorubicin (26) an anthracycline antibiotic, closely related to the natural product daunomycin [Minottin et al., 2004]. It was obtained from a strain of Streptomyces, which was mutated using N-nitroso-N-methyl urethane, and this new strain produced a different red-colored antibiotic, doxorubicin. Doxorubicin showed better activity than daunorubicin against murine tumors, and especially solid tumors. It also showed a higher therapeutic index, yet the cardiotoxicity remained as major side effect [Khan et al., 2005]. To overcome this side effect, doxorubicin is formulated in liposome-encapsulated forms as Doxil and Myocet [Riggiio, 2011].

After daunorubicin and doxorubicin, a series of semisynthetic compounds (e.g. idarubicin (27), epirubicin (28)) followed and entered clinical use. Today, a new series analogues fully synthesized anthracycline are in the advanced phases of clinical studies (e.g. sabarubicin, MEN 10755, nemorubicin). Several hundred structural analogues have been obtained by synthetic modification of daunorubicin and doxorubicin.
All anthracyclines share a quinone containing rigid planar aromatic ring structure (the chromophore) bound by an O-glycosidic bond to an amnio sugar which can readily participate in oxidation-reduction reactions that ultimately generate highly reactive chemical species thought to be responsible for anthracylic-induced cardiotoxicity. Thus small modifications, such as the different orientation of the C-4 hydroxyl group on the sugar in epirubicin compared to doxorubicin are able to reduce cardiotoxicity, preserving the anticancer activity [Cortes-Funes and Coronado, 2007; Robert, 2005].
All anthracyclines are substrates for P-gp mediated drug efflux pump and the overexpression of P-gp represents a major mechanism of cellular resistance to these drugs [Nobili et al., 2006]. Also MRP causes resistance to anthracyclines [Cole et al., 1992]. Drug resistance may also be due to gene mutation or down-regulation of topoisomerase II [Friche et al., 1989]. The side effects of doxorubicin and daunorubicin include bone marrow depression, stomatitis, alopecia and gastrointestinal and dermatological toxicity.

Cardiac toxicity is a peculiar adverse effect observed with these agents. It is characterized by myocardial dysfunction and congestive heart failure. Epirubicin and idarubicin, that have been developed to improve therapeutic and pharmacological properties of the natural compounds, show reduced cardiotoxic effects [Gharib and Burnett, 2002].

1.4. Other natural anticancer compounds
1.4.1. From plant sources

Other examples of plant-derived compounds currently under investigation are flavopiridol, homoharringtonine, 4-ipomeanol, β-lapachone, combrestatin A4.

Flavopiridol (29) (also known as Alvocidib or HMR-1275) is a synthetic N-methylpiperidinyl chlorophenyl flavones derived from the plant alkaloid rohitukine, which was isolated from the leaves and stems of Amoora rohituka and later from Dysoxylum binectariferum [Wang, 2001]. The mechanism of action of flavopiridol involves interfering with the phosphorylation of cyclin-dependent kinases, hampering their activation and blocking cell-cycle progression at the G1/S and G2/M transitions [Blagosklonny, 2004]. This agent is also a competitive inhibitor of adenosine triphosphate activity. In phase I clinical trials with flavopiridol, encouraging response rates were noted in a variety of solid and hematological malignancies and secretory diarrhea was found to be the dose-limiting toxicity. These results led to the initiation of phase II trials in patients with colorectal, prostate, renal cell and non-small-cell lung carcinoma, as well non-Hodgkin's lymphoma and chronic lymphocytic leukemia [Senderowicz et al., 1998]. Administration of flavopiridol after or
concomitant with antineoplastic agents, including mitomycin C (Mutamycin), paclitaxel, gemcitabine (Gemzar), SN-38 (the active metabolite of CPT-11), imatinib, mesylate (Gleevec), and doxorubicin can promote chemotherapy-induced apoptosis [Wright et al., 1998].

Homoharringtonine (30) (also known as Omacetaxine mepesuccinate) is an alkaloid isolated from the Chinese tree *Cephalotaxus harringtonia* (Cephalotaxaceae) [Powell et al., 1970], and has shown efficacy against various hematological cancers [Kantarjian et al., 1996]. In June 2009, results of a long-term open label Phase II study were published, which investigated the use of omacetaxine infusions in chronic myelogenous leukemia (CML) patients. After twelve months of treatment, about one third of patients showed a cytogenetic response [Li et al., 2009]. A study in patients who had failed imatinib and who had the drug resistant T315I mutation achieved cytogenetic response in 28% of patients and hematological response in 80% of patients, according to preliminary data [Quintás-Cardama et al., 2009].

The agent is currently in phase II-III clinical trials. The principal mechanism of action of homoharringtonine is the inhibition of protein synthesis, blocking cell-cycle progression [Zhou et al., 1995]. It has a different point of action than tyrosine kinase inhibitors like imatinib, and has potential therapeutic advantages for patients who have developed resistance to tyrosine kinase inhibitor therapy [Cortes et al., 2012].
4-ipomeanol (31) is a pneumotoxic furan derivative isolated from the sweet potato *Ipomoea batatas* (Convolvulaceae) and has been under clinical evaluation as a lung-cancer-specific antineoplastic agent [Rowinsky *et al.*, 1993]. This compound is converted into DNA-binding metabolites upon metabolic activation by cytochrome P450 enzymes that are present in cells of the lung [Rehm and Devor, 1993].

β-lapachone (32) is quinine obtained from the bark of the lapacho tree (*Tabebuia avellanedae*). It is a DNA topoisomerase I inhibitor that induces cell-cycle delay at G1 or S (synthesis) phase before inducing either apoptosis or necrotic cell death in a variety of human carcinoma cells, including ovary, colon, lung, prostate and breast [Li *et al.*, 1999]. It is currently investigated in phase I-II study [http://www.clinicaltrials.gov].

![Chemical structures](image-url)
Comretastatin A4 (33), a stilbenoid phenol, isolated from the bark of the African tree Combretum caffreum, with vascular disrupting and antineoplastic activities. Combretastatin targets and binds to the colchicine-binding site of tubulin, thereby impairs the polymerization of tubulin dimers and prevents the formation of microtubules in the endothelial cells of tumor. As a result, this may eventually lead to a destruction of the tumor vasculature, disruption of tumor blood flow and tumor cell necrosis [Pettit et al., 1995]. Phase I trials have shown some clinical activity of comretastatin A4 and a favourable toxicological profile [Banerjee et al., 2008].

1.4.2. From microbial sources

Microorganism’s derived new compounds include rapamycin, geldanamycin, wortmannin and L-asparaginase. The immunosuppressant rapamycin (34) (sirolimus) was originally discovered at Wyeth-Ayerst Pharmaceuticals during screening for antifungal agents [Vezina et al., 1975], and was later found to have potent immunosuppressive and antineoplastic properties [Eng et al., 1984]. Rapamycin and its analogs are macrolide compounds obtained from Streptomyces hygroscopicus and inhibit signaling pathways required for T-cell activation and proliferation. Rapamycin acts as a specific inhibitor of m-TOR (mammalian target of rapamycin) that is a downstream mediator of PI3K/PKB, and blocks progression of the cell cycle at middle-to-late G1 phase in T cells and B cells thus selectively blocks transcription activation, leading to tumor cell growth and division [Mita et al., 2003]. These agents are currently in phase I-II studies [http://www.clinicaltrials.gov].

Geldanamycin (35), an analogue of rapamycin, is a benzoquinone ansamycin natural fermentation product that was originally thought to be a direct protein tyrosine kinase inhibitor. However, subsequent studies have revealed that geldanamycin binds to, and inhibits the 90 kDa heat-shock protein-90 (HSP 90) [Schulte and Neckers, 1998]. This agent is currently in phase I-II studies [http://www.clinicaltrials.gov].
Wortmannin (36) is a product of the fungus *Talaromyces wortmanni* and inhibits signal transduction pathways by forming a covalent complex with an active-site residue of phosphoinositide-3-kinase (PI3K), inhibiting PI3K activity [Ferby et al., 1996] and was shown to have detrimental influence on memory and impair spatial learning abilities [Mizuno, 2003]. The anti-cancer properties of the bacterial enzyme L-asparaginase were discovered more than 50 years ago, and since then, L-asparaginase, have been used to treat a variety of lymphoproliferative disorders and lymphomas, in particular lymphoblastic leukaemia, in combination with other anticancer drugs.
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1.4.3. From marine sources

Marine compounds that have reached clinical investigation are trabectedin (37) (also known as ecteinascidin 743 or ET-743) is a tetrahydroisoquinoline alkaloid obtained from sea squirt *Ecteinascidia turbinata*. ET-743 has shown potent antitumor activity in preclinical studies both *in vitro* and *in vivo* on several solid tumors, including ovarian and breast cancer, melanoma, and sarcoma. These preclinical data have been confirmed in several phase II trials in breast and ovarian carcinoma, as well as soft-tissue sarcomas. The biological mechanism of action is believed to involve the production of superoxide near the DNA strand, resulting in DNA backbone cleavage and cell apoptosis. The actual mechanism is not yet known, but is believed to proceed from reduction of molecular oxygen into superoxide via an unusual auto-redox reaction on a hydroxyquinone moiety of the compound. There is also some speculation the compound becomes 'activated' into its reactive oxazolidine form [Cassier *et al.*, 2008]. Various and conflicting reports about whether trabectedin is a substrate for P-gp have been published [Nobili *et al.*, 2006]. The most frequent toxic side effects were neutropenia and
thrombocytopenia. The most common non-haematological side effects were nausea and vomiting, fatigue and biochemical hepatic toxicity [Cassier et al., 2008].

Bryostatins (38) are a group of macrolide lactones isolated from extracts of a species of bryozoan, Bugula neritina. To date 20 different bryostatins have been isolated and these are potent modulators of protein kinase C (PKC) activity, and acts synergistically in combination with other anti-cancer drugs. Drug combination was effective against a large variety of tumor cells including lung, prostate and non-Hodgkin's lymphoma tumor cells. They are currently under investigation as anti-cancer agents and as a memory enhancing agent.

Kahalide F (39) is a cyclodepsipeptide isolated from the marine mollusc Elysia rufescens [Hamann, 1993]. Like other kahalalides, it is probably a secondary metabolite synthesized by the mollusk from peptides produced by a diet of the green algae Bryopsis pennata. KF has potent cytotoxic activity in vitro against cell lines from solid tumors including prostate, breast and colon carcinomas, neuroblastoma, chondrosarcoma, and osteosarcoma [Jimeno et al., 1996; Faircloth et al., 2000; Faircloth et al., 2001; Shao et al., 2001]. The mechanism of action of Kahalide F is not well understood, however, preliminary
evidence suggests specific integrations with lysosomal membranes or proteins [Garcia-Rocha et al., 1996].

The agent is under evaluation in phase I-II studies for treatment of solid tumors [http://www.clinicaltrials.gov]. Didemnin B (40) is a cyclic depsipeptide compounds isolated from the Caribbean tunicate Tridemnum solidum (Didemnidae) acts by interrupting protein synthesis in target cells by binding non-competitively to palmitoyl protein thioesterase [Simmons et al., 2005]. This agent is currently in phase I-II trials [http://www.clinicaltrials.gov].

Similarly, Aplidin (41) (Dehydrodidemnin B) a closely related natural product isolated from a different tunicate species characterized by delayed neuromuscular toxicity that requires careful follow-up but displays promising anti-tumor activity and is currently in phase I-II trials.

Squalamine (42) is a strong naturally derived broad-spectrum antibiotic that is predominantly derived from the livers of dogfish shark Squalus acanthias. It has shown antiangiogenic activity. Because of this function, squalamine lactate is in the process of being tested as a treatment of fibrodysplasia ossificans progressiva, a rare disease where
connective tissue will ossify when damaged. Target of squalamine is the phospholipid bilayer by inhibition of the sodium hydrogen antiporter sodium-proton exchangers. It is currently evaluated in phase II studies [http://www.clinicaltrials.gov].

Neovastat (43) (AE-941) is a derivative of shark cartilage extract having antiangiogenic and anti-tumor properties. AE-941 inhibits the binding of VEGF (vascular endothelial growth factor) to its receptors [Simmons et al., 2005; Singh et al., 2008]. Normally, when VEGF is secreted by tumors it binds to target endothelial receptors and directs the profusion of new capillaries to supply the tumor with nourishment. By blocking the receptor sites, AE-941 preempts the formation of the new blood supply the growing tumor needs to sustain itself. One serious adverse effect was reported, namely a hypoglycemic episode in a known II type diabetic patient. It is now in Phase II-III trials in several countries for renal cell carcinoma, non-small-cell lung cancer and multiple myeloma [http://www.clinicaltrials.gov]. LAF 389 (44), a synthetic analogue of bengamide B, a natural marine product isolated from the Jaspis sponge (Jaspis digonoxea) of the coral reefs near the Fiji Islands and Australia. Target of LAF389 is the methionine aminopeptidase. LAF389 has been studied in phase I trial [http://www.clinicaltrials.gov].
1.5. **Chemopreventive compounds from natural sources**

Chemoprevention is a capable anticancer approach aimed at reducing the morbidity and mortality of cancer by delaying the process of carcinogenesis. Natural products and their isolated constituents have been shown to possess strong chemopreventive activity in animal models. Their effect on apoptotic pathways, signaling pathways, and/or different targets in cancer means that they could be helpful in design and development of novel anti-cancer agents. Many natural dietary compounds have been isolated and have demonstrated health-promoting properties.

1.5.1. **Tea polyphenols**

Tea is one of the most widely consumed beverages produced from leaves of *Camelia sinensis* (Theaceae) and is rich in constituents with antioxidant properties [Cabrera et al., 2006]. Various processing methods produce different kinds of tea. More than 300 different kinds of tea are produced by a variety of manufacturing processes. They are usually divided into three types: green tea (non-fermented), oolong tea (semi-fermented), and black tea (fermented). Among them, black tea and green tea have been studied for their potential
chemopreventive agents, green tea, showed a higher perspective and be more effective against different types of cancer. Experimental and epidemiological studies have linked tea consumption reduces the risk of cancer. These effects were attributed to the polyphenolic compounds in tea [Bushman, 1998]. Catechins are the most abundant polyphenols in green tea. A typical cup of brewed green tea contains, by dry weight, 30-40% catechins, including epigallocatechin-3-gallate (EGCG) (45), epicatechin-3-gallate (ECG) (46), epigallocatechin (EGC) (47) and epicatechin (EC) (48). Epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea and has received the most attention with respect to antitumor activity [Carlson et al., 2007]. Several studies have shown green tea polyphenols preventive and inhibitory effect on tumor formation and growth.

![Diagram of EGCG and ECG](image)

Although, studies are not conclusive, green tea polyphenols, especially EGCG, can be effective in preventing prostate cancer, breast, esophagus, stomach, pancreas and colon cancer [Katiyar et al., 1997]. There is also some evidence that green tea polyphenols may be chemopreventive, or inhibiting the lungs, skin, liver cancer [Lee et al., 1997; Picard, 1996; Hirose et al., 1993]. bladder, ovarian tumors [Sato. 1999; Sugiyama and Sadzuka. 1998]. leukemia [Otsuka et al., 1998], and oral leukoplakia [Khafif et al., 1998 (a)].
Epidemiological studies conducted in different countries and in many studies in animal models have shown promising results in green tea and its components in reducing the risk of human cancer at various sites of the body [Zaveri, 2006]. In xenograft models, green tea polyphenols inhibits tumor growth and suppressed metastasis of metastasis-specific mammary carcinoma 4T1 cells [Baliga et al., 2005] and a decrease in tumor blood vessels in the estrogen receptor-negative breast cancer [Sartippour et al., 2001]. GTP extract reduces the risk of carcinogenesis of the colon after azoxymethane insult in rats [Xiao et al., 2008]. In a case-control study at the Mayo Clinic in patients with chronic lymphocytic leukemia (CLL) and other low-grade lymphoma, which used over-the-counter products containing tea polyphenols showed that four of these patients had clinical signs of the benefits of these products. Based on these findings, the Mayo Clinic started the National Cancer Institute-sponsored phase I/II clinical trials of green tea without caffeine in patients with asymptomatic, early stage CLL [Shanafelt et al., 2006]. Several studies of healthy volunteers were also conducted to identify the main patterns of bio-distribution, pharmacokinetics, and preliminary safety profiles for short-term oral administration of various green tea preparations [Yang et al., 1998; Chow et al., 2001; Chow et al., 2003].

Green tea is generally considered safe, non-toxic beverage and consumption is usually without side effects. The average cup of green tea contains from 10-50 mg of caffeine, and
excessive-consumption can cause irritability, insomnia, nervousness, and tachycardia. A phase I study have shown that up to 1 g green tea solids (equivalent to approximately 900 ml of green tea) can be safely consumed by patients with solid tumors. Because studies on its possible teratogenic effect are inconclusive, caffeine consumption is contraindicated during pregnancy. Lactating women should limit caffeine intake to avoid sleep disorders in infants [DerMarderosian, 1999].

To date, many studies on the anticancer properties of EGCG showed its chemopreventive effects. As with other phytochemicals, EGCG modulates different signaling pathways responsible for its chemopreventive and potential chemotherapeutic activity against cancer cells. EGCG, like other flavonoids, is the prototype of phytochemicals with multiple effects on intra-cellular signaling [Khan et al., 2006]. The anticarcinogenic properties of green tea polyphenols, mainly EGCG, are likely due to inhibition of tumor initiation and promotion, induction of apoptosis and inhibition of cell replication rates, thus slowing the growth and development of neoplasms [Nihal, 1999; Ahmad et al, 1997]. Antioxidant potential of green tea polyphenols is directly related to a combination of aromatic rings and hydroxyl groups that make up the structure, resulting in binding and neutralizing free radicals by the hydroxyl groups. In addition, green tea polyphenols stimulate the activity of hepatic detoxification enzymes, thereby promoting detoxification of xenobiotics, and are also capable of chelating metal ions, such as iron, that can generate radical oxygen species [Serafini et al., 1996; Erba et al., 1999]. Its antioxidant effects in protecting against oxidative damage of DNA, lipids and proteins, potentially associated with cancer development, have been largely investigated. However, this problem is partly contradictory. In fact, EGCG significantly reduces plasma levels of oxidative biomarkers in animal models, but limited efficacy has been proven in humans [Frei and Higdon, 2003, Rietveld and Wiseman, 2003]. In addition, ROS production in cell culture medium by EGCG promoted the DNA damage in rodent macrophage RAW 264.7 and human promyelocytic leukemic HL-60 cell lines. These pro-oxidant effects of the molecule are dose dependent, as human lymphocytes treated with low
concentration (from $10^{-8}$ to $10^{-4}$ M) are protected against DNA strand breakage, whereas the opposite occurs with high doses of EGCG (10$^{-3}$M) [Kanadzu et al., 2006]. This study is another example of conflicting results, when *in vitro* and *in vivo* studies related to natural phytochemicals were compared. In this regard, a recent review suggested a causal relationship between green tea and liver damage. The hepatotoxicity is likely to be attributed to EGCG or its metabolites which, under certain conditions related to the patient's metabolism can cause oxidative stress in the liver [Mazzanti et al., 2009].

EGCG was also found to synergistically increase the effectiveness of other drugs in cell culture and in animals. Studies have shown that EGCG synergistically increased the effectiveness of erlotinib in head and neck cancer models, and can "resensitize" erlotinib-resistant lung cancer cells to erlotinib [Zhang et al., 2008]. Thus, phase I/II trial was open to explore the possibility that, together with GTP erlotinib is more effective than erlotinib only as second-line treatment approach for patients with NSCLC [http://www.clinicaltrials.gov].

On the other hand, a recent study identified adverse effects of EGCG, as an antagonist of bortezomib, a new inhibitor of proteasome in clinical practice for multiple myeloma. This shown that green tea polyphenols may have the ability to block the therapeutic efficacy of certain anti-cancer drugs and suggest that consumption of green tea products may be contraindicated for the treatment of cancer with bortezomib [Golden et al., 2009]. Commenting on the clinical and preclinical studies based on the administration of EGCG, it is necessary to underline that a clear cause-effect relationship between amounts of green tea supplemented and the protective effects have not been established. Encouraged by the results of *in vitro* and *in vivo* models, several clinical studies, now with green tea, alone or in combination with other drugs are under pipeline.

### 1.5.2. Curcumin

Curcumin (49), the hydrophobic polyphenol derived from the rhizomes of *Curcuma longa* plants, is a major yellow pigment present in turmeric, has a wide spectrum of
biological and pharmacological activities [Chattopadhyay et al., 2004]. The medicinal use of this plant has been described in Ayurveda for thousands of years; it was not until the 1980s that curcumin has attracted much attention because of its anti-cancer properties. The anticancer properties of curcumin come from several studies have shown that molecule can induce apoptosis in various cancer cell lines and prevents the formation of tumors in animal models of carcinogenesis [Kunnumakkara et al., 2008].

![Curcumin structure](image)

Curcumin has shown anti-proliferative effect in various cancers, and is an inhibitor of the transcription factor NF-κB and downstream gene products (including c-myc, Bcl-2, COX-2, NOS, Cyclin D1, TNF-a, interleukins and MMP-9) [Jurenka, 2009; Jobin et al., 1999; Plummer et al., 1999; Barnes and Karin, 1997; Wertz et al., 2004]. In addition, curcumin acts on different growth factor receptors and cell adhesion molecules involved in tumor growth, angiogenesis and metastasis [Kunnumakkara et al., 2008]. Curcumin is also reported to reveal synergistic chemopreventive effects with other diet-derived polyphenols, such as genistein [Verma et al., 1997], green tea [Khafif et al., 1998 (b)], and embelin [Sreepriya et al., 2006], and increased the efficacy of many anti-cancer drugs including fluorouracil [Koo et al., 2004], vinca alkaloid [Kunnumakkara et al., 2007], vinorelbine, and gemcitabine [Sen et al., 2005]. Because of these promising in vitro and in vivo results, curcumin has been was brought to clinical trials. A large number of clinical trials have been performed to study the pharmacokinetics, safety and efficacy of curcumin, to show its
therapeutic potential in relation to various cancers, including leukemia and lymphoma, gastrointestinal cancers, genitourinary cancer, breast cancer, pancreatic cancer, lung cancer, melanoma, neurological cancer and others [Kunnumakkara et al., 2008; Aggarwal et al., 2003]. In a pilot study, 100% of patients showed a decreased polyp number and size of an average of 6 months of treatment curcumin and quercetin [Cruz-Correa et al., 2006]. Another phase I clinical trial conducted in patients with high risk or premalignant lesions have shown that curcumin is safe up to 8 g/day [Cheng et al., 2001]. A pharmacokinetic and pharmacodynamic study of oral curcumin extract was conducted in patients with colorectal cancer [Sharma et al., 2001]. The anticancer properties of curcumin are reinforced by the observation that, in many cases, the molecule kills tumor cells without harming normal cells [Syng-Ai et al., 2004]. However, other suggests that curcumin may lead to toxicity under certain conditions.

One possible mechanism is that low concentrations of curcumin induce antioxidant effect, high concentrations of this compound to increase the cellular ROS [Burgos-Moron et al., 2009; Lopez-Lazaro, 2008]. The main problem of curcumin as a therapeutic agent remains its bioavailability. The low plasma and tissue level of curcumin seems due to poor absorption, rapid metabolism, and rapid systemic elimination. To improve the bioavailability of curcumin, various approached have been evaluated: (I) together with an adjuvant like piperine that prevents glucuronidation, (II) liposomal curcumin, (III) curcumin nanoparticles, (IV) curcumin phospholipids complex, (V) structural analogs of curcumin [Anand et al., 2007]. Several phase I and II clinical trials are currently being implemented in several centers for the study of the effectiveness of chemoprevention of curcumin.

1.5.3. Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) (50) is a natural occurring phytoalexin, attracted much attention due to the abundance in grapes and grape products such as wine, long time component of the diet with a variety of bioactivities associated with health promotion
Resveratrol has been found to suppress tumor initiation, promotion, and progression in vitro and reduce tumor incidence and multiplicity of skin through topical application in mice in vivo [Jang et al., 1997].

Topical application of resveratrol in mice, both before and after UVB exposure, inhibited skin damage and decreased skin cancer incidence [Athar et al., 2007]. Resveratrol prevents the development of DMBA-induced mammary carcinogenesis, inhibited the growth of MDA-MB-231 xenografts, and suppressed the progression of prostate cancer in transgenic adenocarcinoma of the mouse prostate mice [Banerjee et al., 2002; Garvin et al., 2006].

Prophylactic use of resveratrol reduced the number and size of esophageal, intestinal, and colon cancers [Athar et al., 2007; Li et al., 2002]. In preclinical studies, resveratrol is also effective against a number of other cancers, including liver, lung, gastric, pancreatic, thyroid, head, neck cancers, ovarian, endometrial tumor, myeloid leukemia and B-cell lymphoma [Khan et al., 2008; Ma et al., 2007; Sun et al., 2002; Carbo et al., 1999; Ding and Adrian, 2002; Kubota et al., 2003]. Resveratrol also exhibited synergistic anti-tumor activity with FU in a marine model of liver cancer [Wu et al., 2004].

The growth inhibitory effects of resveratrol are mediated by induction of apoptosis and cell cycle arrest. In vitro, resveratrol and its analogs cause multiple pathways leading to cell growth arrest. Several molecular targets have been identified in the cell line to support the
anti-tumor activity of resveratrol, including activation of the second phase of detoxification, antioxidant enzymes, inhibition of pro-inflammatory mediators, cell cycle regulation, and activation of pro-apoptotic factors and deactivation of anti-apoptotic factors [Russo et al., 2007]. In some, but not all, cellular models, resveratrol causes cell cycle arrest by up-regulation of p21, p27, p16, and down-regulation of cyclin D1, cyclin E, Cdk2, Cdk4, and Cdk7 in human colon carcinoma cells [Aggarwal and Shishodia, 2006]. Resveratrol has also shown to induce GSH synthesis through activation of Nrf2 [Kode et al., 2008]. In addition, resveratrol induced S phase arrest through the ATM/ATR-Chk1/2-Cdc25C pathway [Tyagi et al., 2005].

As noted by Russo, in vivo bioavailability of the molecule can explain the contradictory data on the mechanism of action of resveratrol. In fact, dietary resveratrol (up to 25 mg) is rapidly absorbed and predominantly present in the plasma as glucuronide and sulfate conjugates [Russo, 2007]. In addition, when administration in food, such as wine or grape juice, resveratrol metabolism is significantly inhibited by other polyphenols because of competitive interactions with metabolizing phase II enzymes, which leads to increased concentration of the free form.

Despite this, the aglycone is almost undetectable in human plasma [Russo, 2007; Wenzel and Somoza, 2005]. Therefore, caution is advised when interpreting the extensive literature on anticancer activity of resveratrol, only on the basis of in vitro studies on cell lines where the molecule is given at pharmacological concentrations (25-50 μM or higher), as aglycone, a form is virtually absent in plasma and urine [Russo, 2007]. Researchers agree on the conclusion that, despite numerous reports describing the pharmacokinetics of resveratrol in animal models systems, there are few similar studies in human, and most of these data regards the administration of pure or dietary resveratrol in healthy individuals to assess its bioavailability in regard of chemopreventive use of the molecule, more than its therapeutic use in cancer patients [Aggarwal et al., 2004; Cucciolla et al., 2007; Bishayee, 2009].
Piceatannol \((51)\), a natural analog of resveratrol, is observed to prevent the proliferation of cancer cell lines via apoptosis and cell cycle arrest [Wolter et al., 2002; Larrosa et al., 2004]. Potter et al., [2002] suggested that the anti-proliferative effects of resveratrol on cancer cells is the result of metabolic conversion of resveratrol into piceatannol by cytochrome P450 1B1 (CYP1B1). CYP1B1 is highly expressed in cancerous tissue of the breast, colon, lung, esophagus, skin, lymph node, brain, and testis, but not in normal tissue [Murray et al., 1997]. The molecular mechanisms associated with anti-proliferative effects in cancer cells are associated with activation of p53 [Huang, et al., 1999] and the suppression of NF-κB and AP-1 [Kundu and Surh, 2004]. A number of clinical trials to study the chemopreventive potential of resveratrol or development with improved bioavailability (eg, SRT501 of Sirtris Pharmaceuticals) are currently ongoing.

1.5.4. Lycopene

Lycopene \((52)\) is the most abundant carotenoid in tomato *Lycopersicon esculentum* L. (Solanaceae) that imparts red color to tomatoes, guava, rosehip, watermelon, and pink grapefruit [Giovannucci, 1999]. Because of its strong antioxidant properties, lycopene has drawn much attention as a cancer preventing agent. A lower risk of a variety of cancers has been inversely associated with high intake of lycopene-containing products. Numerous studies have suggested reduced risk of prostate cancer from the consumption of processed tomato products [Srinivasan et al., 2007].
Initial evidence suggests that tomato products may help to prevent disease progression in benign prostate hyperplasia, and increases apoptosis in benign prostate hyperplasia and carcinoma [Schwarz et al., 2008; Kim et al., 2003]. Several reports have been published on the antiproliferative and pro-apoptotic activity of lycopene on cell lines derived from human prostate cancer [van Breemen and Pajkovic, 2008; Syed et al., 2003]. At non physiological concentration, lycopene triggers apoptosis activating the intrinsic pathway involving mitochondrial function, cytochrome c release and exposure to Annexin V. The antiproliferative activity of lycopene is mostly identified with the ability of the molecule to block the cell cycle at G0/G1 phase. This effect has been observed in prostate cancer, hepatocyte, and breast cancer cell lines [van Breemen and Pajkovic, 2008]. However, a recent study employed various different cancerous and noncancerous cell types, treated with lycopene at different concentrations for different times, indicated that lycopene, at the physiological range does not significantly affect cell proliferation, suggesting more careful investigations [Burgess et al., 2008].

Despite a lot of controversial data in animal model, the consistent number of epidemiological studies showing an inverse relationship between tomato/lycopene intake and risk of incurring in several types of cancer [Giovannucci, 2002], stimulated an investigation from FDA for qualified health claims regarding tomatoes, lycopene and cancer risk. The FDA found no credible evidence to support an association between lycopene intake and a reduced risk of prostate, lung, and colorectal, gastric, breast, ovarian, endometrial, or
pancreatic cancer. The FDA also found no credible evidence for an association between tomato consumption and a reduced risk of lung, colorectal, breast, cervical, or endometrial cancer. The FDA found very limited evidence to support an association between tomato consumption and reduced risks of prostate, ovarian, gastric, and pancreatic cancers [Kavanaugh et al., 2007]. Several phase I and II clinical trials are ongoing worldwide. A phase II randomized clinical trial of lycopene supplementation before radical prostatectomy suggested that lycopene supplementation may decrease the prostate cancer growth. Another phase II trial suggested that the combination of lycopene with soy isoflavones more strongly stabilized serum prostate-specific antigen (PSA) levels than lycopene alone in men with prostate cancer [http://www.clinicaltrials.gov]. Usually, these products are currently available to the market, as herb and vitamin supplements, not regulated by the FDA. Each ingredient has been studied in prostate cancer; no serious side effects have been reported. Result of these studies will hopefully clarify the potential pharmacological use of lycopene in cancer therapy.

1.5.5. Genistein

Genistein (4',5,7-trihydroxyisoflavone) (53), is a phytoestrogen abundant in soybeans and other kinds of legumes. Genistein has been found to be effective scavengers of superoxide and peroxynitrite radicals generated from enzymatic and non-enzymatic systems. Furthermore, several studies have revealed that genistein exhibit protective effect against DNA damage caused by ROS and RNS [Raschke et al., 2006]. Multiple lines of compelling evidence from a number of epidemiological studies support an inverse correlation between dietary soy consumption and the risk of prostate [Hebert et al., 1998], breast [Adlercreutz et al., 1991; Ziegler et al., 1993], and endometrial [Goodman et al., 1997] cancer. The consumption of dietary genistein decreased tumor multiplicity and diminished the incidence of adenocarcinoma in the DMBA-induced mammary cancer in rats [Hilakivi-Clarke et al., 1999].
Genistein inhibits both angiogenesis and the proliferation of endothelial cells in vivo. In addition, genistein inhibits the invasion of MCF-7 and MDA-MB-231 breast carcinoma cells via the down-regulation of MMP-9 and up-regulation of the tissue inhibitor of MMP-1 both in vitro and in vivo. The soybean isoflavone mixture consisting of 74% genistein and 21% daidzein was found to inhibit DMBA-induced adenocarcinoma in the prostate and seminal vesicles in rats [Onozawa et al., 1999] and also prevents the TPA-induced inhibition of Gap-junction intracellular communication. Genistein effectively suppresses the COX-2 promoter activity with and without tumor growth factor-α-stimulation in DLD-1 colon cancer cells. TPA-induced NF-κB DNA binding and the NF-κB-regulated COX-2 promoter activity [Li and Sarkar, 2002], as well as COX-2 expression, are inhibited in human lung epithelial cells by genistein [Mutoh et al., 2000]. Genistein inhibited PCa cell growth in culture by inducing G2/M arrest and apoptosis, inhibits prostate-specific antigen expression in androgen-dependent and independent prostate cancer cell lines and in orthotopic and metastatic in vivo prostate cancer models. In addition, genistein triggers regulative pathways under the control of MAPK, PKB and NF-κB [Banerjee et al., 2008].

Standard soy isoflavone formula contains genistein mainly in the form of genistein (genistein monoglucosides), as well as other isoflavones (daidzin and glycitin). Pharmacological research has developed also synthetic genistein analogs [Ullmann et al., 2005]. The most representative are 1-bonistein and 2-phenoxodvol. 1-Bonistein is a pure synthetic genistein consisting of 99.4% synthetic genistein aglycone. 2-Phenoxodyol is a
synthetic isoflavone characterized by a more pronounced antineoplastic activity with reduced toxicity in cancer-derived cell lines and animal models [Silasi et al., 2009].

On the basis of these observations, several early clinical trials either with genistein or soy products have been completed. A pilot study conducted in patients with prostate cancer and rising serum PSA levels suggested that soy isoflavones may benefit some patient with prostate cancer. Another phase II trial was carried out in PSA-recurrent prostate cancer after previous local therapy, which showed a decrease in serum PSA level from 56% to 20% [Pendleton et al., 2008]. 1-Bonistein has been tested in healthy volunteers in phase I clinical trial to assess safety and tolerability. Tolerability of different doses (from 30 to 300 mg) was good and uptake was also very rapid, as revealed by plasma concentration-time course. 2-Phenoxodyol was tested in phase I/II clinical trial by continuous intravenous infusion in patient with solid cancer. Other clinical trials are ongoing to study the efficacy of soy products and genistein in cancer prevention [http://www.clinicaltrials.gov].

1.5.6. Quercetin

Quercetin (3,3',4',5,7-pentahydroxyflavone) (54) is one of the major antioxidative dietary flavonoid, found in a broad range of fruit, vegetables and beverage such as tea and wine that exhibits significantly inhibitory activity against lipid peroxidation and modulates phase I and phase II enzymes. The anti-oxidant, anti-inflammatory, anti-proliferative or apoptotic effects of the molecule have been largely analyzed in cell culture models [Lamson and Brignall, 2000]. Quercetin blocks the TPA-induced Gap-junction intracellular communication (GJIC) inhibition in rat liver epithelial cells, and protects against the inhibition of GJIC and the phosphorylation of Cx43 and ERK1/2 induced by hydrogen peroxide. Quercetin also inhibits LPS-induced prostaglandin E2 production both in vitro and in vivo, and attenuates prostaglandin E2 biosynthesis in A549 human lung adenocarcinoma cells. Quercetin has also been reported to induce apoptosis in a human breast cancer MDA-
MB-435 cell xenograft model and inhibits azoxymethane-induced colorectal carcinogenesis in F344 rats [Dechsupa et al., 2007].

![Image](image.png)

(54)

Quercetin acts as an anticancer agent by down-regulating the expression of oncogenes (H-ras, c-myc and K-ras) and anti-oncogenes (p53) [Ranelletti et al., 2000], or up-regulating cell cycle control protein (p21WAF1 and p27KIP1) [Casagrande and Darbon, 2001]. In addition, quercetin inhibits different tyrosine and serine–threonine kinases, whose activities are linked to survival pathways (MAPK and PKB) [Spencer et al., 2003]. In animal models, quercetin inhibits cancer growth and induces apoptosis [Mouria et al., 2002]. Consumption of quercetin in onions and apples was found to be inversely associated with lung cancer risk in Hawaii [Le Marchand, 2000]. The effect of onions was particularly strong against squamous cell carcinoma [Boyle et al., 2000].

On the other side, the potential risk associated to quercetin consumption at pharmacological doses has been reported. At 50 mM concentration, quercetin was able to reduce by 35–40% the DNA damage caused by an oxidative insult; however, at the same concentration, quercetin increases the DNA breaks of 4-fold, indicating a clear genotoxic damage [Collins, 2005]. Similarly, the hazard in quercetin administration has been described on cell lines [Rietjens et al., 2005] and animal models [Okamoto, 2005]. A phase I clinical trial indicated that the molecule can be safely administered and its plasma levels are sufficient to inhibit lymphocyte tyrosine kinase activity [http://www.clinicaltrials.gov].