Mohd Sayeed Akhtar
Mallappa Kumara Swamy *Editors*

Anticancer Plants: Clinical Trials and Nanotechnology

Volume 3

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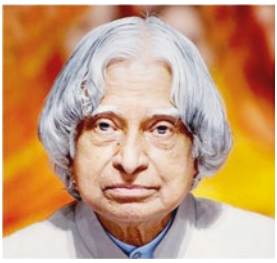

A. P. J. Abdul Kalam
(1931–2015)
A great personality, scientist, missile man, and former president of India

Foreword

Cancer is one of the major life-threatening diseases of the world's population. It is characterized by the formation of abnormal cells that proliferate uninhibitedly and often spread all over the body. About 100 different types of cancers have been categorized on the basis of the affected tissue or organ of the human body. As reported by the World Health Organization, cancer is the second leading cause of human mortality around the globe. Nearly 8.8 million deaths have been reported due to cancer in 2015, and more than 200,000 individuals are being diagnosed with various types of cancers every year. Some of the major causes of cancer include smoking, pollution, dietary imbalances, and chronic infections. Several billions of US dollars are being spent annually toward cancer research in developing effective treatments. Though presently available cancer treatments, such as surgery, chemotherapy, radiotherapy, immunotherapy, and targeted therapies, reduce the growth rate of cancer cells, however they are incapable of complete cure. Many chemodrugs used against this disease cause toxicity and cause serious side effects. Further, the development of resistance to the existing chemotherapeutic drugs has compelled adoption of improved alternative treatment approaches with novel therapeutic agents including nanoproducts. In this regard, plant-based natural anticancer drugs have proven to be more effective with negligible side effects against various cancers. More than 50% of the anticancer drug molecules in clinical use are mainly derived from nature. Plant metabolites such as alkaloids, terpenoids, flavonoids, carotenoids, etc., are reported to have anticancer properties. Major plant-based compounds such as vincristine, vinblastine, camptothecin, paclitaxel, irinotecan, topotecan, and etoposide are widely used in treating cancers. Many other potential plant secondary metabolites have been experimentally proven with anticancer properties and are under clinical or preclinical trial phase. More recently, nanoparticles biosynthesized from plant extracts and their compounds have emerged as promising agents in cancer therapy. Nanotechnology as a herbal therapeutic approach comprising novel nano-based drug delivery systems has been found to be effective in treating cancers. Nano-based drug delivery systems not only increase the therapeutic value but also the bioavailability of the herbal medicine. If the guidelines of

toxicology and clinical pharmacology are followed, more phytocompounds can be made available as an effective anticancer agent.

This book *Anticancer Plants: Clinical Trials and Nanotechnology (Volume 3)* to be published by Springer includes 11 chapters contributed by the potential researchers from various parts of the world. In Chapter 1, Indian authors highlight about the currently practiced cancer therapies, their management, and safety aspects. Chapters 2 and 3 by Indian researchers described in-depth nano-natural products as anticancer agents and natural anticancer compounds and their derivatives under clinical trials. Chapter 4 by Portugal researchers summarized the recent medicinal advancements of natural anticancer compounds and their analogues. Chapters 5 and 6 described the designing of natural cancerous drugs and their delivery system and the applications of nano-based novel drug delivery systems in herbal medicine-mediated cancer therapy, respectively. Similarly, Chapter 7 by collaborative works of Malaysian and Indian authors has discussed on the toxicological and pharmacological use of anticancer compounds, while the recent advancements in the clinical trial of plant-derived anticancer compounds were discussed in Chapter 8. Chapter 9 highlights the past and present studies on organosulfur compounds for exploring their potential as an adjunct in cancer chemotherapeutics and research. Chapter 10 discusses the relevance of traditional Unani system of medicine in cancer treatment. However, Chapter 11 entails the actions and reactions of anticancer plants and their signaling pathways in the cancer therapy. Overall, this book volume gives an overview of various plant-based anticancer compounds in use or under clinical trials and provides a detailed information of the applications of nanotechnology for cancer cure. Also, it discusses on several aspects of toxicology and pharmacology of different anticancer compounds and improved drug delivery systems involving nanostructures. This book volume will be very useful in developing novel therapies against cancers as it provides a wide range of data on the heterogeneity of cancers and chemodrugs and their adverse side effects and in improving the efficacy of cancer treatment approaches by exploring more potential anticancer phytocompounds and nanotechnological applications. I personally congratulate both editors, Dr. Mohd Sayeed Akhtar and Mallappa Kumara Swamy, for their noble academic efforts in bringing out this volume.

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Uma Rani Sinniah

Preface

Cancer is one of the leading death causes of human population increasingly seen in recent times. Plants have been used for medicinal purposes since immemorial times. Though several synthetic medicines are useful in treating cancer, they are inefficient and unsafe. However, plants have proved to be useful in cancer cure. Moreover, natural compounds from plants and their derivatives are safe and effective in treatment and management of several cancer types.

The anticancer plants such as *Catharanthus roseus, Podophyllum peltatum, Taxus brevifolia, Camptotheca acuminata, Andrographis paniculata*, *Crataeva nurvala*, *Croton tonkinensis*, *Oplopanax horridus*, etc., are important source of chemotherapeutic compounds. These plants have proven their significance in the treatment of cancer and various other infectious diseases. Nowadays, several well-known anticancer compounds such as taxol, podophyllotoxins, camptothecin, vinblastine, vincristine, homoharringtonine, etc., have been isolated and purified from these medicinal plants. Many of them are used effectively to combat cancer and other related diseases. The herbal medicine and their products are the most suitable and safe to be used as an alternative medicine. Based on their traditional uses and experimental evidences, the anticancer products or compounds are isolated or extracted from the medicinally important plants. Many of these anticancer plants have become endangered due to ruthless harvesting in nature. Hence, there is a need to conserve these species and to propagate them in large scale using plant tissue culture. Alternatively, plant cell tissue and organ culture biotechnology can be adopted to produce these anticancer compounds without cultivation. The proper knowledge and exploration of these isolated molecules or products could provide an alternative source to reduce cancer risk, anti-tumorigenic properties, and suppression of carcinogen activities.

Anticancer Plants: Clinical Trials and Nanotechnology (Volume 3) provides a timely reviewed effort in this direction. This volume with 11 chapters from various contributors of the globe focuses on the concepts and experimental data in the application of anticancer plants in clinical trials and the use of nanotechnology in cancer therapy as chemotherapeutic agents and formulation of nanoproducts and its relevance in boosting immune system. This book provides an informative data packages

on anticancer compounds and their use as nanomedicine to understand and solve the problems related to various cancers. We hope that this book is helpful for researchers, academicians, clinicians, oncologists, and scholars who are functioning in the fields of oncology, health care, nanotechnology, phytochemistry, toxicology, pharmacology, and herbal research.

We are highly grateful to all our contributors for readily accepting our invitation for not only sharing their knowledge and research but for venerably integrating their expertise in dispersed information from diverse field in composing the chapters enduring editorial suggestions to finally produce this venture. We greatly appreciate their commitment. We are also thankful to Professor Uma Rani Sinniah for his suggestions and writing the foreword for this volume. We also thank the team of Springer Nature, especially Dr. Mamta Kapila, for their generous cooperation at every stage of the book publication.

Shahjahanpur, Uttar Pradesh, India — Mohd Sayeed Akhtar
Serdang, Selangor, Malaysia — Mallappa Kumara Swamy

Contents

About the Editors

Dr. Mohd Sayeed Akhtar is an assistant professor at Gandhi Faiz-e-Aam College, Shahjahanpur, India. He received his PhD degree from Aligarh Muslim University (AMU), India in 2008, prior to conducting postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and Chonbuk National University (CBNU), Republic of Korea, in 2011. He was an assistant professor at Jimma University, Ethiopia (2011–2014), and a fellow researcher at the Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM) (2014–2015). Dr. Akhtar has 14 years of research and 8 years of teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology, and plant nanobiotechnology.

He is the author or coauthor of over a hundred articles in peer-reviewed journals, conference proceedings, and book chapters, and has edited five books with international publishers. His current research focuses on rhizospheric plant-microbe interactions and their molecular biotechnology, bioremediation, biomineralization, nanofertilizers, and nanobiotechnology.

Dr. Mallappa Kumara Swamy is a postdoctoral researcher at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). He received his PhD (Biotechnology) from Acharya Nagarjuna University, Guntur, India, in 2013. He has more than 14 years of teaching and research experience in the fields of plant biotechnology, secondary metabolite production, phytochemistry, and bioactive studies. Dr. Swamy has authored more than 50 research publications in peer-reviewed journals and is an editorial board member and reviewer for several high-impact international journals. His current focus is on cell and tissue culture technology for bioactive compound production and on nanotechnology for medical applications.

Marketing text: Cancer is one of the leading causes of death in human beings. Though several synthetic medicines are used to treat cancer, they are largely inefficient and unsafe. In contrast, plants, which have been used for medicinal purposes since time immemorial, have proved to be useful in fighting cancer, with natural compounds from plants and their derivatives offering safe and effective treatment and management for several types of cancer.

Plants such as *Catharanthus roseus*, *Podophyllum peltatum*, *Taxus brevifolia*, *Camptotheca acuminate*, *Andrographis paniculata*, *Crateva nurvala*, *Croton tonkinensis*, and *Oplopanax horridus* are important source of chemotherapeutic compounds. These plants have proven their value in the treatment of cancer and various other infectious diseases, and several common anticancer compounds such as taxol, podophyllotoxins, camptothecin, vinblastine, vincristine, and homoharringtonine have been isolated and purified from these medicinal plants.

Unfortunately, many of these anticancer plants have become endangered due to ruthless and irresponsible harvesting practices. Hence, there is a need to conserve these species and to propagate them on a large scale using plant tissue culture. Alternatively, plant cell tissue and organ culture biotechnology could be adopted to produce these anticancer compounds without the need for cultivation. A better grasp and continuing exploration of these isolated molecules and products could provide a powerful alternative means of reducing cancer risk.

Anticancer Plants: Volume 3, Clinical Trials and Nanotechnology provides a timely review of concepts and experimental data on the application of anticancer plants and their compounds in clinical trials, and on the use of nanotechnology in cancer therapy.

Chapter 1
Cancer Therapies: Current Scenario, Management, and Safety Aspects

Shivaswamy Santosh, Manasa Deepa Rajagopalan, Bangalore Acharlu Pallavi, Gudepalya Renukaiah Rudramurthy, Valluru Rajashekar, Katta Annaiah Sridhar, and Mallappa Kumara Swamy

Abstract Cancer is a disease state in which abnormal cells proliferate uninhibitedly and might spread all over the body. There are more than 100 types of cancers which are categorized based on the affected tissue or organ of the human body. At present, several methods of cancer treatments including surgery, chemotherapy, radiotherapy, immunotherapy, and targeted therapies are available. These therapies aim to stop or slower the growth rate of cancer cells, however incapable to completely cure cancers. Chemotherapy employs cytotoxic drugs that prevent, weaken, or reduce the reappearance of cancer cells and thereby control the development and progression of cancer. These anticancer compounds work in various ways such as impairing mitosis; targeting the cancer cells' energy source, enzymes, and hormones; preventing the angiogenesis process; and triggering apoptosis activity. Based on the nature of cancer, anticancer compounds are administered either single or in combination through oral, parenteral (intraperitoneal injection, intravenous injection), and other topical routes. These chemo-drugs exert the beneficial pharmacological action on cancer cell, but they also affect the healthy cells and lead to toxicity and clinical side effects in patients. These adverse effects vary depending on the type/group of drugs administered. Some of the common undesirable properties of anticancer compounds include weakness, xeroderma (dry skin), erythema (skin redness), alopecia (hair loss), loss of appetite, weight loss, and

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numbness. This chapter provides an idea about various toxicological side effects and management of these side effects in different types of cancer therapies.

Keywords Drug delivery · Toxicity · Cancer therapy · Permeability retention · Photodynamic therapy · Secondary malignancy · Side effects

1.1 Introduction

Cancer is a disease, associated with a group of more than 100 distinct disorders. Several carcinogenic factors such as genetic, certain microbial infections, pollutants, environmental factors, etc. are involved in the development of cancer. The environmental pollutants and some industrial hazardous chemicals are also well-known causes of cancers. The most common causes of cancer are related to environmental (Sharma and O'Keefe 2007), food (Mathew et al. 2004), lifestyle, and behavioral exposures (Grant et al. 2008). Moreover, other contributing factors of cancer include chemicals (Nasca 2007), some microbial infection (bacteria, virus, and parasites) (Liao 2006), diet (Mathew et al. 2004), radiation (X-rays, γ-rays), hormones (estrogen and progesterone) (Jaiyesimi et al. 1995) and genetic or heredity factors (Kinzler and Vogelstein 1996). The carcinogenic factors induce a definite damage at the genetic and cellular level. However, the exact cause of some cancers is still unknown.

Several endogenous factors promote the release of reactive oxygen species (ROS) such as hydroxyl, superoxide, and peroxyl radicals. Reactive Oxygen Species results in an extensive oxidative cell damages and causes many health-related conditions including atherosclerosis, neurological diseases, and cancers (Halliwell 2009). The absence of apoptosis may lead to uncontrolled proliferation of cancerous cells, which would further allow the cancerous cells to invade nearby tissues (metastasis) through the formation of new blood vessels known as angiogenesis (Weinberg 2007; Garraway and Lander 2013). Mutations at the cellular and molecular level lead to uncontrolled proliferation of normal cells by altering the cell cycle, leading to the development of mass of abnormal cells known as tumor. Carcinogens induce cancer through two distinguishing steps such as initiation and promotion (Caldas 2012). Initiation is a primary rapid phase involved in the formation of cancerous cell; during this step DNA gets damaged due to metabolic activation of genotoxic carcinogen. This initial change in cells may persist for an extensive period, possibly the life span of an individual. Subsequently, initiated cells enter into promotion stage where expansion of cells (clone expansion) takes place through promoters. Changes involved in the epidermal homeostasis provide a favorable tissue environment for the clone expansion. Many agents are known to induce normal cell division. However, promoters induce abnormal/uncontrolled growth leading to the

formation of tumor (Volker 2001). Anticancer agents mainly exhibit a preventative or healing role in an impaired system. Furthermore, an anticancer agent aids to activate the apoptosis (programmed cell death) signaling system to control the cell proliferation process (Dewey et al. 1995; Elmore 2007).

Cancer is due to the uncharacteristic proliferation of different types of body cells and differs markedly. According to cancer pathology, cancer can be either malignant or benign. Malignant forms of tumors possess the capability to invade their nearby normal tissues and organs and spread across the body through the lymphatic or circulatory systems. This is more often called as metastatic state of cancerous cells. While, benign tumors remain localized in their original site of occurrence and do not spread to surrounding regions of the body (Lodish et al. 2000). Cancers are classified on the basis of the type of cells, tissues, or organs that are affected. For instance, carcinomas represent the malignant forms of the epithelial cells, and it is one of the most frequent forms of human cancers (approximately 90%). Sarcomas are tumors of the connective tissues (bone, muscle, cartilage). Lymphomas and leukemias represents the cancers of the lymphatic system and blood-forming cells, respectively. Likewise, tumors are also categorized based on the type of cells involved or origin of tissues, for example, breast or lung malignancies (Lodish et al. 2000; Lahat et al. 2008).

Cancers are treated by using both traditional therapeutic methods (surgery, chemotherapy, and radiation therapy) and complementary and alternative therapeutic approaches. However, current forms of cancer treatments cause side effects and mainly affect the healthy and normal tissues or organs. In addition, side effects may differ from one person to another, even between those patients who received the same therapy. Also, a therapy may show lesser side effects in some patients, but in others, the effects may be many. The type of tumors, progress stages, amount of drugs, treatment frequencies, age, and health conditions of a patient are some of the critical and deciding factors for choosing the right cancer treatment method (NIH-NCI 2017).

The anticancer compounds involved in chemotherapy prevent cancer progress in various ways such as impairing mitosis; targeting the cancer cells' energy source, enzymes, and hormones; preventing the angiogenesis process; and triggering apoptosis activity (Croce 2008). The chemo-drugs are either single or in combination through oral, parenteral (intraperitoneal injection, intravenous injection), and other topical routes. However, the use of chemotherapy is mainly based on the nature of a tumor. Though chemo-drugs effectively inhibit cancerous cells, they also induce toxicity and other adverse health problems in the treated patients (Kroschinsky et al. 2017). Moreover, adverse effects vary with the type/group of drugs administered. Some of the common undesirable effects of anticancer compounds include weakness, xeroderma (dry skin), erythema (skin redness), alopecia (hair loss), loss of appetite, weight loss, and numbness (Shewach and Kuchta 2009; Kroschinsky et al. 2017).

1.2 Cancer Therapy: Side Effects and Management

The choice of cancer treatment depends on origin of primary cancer and stage of illness. Several different strategies are available for the treatment of cancer; the selection and application of the therapy depends mainly on the type of cancer, site of the tumor, stage of the cancerous tissue, and many other factors. The advancement in the field of medical technology has led to the development of innovative and advanced cancer therapies. These advanced therapies are known to have better advantages over earlier strategy which include improvement in the lifestyle of affected individuals, minimizing pain, and many more. The most commonly employed cancer treatment includes surgery, chemotherapy, radiotherapy, immunotherapy, etc. Some of the major anticancer drug types and their clinical effects (NIH-NCI 2017) are mentioned in Table 1.1.

1.2.1 Surgery

The main objective of surgery in cancer treatment includes diagnosis by biopsy, prevention of tumor progression, palliation, and in few cases as a treatment option. The surgical methods may vary based on the tumor and its location (Faries and Morton 2007). Surgical therapy is normally carried out to find the type and stage of the disease. Surgical treatment plays a vital role in soft tissue and bone cancers. However in hematological malignancies, surgery has minimal role. Surgical therapy may be of different types depending upon the modalities and use of therapeutic agents, such as vaporization of tumor using light known as laser therapy (Disaia and Creasman 2002), freezing cancerous cells in liquid nitrogen probe called cryosurgery (Mouraviev and Polascik 2006), laparoscopic surgery (Apgar and Brotzman 2002), electrosurgery (Sheridan and Dawber 2000), thoracoscopic surgery, and micrographic surgery (Grossman and Leffell 2009).

The most common side effects of post-surgery are pain, infections, loss of blood, blood clotting, and impairment of neighboring tissues and/or organs and many more. Apart from these common side effects, some of the long-term effects are also reported after the surgery. For instance, after prostatectomy, male patients may lose to control urine (Hart et al. 2008), loss of limb function following resection of bony tumors, loss of hearing following removal of acoustic tumor, loss of vision after removal of orbital tumors, and many more. Pain and infections are managed with the aid of pain killers such as aspirin, acetaminophen, etc. (Kimberlin et al. 2004) and antibiotics given through oral or intravenous route (Gilbert et al. 2008). Surgeries are more beneficial in preventive, in palliative, and in diagnosis and in certain cases used as a treatment. However, surgery in combination with additional therapies like radiation therapy, immunotherapy, and chemotherapy acts as a powerful tool to cure some of the cancers (Amant et al. 2005; Kantoff et al. 2010).

Table 1.1 Major anticancer drug types and their clinical effects

Anticancer drug types	Example	Mechanism of action	Clinical side effects	Uses	References
Antimetabolites	5-FU	Inhibition of thymidylate synthase and incorporation of its metabolites into RNA and DNA	Photophobia taste changes, metallic taste in the mouth during infusion, low blood counts	In several cancers that includes cancer of colon and rectal, breast, head, and neck, neuroendocrine, cervix, bladder, hepatobiliary, and thymus	Longley et al. (2003)
	Methotrexate	Inhibits the dihydrofolate reductase and thus affects nucleoside metabolism	Kidney toxicity	Acute lymphoblastic leukemia, choriocarcinoma, cancer of the breast, neck, head, lungs, cervix	Chorawala et al. (2012)
			Skin photosensitivity		
	Mercaptopurine	Inhibits the synthetic steps during S-phase of replication	Blood in urine or stool	Acute lymphocytic leukemia	Chorawala et al. (2012)
			Swelling of lower limbs		
			Decreased appetite		
Alkylating agents	Chlorambucil	Interferes with DNA replication and induces cellular apoptosis via the accumulation of cytosolic p53 and subsequent activation of Bax, an apoptosis promoter	Seizure	Leukemia, lymphoma	Peterman and Braunstein (1986)
			Rapid heart rate		
			Trouble concentrating		
	Cyclophos-phamide	Inhibits protein synthesis by introducing alkyl groups into DNA and creates cross-linking between two DNA strands	Shortness of breath	Hodgkin lymphoma	Travis et al. (1995)
			Fast heart beat	Acute lymphoblastic leukemia	
				Retinoblastoma	
				Ovarian cancer	
			Joint pain	Nephritic syndrome	
	Lomustine	Alkylates and crosslinks DNA and also carbamoylates DNA and proteins, thereby inhibiting DNA and RNA synthesis and disruption of RNA processing	Troubled breathing with exertion	Brain tumors	Rahman et al. (2014)
				Breast cancer	
				Colon cancer	
				Melanoma	
			Black, tarry stools	Lung cancer	
			Bleeding gums		

(continued)

Table 1.1 (continued)

Anticancer drug types	Example	Mechanism of action	Clinical side effects	Uses	References
Plant products	Vincristine	Arrest the cell division in metaphase by binding to tubulin	Numbness in fingers and toes	Lungs and breast cancer	Tu et al. (2013)
			Pain in testicles	Neuroblastoma	
			Hallucination	Rhabdomyosarcoma	
				Testicular carcinoma	
				Kaposi's sarcoma,	
	Paclitaxel	Stabilize polymerization of tubulins and inhibit the disassembly of microtubules	Shortness of breath	Cancer of the head and neck, breast, lungs, ovary	Kampan et al. (2015)
			Increased heart beat		
Monoclonal Antibodies	Trastuzumab	Targets against human epidermal growth factor receptor protein-2 (HER-2) and induce the apoptosis in breast cancer	Arthralgia	B-cell lymphoma	Lavaud and Andre (2014)
			UTI	Chronic lymphocytic leukemia,	
			Tachycardia		
			Ear congestion	T-cell lymphoma and breast cancer.	
			Edema		
Antibiotics	Doxorubicin HCl	Intercalation into DNA, leading to inhibition of the DNA synthesis or poisoning of topoisomerase II	Low blood count	Bone sarcoma	Dhanaraj et al. (2014)
			Mouth sores	Breast, kidney, gastric, liver, uterine, endometrial cancer	
				Head and neck cancer	
				Hodgkin lymphoma	
				Non-Hodgkin lymphoma	
				Wilms' tumor	

Vaccine	Sipuleucel-T	An autologous cellular immunological agent is thought to work through APCs to stimulate T-cell immune response targeted against prostatic acid phosphatase (PAP)	Skin rashes, infection, secondary cancer	Hormone-refractory prostate cancer Breast cancer	Goldman and De Francesco (2009), Kantoff et al. (2010)
	Gardasil	The proteins in Gardasil are structural, virus-like proteins (VLP) that resemble the HPV virus. The proteins can activate the immune system but cannot give rise to replicating virus	Skin rashes, infection, secondary cancer	Cervical cancer Anal cancer	Kane (2012)

1.2.2 Chemotherapy

The term "chemo" is described as the chemically induced compound, i.e., the drug which flows through the bloodstream to almost each part of the human system. Chemotherapy is one of the method of treatments followed before/after surgery and/or radiations to cure and/or control/prevent certain cancers. The choice of drug (alone or in combination), and doses (lower to higher) completely depends on cause and category of the cancer and biological factors such as age of patient, past health history, and other treatments taken before the cancer treatment etc. (Hausheer et al. 2006; Kampan et al. 2015). By considering the above facts, several different chemotherapeutic drugs have been studied and formulated for the treatment of various cancers. All these chemotherapeutic drugs are distinct from one another based on their active constituents and their use in cancer therapy. The chemotherapeutic drug is largely administered through different modes such as oral, intravenous, and/or intra-lesional with the aid of special catheters known central venous catheters. Chemotherapeutic agents known as cell-cycle-specific drugs normally act on specific and/or all phases of cancer cell cycle (G_1-, G_2-, M-, S-phase), for example, methotrexate acts during S-phase and vinca alkaloids acts on M phase. However, certain drugs (non-cell cycle specific) such as alkylating agents do not act on the cell division cycle (Nies and Speilber 2006). Hence, an appropriate knowledge of tumor biology and cellular kinetics would be useful in the development of innovative and advanced drugs for cancer therapy (Kroschinsky et al. 2017). Mechanism of action of different chemotherapeutic agents (Payne and Miles 2008) against various types of cancer is listed in Table 1.1.

Earlier studies have reported that the therapeutic efficacy of chemotherapeutic drugs depends on various pathophysiological conditions of cancerous cells/tumors. Conditions such as structural impairment in blood vessels due to angiogenesis, destructive lymphatic drainage system (Lila et al. 2010), suppression of cell signaling, high interstitial fluid pressure in tumor, and large production of phosphoglycoprotein may resist the uptake of drug/s by the cancerous cells (Mahesh et al. 2006). Moreover, previous study on cancer stem cells (CSCs) also reveals that CSC metastasis helps in the development of resistance against anticancer drugs (Visvader and Lindeman 2008; Housman et al. 2014).

Chemotherapeutic drugs used in cancer treatment have major drawback, i.e., they are too toxic and damage the healthy cells surrounding the cancerous cells leading to various side effects. The side effects may remain for short period, perhaps few months to years or may be lifetime, and these side effects vary from drug to drug. The common side effects are gastritis, bleeding, pain, loss of appetite due to damaged cells in the gastrointestinal tract (Honea et al. 2007; Joshi and Ehrenberger 2001); suppression of bone marrow formation due to lowered RBC level; (Balducci et al. 2007; Shayne et al. 2009); difficulty in swallowing due to sore throat and oral ulcers (Bensinger et al. 2008); hair loss and microbial infections due to suppressed immunity (Rolston 2005). However, some of these effects may be reversed after the completion of chemotherapy. In some cases, the comparable side effects may

worsen due to the interactions with other drug. For example, high-dose vitamin consumption may reduce the therapeutic activity of chemotherapeutic drugs; aspirin along with chemotherapeutic drug may reduce the platelet counts and increase risk of severe bleeding (Mora et al. 2008; Mamede et al. 2011; Kampan et al. 2015). Long time side effects may also cause or result in the formation of secondary cancer. Most frequently, after receiving chemotherapy it may cause different forms of leukemia such as acute lymphocytic leukemia and acute myelogenous leukemia. Among the chemotherapeutic agents, anthracycline compounds and topoisomerase inhibitors II are safe enough than the alkylating agent and platinum-based chemodrugs such as cisplatin and paclitaxel (Shayne et al. 2009; Kampan et al. 2015).

1.2.3 Immunotherapy

Immunotherapeutic agents have complex structures comprised of many special cells and work on human immune systems. These types of immunotherapeutic agents known as immunomodulators include vaccines, monoclonal antibodies, cytokines, etc. which induce immune cells to act against cancerous cells or control the cancer cell formation (Cebon J et al. 2007; Kantoff et al. 2010). Comparatively this type of therapy causes fewer side effects than chemotherapy.

1.2.3.1 Monoclonal Antibodies and Cytokines

Monoclonal antibodies (MAbs) are engineered proteins (antibodies) which may be of murine origin or chimeric or humanized (Presta 2006; Ma and Adjei 2009). Several such immunotherapeutic proteins have been developed which are in the form of monoclonal antibodies, cytokines, and hormones (Dranoff 2004; Tayal and Kalra 2008). Among this, MAbs play a remarkable role in the treatment of various cancers. These are widely introduced through parenteral route and few in oral route (Chung 2008). MAbs are generally categorized as naked, radioactive, conjugated, and antibody-drug conjugates (Dubel 2007). Earlier, these were extensively used to treat only hematological malignancies (Mahapatra and Kapoor 2011). However, advanced research studies on these types of proteins showed that they are capable of regulating the signaling through epidermal growth factor receptors (EGFRs) and vascular endothelial growth factor receptors (VEGFRs) and thereby trigger the apoptosis or may produce cellular cytotoxicity (Tonini et al. 2005) in cancer cells. Similarly, the cytokines in the form of interleukins such as interleukins-2 (Liu 2003) and interferons like interferons-α, β, γ (Shankaran et al. 2001) have a major role in various cancer treatment (Besedovsky and Del Rey 2001; Kelley et al. 2003). These nonspecific immunotherapies are mostly administered by parenteral route through the muscle, skin, and vein (Liu 2003).

Development of immunotherapeutic agents is still remaining a challenge for the researchers due to the difficulty in recognizing the relationship between protein

complex structure and its therapeutic activity. The lack of knowledge about MAbs and cytokines may affect the immunogenicity of the product and may become toxic. Different kinds of serious side effects were found in the MAbs immunotherapy based on the antigen it binds (Kelley et al. 2003). Several such used in immunotherapy are known to cause serious problems, for instance, bevacizumab used to treat metastatic conditions of colon and rectal cancer will likely to cause kidney impairment (Miller et al. 2007) and cetuximab used in colorectal cancer treatment is known to induce serious rashes and ocular toxicity (Tonini et al. 2005; Chan et al. 2009; Giro et al. 2009). Moreover, rituximab used in follicular non-Hodgkin lymphoma treatment may cause some serious side effects such as cytokine-related syndrome (CRS), autoimmune hematological disorders, prominent acute infusion reactions, tumor lysis syndrome, progressive multifocal leukoencephalopathy (PML), and renal toxicity (Gianni et al. 2003); interleukins-2 may cause chills and fever (Dutcher et al. 2001; Schwartzentruber 2001). Interferon may cause severe side effects which are intolerable by the patients which include reduction in the blood cell counts, thereby increasing the risk of microbial infections; moreover, the management of side effects caused by interferon is very hard (Tayal and Karla 2008).

1.2.3.2 Vaccines

Vaccines are one of the immunotherapeutic agents used in cancer therapy (Martin A. 'Mac' Cheever 2008; Intlekofer et al. 2012). The word "cancer vaccine" denotes substances that prevent cancer-causing infections, for example, HPV vaccine in cervical cancer and hepatitis B vaccine in liver cancer development, and are also used to cure existing cancers such as breast and prostate cancer, etc. However, the endurable immunogenicity and the duration of activity depend on the type of antigen and adjuvants used in the formulation. Accordingly, the vaccines are categorized into different types (Table 1.1). Furthermore, tumor physiology, patient's age, and health conditions may also modify the effectiveness of vaccines (Dunn et al. 2004; Lollini et al. 2006; Pejawar-Gaddy and Finn 2008). Vaccines need to be produced with long-term memory without causing any autoimmune disorders for effective use in therapy (Lotze and Rees 2004). Last decades of hypothetical reports on vaccines in various cancers reveal that vaccines help in maintaining a healthy immune system in individuals and destroy cancer cells consistently whenever they arise (Dunn et al. 2004). Several clinical trials are in process to investigate the vaccines, as prospective treatment in non-small cell lung cancer (NSCLCs) and also against other types of cancer (Jaffee et al. 2001; Ringden et al. 2009). Examples of vaccine in clinical trials include tumor-cell vaccine (belagenpumatucel-L), melanoma antigen, and mucin-1vaccine/liposomes (Goodyear and Hossein 2011). Several other advanced vaccines such as DNA vaccines, antigen vaccines, viral-vector-based vaccines, tumor-cell vaccines, and dendritic cell vaccines are also in the clinical trials aimed to improve the effectiveness and minimize the toxicity. For instance, sipuleucel-T is a type of therapeutic cancer vaccine used in cellular immunotherapy consisting of

PBMCs (autologous peripheral-blood mononuclear cells) like APCs (antigen-presenting cells) activated by recombinant fusion proteins (PA2024) ex vivo. PA2024 protein contains prostate-specific antigens (prostatic acid phosphatase) which are attached to an immune-cell activator (granulocyte-macrophage colony-stimulating factor) (Goldman and De Francesco 2009; Kantoff et al. 2010). The common side effects of immunotherapy include fatigue, chills, dizziness, fever, and many more. However, additional serious side effects like systemic lupus erythematosus, pelvic inflammatory disease, and autoimmune diseases, including arthritis, asthma, and appendicitis, have been reported in patients undergoing immunotherapy. Moreover, vaccines may also interact with similar kind of drugs and cause serious life-threatening effects which are quite difficult to manage (Becker et al. 2010; Allen and Giridhar 2014).

1.2.3.3 Stem Cells and Stem Cell Therapy

Stem cells are immature blood cells, formed by the process of hematopoiesis. Generally, these are abundant in the bone marrow and few are in the bloodstream. After maturation into a complete cell in the bone marrow, they move into the systemic circulation. These stem cells have been identified to play significant role in the treatment of certain cancer and are commonly called as stem cell therapy and bone marrow transplant therapy (Bashey 2000). Generally, stem cells comprise three diverse components such as red blood cells (RBCs), white blood cells (WBCs), and platelets, which have a definite role. RBCs help in the respiration process, while WBCs is comprised of various cell components such as neutrophils and lymphocytes (natural killer cells, T and B lymphocytes) which have a wide role against various infections (Bacigalupo 2003). Platelets, one of the important blood components, help to stop bleeding if it occurs either from known or unknown injuries (George 2000; George and Colman 2006). In most chemotherapy or radiation therapy cases, bone marrow destruction is vivid. For instance, in multiple myeloma or in leukemia after the combination of chemotherapy and radiation therapy, huge suppression in bone marrow cells takes place (Barkholt et al. 2008).

In these circumstances, the right choice of therapy is stem cell transplant. During stem cell transplant, individual's system stem cells can be replaced with new body stem cells or also it may work as graft-versus-cancer effect, i.e., it can find and kill the cancerous cell than the individual's immune cells. However, patients with stem cell transplant are under high risk due to related complications. Generally, the physician will decide the necessity of stem cell transplant depending upon the age factor of an individual, condition of disease, type of donor, etc. Collectively some of the common side effects include mouth and throat pain (short period), nausea and vomiting, and infection caused by certain bacteria, virus, and fungi (Bashey 2000). Bacterial and fungal infections can be generally managed with some antibiotics and antifungals (Perrotta and Synder 2001). Cytomegalovirus can often cause infection such as pneumocystis pneumonia in stem cell transplanted patients, and this is a little risky to manage. Hence, patients are advised with precautionary/prevention

methods for the viral infections. All these serious side effects may also occur due to graft rejection (tissue mismatching of donor and recipient). If the patients are not treated on time during infection, they may further have the risk of graft-versus-host disease (Brecher et al. 2000; Firestone and Pitocco 2005; Villa et al. 2016). Platelet counts are reduced generally after the first 3 weeks of stem cell transplant; nonetheless, if the number of platelet in the blood becomes very low in the patient, it may be difficult to treat internal organ bleeding or bruising, which needs platelet transplant (Schiffer et al. 2001). Various other infections such as infections in the skin, lungs, and gastrointestinal tract are also observed due to graft-versus-host disease (Chemaitilly and Sklar 2007). Some of the other serious side effects of stem cell transplants include secondary cancers (Holman 2000), imbalanced hormone secretion in the thyroid and pituitary gland, vision loss (Firestone and Pitocco 2005), and many more.

1.2.4 Photodynamic Therapy

Photodynamic therapy also termed as photo-chemotherapy involves injection of photosensitizing agents through the vein or administered under the skin. After certain duration of intervals from hours to days, these agents are activated in the system by means of passing different wavelengths of light depending upon the type of photosensitizing agent leading to sensitization of cancerous as well as normal cells. The normal cells get rid of these agents within a couple of days; however cancer cells may hold these agents for many days to weeks. This therapy plays a significant supportive role in treating mouth and skin cancers as well as precancerous cells (van Straten et al. 2017). Specificity photodynamic therapy allows it to be used in many localized cancers such as skin and mouth cancers without pain (Querfeld et al. 2003). At present, there are only few photodynamic therapeutic agents such as porfimer sodium (aminolevulinic acid) that have been approved for the treatment of Barret's esophagus (Posner et al. 2005), NSCLCs (Ross 2003), and acute keratosis (De Berker et al. 2007). As mentioned earlier, various wavelengths of lights have been used to activate the photosensitizing agent, for instance, blue light for the activation of aminolevulinic acid (Piacquadio et al. 2004) and red laser light to activate the porfimer sodium, through a thin fiber-optic glass filament. In Barrett's esophagus the glass filament has been introduced through a flexible tube called endoscope (Phan et al. 2005). Similarly, bronchoscope is used in lung cancer (Vachani et al. 2008). Photodynamic therapy has shown more patient compliance than radiation therapy, chemotherapy, and surgery. The advantages of photodynamic therapy include the following: it is less intrusive, it has no extended period of side effects, and only minute scars occur after the treatment (Phan et al. 2005). Some of the current research studies on photodynamic therapy showed that this therapy is useful even for deeper tissues and the organs. Phototherapeutic agent such as Photochlor is found to be very effective against throat, lung, skin, oral, and esophageal cancers.

Advanced level studies on photodynamic therapy may be focused on combinatorial therapy, as well as imaging and treatment of solid tumors (Bellnier et al. 2006).

Though the therapy has wide range of advantages, it carries certain limitations such that phototherapeutic agents cannot pass into larger and deeper tissue effectively and can act only at the peripheral level. In certain conditions photodynamic therapy is not suitable to patients who have past history of illness like fistula (between the trachea and bronchus), porphyrias (the presence of tumors at the foremost blood vessels in the esophagus and stomach increases vein size above the normal level), etc. Depending upon the part of cells/tissue treated, several common specific side effects were observed post porfimer sodium treatment, for example, severe cough, difficulties in swallowing in the case of esophageal cancer treatment with porfimer sodium (Phan et al. 2005), pneumonia, coughing with blood, and fluid collection in the case of lung cancer treatment (Karp and Thurer 2003). Photodynamic agents such as aminolevulinic acid cause side effects like photosensitivity reactions on the skin (swelling and reddening of the skin) induced by exposure of sunlight, immunosuppression, porphyria, flaking, and itching (usually gets rid third week after treatment). Some of the other side effects caused by aminolevulinic acid are eczema and hives which sometimes turn into serious allergic reactions.

1.2.5 Hormone Therapy

Modulations in hormone secretion are responsible for some cancer formations such as breast, prostate (Group PC 2000), and uterine cancer. Therapeutic agents belonging to this category will either stop production of such sex hormones or block the cells to utilize this hormone for their further growth. For example, tamoxifen is one of the most common hormone therapies for breast cancer which controls cell proliferation by blocking the estrogen receptors (Ellis and Perou 2013). In prostate cancer treatment, GnRH agonists such as goserelin, leuprorelin, and triptorelin stop the testicles from producing testosterone (Jaiyesimi et al. 1995; Fisher et al. 2005). Moreover, earlier reports show that hormonal treatments also work well with uterine cancers, such as endometrial stromal sarcoma, and these include tamoxifen (Jaiyesimi et al. 1995), letrozole (Elisaf et al. 2001), megestrol acetate, medroxyprogesterone (Loprinzi et al. 1990); these drugs either stop the production of respective hormones or block the cells to utilize the hormone for further growth. The side effects of hormone therapy are specific to individuals and are distinctive to the sex of the patient. Few side effects seen in women are menopausal symptoms such as genital irritation and vaginal discharge, fatigue, gastroenterological disorders, alopecia (hair thinning), muscle and bone change, increase in body weight, depression, and blood coagulation (Elisaf et al. 2001). In men the side effects observed were tiredness and impotence (Hellestedt and Pienta 2002). In women, some worst side effects seen in high dose of bicalutamide (casodex) are tumor flare and breast tenderness. In men, certain drugs like leuprorelin and goserelin may lead to depression

and mood swings, osteoporosis (Elisaf et al. 2001), etc. Physiological side effects are managed with counseling, while bone-related effects may be treated with vitamin D and calcium (Estilo et al. 2008; Mora et al. 2008).

1.2.6 Radiation Therapy

Radiation is a choice of treatment to shrink the solid tumors and kill the cancer cells using high radiation with various radioactive substances (Halperin et al. 2004). Radioactive substances are physical agents which radiate the electrically charged ions. This high energy radiation such as gamma rays and X-rays causes huge damage in the genetic content of the cell/DNA thereby killing the cancer cell. Radiation can be activated through radioactive substances in the human system, for example, brachytherapy (Akbal et al. 2008) or by external source, i.e., by external-beam radiation therapy (Hall and Cox 2003). Some of the radioactive substances that are used in the treatment of different cancer are calcium (^{44}Ca and ^{45}Ca) in bone cancer,^{131}I in thyroid cancer (Davies and Gilbert Welch 2014),^{59}Fe in blood cancer, and many more.

The side effects of radiotherapy may be acute/chronic and may vary in every radiation process. Radiation therapy used in the stomach or abdomen region may cause diarrhea, nausea, and vomiting, whereas patients undergoing radiation in the head and neck area may develop mouth blisters (Bensinger et al. 2008). Acute side effects may be shorter or last for weeks and may become extinct. For instance, during head and neck radiation therapy, patients may experience hair loss. Some of chronic effects caused after radiation therapy will last for weeks to years or may persist for a lifetime (Carper 2007). The side effects observed after the radiation therapy in the abdomen or pelvis are bowel dysfunction, infertility, or sexual-related complications (Miller et al. 2007). In certain cases lymphedema (fluid buildup and swelling in parts of the body) was also observed after the radiation therapy (Cheville 2007). Radiation therapy may also lead to the development of secondary cancers, for instance, the radiation treatment for Hodgkin lymphoma in young women was later found to develop secondary breast cancers (Horning 2008).

1.2.7 Targeted/Nanoscale Therapy

Targeted delivery involves delivery of drugs specifically to cancer cells rather than the normal cells. The targeted drugs work on cancer cells by means of blocking the signals, activating the immune system, altering the 3D structure of cancer cell protein molecules, blocking the angiogenesis (new blood vessel formation), and taking the toxins from the cancer cells (Croce 2008). The specific delivery has allowed this therapy to be used as main a course of treatment or as co-therapy. Moreover, pathophysiological conditions of cancer tumors such as damaged lymphatic drainage, leaky vascular system, and enhanced permeability and retention effect affect the

traditional chemotherapeutic drug efficacy (Jong and Borm 2008). Drug distribution and drug specificity can be achieved by means of reducing the particle size ranging from micro- to nanoscale known as nano-medicines (Couvreur 2013). Nanotechnology includes designing, synthesis, and characterization of bulk material into nanoscale range which involves engineering, and technology (Akhtar et al. 2015; Swamy et al. 2015a,b; Rudramurthy et al. 2016). These engineered nanomaterials must be safe and biocompatible to use in biology and medicine. Several different techniques have been developed for the synthesis and characterization of different nanoparticles (Akhtar et al. 2013; Akhtar and Ahmad 2014; Rudramurthy et al. 2016). Nanomaterials or nanoparticles used in diagnostic images, or in cancer treatment act by regulating the cell proliferation or kill the cell and/or block the new blood vessel formation in cancer cells (Lila et al. 2010). Nano-therapeutic agents are widely used as biosensors, diagnostic imaging and to deliver the drug specifically to cancer cells. Nano-engineered drug substances can withstand for longer period in the cell and enhance the biological half-lives of therapeutic effects which help to reduce the dose and dosage regimen (multiple administration). Moreover, this also assists the nano-sized material to avoid quick clearance and metabolism from the individual's system (Ruoslahti et al. 2010; Das et al. 2015).

Cancer therapy using chemotherapeutic agents may damage the healthy cells also; hence, dose and dose frequency during cancer chemotherapy should be optimum and otherwise may lead to lower the therapeutic efficacy or increase the toxicity (Mukherjee et al. 2008). To balance these difficulties, nanoscale delivery is a favorable choice of therapy to target the cancer cells. Nanoscale delivery systems have been well progressed and developed as a promising tool in the improvement of the therapeutic efficacy, minimization of side effects, and lowering of the doses of drugs (Saad et al. 2008; Subbiah et al. 2010). Various types of nanoscale drug delivery systems have been developed to use in cancer therapy and are in the form of quantum dots (Fang et al. 2012), nanoliposomes (Rudra et al. 2010), nanoparticles (Desai et al. 2003; Kanagesan et al. 2016; Aziz et al. 2016), nanocarriers (Kumari et al. 2016), nanorobotics (da Silva Luz et al. 2016), nanospheres (Dhanaraj et al. 2014), nanoshells (Zhao et al. 2014), dendrimers (Vieweg and Dannull 2003), and fullerenes (Prylutska et al. 2014). Nanomaterials differ from one another on the basis of method of fabrication, such as site specificity, dose, and dose frequency.

Similar to regular chemotherapeutic agents, targeted/nanoscale delivery do not harm the healthy cells. However, some side effects have been observed depending upon the drug and its target site and, may vary from individual to individual. Since the past two decades of research and clinical trials on targeted cancer therapies, very few drugs have been approved by US FDA for safe therapy. Consequently, the serious side effects caused by each nanoscale system are not exactly observed as same as in traditional chemotherapeutic drugs. Nanoscale/targeted therapy is also known to cause some side effects, for instance, the drugs act as epidermal growth factor receptor inhibitors that block the protein production both in normal and cancer cells (Kuan et al. 2001). Hence, this drug may affect the normal skin protein formation and may change the skin color of patients. Likewise, angiogenesis inhibitors may block the blood vessel formation in and around the cancer tissue and thereby affect the small

blood vessels present in the foot and hands; thereby it may cause skin rashes like foot, hand, and mouth syndrome (Cooney et al. 2005). Some of the common side effects are changes in hair growth and swelling around the lips and eyes in certain targeted therapies (Basti 2007). Skin rashes have been managed with broad-spectrum sunscreen lotion with SPF of at least 30, zinc oxide, or titanium dioxide, and foot and hand syndrome has been treated with antibiotic gel (Timoney et al. 2003).

1.3 Management of Cancer Therapy Side Effects

Pain is one of the major challenges to treat as it can be due to the disease process or can also arise from the side effects of treatment (Shute 2013). Pain relief can be achieved by medications which are administered by various routes like oral, parenteral, or transdermal opiates. In addition sedatives, nerve blocks, and relaxation therapy may also be helpful (Lang and Patt 2004; Campbell 2011; Shute 2013). Nausea and vomiting is the second most common side effect and is managed by the use of antiemetics (Rao and Faso 2012). Various molecules like metoclopramide, megestrol acetate, and cannabinoids are used in this treatment. Adequate nutritional supplement intake can help in the patient's speedy recovery and in turn will reduce the hospital stays, reduce costs and severity of the side effects, and improve patient's well-being. Somatostatin and its analogues are used in the treatment, as it reduces intestinal secretions; pro-kinetic agents may also be of help in such patients. Opiate-induced bowel disturbance in the form of constipation is quite common as it is used regularly for pain relief. It is managed by the use of laxatives, increase of water intake/hydration, and regular bowel habit. During or post radiation therapy, local effect on the skin is associated in the form of dry skin, hyperpigmentation, and severe pruritus. Care of the skin is important to avoid ulcer formations; patients are educated on this front by using mild soap and oil-based lotions to reduce dryness. In pruritus, patients are advised to use calamine lotion, or cocoa butter, or to bathe in sodium bicarbonate (Bolderston et al. 2006; Chan et al. 2009; McQuestion 2011). Mostly chemotherapy is administered by the intravenous route; as a result of which, care of catheter/venous line is important to avoid inflow failure, catheter kinks, fibrin blood in catheter, and catheter migration. Using heparin flushing before and after drug administration can avoid blocks, and it is mandatory to check the catheter placement by taking an x-ray or dye studies to avoid associated complication (Goossens 2015). Moreover, post-chemotherapy patients become very weak and are very susceptible to systemic infection due to the development of neutropenia.

Diagnosing infections in cancerous patients is problematic because distinguishing between infections and fever of unknown origin is very difficult. This clinical challenge can be detected by identifying some biochemical markers, for example, increased concentration of blood procalcitonin (PCT) is linked with microbial infection. Thus, detecting PCT levels (normal = 0.5 ng/ml) will be a very useful parameter to detect severe infections, specifically a bloodstream infection in cancer patients (Durnas et al. 2016). Patients must be educated about the red flag signs and

symptoms and also consult the doctor immediately. Patient education about adverse effects plays a major role in the management and also alleviates the serious or life-threatening treatment complications (Green et al. 2015; Tudor Car et al. 2016).

1.4 Conclusions and Future Prospects

The chemotherapeutic agents (either synthesized or from plants) may cause cell death both in healthy and cancerous cell. If normal cell proliferation is affected, it may produce unwanted effects which may be a little and/or very common after the therapy. However, most of the synthesized chemotherapeutic drugs alone or in combination with therapy lead to serious side effects. Advancement in the field of science and medicine led to the development of innovative treatment methodology known as nanoscale or targeted delivery. Many recent clinical trials have proved that targeted drug delivery system may target the cancerous cell with less harm and may help patient to live quality life. Several naturally derived anticancer molecules with minor side effects have been under consideration for clinical treatment of cancers. Even then, oncologists face the problems of new adverse effects linked to these new therapies, thus forcing to develop novel strategies against cancers. The knowledge of the pathophysiological effect of new therapies and their side effects are yet to be understood in detail. Moreover, the recommended use of new therapies still requires validation. Information about the clinical management of cancer is inadequate, hence, requiring more clinical investigations in this regard. Future direction of research should focus on identifying and overcoming adverse side effects in cancer patients by adopting different strategies. Thus, added preclinical and clinical investigations on toxicity and cancer management are immediately needed. Promoting public awareness, educating patients, effective diagnosis, and easy access to oncologists are the best ways to control and prevent cancers. Further, educating patients, caretakers, or medical staffs on the possible severe complications of cancer therapies and adopting suitable measures to overcome during the course of treatment process would be more useful.

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Chapter 2
Nano-natural Products as Anticancer Agents

Atish Tulshiram Paul and Anil Jindal

Abstract Cancer is one of the noxious diseases and is a major public health problem worldwide. The clinical management of cancer involves various approaches, but the most common one is chemotherapy. Natural products such as paclitaxel, camptothecin, podophyllotoxin, etc. have been used as major sources of anticancer drugs in many clinical trials. In spite of availability of these drugs for treatment of cancer, failure in chemotherapy is very common due to dose-limited toxicities and occurrence of drug resistance. In this regard, the nano-delivery systems directly target and deliver the selective drug to the cancerous sites and increase the permeability and intracellular accretion of anticancer drugs. Thus, the aim of this chapter is to focus on the application of nanotechnology to develop nano-natural products for effective treatment of cancer.

Keywords Cancer · Cell permeability · Nanoparticle · Nanotechnology · Natural products

2.1 Introduction

Cancer is one of the noxious diseases and a major public health problem worldwide. Despite genetic causes, there are several reasons that cause cancer in an individual which include lifestyle (such as consumption of tobacco and alcohol), exposure to infectious agents (e.g., *H. pylori*), exposure to carcinogens (e.g., chemicals and radiation), ethnicity, etc. As per World Health Organization (WHO) statistics, cancer has been responsible for 8.8 million deaths in 2015 (WHO 2017). As predicted, by 2030, the figure of deaths due to cancer around the globe will be 13.2 million with global burden of new cancer cases being estimated to rise to 20.3 million by 2030 (Ferlay et al. 2010; Bray et al. 2012). Even though many advances have been

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made in tackling cancer, but still it remains as a major challenge in developed countries. In the case of low- and middle-income countries, the cases of cancer increased due to the change in lifestyle. Due to its devastating effect in terms of premature deaths and morbidity, it has caused a 20% global economic burden when compared to other diseases. Hence, in 2011, United Nations has included cancer in the list of noncommunicable diseases and has pledged to reduce death by 25% till the year 2025. There are many hurdles and challenges in cancer treatment with respect to clinical as well as from drug discovery point of view. After many decades of basic research work, we have been able to understand cancer. The modern-day technology has provided insights into the numerous factors that are relevant to cancer development and progression. However, still today in most of the cases, diagnosis of cancer is found only in its late phase. This issue of late diagnosis has defeated modern-day research, wherein newly developed drugs are not able to save lives of people even after their availability. The recent data, research, and facts need to be translated into new clinical approaches, as well as public education and policy initiatives. People need to be made aware about various factors that may lead to cancer development. Also there is an urgent need to strengthen the global cancer surveillance and cancer control programs (John and Ross 2010). At drug discovery and development levels, newer classes of compounds, biological methods, etc. need to be developed for achieving higher success rate of clinical useful drugs. Natural products, in spite of being the major source of drugs for millennia, face higher failure rate due to poor pharmacokinetics, bioavailability, poor efficacy, chemical instability, first-pass metabolism, and unwanted biodistribution in the organs of the body.

In the recent era, the nanotechnology has become a mainstay for the field of drug discovery and development. Nanoparticles (NPs) are defined as particulate matter ranging in the size of 1–100 nm, with an ability to carry or deliver medicinally active drugs, nutritional materials, diagnostic aids, etc. (Akhtar et al. 2013; Akhtar and Ahmad 2014; Swamy et al. 2015a, b). Major reasons for the development of nanotechnology platforms for pharmaceutical purpose have been attributed to the following reasons: reduction of toxicity of existing drugs, improvement of pharmacokinetic properties, formulation stability (shelf life), biocompatibility, etc. Various structural NPs such as liposomes (natural and synthetic lipids), micelles (amphiphilic biomaterials), dendrimers (branched biomaterials), exosomes (biomembrane), polymers (polylactic acid, polycaprolactam, polycyanoacrylates), carbon derivatives (various carbon allotropes), and inorganic derivatives (gold, silver, silica, Cd/Zn-selenides) have been used for development of nanoparticulate delivery (Fig. 2.1). Over a decade, nanotechnology has significantly contributed in the development of new anticancer products. The first nanoanticancer product to be approved by FDA was PEGylated liposomal doxorubicin in 1995 for treatment of HIV-related Kaposi sarcoma, ovarian cancer, and multiple myeloma. Since then many nanoparticulate delivery systems have been discovered and developed for tackling the menace of cancer (Table 2.1). Thus, the aim of the present chapter is to highlight the application of nanotechnology as an anticancer agent and also to develop nano-natural products for effective treatment of cancer.

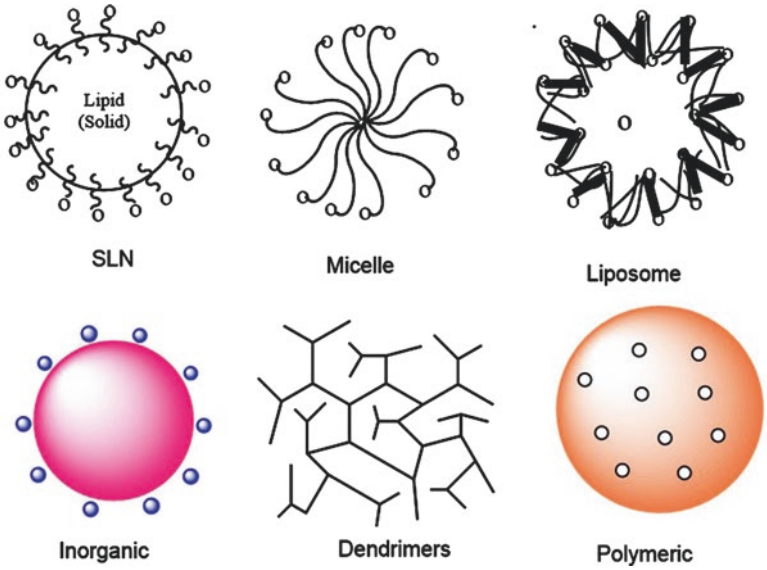

Fig. 2.1 Nanoparticulate systems available for delivery of chemicals

Table 2.1 Summary of some approved nanoparticulate systems used in cancer therapy

Active ingredient	Brand name	Platform technology	Cancer types
Doxorubicin	Doxil	PEGylated liposome	Kaposi sarcoma, ovarian cancer, and multiple myeloma
	DaunoXome	Liposome	Kaposi sarcoma
	Myocet	Liposome	Metastatic breast cancer
Irinotecan	Onivyde	PEGylated liposome	Metastatic pancreatic cancer
Paclitaxel	Abraxane	Albumin nanoparticles	Breast, lung, and pancreatic cancer
Vincristine sulfate	Marqibo	Liposome	Acute lymphoblastic leukemia

2.2 Natural Products and Anticancer Drug Discovery

Since primeval times, nature is recognized for being a source of diverse and unique chemical compounds used by mankind. The natural products or secondary metabolites are obtained from a wide range of naturally occurring sources like plants, minerals, animals, microbes, and marine fauna. These may serve as the basis of drug discovery programs for the past several decades due to their structural uniqueness and functional diversity (Newman 2008). The total number of natural products that have been identified from the various sources is approximately ~200,000 or a bit more. However, these may not be the actual figure, and a very small percentage

(about 15%) of higher plants have been explored, and a vast majority still remains to be explored. The major contribution of the above natural sources in drug development has been mainly in the area of cancer (60%) and infectious diseases (75%) (Cragg et al. 1997; Newman et al. 2003; Newman and Cragg 2007, 2012). Recently, Newman and Cragg (2016) have highlighted the contribution of natural products in the area of anticancer drugs. Among the various anticancer drugs discovered till date, the most successful are Taxol®, vincristine, camptothecin, etc. Some of the other molecules include temsirolimus (Torisel™), ixabepilone (Ixempra™), romidepsin (Istodax®), and amrubicin hydrochloride (Calsed®).

2.3 Nano-natural Products as Anticancer Agents

2.3.1 Phenolics

Phenolic compounds are one of the most abundant classes of secondary metabolite (Fig. 2.2). These compounds bear one or more hydroxyl groups on aromatic ring(s) and are referred to as polyphenolics at times. They are abundantly present in fruits and vegetables and exhibit a wide range of different biological activities such antioxidant, anti-inflammatory, antimicrobial, etc. They are known to possess the ability to prevent different types of cancers. Some of them are described below.

2.3.1.1 Quercetin and Citropten

Gismondi et al. (2015) reported the nanodiamond conjugation of citropten and quercetin to improve their antiproliferative activity on human (HeLa) and murine (B16F10) tumor cells. Nanodiamonds were synthesized by three different protocols (oxidation, chemical, and plasma reduction). The citropten and quercetin content in each mg of nanodiamond adduct was 0.5 mmol, respectively. However, citropten-loaded samples that were prepared by plasma reduction method contained exceptionally higher content of the drug. Treatment of HeLA cell line for 48 and 72 h resulted in great antiproliferative effects especially for citropten adduct prepared by oxidation and plasma reduction, which reduced cell growth better than pure citropten. On the other hand, nanodiamonds functionalized with quercetin similarly inhibited proliferation rate with respect to pure quercetin. In both the cell lines, citropten bioactivity was enhanced after conjugation with nanodiamonds prepared by oxidation and plasma reduction, while antiproliferative effects of quercetin were increased after conjugation with nanodiamonds prepared by chemical and plasma reduction. The observed effect may be due to the ability of nanodiamond adducts to stimulate cell processes, such as cell cycle differentiation, cell cycle arrest, etc. Various nanoparticulate delivery systems have been developed for effective delivery of quercetin for anticancer effect (Table 2.2).

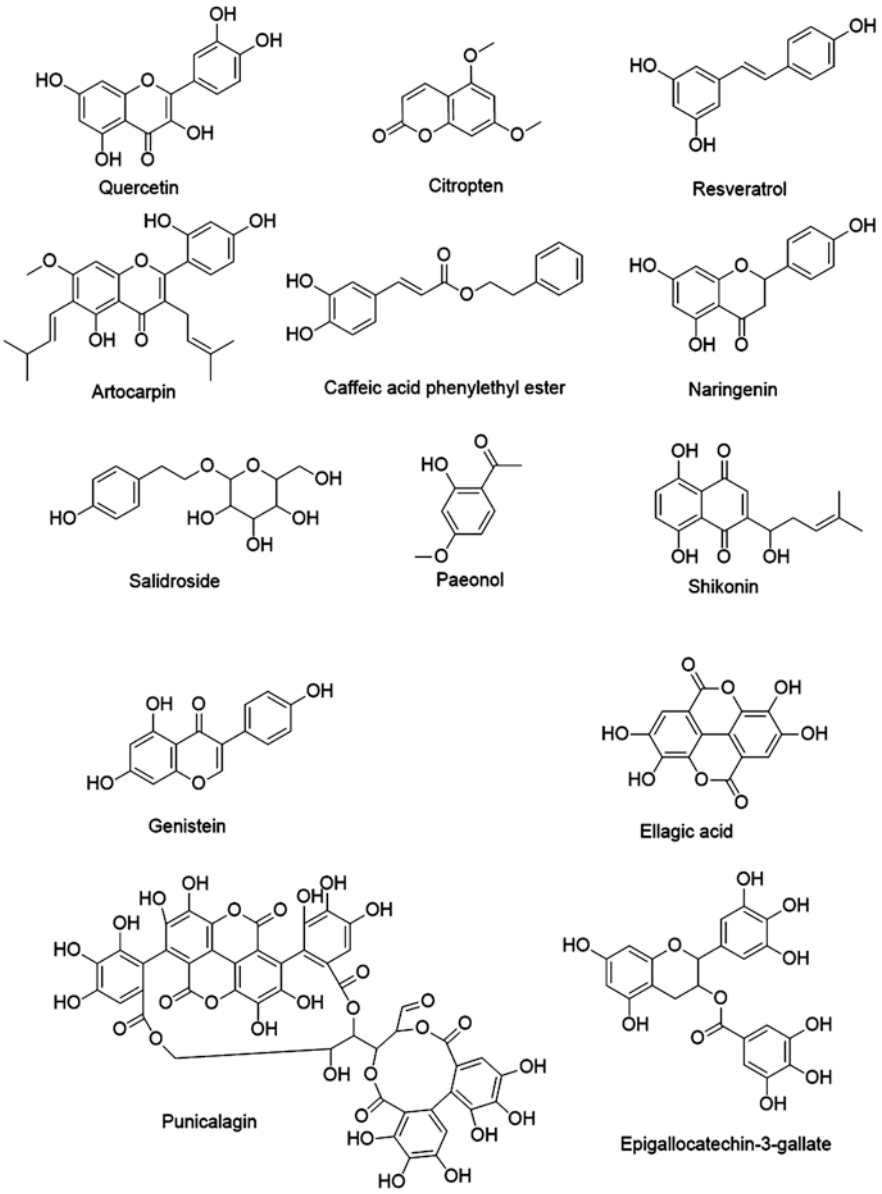

Fig. 2.2 Structures of polyphenolic class of compounds

Table 2.2 Quercetin-based nanoparticulate delivery systems and their anticancer effect in various cancer models

Carrier type	Modification	Cancer models	References
Nanoliposomes	Nil	C6 glioma cell	Wang et al (2012)
Nanomicelles	Nil	Lung tumor mice	Tan et al. (2012)
Folic acid-PEG-PLGA	Folic acid conjugation	HeLa cells; HeLa or IGROV-1tumor-bearing mice	El-Gogary et al. (2014)
NLCs	Nil	MCF-7 and MDA-MB-231 breast cancer cells	Sun et al. (2014)
Liposomes	Nil	MCF-7 breast cancer cells	Rezaei-Sadabady et al. (2016)

Table 2.3 Resveratrol-based nanoparticulate delivery systems and their anticancer effect in various cancer models

Carrier type	Modification	Cancer models	References
Bovine serum albumin	Nil	Human primary ovarian carcinoma cells in nude mice	Guo et al. (2010)
Dequalinium polyethylene glycol-distearoylphosphatidyletheanolamine conjugate	Nil	Human lung adenocarcinoma A549 cells, resistant and tumor spheroid A549/cDDP cells, and xenografted resistant A549/cDDP cancers in nude mice	Wang et al. (2011a)
Chitosan	Nil	HepG2 cells and Kunming mice	Bu et al. (2013)
Gelatin	Nil	Lung cancer (NCI-H460) cells	Karthikeyan et al. (2013)
PEG-PLA	Nil	CT26 colon cancer cells and CT26 tumor-bearing mice	Sanna et al. (2013)
SLN	Nil	C6 glioma cells	Jose et al. (2014)

2.3.1.2 Resveratrol

Teskac and Kristl (2010) have investigated solid lipid NPs (SLNs) of trans-resveratrol. The prepared SLNs were compared for their effects on the internalization, morphology, growth, metabolic activity, and genetic material of keratinocytes using NCTC2544 cell line. It was observed that SLNs with a size below 180 nm move promptly through the cell membrane, distributing throughout the cytosol and finally localizing in the perinuclear region without inducing cytotoxicity. An increase in solubility, stability, and intracellular delivery was observed for SLNs of resveratrol, wherein resveratrol was released in a biphasic pattern. Resveratrol SLNs were found to concentrate around nuclei and released resveratrol in a constant way to express its cytostatic effect with prominent S-arrest of cell cycle and a large drop of G2/M phase. Resveratrol has been delivered effectively through different NPs as summarized in Table 2.3.

2.3.1.3 Artocarpin

Artocarpin, a prenylated flavonoid isolated from *Artocarpus communis* extracts, has been found to induce death in human hepatocellular carcinoma cells. Tzeng et al. (2016) developed the PVP nanoparticulate system to overcome the poor water solubility and investigated the improvement in therapeutic efficacy of artocarpin against HepG2 and PLC/PRF/5 cell line. Artocarpin-PVP NPs enhanced the solubility of artocarpin by reducing particle size and forming hydrogen bonds. The NPs also showed superior autophagic cytotoxicity as indicated by increased autophagy-related protein LC3-II expression and the ratio of LC3-II/LC3-I after 24 h treatment. However, artocarpin was unable to induce autophagy in hepatoma cells and did not alter the LC3 expression.

2.3.1.4 Caffeic Acid Phenethyl Ester

Caffeic acid phenethyl ester (CAPE) NPs were prepared using methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone) copolymer, and their antitumor activity against pulmonary metastasis model of CT26 colon carcinoma cells was studied. CAPE NPs had particle size of <300 nm, and they inhibited proliferation, induced apoptosis, and showed anti-invasive and antimigrative effect against CT26 cells as compared to CAPE. CAPE NPs also showed superior antimetastatic efficacy in pulmonary metastasis model of CT26 cells in nude mouse (Lee et al. 2015).

2.3.1.5 Naringenin

Chemopreventive efficacy of naringenin-loaded NPs was described in 7,12-dimethylbenz[a]anthracene (DMBA)-induced experimental oral carcinogenesis in Syrian hamsters. Oral administration of naringenin-loaded NPs completely prevented tumor formation as compared to free naringenin and significantly reduced the degree of histological lesions. Additionally, in the buccal mucosa of DMBA-exposed animals, NPs downregulated the expression of proliferating cell nuclear antigen (PCNA) and p53 and exerted higher anti-lipid peroxidative and antioxidant activities, when compared with free naringenin (Sulfikkarali et al. 2013).

2.3.1.6 Salidroside and Paeonol

Peng et al. (2014) constructed a novel nanosphere gel for sequential delivery of salidroside and paeonol for anti-melanogenic activity. Nanospheres containing both paeonol and salidroside were incorporated in carbomer hydrogel to form a dual drug releasing nanosphere gel. Dynasan 116 and Miglyol 812 in a ratio of 6:4 gave the best nanoparticle (particle size was 275 nm) with a low PDI index (0.208). With this nanosphere gel, rapid release of salidroside from the hydrogel followed by

sustained release of paeonol from the nanosphere was achieved. The anti-melanogenesis effects were evaluated using melanogenesis in UVB-irritated guinea pig skin. Salidroside was released efficiently in the first hour (36.9%) with over 91% released over 12 h, while 67% paeonol was released in a sustained manner over 72 h in the nanospheres.

2.3.1.7 Shikonin

Matthaiou et al. (2014) formulated shikonin-loaded biodegradable NPs of poly(lactic-co-glycolic acid) (PLGA) using single/double emulsion-solvent evaporation/diffusion technique with sonication. The NPs were evaluated in tumor-associated endothelial MS1 cells, primary lymphocytes, and epithelial ovarian cancer OVCAR-5 cells. The surface of NPs was decorated with polyethylene glycol (PEG) and tumor endothelial marker 1 (TEM1)/endosialin-targeting antibody (Ab). The developed NPs showed a smooth spherical shape (120–250 nm), with final drug entrapment efficiency of ~60%. Shikonin was released in a sustained release manner from NPs. Ab-armed NPs interacted with TEM1-positive MS1 cells, but not with TEM1-negative MS1 cells. Exposure of the Ab-armed and PEGylated NPs to TEM1-positive MS1 cells and OVCAR-5 cells for long term proved to be significantly toxic.

2.3.1.8 Genistein

Genistein-loaded PLA nanocapsules were prepared by nanoprecipitation method and were incorporated into semisolid formulations. The optimized formulation contains nanocapsule of mean diameter of 139 nm, PDI index of 0.128, encapsulation efficiency of 89%, and drug loading from 0.6 to 1.4 w/w%. The developed nanocapsules were stable during the 90 days of the assay; however, a drop in encapsulation efficiency was observed in the first 10 days. Permeation experiments were carried out using porcine ear skin, where it was found that higher amount of genistein reached deeper layers of the skin with the help of nanocapsules (Zampieri et al. 2013). Different genistein NPs that have been developed by various research groups are listed in tabular form (Table 2.4).

Table 2.4 Genistein-based nanoparticulate delivery systems and their anticancer effect in various cancer models

Carrier type	Modification	Cancer models	References
NLCs	Nil	Prostate cancer cells	Aditya et al. (2013)
Liposomes	Nil	PC-3 and OVCAR-3 cancer cells	Phan et al. (2013)
Lipidic micelles and nanoemulsions (NEs)	Nil	CT26 and HepG2 cells	Pham et al. (2013)
Polymeric nanocapsules	Nil	Ehrlich ascites tumor (EAT)-bearing mice	Mendes et al. (2014)

2.3.1.9 Pomegranate Polyphenolics

Shirode et al. (2015) developed a novel formulation of poly(d,l-lactic-*co*-glycolic acid)-polyethylene glycol NPs loaded with pomegranate extract (PE) and individual polyphenols, namely, punicalagin (PU) and ellagic acid (EA). Using double emulsion-solvent evaporation method, monodispersed, spherical (150–200 nm) NPs were prepared. NPs of PE, PU, and EA had a 2- to 12-fold enhanced effect on cell growth inhibition compared to their free counterparts, while void NPs did not affect cell growth. PU-NPs were the most potent nanoprototype of pomegranates with IC_{50} values of 4.45 μg/ml (Hs578 T cells) and 8.13 μg/ml (MCF-7 cells), respectively.

2.3.1.10 Tea Polyphenolics

Polyphenolics found in green tea are of great interest to the scientific community due to a myriad of biological properties such as cardioprotective, neuroprotective, and anticancer effects. But their potential is limited by their low oral bioavailability and poor stability. Various research groups have investigated this class of molecules and developed nanoparticulate delivery systems. Khan et al. (2014) developed epigallocatechin-3-gallate-loaded (Chit-nano EGCG) chitosan nanoparticle-based oral formulation for the treatment of prostate cancer. The developed formulation resulted in an initial release profile of ~20%, due to the release of the desorbed EGCG from the particle surface, which was followed by a slow release of ~50% up to 24 h. The antitumor efficacy of Chit-nano EGCG was assessed in subcutaneously implanted 22Rν1 tumor xenografts in athymic nude mice. Chit-nano EGCG significantly inhibited the growth of tumor and secretion of prostate-specific antigen levels compared with EGCG and control groups. Chit-nano EGCG caused induction of poly(ADP-ribose) polymerases cleavage, increase in the protein expression of Bax with concomitant decrease in Bcl-2, activation of caspases, and reduction in Ki-67 and proliferating cell nuclear antigen in tumor tissues of mice treated with Chit-nano EGCG.

Singh et al. (2015) developed PLGA-based nanoparticulate formulation of theaflavin (TF) and EGCG with 26% and 18% encapsulation efficiency, respectively. The encapsulated TF-PLGA NPs exhibited a pronounced antiproliferative effects (IC_{50} value of 6 μM in A549 cells, 7 μM in HeLa cells, and 15 μM in THP-1 cells), while EGCG-PLGA-NPs inhibited cell proliferation, with an IC_{50} dose of 9 μM (A549 cells), 12 μM (HeLa cells), and 27 μM (THP-1 cells). It also enhanced the anticancer potential of cisplatin (CDDP) in A549 (lung carcinoma), HeLa (cervical carcinoma), and THP-1 (acute monocytic leukemia) cells. TF/EGCG NPs were more efficient than bulk TF/EGCG in sensitizing A549 cells to CDDP-induced apoptosis, with a dose advantage of up to 20-fold. The developed NPs effectively inhibited NF-κB activation and suppressed the expression of cyclin D1, matrix metalloproteinase-9, and vascular endothelial growth factor. They also induced the cleavage of caspase-3 and caspase-9 and Bax/Bcl2 ratio in favor of apoptosis. Combination study of these

Table 2.5 EGCG-based nanoparticulate delivery systems and their anticancer effect in various cancer models

Carrier type	Modification	Cancer models	References
PLGA-PEG	Nil	Prostate cancer (LNCaP) cells	Sanna et al. (2011)
Nanoliposomes	Chitosan-coated nanoliposomes	Breast cancer (MCF-7) cells	de Pace et al. (2013)
Micellar nanocomplexes	Herceptin: oligomerized EGCG: PEG-EGCG	Breast cancer (BT-474) cells and athymic nude-Foxn1nu female mice inoculated with BT-474 cells	Chung et al. (2014)
Chitosan	Nil	Human melanoma cells; athymic (nu/nu) male nude mice treated with 1XPBS	Siddiqui et al. (2014)

NPs with CDDP resulted in an increase of life span in mice bearing Ehrlich ascites carcinoma cells. Hsieh et al. (2011) physically attached EGCGs onto the surface of nanogold particles (pNG) and investigated their anticancer potential in C3H/HeN mice subcutaneously implanted with MBT-2 murine bladder tumor cells. EGCGepNG inhibited the tumor cell growth by induction of cell apoptosis via activation of caspase cascade through the Bcl-family proteins in the mitochondrial pathway. They also suppressed growth of bladder tumors when injected directly into the tumor site through downregulation of VEGF in model mice.

Rocha et al. (2011) have prepared polysaccharide (gum arabic and maltodextrin) NPs for delivery of ECGC with an encapsulation efficiency of approximately 85%. The encapsulated EGCG at concentrations of 1–2 μM caused inhibition of cell proliferation (10–20%) compared with free EGCG. It also reduced the cell viability and induced apoptosis of Du145 prostate cancer cells. Various EGCG NPs having anticancer activities are summarized in tabular form (Table 2.5).

2.3.2 Alkaloids

Alkaloids are naturally occurring nitrogenous compounds found in plants, microbes, marine organisms, and to a lesser extent animal sources. They contain basic nitrogen which imparts reactivity to this class of molecules (Fig. 2.3) and tend to exhibit mainly pharmacological activities such as anticancer, anti-inflammatory, and analgesic activities.

2.3.2.1 Paclitaxel and Docetaxel

Liu et al. (2015) developed a paclitaxel-rubusoside (PTX-RUB) nanomicellar formulation. Rubusoside is a steviol glycoside with unique solubilizing properties. PTX-RUB complexes were dried to a powder which was subsequently reconstituted in physiological solutions. PTX-RUB exhibited cytotoxicity against three human

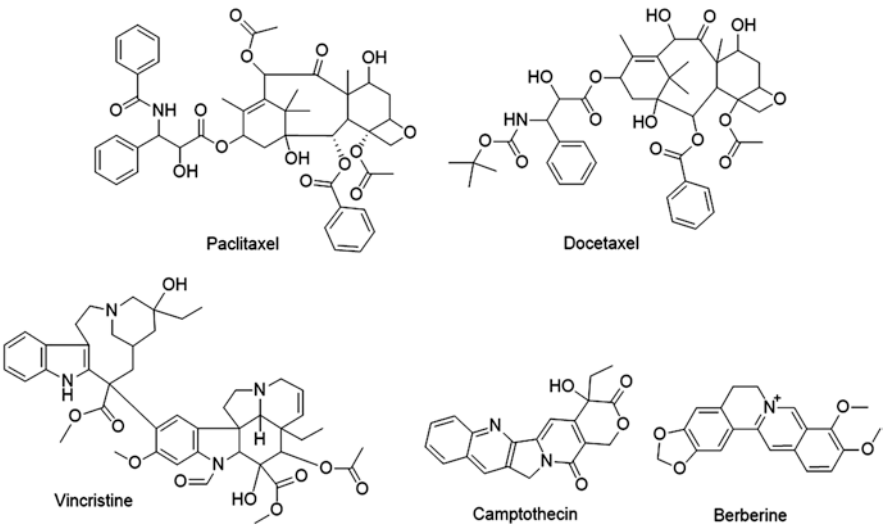

Fig. 2.3 Structures of alkaloid class of compounds

cancer cell lines with IC_{50} values ranging from 4 to 20 nM when compared to DMSO-solubilized PTX. PTX-RUB NPs were nearly four times more permeable in Caco-2 cell monocultures as compared to Taxol®. At concentration of 5 nM, tubular formation and migration of HUVECs were inhibited by PTX-RUB NPs. In another attempt to decrease severe side effects of paclitaxel, Lu et al. (2013) developed a micelle-based PTX formulation by conjugating it with PEG-5000 and embelin (EB). Embelin is a natural antitumor principle present in *Embelia ribes* and acts via blocking the activity of X-linked inhibitor of apoptosis protein (XIAP). Cell uptake study showed that the PEG5K-EB2 micelles were selectively taken up by tumor cells. The same was confirmed through total body near-infrared fluorescence (NIRF) imaging. PTX-PEG5KEB2 micelles showed more potent cytotoxicity than Taxol in several cultured tumor cell lines and superior antitumor activity in murine models of breast and prostate cancers.

Najlah et al. (2016) investigated PTX-loaded Clinoleic 20% and Intralipid 20% nanoemulsion for cytotoxicity against U87-MG glioma cell. The PDI values of both the Clinoleic and Intralipid formulations were less than 0.2. Loading efficiency for PTX for Clinoleic formulation was 70.4–80.2%, while that for Intralipid formulation was 44.2–57.4%, respectively. The viability of U87-MG cell was decreased to 6.4% by PTX-loaded Clinoleic formulation as compared to PTX-loaded Intralipid formulation (21.29%). Thus, the study highlights the potential of PTX formulation for treatment of glioma. However, Kim and Park (2017) investigated hyaluronan-coated nanoemulsions (HNEs) for enhancing PTX solubility and tumor cell targeting. The HNEs consisted of dl-α-tocopheryl acetate, soybean oil, polysorbate 80, and ferric chloride. This composition was coated with hyaluronic acid (HA) as a targeting moiety. High-pressure homogenization with a microfluidizer was used to

prepare the NEs with particle size of 65 nm. HNEs displayed a tenfold higher targeting capability in SKOV-3 (cluster of differentiation 44 [CD44]+) and OVCAR-3 (CD44−) cells.

Verma et al. (2016) developed stable, effective, and safe nanoemulsion (NE) of docetaxel (DTX) using hot homogenization followed by ultra-sonication and soybean oil, lecithin, Pluronic F68, PEG 4000, and ethanol as excipients. Among the various formulation batches, NE F11 displayed 2.83 times higher cell uptake than control and strong cytotoxic activity (IC_{50} 13.55 μg/mL) against MCF-7 cancer. Similarly, Jin et al. (2016) studied the advantage of docetaxel-loaded PEG-albumin NPs (PEG-DANPs) against non-small cell lung cancer (NSCLC). It was found that PEG-DANPs showed a dose- and time-dependent efficacy in the *in vitro* cytotoxicity studies. At a dose of 20 mg/kg, it also reduced the tumor's growth and metastasis in the livers of NSCLC-bearing nude mice, while Wang et al. (2011b) designed and studied the clinical effects of a lipid-based nanosuspension (LNS) of docetaxel (DTX). DTX-LNS were prepared by high-pressure homogenization method, with a particle size of 200 nm. DTX-LNS showed antitumor efficacy and increased survival rate in B16 melanoma-bearing mice. The *in vitro* cytotoxic activity was assessed by MTT against SKOV-3 and malignant melanoma B16 cells. The *in vivo* pharmacokinetics, tissue distribution, and antitumor efficacy were investigated in B16 melanoma-bearing Kunming mice. The zeta potential of DTX-LNS was −11.15 mV. Together these results suggested that DTX-LNS could effectively inhibit tumor growth, reduce toxicity during the therapeutic procedure, and hold the potential to be an appropriate choice for the clinical administration of DTX.

2.3.2.2 Camptothecin

Camptothecin, a cytotoxic alkaloid from *Camptotheca acuminata*, was loaded in iron oxide-based superparamagnetic NPs (particle size 14 nm). The developed NPs displayed proapoptotic activity in H460 lung cancer cell line (Castillo et al. 2014). In another study, poor solubility of camptothecin analog (CA) was addressed by formulating a PEG-based nanoparticulate system using high-pressure homogenization technique. The nanoparticulate formulation showed equivalent antitumor activity to that of standard irinotecan in human tumor xenografts (NCI-H460 cell lines) grown in athymic nude mice (Nekkanti et al. 2011).

2.3.2.3 Berberine

A liquid and solid form of self-nanoemulsifying drug delivery system (SNEDDS) of berberine was developed using Acrysol K-150, Capmul MCM, and polyethylene glycol 400 (Pund et al. 2014). The developed SNEDDS possessed globule size of 17–45 nm and exhibited higher extent of release of berberine than berberine alone. The berberine SNEDDS exhibited potent anti-angiogenic activity in chick chorioallantoic membrane assay.

2.3.2.4 Vincristine

To overcome the multidrug resistance (MDR) challenge, multifunctional nanoassemblies (MNAs) were developed for vincristine sulfate (VCR) (Zhang et al. 2011). An encapsulation efficiency of up to 94.4% was achieved for VCR in MNAs by addition of phosphatidylserine (PS). A 36.5-fold increase in cytotoxicity of VCR-MNAs was observed in MCF-7/Adr cells as a result of increased (12.6-fold) cellular accumulation of VCR via clathrin- and caveolae-mediated endocytosis pathways that escaped the efflux induced by P-gp.

2.3.3 Phenyl Propanoids

Phenyl propanoids are structurally simple but unique organic compounds that contain a three-carbon-length propene group attached to a six-carbon aromatic phenyl group. These compounds are synthesized in plants from the amino acids phenylalanine and tyrosine, respectively. They also serve as the starting material for the biosynthesis of a number of structurally and functionally diverse classes of compounds such as lignans, polyphenols, etc. (Fig. 2.4).

2.3.3.1 Salvianolic Acid B

Salvianolic acid B (SalB) is an anticancer compound that has been isolated from *Salvia miltiorrhiza* Bge (Danshen or Tanshen). Li et al. (2016) developed phospholipid complex-loaded NPs (PLC-NPs) encapsulating SalB (particle size 112 nm) for

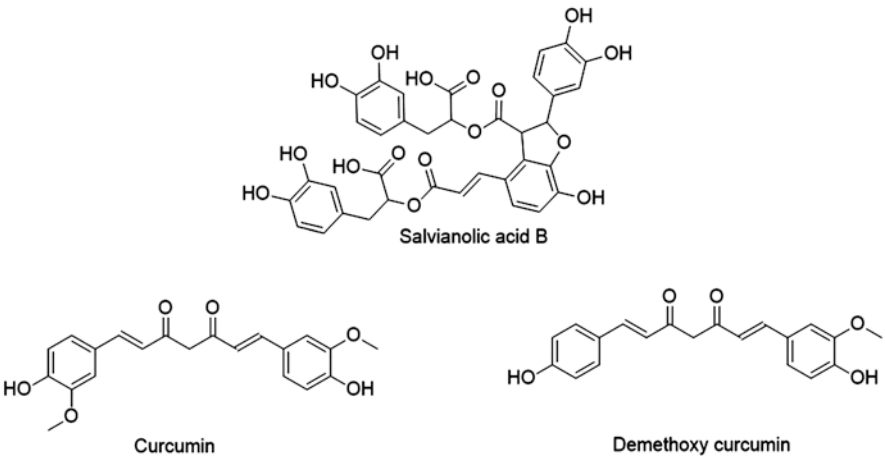

Fig. 2.4 Structures of phenyl propanoid class of compounds

investigation against human HNSCC (HN13, HN30) cells and oral precancer Leuk1 cells. Nano-SalB significantly inhibited the cell growth of HN13 and HN30 cells to 56.1% and 29.3%, respectively, as compared to equivalent amount of free SalB. Nano-SalB inhibited the proliferation of HN13 cells via a combination of cell cycle arrest and induction of apoptosis, while in HN13 cells inhibition of proliferation was only the result of cell cycle arrest.

2.3.3.2 Curcumin and Demethoxycurcumin

Curcuminoids are the active principal components of the popular Indian spice, turmeric, which consists of rhizomes of *Curcuma longa* (Zingiberaceae). Among the various curcuminoids, curcumin is the principal component responsible for the observed biological activities including anticancer activity against a wide range of cancers such as colon and pancreatic cancer (Half and Arber 2009; Johnson and Mukhtar 2007), myelodysplastic syndromes (Hatcher et al. 2008), and multiple myeloma (Ghoneum and Gollapudi 2011; Park et al. 2008; Sung et al. 2009). Various types of nanoparticulate systems such as liposome/phospholipid, solid lipid NPs, polymer NPs, nano/microemulsions, polymeric micelles, nanogels, conjugates, etc. have been developed and tested to solve low solubility, bioavailability, stability, and delivery issues of curcumin (Table 2.6).

2.3.4 Saponins and Their Aglycones

Saponins are often referred to as the class of compounds that often produce soap-like foaming when shaken in aqueous solutions. Structurally they contain two parts, namely, the "glycone" (one or more hydrophilic sugar moieties) and a glycone (a lipophilic triterpene or steroid derivative). Various saponin derivatives have been isolated from natural sources (Fig. 2.5), and studies on their nanoparticulate delivery have been investigated.

2.3.4.1 Saikosaponin

Polyvinylpyrrolidone (PVP)-based NPs of saikosaponin-d were prepared by grinding through a planetary ball mill. The developed NPs possessed an average size of 373.6 nm with polydispersity index of 0.76. The NPs showed enhanced antiproliferative activity against human A375.S2 melanoma cells and induced apoptosis through the mitochondrial pathway. The anti-melanoma activity was mediated by increasing the phosphorylation of JNK and p38. NPs also increased the activation of p53, level of cytochrome-c, and activation of caspase 9 (Hu et al. 2016).

Table 2.6 Curcumin-based nanoparticulate delivery systems and their anticancer effect in various cancer models

Carrier type	Modification	Cancer models	References
MPEG-PCL micelles	Nil	Colon cancer model using BALB/c mice	Gou et al. (2011)
Bovine serum albumin	Nil	Breast cancer (MDAMB231) cell line	Jithan et al. (2011)
Den O400		Normal mouse embryonic fibroblast (MEF), human epidermoid carcinoma (A431) cell lines, and BALB/c mouse fibrosarcoma (WEHI-164)	Babaei et al. (2012)
PLGA	Nil	Liver cancer (HeLa) cells	Nair et al. (2012)
PLGA	Nil	Multidrug-resistant cervical carcinoma (KB-V1) and drug-sensitive (KB-3-1) cells	Punfa et al. (2012)
Liposomes	Nil	Human pancreatic cancer (MiaPaCa) cells and pancreatic tumor xenograft	Ranjan et al. (2013)
Solid lipid NPs	Nil	Breast cancer (MCF-7) cells and male Sprague-Dawley rats	Sun et al. (2013)
Solid lipid NPs	Nil	Lung cancer (A549 cells) and nude mice bearing A549 cell xenografts	Wang et al. (2013)
Solid lipid NPs	Nil	Liver cancer (SMMC-7721) cells	Zhu et al. (2013)
Chitosan	Thiolation	Colon cancer (HT29) cells in Swiss albino mice	Anitha et al. (2014)
PVP	Loading recombinant IκBα on curcumin NPs	Cervical carcinoma (HeLa) and glioblastoma cells (U87MG)	Banerjee et al. (2014)
PLGA, soybean lecithin, and DSPE-PEG 2000/curcumin encapsulated bioconjugates	EpCAM Apt	Human colon cancer (HT29, EpCAM+) and human embryonic kidney (HEK293T, EpCAM−) cells	Li et al. (2014)
Lipid	Nil	MDA-MB-231 breast cancer cells	Palange et al. (2014)
Nanostructured lipid carriers	Nil	Prostate cancer PC3 cells	Yallapu et al. (2014)
Chitosan and gum arabic	Nil	Human colon carcinoma (HCT116)-BCRC 60349 and human colorectal adenocarcinoma (HT29)-BCRC 60157 cell lines	Udompornmongkol and Chiang (2015)

(continued)

Table 2.6 (continued)

Carrier type	Modification	Cancer models	References
Nanohybrids	Nano-Ag and chitosan	Mouse fibroblast (L929) and human colorectal adenocarcinoma (HT-29) cells	Barbinta et al. (2016)
Dendritic mesoporous silica NPs	Calcium doping of dendritic mesoporous silica NPs modified with folic acid	Breast cancer (A549 and MCF-7) cells; BALB/c nude mice	Wang et al. (2016)
PLGA	Nil	HPV-infected cervical cancer (Caski and SiHa) cell lines and NOD-SCID gamma (NSG) orthotopic xenograft mouse model	Zaman et al. (2016)
PEG	Transferrin conjugation	Breast cancer (MCF-7) cells and BALB/c nude mice	Cui et al. (2017)
Polyamidoamine dendrimer	Nil	Breast cancer cells (MCF-7) cells	Falconieri et al. (2017)
Curcumin nanosuspension	mPEG2000-DSPE and soybean lecithin as stabilizer	Hela, 4T1, HCT-8, and HepG2 cell lines and H22 tumor-bearing mice	Hong et al. (2017)

2.3.4.2 3α,24-Dihydroxyurs-12-Ene and 3α, 24-Dihydroxyolean-12-Ene (TPD)

It is obtained from boswellic acids loaded in PLGA (TPDPLGA-NPs) to develop NPs using emulsion-solvent evaporation method. The developed TPDPLGA-NPs had a particle size distribution of 152.56 nm and zeta potential of −17.36 mV. 51.03 μg/mg of TPD could be loaded in the optimized formulation, which exhibited a sustained release profile. TPDPLGA-NPs inhibited OVCAR-5 cells with IC_{50} values 32.8 μM after 48 h. The developed TPDPLGA-NPs also exhibited synergistic interaction effect in combination with cisplatin (CI <1), where it caused a 3.8-fold reduction of cisplatin (Alam et al. 2016). In another study, the same TPD from *Boswellia serrata* was formulated into solid lipid NPs (SLNs) by the microemulsion method. The TPD-SLNs obtained were solid, and spherical particles in the range of 100–200 nm, which inhibited the HL-60 cells, cells with an IC_{50} value of 9 μg/ml. TPD-SLNs induced apoptosis by intrinsic and extrinsic apoptotic pathways via activation of both TNF-R1 and cytochrome-c in HL-60 cells. TPD-SLNs were 12 times more active than TPD alone in sarcoma-180 solid tumor-bearing mice exhibiting and proving their *in vivo* potential (Bhushan et al. 2013).

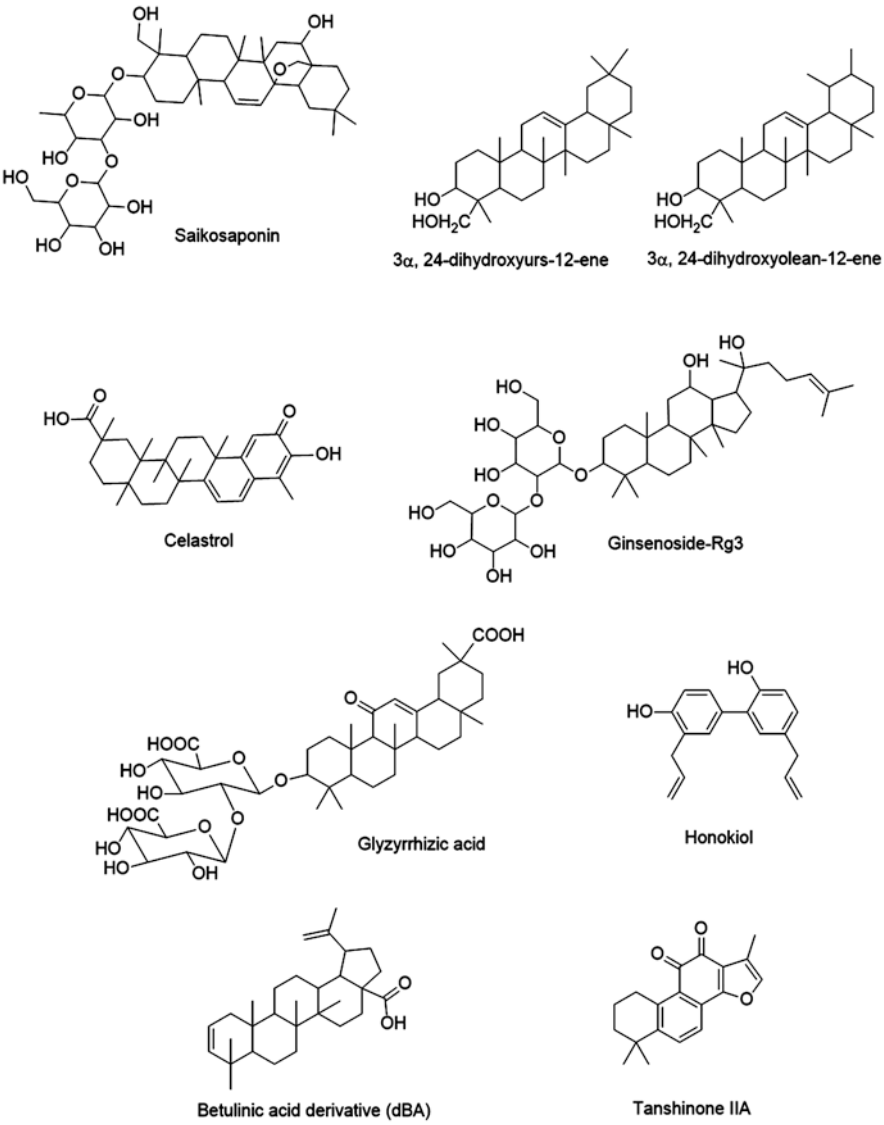

Fig. 2.5 Structures of saponin class of compounds

2.3.4.3 Celastrol

Celastrol is a triterpene, which is extracted from *Tripterygium wilfordii* Hook F. Poly(ethylene glycol)-block-poly(ε-caprolactone) nanopolymeric micelles were developed incorporating celastrol to study the inhibitory effect of these NPs on SO-Rb 50 cell proliferation. It was found that CNPs inhibited the proliferation of

SO-Rb 50 cells in a dose- and time-dependent manner with an IC_{50} of 17.73 μg/mL via decreasing the expression of Bcl-2, NF-κB p65, and phospo-NF-κB p65 at a concentration of 54.4 μg/ml for 48 h. However, the Bax/Bcl-2 ratio was found to increase, while the expression of Bax was not significantly changed. The *in vivo* potential of CNPs was evaluated using a human retinoblastoma xenograft model in NOD-SCID mice. CNP at a dose of 27.2 mg/kg/2 days successfully inhibited the growth of retinoblastoma and induced apoptosis in retinoblastoma cells in mice (Li et al. 2012).

2.3.4.4 Ginsenoside Rg3

Ginsenoside Rg3 is a saponin derived from the Chinese herb *Panax ginseng*. A VEGFR-3 antibody-conjugated ginsenoside Rg3 nanoemulsion (VRIN) was prepared and evaluated in an orthotopic mouse model of human gastric cancer. VRIN (1 mg/kg every other day, i.v.) was administered to the tumor-bearing mice. Both VRIN and the standard drug 5-FU significantly inhibited primary tumor growth as compared to vehicle control, while VRIN-treated group showed significant inhibition of lymph-node metastasis. VRIN-treated group also showed inhibition of VEGF-C, VEGF-D, and VEGFR-3 expression in the tumor (Dai et al. 2017).

2.3.4.5 Glycyrrhizic Acid

Chandrasekharan et al. (2011) developed a silver NP (SN)-complexed glycyrrhizic acid (GLY)-based delivery system for radioprotective application. On oral administration, the SN-GLY complex (50 mg GLY/kg body weight) was found to reduce the radiation-induced damage in peripheral blood leucocytes, bone marrow cells, and spleen cells of Swiss albino mice after 1 h of radiation exposure. Also SN-GLY administration resulted in reduction in micronucleus formation and chromosomal aberrations induced by exposure of mice to whole-body gamma irradiation.

2.3.4.6 Honokiol

Honokiol is a constituent of Chinese medicinal herb *Magnolia officinalis* or *M. grandiflora*. PCL-PEG-PCL (PCEC)-based monodispersed NPs (<200 nm) were prepared by solvent diffusion method without using any surfactants. The prepared PCL-PEG-PCL NPs showed poor cytotoxicity (IC_{50} > 5 mg/mL) toward HEK293 cells. They also inhibited the growth of cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) human ovarian cancer cells at various concentrations used (Gou et al. 2009).

2.3.4.7 *Phytolacca decandra*

PLGA-based NPs of a betulinic-acid derivative (dBA) that was isolated from an ethanolic extract of *Phytolacca decandra* were developed. The developed NPs of dBA (NdBA) were tested for anticancer activity using lung adenocarcinoma A549 cancer cells. NdBA induced apoptosis in A549 cancer cells via ER stress and Ca^{+2}-associated mitochondria-dependent pathway at IC_{50} values of 50 mg/ml and 100 mg/ml, respectively. NdBA also caused the breakdown of the oxidative phosphorylation system by depletion of ATP/ADP ratio and elevation of ROS (Das et al. 2014).

2.3.4.8 Tanshinone IIA

Tanshinone IIA (TA) is a major active component of Danshen (*Salvia miltiorrhiza* bunge), a well-known traditional Chinese medicine. Various NEs (2^3 factorial design) of TA were prepared using emulsification/high-pressure homogenization method and ratios of Tween 80, lecithin, and water. TA-NE-F4 (particle size 95 nm, spherical and intact; entrapment efficiency 99.3%) exhibited time- and dose-dependent manner cytotoxicity against T24 human bladder cancer cells that was 103-fold greater than pure TA alone (Chang et al. 2011).

2.4 Conclusions and Future Prospects

Various factors such as environmental pollution and changed lifestyle are always associated with the increased risk of cancer. In this regard, the natural products have proven their anticancer potential for the drug discovery. Nowadays, these drugs are successful at clinical level. In addition to these drugs, many candidates are still in clinical trial phases. In spite of such contribution, there has not been a decline in the incidences and demands to detailed study in both the disease understanding and drugs acting on them. Some major hurdles in the successful development of anticancer agents are poor solubility, development of resistance, unwanted side effects, etc. Nanotechnology platform has been successful in recent times to answer and solve the above hurdles via "nano-natural products" and has provided a new dimension and face to natural products in treatment of cancer.

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Chapter 3
Natural Anticancer Compounds and Their Derivatives in Clinical Trials

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Abstract Cancer continues to be a global challenge to both clinicians and researchers with an increasing mortality rate. Despite the enormous progress made in the anticancer drug discovery, there is a constant demand for novel therapeutic agents, because of the development of resistance to the existing chemotherapeutic drugs and their adverse side effects. The anticancer drugs derived from the natural sources have shown to be effective and safe in the treatment of cancers. Secondary metabolite compounds from plants such as alkaloids, flavonoids, and carotenoids are known for their cancer prevention and antitumor properties. Peptides produced from marine organisms and anthracyclines synthesized by microbes as secondary metabolites are also known for their anticancer properties. Some of these natural compounds are widely used in cancer therapy, and some are under clinical or preclinical trials. Some of the potential anticancer agents from plants (paclitaxel, vincristine, vinblastine, irinotecan, etoposide, topotecan, and camptothecin), marines (dolastatin 10, cytarabine, and aplidine), and microorganisms (bleomycin, doxorubicin, and dactinomycin) have been used in cancer therapy. Cancers are characterized by the alterations in the cell signaling pathways. Most of the current anticancer therapies involve the modulation of altered signaling targets in cancers. The advantage of using natural compounds with antitumor properties for cancer therapy is that the compounds have well-defined signaling targets with a minimal toxicity. Natural anticancer drugs have been categorized based on their target-specific signaling pathways, which include DNA-damaging drugs, methyltransferase inhibitors, mitotic

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disrupters, and histone deacetylase inhibitors. Thus, the present chapter highlights the natural anticancer compounds and their derivatives which are under clinical trials and their mechanism of action in cancer therapy.

Keywords Anticancer · Clinical trial · Natural compound · Toxicity · Therapy

3.1 Introduction

Cancer is a major cause of death globally. Despite the progress in the field of cancer drug development, still there is a need for the discovery of anticancer therapeutic agents. Cancer is an abnormal growth of cells in the body that can lead to death. The causes for cancer may include environmental factors, genetic predisposition, and improper diet. Environmental factors that increase cancer risks include smoking, chemicals, and radiation in our surroundings along with polluted food, water, and air. Other factors which are likely to affect include unhealthy diet, use of tobacco, as well as infectious diseases. Cancer begins with gene mutation, which regulates the cell division (Dizon et al. 2016). Normal cells repair most of the mutations in their DNA, but the unrepaired mutations lead to cancer. In most of the cases, cancer cells invade and destroy normal cells. Estimates from the American Society and the International Union Against Cancer reported that 12 million new cases of cancer were reported in the year 2014, with 7 million deaths worldwide. However, the current annual statistical estimate from the American Cancer Society has reported that the death rate from cancer in the USA has declined steadily over the past two decades. The cancer death rate fell 23% from its peak in 1991–2012 for men and women combined. The most recent 4-year (2009–2012) data showed that the rate of new cancer diagnoses decreased by 3.1% per year in men and no change in women. Approximately, a total of 16,88,780 new cancer cases and 6,00,920 deaths from cancer in the USA is projected to occur in 2017 (Siegel and Miller 2017). By the year 2030, 27 million cases of cancer with 17 million deaths are estimated worldwide. The current cancer treatments like chemotherapy and radiotherapy can put patients under a lot of strain and further damage their health. Therefore, researchers are focusing on using natural compounds for therapies against cancer (Bhanot et al. 2011; Rahib et al. 2014; Greenwell and Rahman 2015). Natural products from plant source have been used in the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in China, Egypt, Greece, and India from ancient times. Although a number of medicines are available in the market to treat cancer, there is no single drug found to be completely effective and safe. Therefore, the natural lead compound constituents from plants, microbes, and marine fauna and flora are being investigated for their anticancer activities in recent years. Many naturally occurring compounds and their derivatives have been identified as potent anticancer agents. Natural compounds have played a significant role in the development of more than 60% of clinically useful anticancer agents. Among them, a considerable number of natural compounds are in clinical and preclinical

development. The consumption of foods rich in anticancer compounds such as fruits, vegetables, and spices has shown a lower incidence of cancers (oral cavity, stomach, esophagus, lung, pharynx, endometrium, pancreas, and colon) (Cragg and Newman 2005; Bhanot et al. 2011; Greenwell and Rahman 2015; Kinghorn 2015). In the present chapter, some of the most potent naturally occurring anticancer agents from plants, marines, and microorganisms and their derivatives which are in clinical trials for cancer therapy are discussed. In addition, the targets of these anticancer agents and the mechanism of action in killing cancer cells in cancer therapy are briefly discussed. The listed natural compounds in this chapter have current or recent clinical trial status shown mostly in the NIH (https://clinicaltrials.gov/) or the corresponding EU clinical trial database.

3.2 Anticancer Compounds from Plant Source

Medicinal plants are used for their health benefits for thousands of years in folk medicines of Asia and Africa. Plants have a long history of use in the treatment of cancer. Lists of more than 3000 plant species have been reportedly used in the treatment of cancer. The discovery of vinca alkaloids (vinblastine and vincristine) and podophyllotoxin in the 1950s has encouraged scientists in the development of anticancer compounds from plants (Cragg and Newman 2005). These discoveries drive the United States National Cancer Institute (NCI) to initiate a widespread plant collection program in 1960, leading to the discovery of several novel chemotypes showing a range of cytotoxic activities. These include taxols and camptothecins, but it took 30 years, from the early 1960s to the 1990s, to develop these compounds into clinically active agents. Later in 1986, NCI developed the plant and other organism collections and new drug screening technologies. Through this program, several novel lead compounds have been discovered and are used in various stages of clinical and preclinical development (Kinghorn 2015). Vinca alkaloids, such as vinblastine (VLB), vincristine (VCR), vinorelbine, and vindesine isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae), were the first agents to advance into clinical use. These compounds are used in combination with other drugs for the treatment of a variety of cancers, including leukemias, advanced testicular cancer lymphomas, breast and lung cancers, and Kaposi's sarcoma. Podophyllotoxin, a lignan isolated from *Podophyllum peltatum*, has a long history for treatment of skin cancer and warts. Podophyllotoxin was failed under clinical trial because of high toxicity, but its semisynthetic derivatives such as etoposide and teniposide have shown anticancer property with low toxicity. Currently, etoposide and teniposide are being used for the treatment of lymphoma and bronchial and testicular cancer. These compounds act by inhibiting topoisomerase II, an important enzyme involved in the replication pathway of DNA during cell cycle progression (Newman et al. 2003; Greenwell and Rahman 2015; Kinghorn 2015). Anticancer compounds from secondary metabolite of plants such as alkaloids, terpenes and sterols, and phenols are discussed below for their clinical status and mechanisms of action (Table 3.1; Figs. 3.1a and 3.1b).

Table 3.1 Anticancer compounds from plant sources

Class	Compound	Plant source	Mode of action	Clinical trial stage	References
Alkaloids	Vinca alkaloids	*Catharanthus roseus*	Microtubule inhibitor	VCR, VLB approved for ALL, AML, and VRL, VDL approved for breast and small cell lung cancer	Mukhtar et al. (2014) and Kaur and Arora (2015)
	Vincristine (VCR)			High-risk vascular tumors, AML – phase II for VCR	
	Vinblastine (VLB)			Low-grade glioma – phase II for VLB	
	Vinorelbine (VRL)			Metastatic breast cancer, NSCLC – phase II (r) ovarian cancer, melanoma – phase II (c) for VRL	
	Vindesine (VDL)				
	Camptothecin	*Camptotheca acuminata*	Topoisomerase inhibitor	Pancreatic, colorectal, and ovarian cancers – phase I/II/III (c) and relapsed SCLC – phase I/II (r)	Pommier (2006), Venditto and Simanek (2010), and Wahid and Bano (2014)
	Piperine	*Piper nigrum*	Pro-apoptotic	Bladder cancer – phase I (r)	Singh and Duggal (2009) and Chinta et al. (2015)
	Homoharringtonine	*Cephalotaxus harringtonia*	Translational inhibitor	AML and CML – phase I/II (r)	Lü and Wang (2014) and Badgujar et al. (2015)
	Berberine	*Berberis vulgaris*	Cell cycle arrest, apoptosis, and autophagy	Colorectal cancer – phase II/III (r)	Sun et al. (2009), and Raza et al. (2015)
Terpenes and sterols	Ursolic acid	*Mirabilis jalapa*	Pro-apoptotic	Advanced solid tumors – phase I/II	Tu et al. (2009), Li et al. (2014b), and Wozniak et al. (2015)
	Limonene	Citrus fruits	Pro-apoptotic and antiangiogenesis	Pancreatic cancer – phase I (r)	Sun (2007) and Bayala et al. (2014)
	Lycopene	*Solanum lycopersicum*	ROS induction and cell cycle arrest	Prostate cancer – phase II (r)	Bhuvaneswari and Nagini (2005) and Wei and Giovannucci (2012)
				Colorectal cancer – phase II (r)	

Paclitaxel	*Taxus brevifolia*	Microtubule inhibitor, cell cycle arrest, and apoptosis	Breast, ovarian cancers, NSCLC, AIDS-related Kaposi's sarcoma – approved	Breast, ovarian cancers, NSCLC, and pediatric solid tumors – phase I/II (r)	Ganguly et al. (2010), and Priyadarshini and Keerthi (2012)
	Digoxin	*Digitalis purpurea*	Cell cycle arrest	Kaposi's sarcoma, breast cancer – phase II, head and neck cancer – phase I/II (r)	Lopez-Lazaro (2009) and Biggar et al. (2012)
				Metastatic breast cancer, melanoma – phase I (c)	
Phenols	Genistein	*Flemingia vestita*	Tyrosine kinase inhibitor and autophagy	Pediatric malignancies – phase I/II (r)	Pavese et al. (2010), Spagnuolu et al. (2015), and Russo et al. (2016)
				Prostate and bladder – phase II (r)	
				Metastatic colorectal cancer – phase I/II (c)	
	Quercetin	*Trigonella foenum*	Apoptosis and cell cycle arrest	Prostate cancer – phase I (a)	Baghel et al. (2016)
				Renal cancer – phase I/II (r)	
	Silymarin	*Silybum marianum*	DNA damage and cell cycle arrest	Metastatic colorectal cancer – phase IV (r) prostate cancer – phase II (c) gastrointestinal cancer – phase II/III (c)	Ramasamy and Agarwal (2008) and Mastron et al. (2015)
	Chlorogenic acid	*Phyllostachys edulis*	Pro-apoptotic	Glioblastoma and advanced cancers – phase I (r)	Del Rio et al. (2010) and Rocha et al. (2012)
	Combretastatin	*Combretum caffrum*	Tubulin depolymerization	AML – phase I/II, ovarian cancer – phase II/III (r)	Arora et al. (2013)
				Gastrointestinal cancer – phase II/III (c)	
	Flavopiridol	*Amoora rohituka*	CDK inhibitor	AML – phase II (r), metastatic solid tumors – phase I (c), and pancreatic cancer – phase II (c)	Tan and Swain (2002) and Raju et al. (2003)
	Curcumin	*Curcuma longa*	Cell cycle arrest and apoptosis	Prostate cancer – phase III (r) and cervical neoplasms, metastatic breast cancer – phase II (r), and advanced colorectal cancer – phase I (c)	Wilken et al. (2011) and Rahmani et al. (2014)

(continued)

Table 3.1 (continued)

Class	Compound	Plant source	Mode of action	Clinical trial stage	References
	Podophyllotoxins	*Podophyllum peltatum*	Topoisomerase inhibition	Leukemia; brain, ovarian, and breast cancer; and SCLC – phase I/II (r) for etoposide	Gordaliza et al. (2000) and Choi et al. (2015)
	(i) Etoposide – ETP			Leukemia and esophageal cancers – phase III/IV (r) for teniposide	
	(ii) Teniposide – TNP				

The clinical trial stage shown in table is mostly from the NIH (https://clinicaltrials.gov/) or the corresponding EU clinical trial database
r recruiting participants, *a* active participants, *c* completed trial

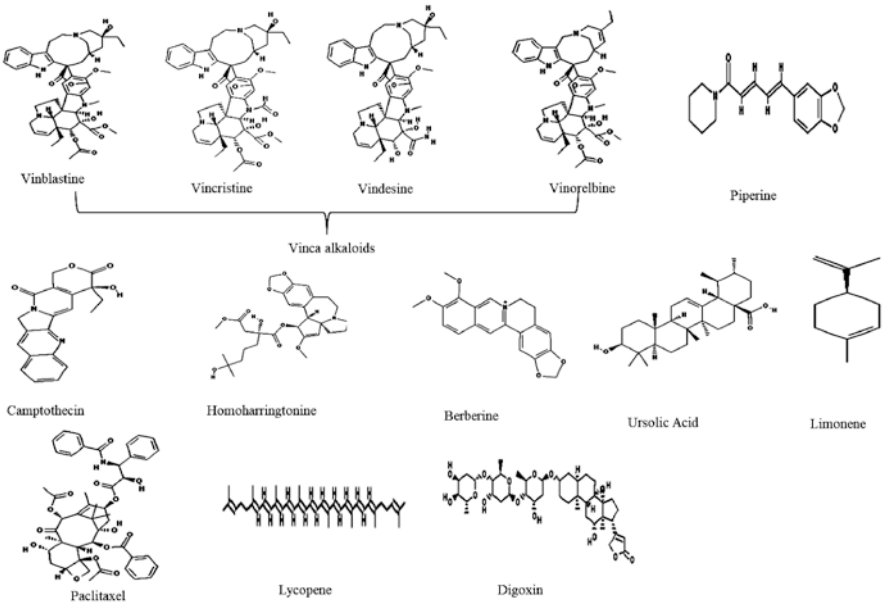

Fig. 3.1a Anticancer compounds from plant under clinical trial

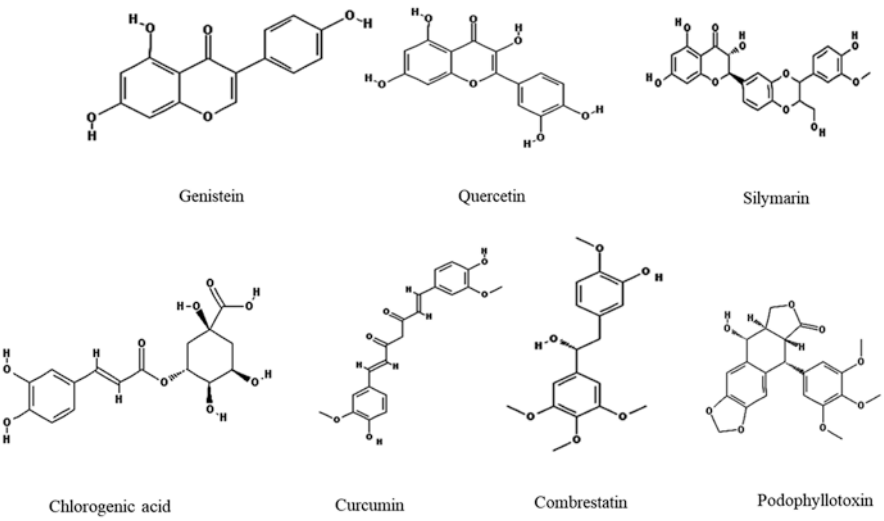

Fig. 3.1b Anticancer compounds from plant under clinical trial

3.2.1 Alkaloids

Alkaloids are found mostly in the plant kingdom compared to any other natural resources. They usually exist in higher plants such as Menispermaceae, Leguminosae, and Ranunculaceae. Alkaloids constitute an important class of secondary metabolites having heterocyclic ring containing nitrogen atom (Lu et al. 2012). Alkaloids form around 20% of plant secondary metabolites and have low-molecular-weight structures possessing various medicinal properties with relatively low toxicity (Ziegler and Facchini 2008). Major biological functions of alkaloids include antibacterial, antidiabetic, anti-inflammatory, and anticancer activities (Souto et al. 2011; Tiong et al. 2013; Cushnie et al. 2014; Kaur and Arora 2015). Well-known alkaloids with anticancer potential include vinblastine and vincristine (Jordan and Wilson 2004; Moudi et al. 2013). These alkaloids are the most important active ingredients in the traditional medicines and have also been approved for cancer therapy in recent times.

3.2.1.1 Vinca Alkaloids

Vinca alkaloids are isolated from *Vinca rosea* (*Catharanthus roseus*) belonging to the family Apocynaceae and better known as "Madagascar periwinkle." The plant grows throughout the world and is known for centuries as a traditional medicinal source for diabetes, high blood pressure, ulcer, and scurvy diseases. Vinca alkaloids are one of the oldest classes of plant alkaloids used to treat the cancer, and it was first found out by the Canadian scientists Robert Noble and Charles Beer in the 1950s (Moudi et al. 2013; Anitha Sri 2016). There are four major vinca alkaloids used for cancer treatment, which include vincristine, vinblastine, vinorelbine, and vindesine (Anitha Sri 2016). The mechanism of action of vinca alkaloids is by arresting the cell cycle at M phase by the interaction and disruption of microtubule which consists of mitotic spindles (Jordan and Wilson 2004; Moudi et al. 2013). Vinblastine blocks the endothelial proliferation and spreading of fibronectin and inhibits the malignant angiogenesis by binding to microtubule (Mukhtar et al. 2014). Vincristine destabilizes the microtubules by binding tubulin and blocking polymerization leading to cell cycle arrest and apoptosis (Moudi et al. 2013). Vinorelbine has demonstrated better anticancer property in patients suffering from breast and small cell lung cancer compared to others (Brunello et al. 2010). Vinorelbine and vindesine inhibit tubulin polymerization by disrupting the spindle formation and thereby arrest the cell cycle (Moudi et al. 2013). In 1989 and 1991, France approved vinorelbine for the treatment of small cell lung carcinoma (SCLC) and breast cancer. The USA approved vinorelbine for the treatment of SCLC in 1994 (Bennouna et al. 2008; Schutz et al. 2011). Currently, vinorelbine and vindesine in combination with other drugs such as lapatinib, epirubicin, bevacizumab, gefitinib, cetuximab, and gemcitabine are under the clinical trial phase I, II, and III for the treatment of breast, lung, colon, and skin cancers. Patients are being recruited

for phase II clinical trials for the treatment of metastatic breast cancer and non-small cell lung cancer (NSCLC) using vinorelbine. Vincristine has been approved for the treatment of leukemia, Hodgkin's disease, and SCLC. Vinblastine is used to treat a number of cancers such as testicular, brain, and bladder cancers, Hodgkin's lymphoma, and lung cancer. Vinblastine acts by two mechanisms; at low concentrations, it suppresses the microtubule action, whereas, at higher concentrations, it reduces the microtubule mass, which is currently in the recruitment stage of phase II trials for the treatment of low-grade glioma. Currently, vincristine and vinblastine with other compounds such as sirolimus, alisertib, bryostatin I, volasertib, nilotinib, cytarabine, bevacizumab, and doxorubicin are under the clinical trial phase I/II/III for the treatment of acute lymphoblastic leukemia (ALL), sarcoma, and lymphoma.

3.2.1.2 Camptothecin

Camptothecin is a quinoline alkaloid isolated from the stem and bark of *Camptotheca acuminata* and *Mappia foetida* trees commonly available in China. These trees were used for the treatment of cancer in traditional Chinese medicine. Camptothecin was first isolated by Monroe Wall and Mansukh Wani in 1966 from *C. acuminata* (Efferth et al. 2007). The use of camptothecin has gained its importance in the 1980s when they found its molecular target as topoisomerase I. Camptothecin binds to topoisomerase I and forms a DNA complex, which prohibits the DNA re-ligation and finally results in apoptosis. The use of camptothecin suffers from many restrictions, because of its low stability and solubility. To overcome this issue of camptothecin, several derivatives such as irinotecan, topotecan, silotecan, cositecan, exatecan, lurtotecan, and belotecan have been developed. Among them, the Food and Drug Administration (FDA) agency approved topotecan for the treatment of small cell lung cancer and ovarian cancer and irinotecan in combination with 5-fluorouracil for metastatic colorectal cancer (Pommier 2006; Venditto and Simanek 2010; Wahid and Bano 2014). Camptothecin has completed phase I/II/III clinical trials for the treatment of pancreatic, colorectal, and ovarian cancers, and patients are being recruited for phase I/II trials for the treatment of relapsed NSCLC.

3.2.1.3 Piperine

The alkaloid piperine is isolated from the fruits of *Piper nigrum* (black pepper) and from the plant *P. longum* (long pepper). Piperine exhibits antioxidant, anti-inflammatory, antidiarrheal, anticonvulsant, and antimutagenic activities (Vasavirama and Upender 2014). Piperine induces apoptosis by inhibiting the transcription factors and cofactors such as NF-κB, CREB, ATF-2, and ap-1function in various cancer cells. These factors play an important role in the production of pro-inflammatory cytokine and matrix metalloproteinase that promote tumor growth and metastasis. It inhibits proliferation and induces apoptosis in androgen-dependent

prostate cancer by activating capsase-3 and cleavage of PARP-1 proteins. Piperine arrests the cell cycle at different phases such as G0/G1, G1/S, and G2/M in various cancer cells (Singh and Duggal 2009; Chinta et al. 2015). Currently, piperine in combination with the curcumin is in phase I clinical trial for the treatment of bladder cancer.

3.2.1.4 Homoharringtonine

Homoharringtonine was isolated from the tree *Cephalotaxus harringtonia* generally known as Japanese/Chinese plum yew. The Earl of Harrington was the first person to grow plum yew plant in European garden centuries ago. To honor his effort, plum yew plant was named after him as *C. harringtonia.* In 1963, Paudler and his team were the first to isolate harringtonine and cephalotaxine from this plant. Later, some alkaloid esters known as harringtonine, isoharringtonine, deoxyharringtonine, and homoharringtonine were also isolated from the same plant. Among these esters, homoharringtonine showed better anticancer property (Bhanot et al. 2011; Badgujar et al. 2015). The mechanism of action of homoharringtonine is inhibition of protein translation. It inhibits translation in the elongation step by interaction with ribosomal A-site and prevents exact positioning of amino acid side chains of incoming aminoacyl-tRNAs. Homoharringtonine decreases the protein efficiency especially proteins with short half-lives such as c-Myc, Mcl-1, and cyclin D1 which are related to cell survival and proliferation, resulting in cell apoptosis. In 2012, homoharringtonine was approved for the treatment of chronic myeloid leukemia (CML) (Lu and Wang 2014; Cao et al. 2015). Currently, homoharringtonine in combination with other drugs such as quizartinib, sorafenib, and cytarabine is under phase I/II clinical trials for the treatment of acute myeloid leukemia (AML) and myelodysplastic syndrome.

3.2.1.5 Berberine

The isoquinoline alkaloid, berberine, is usually isolated from *Berberis vulgaris*, and rhizomes of coptidis (*Rhizoma coptidis* RC). It possesses various biological activities such as antibacterial, antidiabetes, antiulcer, and anti-inflammation (Bhanot et al. 2011; Lu et al. 2012; Kaur and Arora 2015). Several studies have shown the anticancer property of berberine in *in vitro* and *in vivo* experiments. Berberine interacts with DNA and RNA thereby inhibiting the cell proliferation by inducing cell cycle arrest at G1 or G2/M phase in various cancer cell lines. Berberine induces apoptosis in cancer cells by expressing proteins such as Bax, Bcl-2, and Bcl-xl and caspases and regulation of cyclin-dependent kinase proteins. Berberine inhibits COX-2 and telomerase and induces autophagy and endoplasmic reticulum stress. The effect of berberine on metastasis and angiogenesis is through the reduction of

COX-2 and prostaglandin receptors, inhibition of matrix metalloproteinases 2 and 9, and downregulation of pro-inflammatory mediators and vascular endothelial growth factor (Sun et al. 2009; Lu et al. 2012; Raza et al. 2015). Currently, berberine is undergoing phase II/III clinical trials to evaluate its safety and adverse effects in patients suffering from colorectal cancer.

3.2.2 Terpenes and Sterols

Terpenes are secondary metabolites produced by plants, known to have various functions in industrial and medical applications. Majority of the flavors, fragrances, and spices are isolated from the plant terpenes that usually have a strong odor to help the plant in pollination and to fight against parasites. The oxidized products of terpenes are usually called terpenoids. However, more research efforts have led to the identification of various terpenes with anticancer potential which act by inhibiting the cell proliferation and inducing metastasis by various mechanisms (Martin 2003; Pichersky 2006; Huang et al. 2012).

3.2.2.1 Ursolic Acid

Ursolic acid is a pentacyclic triterpenoid isolated from *Mirabilis jalapa*, and it is widely found in the peels of fruits like apple, basils, bilberries, and cranberries and in flowers like peppermint, rosemary, lavender, and others (Bhanot et al. 2011; Huang et al. 2012; Singh and Sharma 2015). Ursolic acid was designated as a multitasking agent as it induces several cell signaling pathways simultaneously to protect cells against carcinogenic agents. Novel pharmacological strategies of ursolic acid are not only on the destruction of cancer cells but also modulation of their metabolism to prevent angiogenesis and metastasis to protect healthy tissues against inflammation and oxidative stress that may lead to neoplasm formation. The ability to suppress communication through MAPK/ERK and PI3K/AKT/mTOR signaling pathways is one of the most important anticancer activities of ursolic acid. Apoptosis induction is the most important anticancer property of ursolic acid and has been reported in several cancer types both *in vitro* and *in vivo*. Ursolic acid suppresses the activity of anti-apoptotic protein Bcl-2 and Bcl-xl expression while enhancing the activity of pro-apoptotic protein BAX. Ursolic acid is proved to be an efficient COX-2 inhibitor which is able to suppress progression of inflammation (Tu et al. 2009; Shan et al. 2011; Prasad et al. 2012; Wang et al. 2012; Li et al. 2014b; Wozniak et al. 2015). Ursolic acid has entered phase I/II clinical trials for the treatment of advanced solid tumors (Yan et al. 2013; Wang et al. 2013).

3.2.2.2 Limonene

The monoterpene, limonene, is usually isolated from the citrus fruits and other plants. Limonene occurs in two forms, i.e., L-limonene and D-limonene, which are mirror images to each other. D-limonene is the major constituent of citrus fruits like orange, lemon, mandarin, and grapefruit. It is known for its anti-inflammatory, anti-invasive, and antiangiogenic properties (Sun 2007; Bayala et al. 2014). Although D-limonene is a well-studied monoterpene against different types of cancer, its exact mechanisms of actions are still not understood. However, it is reported to protect tumor growth mainly by pro-apoptotic, antioxidant, and antiangiogenic properties. D-limonene cleaves caspase-3 and caspase-9 by upregulation of Bax protein and helps to release cytochrome c from mitochondria. D-Limonene binds to 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and inhibits its activity, which leads to the inhibition of small G proteins which helps in the chemo-prevention and cancer therapy. D-Limonene initiates the induction of hepatic detoxification enzymes such as glutathione-s-transferase and uridine diphospho-glucuronosyltransferase in order to protect from carcinogens (Bayala et al. 2014; Sobral et al. 2014). Currently, D-limonene along with gemcitabine or paclitaxel is under phase I clinical trial for the treatment of pancreatic cancer.

3.2.2.3 Lycopene

Lycopene is a red-colored carotenoid terpene, majorly found in tomato (*Solanum lypersicum*) and other red fruits/vegetables such as watermelons, red carrots, and papaya. Lycopene is also present in foods which are not red colored such as asparagus and parsley. Because of its red color, lycopene is approved for food coloring agent in the USA, Australia, New Zealand, and Europe. Lycopene showed very good antioxidant activity *in vitro*. Lycopene is majorly used for cardiovascular and prostate cancer therapy (Bhuvaneswari and Nagini 2005; Wei and Giovannucci 2012). The important mechanisms underlying the anticancer property of lycopene are by ROS scavenging, upregulation of detoxification enzymes, cell proliferation interference, cell cycle arrest, and alteration in signal transduction pathways (Bhuvaneswari and Nagini 2005; Lippi and Targher 2011; Wei and Giovannucci 2012; Chen et al. 2013). Currently, lycopene is in phase II clinical trial for the treatment of hormone-resistant prostate cancer in combination with docetaxel and also for treating colorectal cancer.

3.2.2.4 Paclitaxel

Paclitaxel, a diterpenoid, was first isolated from the bark of Pacific yew tree, *Taxus brevifolia*, in 1971. In 1993, paclitaxel was approved for the cancer treatment, commonly used for the treatment of breast, ovarian, pancreatic, and cervical cancer. The World Health Organization (WHO) declared that paclitaxel is an essential lifesaving

drug. The mechanism of action of paclitaxel is mainly tubulin binding related. Upon binding to tubulin, paclitaxel disrupts the spindle assembly, chromosome segregation, and cell divisions in cancer cells, resulting in cell cycle arrest and apoptosis. Apart from tubulin binding, paclitaxel induces gene expressions which are responsible for apoptosis and also induces antiangiogenesis by regulating vascular endothelial growth factor (Jordan and Wilson 2004; Ganguly et al. 2010; Fauzee et al. 2011; Priyadarshini and Keerthi 2012). Commercially, paclitaxel has been sold under the name Taxol. Several paclitaxel analogs have been synthesized and tested for their anticancer properties. Paclitaxel and its derivatives such as nanopaclitaxel (Abraxane) and docetaxel with other compounds such as carboplatin, rituximab, doxorubicin, ibrutinib, durvalumab, neratinib, gemcitabine hydrochloride, cisplatin, and others are under clinical trials of phases I/II/III and IV for the treatment of various cancers, mainly breast, ovarian, and endometrial cancers. Currently, patients are being recruited for phase I/II clinical trials for the treatment of pediatric solid tumors using paclitaxel.

3.2.2.5 Digoxin

Digoxin was first isolated from the plant *Digitalis lantana*, and it belongs to the class cardiac glycosides, a sterol. The key mechanism of action of digoxin is the inhibition of sodium potassium ATPase. It is currently used for the treatment of cardiac disease, and WHO declared digoxin as an essential medicinal drug. The anticancer property of digoxin mainly involves cell proliferation and induces apoptosis in cancer cells by mitochondrial pathway which involves cytochrome c, caspase 8, and caspase 3 (Lopez-Lazaro 2009; Biggar et al. 2012; Kepp et al. 2012; Biggar et al. 2013; Calderón-Montaño et al. 2014; Newman and Cragg 2016a). Currently, digoxin is under phase II clinical trial for patients suffering from stage I–III breast cancer and phase I/II trials for treating head and neck cancer. Digoxin has completed phase I clinical trials for the treatment of metastatic melanoma which cannot be removed by surgery and also for metastatic breast cancer.

3.2.3 Phenols

Phenols and phenolic compounds are the largest classes of plant secondary metabolites, usually present in flowers, fruits, vegetables, and seeds. Phenols are derived from pentose phosphate, shikimate, and phenylpropanoid pathway in plants. Based on the number of phenolic groups, phenols are classified as simple phenol and polyphenol. The major biological activity of polyphenol is the antioxidant property as they have the ability to interact with proteins, lipids, and other molecules. The phenolic compounds are broadly classified as hydroxycinnamic acid, hydroxybenzoic acid (tannins), flavonoids, stilbenes, xanthones, chalcones, lignins, and lignans. Majority

of the phenolic compounds have anticancer property as they induce apoptosis, cell cycle arrest, and DNA damages (Dai and Mumper 2010; Ozcan et al. 2014).

3.2.3.1 Genistein

Genistein is a flavonoid usually found in plants such as beans, kudzu, psoralea, and lupin and also in some medicinal plants such as *Flemingia vestita* and *F. macrophylla*. Genistein is known for its antioxidant, antihelmintic, antiproliferative, antiangiogenesis, immunosuppressive, and phytoestrogen properties. The anticancer property of genistein is by inhibition of tyrosine kinase and topoisomerase, stimulation of autophagy, activation of Nrf2 antioxidant and peroxisome proliferator-activated receptors (PPARs), DNA fragmentation, and cell cycle arrest (Pavese et al. 2010; Spagnuolu et al. 2015; Russo et al. 2016). Currently, genistein is under phase II clinical trial for the treatment of bladder and prostate cancers and also under phase I/II clinical trials for the treatment of pediatric malignancies. Genistein has completed phase I/II clinical trials for the treatment of metastatic colorectal cancer.

3.2.3.2 Quercetin

Quercetin is a natural polyphenolic flavonoid, usually found in fruits and vegetables such as capers, radish, coriander, red onions, radicchio, cranberry, Hungarian wax pepper, fennel leaves, cow peas, sweet potato, broccoli, bilberry, and red kidney beans and also in green tea. Quercetin is a powerful antioxidant and also possesses anti-inflammatory activity. It generates pro-inflammatory cytokines in patients suffering from chronic inflammatory diseases. The anticancer property of quercetin is by the induction of apoptosis, cell cycle arrest, tyrosine kinase inhibition, inhibition of heat shock proteins, and expression of Ras proteins in various cancers (Gibellini et al. 2011; Baghel et al. 2016; Srivastava et al. 2016). At present, quercetin with other compounds such as green tea and genistein is in phase I, active recruiting phase for the treatment of prostate cancer, and also in phase I/II trials for the treatment of renal cancer.

3.2.3.3 Silymarin

Silymarin is a complex mixture of flavolignans and flavonoids, usually isolated from the seeds of *Silybum marianum* L. The mixture of flavolignans includes silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin, and the flavonoid, taxifolin. It is extensively used for the treatment of chronic viral hepatitis and alcoholic cirrhosis. The anticancer property of silymarin is by inhibiting the cell proliferation and inducing apoptosis through the expression of cell cycle regulators. In most of the cancers, silymarin induces apoptosis by cell cycle arrest at G_1

and G_2 checkpoint and activates the MAPK/JNK pathway. In prostate cancer, silymarin inhibits the epidermal growth factor receptor (EGFR) pathway through transforming growth factors (TGF). In umbilical cancer, silymarin inhibits the growth and survival of human umbilical vein endothelial cell by inhibiting capillary tube formation and induction of apoptosis by arresting the cell cycle (Ramasamy and Agarwal 2008; Mastron et al. 2015). Currently, silymarin is in recruitment stage for phase IV clinical trials for the treatment of metastatic colorectal cancer. It has completed phase II clinical trials for prostate cancer and phase II/III trials for gastrointestinal cancer treatment.

3.2.3.4 Chlorogenic Acid

Chlorogenic acid is a polyphenol and an ester of caffeic acid and quinic acid, usually isolated from bamboo (*Phyllostachys edulis*) and also from the shoots of common heather (*Calluna vulgaris*). Chlorogenic acid is also found in the leaves of *Hibiscus sabdariffa*, flesh of eggplant, peach, prunes, and green tea. It is majorly used as a food additive in coffee products, chewing gum, and mints. Currently, chlorogenic acid is available in the market as Svetol. Chlorogenic acid inhibits DNA damage by scavenging ROS and protecting against carcinogenesis. Chlorogenic acid inhibits matrix metalloproteinase activity and upregulates the genes involved in the immune system by enhancing the activation of macrophages and natural killer cells (Luthria and Mukhopadhyay 2006; Del Rio et al. 2010; Rocha et al. 2012; Zhen et al. 2016). Currently, chlorogenic acid is in phase I clinical trial, recruiting patients for the treatment of glioblastoma and to study the pharmacokinetic properties in advanced cancers.

3.2.3.5 Combretastatin

Natural phenol combretastatin is formed from the backbone of dihydrostilbene (class stilbenes). Combretastatin is usually isolated from the bark of South African bush willow *Combretum caffrum*, which belongs to the family of Combretaceae. The mechanism of action of combretastatin is the inhibition of tubulin polymerization in cancer cells. There are five combretastatin isoforms found in *C. caffrum,* and they are combretastatin A-4, combretastatin A-1, combretastatin B, combretastatin C, and combretastatin D. Among them, combretastatin A-4 has showed better anticancer activity. It binds to tubulin at colchicine site and inhibits the polymerization of the microtubules leading to the arrest of cell cycle at M phase. Apart from tubulin depolymerization, combretastatin A-4 also shows antiangiogenesis by inducing the morphological changes in endothelial cells by a direct damage to the vasculature (Jordan and Wilson 2004; Siemann et al. 2009; Arora et al. 2013). At present, combretastatin A-4 is under phase II/III clinical trials for the treatment of platinum-resistant ovarian cancer and phase I/II clinical trials for the treatment of AML. It has completed phase II clinical trials for the treatment of advanced solid tumors and NSCLC.

3.2.3.6 Flavopiridol

Flavopiridol is a flavonoid isolated from *Amoora rohituka*. It is being used for the treatment of arthritis, atherosclerosis, and cancer. Flavopiridol is a potent inhibitor of CDK 1, 2, 4, 7, and 9, thereby arresting cell cycle at G1 or G2 phase. It induces apoptosis by downregulation of Bcl-2 in cancer cells. In 2014, flavopiridol was approved for the treatment of acute leukemia. Commercially flavopiridol is known as alvocidib (Tan and Swain 2002; Raju et al. 2003; Mariaule and Belmont 2014). Currently, flavopiridol is undergoing phase II clinical trials for the treatment of AML and has completed phase I and phase II clinical trials for treating metastatic solid tumors and pancreatic cancer, respectively.

3.2.3.7 Curcumin

Curcumin is a polyphenolic compound, isolated from *Curcuma* plant species, which belongs to the family Zingiberaceae. It is extensively used in Ayurveda, Unani, Siddha, and Chinese medicine for treating cough, wound healing, inflammation, hepatitis, and cancer. Anticancer property of curcumin is based on its multiple actions such as cell cycle regulation, regulation of apoptosis, proliferation, and metastasis inhibition. Curcumin downregulates the expression of p53, Egr1, and Bcl-xl in cancer cells which results in inhibition of cell cycle progression and leads to apoptosis. Curcumin induces the expression of NF-κB and IKK and inhibits MMP, PKA, PKC, and STAT3 proteins to suppress angiogenesis, tumor invasion, tumor growth, and proliferation in cancer cells. Curcumin suppresses the TNF expression which results in impaired cell proliferation in cancer cells (Wilken et al. 2011; Mehta et al. 2014; Rahmani et al. 2014; Salem et al. 2014). At present, curcumin is under phase II clinical trials for the treatment of metastatic breast cancer and cervical neoplasms and phase III trials for treating prostate cancer. Also, it has completed phase I trials for treating advanced colorectal cancer.

3.2.3.8 Podophyllotoxins

The naturally occurring lignin podophyllotoxin is usually isolated from *Podophyllum peltatum* L. and *P. emodi* Wall. Podophyllotoxin is known for its immunosuppressive and antiviral activities specifically for treating herpes, measles, influenza, and warts and also for its anticancer activity. The anticancer mechanism of podophyllotoxins is by arresting the cell cycle through the inhibition of microtubule polymerization. Podophyllotoxin was found to be unsuitable for clinical use because of its toxic side effects. However, based on its biological activity, semisynthetic compounds such as etoposide, etopophos, and teniposide were derived from podophyllotoxin. These derivatives showed anticancer property by inhibiting DNA topoisomerase II rather than inhibiting tubulin polymerization (Gordaliza et al. 2000; Choi et al. 2015). Currently etoposide and teniposide derivatives are under

clinical trials for various cancer treatments. Etoposide with other drugs such as ciprofloxacin, vorinostat, apatinib, dexamethasone, and methotrexate is currently in phase I/II clinical trials for the treatment of leukemia, brain, ovarian, breast, and SCLC. Teniposide with other drugs such as dexamethasone, fotemustine, vindesine, irinotecan, and cladrizine is currently under phase III/IV clinical trials for the treatment of leukemia and esophageal cancers.

3.3 Anticancer Compounds from Microbial Source

Microorganisms are an important source of several clinically important metabolites, which have substantially contributed for the development of pharmaceutically significant compounds. They have played an important role in finding natural compounds with therapeutic benefits against cancer. Screening of natural compounds has led to the discovery of a wide range of anticancer drugs of microbial origin. The most important compounds used in the cancer chemotherapy are microbial origin antibiotics or their derivatives (Demain and Vaishnav 2011). Anticancer drugs such as doxorubicin, dactinomycin, bleomycin, and mitomycin are approved and widely used in the treatment of a variety of cancers. In addition, several derivatives have been developed based on the scaffolds of these microbial compounds. Some of them have demonstrated potent anticancer activity and have entered clinical trials and also in therapeutic stages. Among these antitumor antimicrobial compounds, anthracyclines such as doxorubicin and daunorubicin and glycopeptide antibiotics such as bleomycin are approved for the treatment of numerous cancers as monotherapy or in combination with other drugs. These compounds which belong to these classes cause cytotoxicity in cancer cells by targeting DNA damage response pathway, of which cancer cells are mostly deficient whereas, normal cells are not (Kathiravan et al. 2013).

It all began in the 1940s with the discovery of streptomycin and many other milestone compounds with antimicrobial activity from a variety of microorganisms (Giddings and Newman 2013). Actinomycetes, an important class of antibiotics, were discovered in the 1950s at Abraham Waksman's laboratory, which subsequently led to the discovery of several antimicrobials which were proven effective against a wide range of microbial infections (Waksman and Woodruff 1941). In the 1980s, screening of antibiotics to explore their additional properties other than targeting the microbial infections that included antitumor property was initiated. In the laboratory screening test, several microbial compounds which were considered as failed antibiotics were identified as potent toxins for tumors, and most of the important compounds of microbial origin and their derivatives were successfully used against a variety of cancers (Demain and Vaishnav 2011). Despite their proven antimicrobial action, some antibiotics such as doxorubicin, daunorubicin, and bleomycin have already been used in the treatment of human cancers (Kumar 2015). In addition, many other microbial origin compounds are also under investigation for their antitumor activity. While most of the compounds target DNA (Bhattacharya

and Mukherjee 2015), some target tubulin (Jordan and Wilson 2004) and some other compounds induce apoptotic mode of cell death in cancer cells (Chung et al. 2008). In the last few decades, natural products predominately from the actinomycetes and Eukarya or their derivatives have played extremely important roles in cancer drug discovery (Giddings and Newman 2013). Notably, microbial secondary metabolites are the major source of drugs not only used unchanged for the treatment but served as basic chemical structures which can be selectively modified to control tumor cell growth (Cragg et al. 2009; Bhattacharya and Mukherjee 2015). With the advent of high-throughput screening technology, several programs were initiated to discover the small molecules which have anticancer properties (Florento et al. 2012; Hoelder et al. 2012). These research programs focused mainly on quickening the drug development and reducing costs. Several pharmaceutical companies have introduced many small molecules which resemble the core scaffolds of natural products (Giddings and Newman 2013). Yet, there is an inadequacy of lead compounds making their way into the clinical trials. Approximately 50% of all small molecules that received approval by FDA from 2000 to 2006 were not newly synthesized chemical compounds instead derived from combinatorial chemistry but mostly based upon the natural products (Newman and Cragg 2007). Microbial origin anticancer compounds and their derivatives which are under clinical trial for cancer treatment are discussed in this section (Table 3.2; Figs. 3.2a and 3.2b).

3.3.1 Actinomycins

The actinomycins are a group of chromopeptide antibiotics that exhibit high antibacterial and antitumor activity. Several milestone antimicrobial compounds such as streptomycin, neomycin, actinomycin, and others were first discovered by Waksman's group. Actinomycins were discovered in several soil bacterial species of *Streptomyces*, which showed potent inhibition properties against a wide range of microbial infections (Waksman and Woodruff 1941). In 1963, actinomycin D was approved for the treatment of highly malignant pediatric tumors such as rhabdomyosarcoma and Wilms tumor as a single agent and also in combination with other anticancer compounds (Giddings and Newman 2013). Actinomycin D is a DNA intercalating agent that interferes with the binding of transcription factor to DNA, thereby inhibits RNA synthesis and eventually protein translation (Lo et al. 2013). The efficacy of actinomycin D is restrained by its severe cytotoxicity. Therefore, its derivatives from various soil and marine *Streptomyces* species and several synthetic analogs have been developed to modulate the cytotoxicity (Giddings and Newman 2013). Actinomycin D is FDA approved for the treatment of Wilms tumor, advanced breast cancer, and testicular and ovarian cancers (NCI). It has completed phase IV clinical trials for the treatment of various pediatric cancers. Currently, patients are being recruited for phase II clinical trials involving actinomycin D as a single agent and/or in combination with other cytotoxic drugs for the treatment of germ cell tumors and high-risk rhabdomyosarcoma.

Table 3.2 Anticancer compounds from microbial source

Class	Compound	Source	Mode of action	Clinical trial stage	References
Actinomycins	Actinomycin D	*Streptomyces* sp.	DNA damage	Advanced breast cancer, ovarian and testicular cancer, and Wilms tumor approved	Waksman and Woodruff (1941) and Giddings and Newman (2013)
				Germ cell tumors and high-risk rhabdomyosarcoma – phase II (r)	
				Pediatric cancer phase IV (c)	
Anthracyclines	Doxorubicin	*S. peucetius*	DNA damage	Breast, bladder cancers, Kaposi's sarcoma, lymphoma, ALL, and AML – approved triple-negative breast cancer, Ewing's sarcoma – phase III metastatic sarcoma – phase I/II (r) ovarian cancer – phase IV (c)	Khazir et al. (2014) and Cheung-Ong et al. (2013)
	Daunorubicin	*S. caeruleorubidus*	DNA intercalation and topoisomerase inhibition	AML, ALL, CLL – approved	Khazir et al. (2014)
				AML – phase I/II/III (r)	
	Epirubicin	*S. peucetius*	DNA intercalation	Node-positive breast cancer – approved	Bhanot et al. (2011) and Newman and Cragg (2016a)
				DLBCL – phase III/IV (r)	
				Bladder cancer – phase IV (r)	
	Idarubicin	*S. peucetius*	Topoisomerase II inhibition	AML approved	Bhanot et al. (2011) and Newman and Cragg (2016a)
				Metastatic hepatocellular carcinoma – phase I (c)	
Glycopeptides	Bleomycin	*S. verticillus*	DNA damage	Testicular, cervical, head, and neck cancers, Hodgkin's and non-Hodgkin's lymphoma – approved Kaposi's sarcoma – phase III (c) Rectal cancer – phase II and germ cell tumors – phase III (r)	Hecht (2000) and McKinney et al. (2013)
Enediynes	Calicheamicin (an ADC – gemtuzumab ozogamicin)	*Micromonospora echinospora*	DNA damage	AML – phase I/II (a/c)	Nicolaou et al. (1993) and Shen et al. (2015)
				Non-Hodgkin's lymphoma – phase II (c)	
				ALL – phase III (a)	
	Neocarzinostatin	*S. carzinostaticus*	DNA intercalation	Recurrent hepatocellular carcinoma – phase III (r)	Giddings and Newman (2013)

(continued)

Table 3.2 (continued)

Class	Compound	Source	Mode of action	Clinical trial stage	References
Epothilones	Epothilone B	*Myxobacterium*, *Sorangium cellulosum*	Microtubule inhibition	Metastatic breast cancer, NSCLC, colorectal and ovarian cancer – phase II (c)	Reichenbach and Höfle (2008), Ferrandina et al. (2012), and Fanale et al. (2015)
				Advanced solid tumors – phase I (c)	
				Ovarian and breast cancer – phase II (r)	
	Epothilone D	*Myxobacterium*, *Sorangium cellulosum*	Microtubule inhibition	Metastatic breast cancer – phase III (r)	
				Advanced solid tumors – phase I (c)	
				Ovarian cancer and urothelial carcinoma – phase II (r)	
	Ixabepilone	Semisynthetic analog of Epothilone B	Microtubule inhibition	Advanced solid tumors – phase I (c)	
				Ovarian cancer and urothelial carcinoma – phase II (r)	
				Metastatic breast cancer – phase III (r)	
Geldanamycins	Tanespimycin	*S. hygroscopicus*	HSP90 inhibition	Metastatic thyroid cancer – phase II (c)	Fukuyo et al. (2010) and Bhanot et al. (2011)
				AML, ALL, CML – phase I (c)	
				Multiple myeloma – phase I/II/III (c)	
Rapamycin	Rapamycin or sirolimus	*S. hygroscopicus*	Inhibits TOR, induces autophagy/apoptosis	NSCLC, ALL, breast cancer, glioma, AML – phase I/II (r) bladder cancer – phase I (r)	Ballou and Lin (2008), Seto (2012), and Li et al. (2014a)
	Everolimus (RAD001)	Synthetic analog of rapamycin	Inhibits TOR, induces autophagy/apoptosis	Glioma – phase II (c) relapsed osteosarcoma – phase II (u)	
				Triple-negative breast cancer – phase I (r)	
				Metastatic renal cell carcinoma – phase II (r)	
	Temsirolimus (CCI-779)	Synthetic analog of rapamycin	TOR inhibition, autophagy, and apoptosis	Renal carcinoma – approved	
				Glioma – phase II (r), advanced cancers and solid tumors – phase I (r)	
	Ridaforolimus	Synthetic analog of rapamycin	TOR inhibition, autophagy, and apoptosis	Metastatic soft tissue and bone sarcomas – phase III (c), advanced solid tumors – phase I (a), and head and neck cancer and NSCLC – phase I (c)	

Romidepsin, Trichostatin A	Romidepsin	*Chromobacterium violaceum*	HDAC inhibition	Advanced colorectal cancer – phase II (r)	Xu et al. (2007), Butler et al. (2014), and Foss et al. (2016), and Newman and Cragg (2016a)
				T-cell lymphoma, CLL, and solid tumors – phase II (a)	
	Vorinostat	*Streptomyces hygroscopicus*	HDAC inhibition	Lymphoma – approved	
				Metastatic soft tissue sarcoma – phase I/II (r), advanced melanoma – phase II (c), advanced solid tumors, NSCLC – phase I (r), and brain metastases – phase I (c)	
Staurosporines	7-Hydroxy staurosporine	*Streptomyces staurosporeus*	PKC inhibition	Advanced solid tumors, triple-negative breast cancer, advanced lymphoma/leukemia – phase I (c) and relapsed SCLC and metastatic pancreatic cancer – phase II (c)	Giddings and Newman (2013)
	Midostaurin	Analog of staurosporine	PKC inhibition	Mast cell leukemia, AML – phase II (a) advanced rectal cancer – phase I (r)	
	Becatecarin	Analog of rebeccamycin	PKC inhibition	AML, CML – phase I (c) NSCLC, stage IIIB/IV breast cancer, non-Hodgkin's lymphoma – phase II (c)	
Nitrosoureas	Streptozocin	*Streptomyces achromogenes*	DNA alkylation and DNA replication inhibition	Metastatic pancreatic cancer – approved	Cheung-Ong et al. (2013), Blasiak et al. (2004), and Newman and Cragg (2016a)
				Metastatic neuroendocrine tumors, recurrent brain neoplasms – phase II (c)	
				Advanced adrenocortical carcinoma – phase III (c) and pancreatic neuroendocrine tumors – phase III (r)	
Mitomycins	Mitomycin C	*Streptomyces caespitosus*	DNA cross linker	Bladder cancer – phase III (c)	Tomasz (1995)
				Metastatic breast cancer – phase II (c)	
				Advanced colorectal cancer – phase II/III (r) and anal carcinoma – early phase I	
Mithramycin	Mithramycin A	*Streptomyces plicatus*	DNA methylation and TRAIL-mediated apoptosis	Malignancies of lungs and esophagus – phase I/II (a)	Lee et al. (2006) and Lin et al. (2007)

The clinical trial stage shown in table is mostly from the NIH (https://clinicaltrials.gov/) or the corresponding EU clinical trial database
ADC antibody-drug conjugate, *TOR* target of rapamycin, *PKC* protein kinase C, *TRAIL* TNF-related apoptosis-inducing ligand, *c* completed, *r* recruiting participants, *a* active

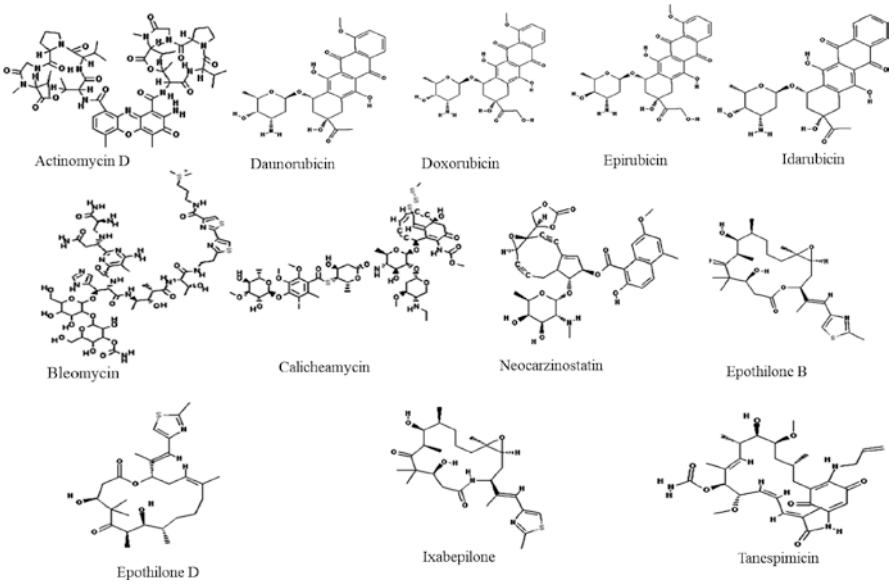

Fig. 3.2a Anticancer compounds from microbes under clinical trial

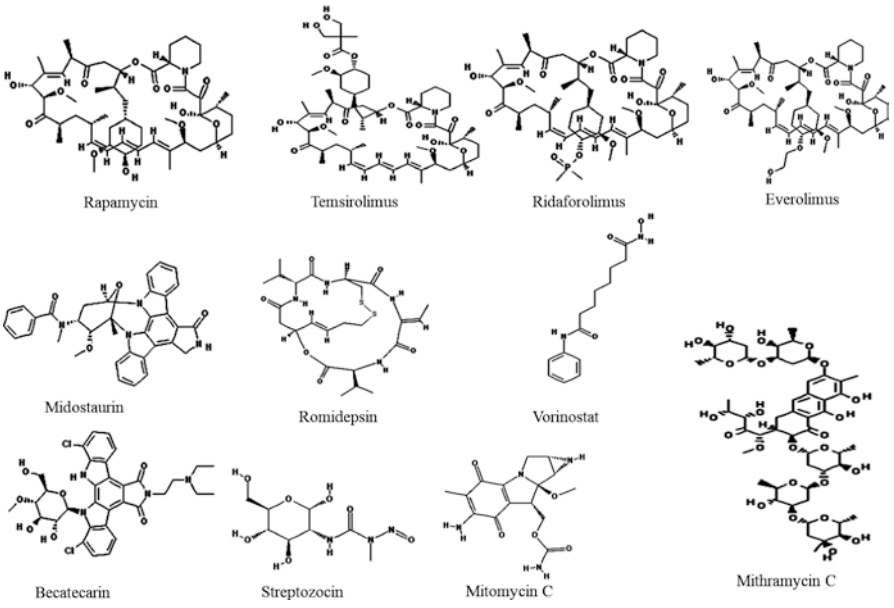

Fig. 3.2b Anticancer compounds from microbes under clinical trial

3.3.2 Anthracyclines

Anthracyclines are a class of antitumor compounds widely used in the treatment of a variety of cancers. The two most useful anthracyclines are doxorubicin (DOX) and daunorubicin (DAN) which were first isolated from *Streptomyces peucetius* and *S. caeruleorubidus*, respectively (Ramasamy and Agarwal 2008; Cheung-Ong et al. 2013; Bhattacharya and Mukherjee 2015). Both DOX and DAN were approved by FDA for cancer therapy in the 1960s (Khazir et al. 2014). DOX is already in clinical use in the treatment of ALL, AML, breast cancer, gastric cancer, Hodgkin's and non-Hodgkin's lymphoma, neuroblastoma, SCLC, soft tissue and bone sarcoma, solid tumors in children, cancers of the bladder and thyroid, and Wilms tumor. Further, it is being studied in combination with other drugs (phase III) against non-metastatic extracranial Ewing's sarcoma, T-cell ALL (phase I), and solid tumors. DOX along with other drugs such as cyclophosphamide and paclitaxel with or without carboplatin is in phase III clinical trial against triple-negative breast cancer. DOX has completed phase IV clinical trials for the treatment of ovarian cancer. DAN is used in the treatment of acute lymphoblastic or myeloblastic lymphoma. DAN is currently undergoing clinical trials ranging from phase I to phase III of combination therapy against AML and ALL.

Both DOX and DAN intercalate between the base pairs of DNA, also inhibit topoisomerase II, and generate free radicals which eventually damage DNA and proteins (Cheung-Ong et al. 2013). Both anthracyclines inhibit DNA and RNA synthesis and cause apoptotic cell death (Kumar 2015). DNA-damaging agents specifically target cancer cells which have impaired DNA damage response and cell cycle checkpoint pathways without causing much harm to the normal cells. The major limitations of these compounds are acute cardiotoxicity and development of resistance. This has led to the investigation of numerous natural, biosynthetic, and synthetic derivatives. Among them are clinically useful analogs such as epirubicin, pirarubicin, idarubicin, amrubicin, valrubicin, aclarubicin, and mitoxantrone hydrochloride (Giddings and Newman 2013). Epirubicin, a semisynthetic anthracycline, is a potent DNA intercalating agent and has been approved by the FDA for the treatment of node-positive breast cancer. Interestingly, epirubicin is reported to cause substantially less cardiotoxicity than DOX (Robert 2007). Patients are being recruited for phase III and IV clinical trials for the treatment of diffuse large B-cell lymphoma (DLBCL) in combination with rituximab and other anticancer drugs which are also in phase IV clinical trials for bladder cancer treatment. Idarubicin, a semisynthetic 4-demethoxy analog of DAN, is an inhibitor of topoisomerase II and has been approved for the treatment of AML (Wellington 2015; Newman and Cragg 2016a). Currently, it is undergoing investigations for the treatment of AML in combination with other cytotoxic agents. It has also completed the phase I clinical trial for the treatment of metastatic hepatocellular carcinoma.

3.3.3 Glycopeptide Antibiotics

Another class of extremely important molecules from actinomycetes is glycopeptide antibiotics, which include bleomycin. Bleomycin A2 is generally used as the representative structure for bleomycin. They all share a core structure but differ in the various functional groups and disaccharides (Cragg and Newman 2013). Bleomycins were originally isolated from *Streptomyces verticillus* and were later developed as antitumor agents (Hecht 2000; Giddings and Newman 2013). Bleomycin is known for its unique mechanism of binding to GC-rich regions of DNA which directly induces strand breaks in DNA. Cleavage of DNA by bleomycin may be due to the chelation of metal ions, particularly iron, resulting in the formation of free radicals which induce single-strand breaks in DNA (McKinney et al. 2013; Shapiro 2015). Nevertheless, bleomycin efficacy has been limited by its acute pulmonary toxicity (Giddings and Newman 2013; Bhattacharya and Mukherjee 2015; Rida et al. 2015). Another similar glycopeptide antibiotic closely related to bleomycin is NC-0604, which was isolated from the fermentation broth of *S. verticillus* var. *pingyangensis* n. sp. and has been reported to possess stronger antitumor action than that of bleomycin and also lower pulmonary cytotoxicity. NC-0604 is currently in preclinical trials (Giddings and Newman 2013).

Bleomycin is known to induce DNA damage in cancer cells with defective DNA repair genes (Simon et al. 2000; Melorose et al. 2008; McKinney et al. 2013). Bleomycin is approved by the FDA for the treatment of Hodgkin's and non-Hodgkin's lymphoma, penile testicular and cervical cancers, vulva carcinoma, and head and neck cancer. It has completed phase III clinical trial for the treatment of AIDS-related Kaposi's sarcoma. It is currently in the phase III clinical trial in combination with other compounds such as taxol and etoposide in treating germ cell cancers. In addition, electrochemotherapy involving administration of bleomycin is being investigated in the phase II clinical trials against locally advanced rectal cancer, inoperable vulva carcinoma, and advanced germ cell cancers.

3.3.4 Enediynes

Enediynes are a class of naturally occurring compounds with potent antitumor property. The limited clinical use of the naturally occurring enediynes is because of their severe cytotoxicity for both tumor and normal cells (Giddings and Newman 2013). Enediynes are structurally unique antitumor antibiotics, whose core structure was first elucidated in 1985, and since then, only 11 enediynes have been discovered, some of which include calicheamicins, dynemicin A, esperamicin, kerdarcidin, and neocarzinostatin. The enediynes contain two acetylenic groups conjugated by a double bond within either a nine- or ten-membered ring based on the structure of the core (Shen et al. 2015). The enediynes are some of the most cytotoxic molecules known today (Giddings and Newman 2013; Shen et al. 2015). Their cytotoxic

property is mostly attributed to their ability to cause DNA double-strand breaks upon activation followed by cell death. An alternative mode of action of enediynes has been proposed, which is through the formation of inter-strand cross-links. This property of enediynes has been exploited to target solid tumors or other cancer cells under hypoxic environments which do not respond well to those enediynes that predominantly induce oxygen-dependent DNA double-strand breaks. Due to their very high cytotoxicity, enediynes are used in conjugation with monoclonal antibodies for selectively targeting the cancer cells. This significant mechanism of action of enediynes has made them an excellent payload candidate for antibody-drug conjugates in targeted cancer therapy (Damelin et al. 2015).

Calicheamicin-γ1, the most prominent member of calicheamicin, has been approved for clinical use and is known to possess remarkable anticancer properties (Demain and Vaishnav 2011; Giddings and Newman 2013). In 2000, gemtuzumab ozogamicin, an anti-CD33 humanized antibody linked to a calicheamicin derivative, was approved by the FDA as the first antibody-drug conjugate to use against CML. Later, it was withdrawn in 2010 from the US market because of its cytotoxicity. However, it is still in clinical use in Japan and other parts of world. Calicheamicin is in clinical trials ranging from phase I to III for investigating its cytotoxicity against AML. The antibody conjugated with calicheamicin has completed phase II trial for the treatment of indolent non-Hodgkin's lymphoma and undergoing phase III clinical trial in combination with rituximab and other cytotoxic drugs such as cytarabine and mitoxantrone for the treatment of ALL (Wondrak 2009; Giddings and Newman 2013; Butler et al. 2014; Khazir et al. 2014). Patients are being recruited for phase III clinical trials involving neocarzinostatin for the treatment of recurrent hepatocellular carcinoma. However, research is in progress to produce nontoxic enediyne-based anticancer compounds which are more selective in their action.

3.3.5 Epothilones

Epothilones are a class of naturally occurring anticancer compounds which mimic the mechanism of taxol. They are 16-membered polyketide macrolide lactones isolated from myxobacterium *Sorangium cellulosum* (Zhou and Giannakakou 2005; Behrangi et al. 2012). Epothilones were originally used as antifungal agents, but later their antitumor activity was discovered. They promote tubulin polymerization and stabilization which in turn prevent disassembly of microtubules and arrest the cells in G2/M phase and eventually cause apoptosis (Jordan et al. 1993). Though the mechanism of action of epothilones is similar to that of taxanes, they are more soluble in water than taxanes and bind to a distinct region on tubulin (Demain and Vaishnav 2011). Epothilones have been effective against multidrug-resistant cancer cell lines and also those which are resistant to paclitaxel therapy mainly due to specific β-tubulin mutations (Escuin et al. 2005; Stanton et al. 2011). Thus, epothilones have become attractive lead molecules for developing many clinically important

derivatives (Ferrandina et al. 2012). Among numerous epothilones identified so far, epothilones A, B, and D are the most important variants (Escuin et al. 2005; Reichenbach and Höfle 2008). Epothilone B has completed phase I and phase II clinical trials for the treatment of advanced breast cancer, advanced colon cancer, advanced melanoma, glioblastoma, metastatic hepatocellular carcinoma, and NSCLC. It has also completed phase III trials for the treatment of a variety of cancers in combination with doxorubicin. Epothilone D has completed phase II clinical trials for the treatment of Her2-positive advanced breast cancer, stage IV NSCLC, and metastatic refractory colorectal cancer in combination with herceptin and phase I trial for the treatment of advanced solid tumors.

Ixabepilone is a semisynthetic analog of epothilone B which is orally bioavailable and effective against taxane-resistant tumors (Harvey 2008; Stanton et al. 2011). Ixabepilone has received FDA approval in 2007 for the treatment of aggressive metastatic or locally advanced breast cancer, which no longer responds to the currently available chemotherapies (Stanton et al. 2011; Scully et al. 2012; Cragg and Newman 2013). Ixabepilone has entered phase I clinical trial for advanced solid tumor treatment. Patients are being recruited for clinical trial phase II for ovarian and phase III for breast cancer treatment using ixabepilone with other cytotoxic agents. In addition, recruitment is being done for the phase II clinical trial based on genomic alteration in advanced urothelial carcinoma involving ixabepilone.

3.3.6 Geldanamycins

Geldanamycins are the naturally occurring benzoquinone ansamycin antibiotics isolated from *Streptomyces hygroscopicus*. Their anticancer property is through the inhibition of HSP90 chaperone function (Li et al. 2009; Fukuyo et al. 2010). Inhibition of HSP90 leads to the proteasomal degradation of cancer-relevant client proteins. HSP90 proteins are expressed in cancer cells two- to tenfold higher than that in normal cells, which makes them a potential target for anticancer therapy (Neckers 2002). Despite of their anticancer properties, the clinical use of geldanamycins is limited by acute hepatotoxicity at clinically relevant doses, poor water solubility, and metabolic instability. Several variants of geldanamycins have been developed with quinone ring modification, which resulted in improved potency, tolerance, metabolic stability, and water solubility (Neckers 2002; Li et al. 2009). Geldanamycin's analog tanespimycin/17-AAG/DMAG has been investigated in a number of clinical trials for the treatment of various cancers as a single agent or in combination with other anticancer drugs (Giddings and Newman 2013). Some of them are in phase III clinical trial for the treatment of relapsed and refractory multiple myeloma; phase I clinical trial for the treatment of AML, ALL, and CML; and phase II clinical trial for the treatment of metastatic thyroid cancer and metastatic breast cancer (with trastuzumab) and stage IV pancreatic and ovarian cancers (with gemcitabine hydrochloride).

3.3.7 Rapamycin

Rapamycin is a macrolide antibiotic isolated from *Streptomyces hygroscopicus* from the soil samples of Rapa Nui Island. It was originally reported to be a potential antifungal agent in 1975, but later, it was found to possess powerful immunosuppressant and antiproliferative activity against cancer cells (Giddings and Newman 2013). Rapamycin is a potent inhibitor of mammalian target of rapamycin (mTOR) in mammalian cells, which is a key regulator of cell growth and metabolism, and increased activation has been implicated in many human cancers (Ballou and Lin 2008; Seto 2012; Li et al. 2014a). In the 1990s, TOR in yeast and its mammalian homolog mTOR were discovered, and it was shown that TOR is involved in the regulation of cell growth by controlling transcription, translation, and ribosome biogenesis (Giddings and Newman 2013). Increased activation of mTORC1 is observed in many human cancers due to mutations in upstream regulators of mTORC1 (Ballou and Lin 2008; Li et al. 2014a). Gain-of-function mutations in oncogenes such as PI3K, AKT, or Ras and/or loss-of-function mutations in tumor suppressor genes such as PTEN, LKB1, or TSC1/2 lead to overexpression of mTORC1. These mutations provide cancer cells with a selective growth advantage in comparison to normal cells. In order to meet the high demands of cell proliferation, cancer cells generally have altered processes such as nutrient uptake and energy metabolism, which are directly controlled by the mTORC1 pathway. Rapamycin and other drugs which selectively target mTORC1 and impair cancer metabolism are considered potential and promising anticancer compounds. Rapamycin was approved as an immunosuppressive agent in 1999, and it has now completed phase I and II clinical trials for the treatment of a wide range of cancers (Giddings and Newman 2013) such as NSCLC, recurrent glioma, advanced ALL, neuroblastoma, advanced sarcoma, and metastatic breast cancer as a single drug and/or in combination with other anticancer compounds. Presently, patients are being recruited for phase IV clinical trials for investigating the efficacy of rapamycin against refractory solid tumors and pancreatic cancer.

Everolimus (RAD001) is a derivative of rapamycin which was initially used as an immunosuppressive agent and was later approved for the treatment of kidney, brain, pancreatic, and breast cancers. It continues to be investigated in combination with other cytotoxic drugs in a huge number of clinical trials for the treatment of various types of cancer (Giddings and Newman 2013). Currently, patient recruitment has been initiated for investigating everolimus in phase II clinical trials for treating metastatic thyroid cancer and renal cell carcinoma and in phase I clinical trials for the treatment of triple-negative breast cancer in combination with other anticancer drugs. Temsirolimus (CCI-779) is another derivative of rapamycin which has been approved by the FDA for the treatment of renal carcinoma. It has completed phase II clinical trials for the treatment of glioma and phase I clinical trials for treating pediatric solid tumors (Cragg and Newman 2013; Kinghorn et al. 2016). At present, it has been investigated in phase I trial for treating advanced cancers and solid tumors. Also, there are phase I clinical trials for the treatment of advanced

breast, endometrial, and ovarian cancer and phase II clinical trials for the treatment of hepatocellular carcinoma and Hodgkin's lymphoma using temsirolimus. Ridaforolimus/deforolimus is another analog of rapamycin, which has completed many clinical trial phases for treatment of various cancers (Seto 2012; Giddings and Newman 2013; Li et al. 2014a). Some of the clinical trials include phase III trial for the treatment of metastatic soft tissue and bone sarcomas and phase II trial for the treatment of metastatic endometrial and breast cancers (in combination with trastuzumab). It is also being investigated in phase I clinical trial for the treatment of advanced solid tumors.

3.3.8 Romidepsin and Trichostatin A (HDAC Inhibitors)

Romidepsin, a bicyclic depsipeptide, was first isolated from a gram-negative bacterium, *Chromobacterium violaceum*, isolated from the soil sample of Japan in 1994 (Butler et al. 2014). Romidepsin belongs to class I histone deacetylase (HDAC) inhibitors and forms an unusual disulfide bond between a thiol and D-cysteine. Unlike conventional chemotherapeutic agents, HDAC inhibitors target epigenetic abnormalities responsible for cancer development. In 2009, romidepsin was approved by the FDA for the treatment of cutaneous and peripheral T-cell lymphoma (Mottamal et al. 2015). Romidepsin has completed early stage of clinical trials for the treatment of solid tumors; lung, thyroid, and prostate cancers; and metastatic soft tissue sarcoma (Giddings and Newman 2013; Zwergel et al. 2015). Currently, patients are being recruited for phase I/II clinical trials of romidepsin for the treatment of advanced colorectal cancer and also for phase II trials for the treatment of relapsed or refractory B-cell or T-cell lymphomas, CLL, and solid tumors in combination with other drugs. Similarly, trichostatin A is another potent HDAC inhibitor and was isolated from *S. hygroscopicus* in 1976 and determined to be an antifungal agent at much lower IC_{50}, i.e., less than 10 nM, and showed over 300-fold selectivity for inhibition of class II HDACs. Trichostatin A is currently being investigated for its potential as an anticancer drug, and it has been reported to be effective against hepatocellular carcinoma cells (Mottamal et al. 2015). Suberoylanilide hydroxamic acid (SAHA) also known as vorinostat was synthesized based on the structure of trichostatin A and has been approved by the FDA for the treatment for lymphoma and is currently in clinical trials for the treatment of a variety of cancers (Xu et al. 2007; Giddings and Newman 2013; Foss et al. 2016). Vorinostat has completed phase II clinical trials for treating metastatic soft tissue sarcoma and phase I clinical trials for treating brain metastases. Patients are being recruited for phase I/II clinical trials for the treatment of advanced melanoma and solid tumors using vorinostat.

3.3.9 Staurosporines

Staurosporine is an indolocarbazole alkaloid, which was first isolated from *Streptomyces staurosporeus* in 1977, but its potential as an anticancer compound came to light only a decade after its discovery. It is a potent protein kinase C (PKC) inhibitor with antitumor activity and has led to the development of several analogs including 7-hydroxystaurosporine and midostaurin (Giddings and Newman 2013). 7-Hydroxystaurosporine has completed phase I clinical trials for the treatment of advanced solid tumors and triple-negative breast cancer and phase II clinical trials for treating relapsed SCLC, metastatic pancreatic cancer, and advanced lymphoma/leukemia. Midostaurin is being investigated in the ongoing phase II clinical trials for the treatment of mast cell leukemia and phase II clinical trials for treatment of AML. Patients are being recruited for phase I clinical trial for treating advanced rectal cancer. Indolocarbazole rebeccamycin is another naturally occurring staurosporine. Its derivative, becatecarin, has completed the phase II clinical trials for the treatment of advanced lung cancer, stage IV breast cancer, and non-Hodgkin's lymphoma and phase I clinical trials for treating AML and CML.

3.3.10 Nitrosoureas

Streptozocin (streptozotocin) is a glucosamine nitrosourea compound produced from *Streptomyces achromogenes* (Reusser 1971; Simon et al. 2000). It was originally identified as an antibiotic in the 1950s and approved by the FDA as a therapeutic agent for the treatment of metastatic pancreatic cancer in 1982. It selectively binds to GLUT2 glucose transporter and enters the cells. Like other nitrosoureas, streptozocin induces DNA damage and inhibits DNA synthesis (Blasiak et al. 2004; Cheung-Ong et al. 2013). This could be the possible underlying mechanism by which it causes diabetes in experimental animals by causing DNA alkylation and cell death of pancreatic islet cells (Simon et al. 2000; Lee et al. 2005). However, the DNA alkylating ability of streptozocin has been exploited to test its potential as an anticancer agent against a wide range of cancers including insulinoma, myeloma, glioma, lymphoma, melanoma, and SCLC. Streptozocin has completed phase II clinical trials for the combined chemotherapy against metastatic neuroendocrine tumors and recurrent brain neoplasms and also a phase III clinical trial for advanced adrenocortical carcinoma. Currently, patients are being recruited for the phase III clinical trial in which streptozocin is being evaluated for the treatment of pancreatic neuroendocrine tumors (pNET).

3.3.11 Mitomycin C

Mitomycin C is an aziridine-containing antibiotic isolated from *S. caespitosus* or *S. lavendulae* in 1956 that possesses antitumor properties (Bhattacharya and Mukherjee 2015). It is a strong DNA cross-linking agent, known to cause inhibition of DNA synthesis in mammalian cells (Nijwening et al. 2011; Cheung-Ong et al. 2013). Several synthetic DNA cross-linking agents have been developed based on the structure of mitomycin C. As such, mitomycin C does not react with DNA; however it gets activated upon enzymatic or chemical reduction, which converts mitomycin C into a highly reactive bifunctional alkylating agent that binds to DNA. Thus, it selectively targets and suppresses the growth of the solid tumors which predominantly have hypoxic conditions, while, normal cells do not (Tomasz 1995). Moreover, it has been reported to synergistically work with anticancer drugs such as doxorubicin and 5-fluorouracil in the treatment of gastric adenocarcinoma and a variety of cancers. It has also been reported to be effective in the treatment of lymphoma and leukemia. It has completed phase II trial for metastatic breast cancer. Currently, it is in phase II and phase III clinical trials for the treatment of colorectal cancer and urinary bladder neoplasms respectively.

3.3.12 Mithramycin/Mithramycin A

Mithramycin or plicamycin is an antitumor antibiotic originally isolated from *Streptomyces plicatus*. This anticancer drug has been introduced in the year 1961 for the treatment of bone cancer (Paget's disease), testicular cancer, and tumor-related hypercalcemia. Mithramycin binds to GC-rich region of DNA and inhibits RNA and protein synthesis. A preclinical report suggests that mithramycin may interfere with the DNA methyltransferase activity in CpG hypermethylation of major tumor suppressor genes, which is observed in many human cancers (Lin et al. 2007). It induces TRAIL-mediated apoptosis in cancer cells (Lee et al. 2006). It has been investigated in phase I/II clinical trials for the treatment of Ewing's sarcoma, thoracic malignancies, and germ cell neoplasms. However, its clinical use is limited because of its severe toxicity (Lin et al. 2007).

3.4 Anticancer Compounds from Marine Source

The oceans constitute over 70% of the earth's surface and 95% of its tropical biosphere. Marine organisms cover 50% of the total biodiversity on the earth that represents 34 of the 36 phyla (Thakur et al. 2005; Hussain et al. 2012). Marine flora constitutes most of the oceanic organisms which include marine fungi, bacteria, algae, cyanobacteria, sponges, and seaweeds (Li et al. 2015; Rangel and Falkenberg

2015). With the taxonomical diversity and biological properties, these marine sources have provided an enormous opportunity for the discovery of novel anticancer compounds. Recently, a considerable number of marine compounds with robust anticancer properties have been discovered which have proved their efficiency clinically. Most of them are polyphenols and sulfated polysaccharides, which are known for their potent antioxidant, antitumor, and immunostimulatory activities (Dixit and Suseela 2013; Pangestuti and Kim 2017). Marine origin compounds have also demonstrated other biologically and clinically important properties such as antimicrobial, anti-inflammatory, anticoagulant, photoprotective, and others. Some of the marine organisms such as sponges symbiotically associate with actinomycetes and have also been investigated for anticancer potential. For the past three decades, marine pharmaceutics has developed a number of antitumor drugs (Schwartsmann et al. 2001; Hussain et al. 2012). However, the marine environmental sources have been underexplored for the production of novel antitumor compounds. It is mainly because of the shortfalls in this field (Bhatnagar and Kim 2010). Although there are only a few marine-derived products currently in the market, most of them are in the clinical trials and drug development stage (Newman and Cragg 2014). The drugs from marine sources such as marine alkaloid trabectedin were introduced in 2007 for the treatment of cancer. About 2500 metabolites with antiproliferative activity have been reported during the last decade (Jimeno et al. 2004). Anticancer compounds from marine source and their derivatives under clinical trial and for their clinical status are discussed below (Table 3.3; Figs. 3.3a and 3.3b).

3.4.1 Bryostatins

Bryostatins are a class of naturally occurring macrolides with an immense therapeutic value, originally isolated from the marine invertebrate *Bugula neritina*. They generally share a 20-membered macrolactone core and 3 remotely functionalized polyhydropyran rings. Bryostatins differ from each other in substitution at C7 and C20 and placement of the c-lactone at either C19 or to C23 in the polyhydropyran ring. Several bryostatin analogs have been synthesized such as bryostatin 1, 2, 3, and 7 with several features and activities. Among the bryostatin analogs, the most studied member is bryostatin 1 and is a powerful PKC modulator to which its antitumor activity is attributed. Interestingly, bryostatin 1 has minimal tumorigenic properties unlike most of the PKC modulators (Irie et al. 2012; Giddings and Newman 2013; Newman and Cragg 2016b). Bryostatin 1 is being evaluated for its efficacy against a variety of cancers as a single agent and in combination with other cytotoxic compounds such as vincristine and paclitaxel. Bryostatin 1 has completed phase I clinical trials for the treatment of recurrent HIV-related lymphoma in combination with vincristine and metastatic solid tumors with temsirolimus. It has also completed phase II trials for the treatment of advanced pancreatic cancer, metastatic prostate, and kidney cancers.

Table 3.3 Anticancer compounds from marine source

Class	Compound	Source	Mode of action	Clinical trial stage	References
Bryostatins	Bryostatin 1	*Bugula neritina* (bryozoan)	PKC inhibition and apoptosis	Advanced pancreatic, metastatic prostate, and kidney cancer – phase II (c)	Schwartsmann et al. (2001), Singh et al. (2008), and Butler et al. (2014)
				HIV-related lymphoma, metastatic solid tumors – phase I (c)	
Dolastatin	Dolastatin 10	*Dolabella auricularia/ Symploca* sp.	Microtubule inhibition	Metastatic pancreatic cancer, kidney and ovarian cancers, lymphoma – phase II (c), metastatic soft tissue sarcoma – phase II (c)	Ray et al. (2007), and Singh et al. (2008)
	Soblidotin/ auristatin (TZT-1027)	Synthetic derivative of dolastatin 10	Microtubule inhibition	Metastatic soft tissue sarcoma and NSCLC – phase II (c)	Ray et al. (2007), Newman and Cragg (2014), and Fanale et al. (2015)
	Synthadotin/ tasidotin	Synthetic derivative of dolastatin 15	Mitosis and topoisomerase inhibition	Malignant melanoma – phase I/II (c)	
				NSCLC and prostate cancer – phase II (c) solid tumors – preclinical (c)	
	Monomethyl auristatin E (MMAE)/Vedotin	Synthetic variant of auristatin (as ADC)	Microtubule inhibition	Lymphoma – phase II melanoma, triple-negative breast cancer – phase III and solid tumors – phase I/II	
	Monomethyl auristatin F (MMAF)	Synthetic variant of auristatin (as ADC)	Microtubule inhibition	Relapsed/refractory multiple myeloma – phase I (r) and hematologic malignancies – phase I	

Ecteinascidin	Trabectedin (ET-743)	*Ecteinascidia turbinate*	Double-strand DNA damage by minor groove binding	Solid tumors, pediatric cancers – phase II (r)	D'Incalci and Galmarini (2010), Romano et al. (2013), and Petek and Jones (2014)
				Breast and advanced prostate cancer – phase II (c)	
				Soft tissue sarcoma and ovarian cancer – approved	
	Lurbinectedin (PM01183)	Structural variant of trabectedin	Double-strand DNA damage by minor groove binding	Osteosarcoma – phase II (c) NSCLC – phase III (r) and advanced solid tumors, metastatic breast cancer – phase II (r)	
	Zalypsis (PM 00104)	Structural variant of trabectedin	DNA minor groove binding	Metastatic Ewing's family of tumors – phase II (c)	
Kahalalide	Kahalalide F	*Elysia rufescens/ Bryopsis* sp.	Lysosome target and promotion of oncosis	NSCLC – stage IIIB/IV – phase II	Jimeno et al. (2004), Hussain et al. (2012), and Cragg and Newman (2013)
	Elisidepsin (PM02734)	Synthetic derivative of kahalalide F	Oncosis and necrosis promotion	Advanced malignant solid tumors – phase I (c)	
Aplidine	Aplidine	*Aplidium albicans*	Apoptosis and angiogenesis inhibition	Refractory/relapsed multiple myeloma – phase III (r), leukemia, lymphoma – phase II (c) advanced solid tumors – phase I (r)	Schwartsmann et al. (2001), Jimeno et al. (2004), Munoz-Alonso et al. (2008), and Butler et al. (2014)
Epoxomicin	Carfilzomib	Synthetic derivative of epoxomicin	Proteasome inhibition	Multiple myeloma – approved NSCLC; ovarian, renal, and prostate cancers; and hematological malignancies – phase I/II (r)	Moore et al. (2008) and Butler et al. (2014)
Halichondrin	Eribulin mesylate (E7389)	Synthetic derivative of halichondrin B	Microtubule inhibition	Metastatic breast cancer, liposarcoma – approved NSCLC, advanced solid tumors, prostate cancer – phase II (c), soft tissue sarcomas, pediatric, cervical cancers – phase I/II (r)	Towle et al. (2001) and Newman and Cragg (2014)

(continued)

Table 3.3 (continued)

Class	Compound	Source	Mode of action	Clinical trial stage	References
Salinosporamides	Salinosporamide A (NPI-0052)	*Salinispora* sp. (bacterium)	20 S proteasome inhibition	Grade IV malignant glioma – phase I/II (r)	Moore et al. (2008) and Butler et al. (2014)
				Solid tumors, lymphomas, leukemia, multiple myeloma, pancreatic cancer, melanoma, NSCLC – phase I (c)	
Cytarabine	Ara-C	*Cryptotheca crypta*	DNA synthesis inhibition	AML, ALL and CML – approved meningeal metastasis of breast cancer – phase III (r), medulloblastoma and metastatic solid tumors – phase II (r)	Schwartsmann et al. (2001), Jimeno et al. (2004), and Hussain et al. (2012)
	Gemcitabine	Derivative of cytarabine	DNA synthesis inhibition	Metastatic breast, ovarian, pancreatic cancers, and NSCLC – approved	
				Urothelial cancer – phase III (r), T-cell lymphoma, biliary tract cancers, and metastatic colorectal cancer – phase II (r), solid tumors and bladder cancer – phase I (r)	
Halimide	Plinabulin (NPI-2358)	*Aspergillus* sp.	Microtubule inhibition	Metastatic NSCLC – phase III (r) and advanced solid tumors – phase II/III (r)	Gomes et al. (2015)
Hemiasterlin	E7974	*Hemiasterellaminor* sponge	Microtubule inhibitor	Solid tumors – phase I (c)	Singh et al. (2008 and Kuznetsov et al. (2009)

The clinical trial stage shown in table is mostly from the NIH (https://clinicaltrials.gov/) or the corresponding EU clinical trial database
PKC protein kinase C, *ADC* antibody-drug conjugate, *DDR* DNA damage response, *c* completed, *r* recruiting participants, *a* active

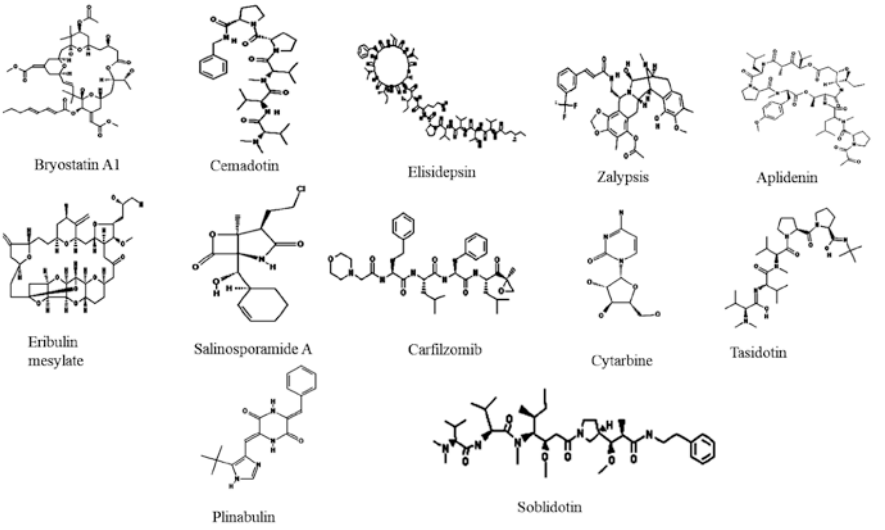

Fig. 3.3a Anticancer compounds from marine under clinical trial

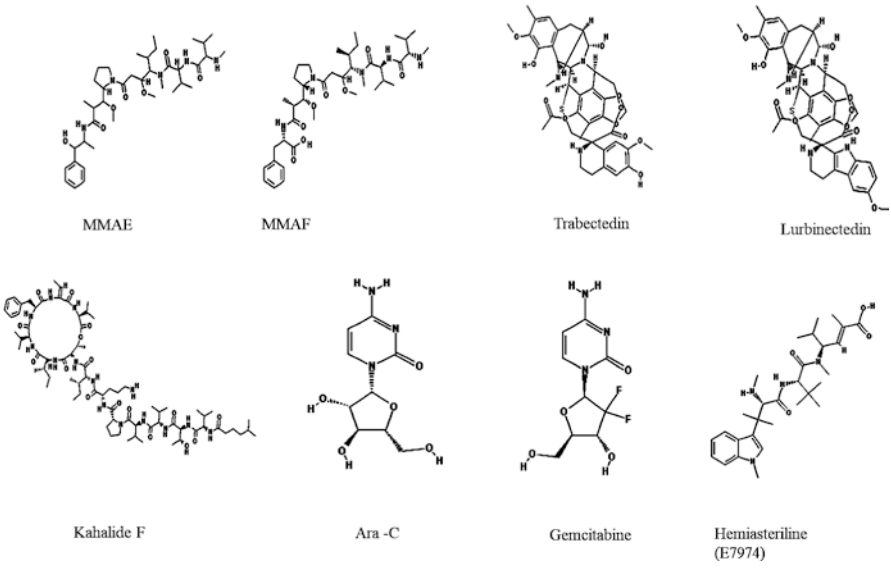

Fig. 3.3b Anticancer compounds from marine under clinical trial

3.4.2 Dolastatins

Dolastatins are pentapeptides originally isolated from the sea hare *Dolabella auricularia* in the 1970s from the Indian Ocean and later from cyanobacterium of the genus *Symploca*, which has been recently renamed as *Caldora penicillata* (Newman and Cragg 2016b). Dolastatin 10 is the most active compound of this class with its ED_{50} being 4.6×10^{-5} μg/ml against murine PS leukemia cells (Singh et al. 2008). Dolastatin 10 is a potent antimitotic agent which is similar to the mechanism of action of taxanes and vinca alkaloids (Simmons et al. 2005). Reports suggest that it binds to β-subunit of tubulin, forms tubulin bundles, and inhibits the formation of microtubules which arrests the cells in G_2/M phase and causes Bcl2 mediated apoptotic cell death (Pangestuti and Kim 2017). Also, it acts synergistically with vinca alkaloids and bryostatin 1 against certain cancers (Singh et al. 2008). Dolastatin 10 was evaluated in several phase I and phase II clinical trials for the treatment of solid tumors of the pancreas and kidney and also a multi-institutional phase II clinical trial for the treatment of metastatic soft tissue sarcoma. However, due to minimal responses and side effects like peripheral neuropathy in cancer patients, it was withdrawn from further clinical trials. Nevertheless, because of its antimitotic action against tumors, dolastatin 10 has prompted the development of its synthetic derivatives (Simmons et al. 2005).

3.4.2.1 TZT-1027 (Soblidotin and Auristatin PE)

Soblidotin is a synthetic analog of dolastatin 10, which has been developed by retaining the anticancer property of the lead compound and reducing its toxicity. The only difference between soblidotin and dolastatin 10 is the replacement of the terminal dolaphenine amino acid residue unit of dolastatin with a phenylethylamine group in soblidotin (Simmons et al. 2005). Soblidotin is a microtubule-disrupting agent and has demonstrated to have potency against p53 mutant cell lines, against which DNA-damaging agents are generally less effective. Soblidotin is also known to be a potent tumor-vasculature-disrupting agent due to its ability to interact with VEGF. It entered phase II trials for the treatment of soft tissue sarcoma and lung cancer (Fanale et al. 2015). Due to extreme hematological toxicities and lack of expected cytotoxicity against cancer cells, soblidotin has not progressed beyond phase II clinical trials (Singh et al. 2008; Newman and Cragg 2016b).

3.4.3 Dolastatin 15

Dolastatin 15 is a linear depsipeptide, closely related to dolastatin 10, and was also isolated from the sea hare *D. auricularia*. The initial investigations on dolastatin 15 demonstrated that its ED_{50} was 2.4×10^{-3} μg/ml and it binds directly to vinca

domain of tubulin. However, due to poor water solubility and low yield, it could not enter clinical trials. Nevertheless, it has encouraged the development of its synthetic analogs such as synthadotin and cematodin with enhanced pharmaceutical properties (Simmons et al. 2005).

3.4.3.1 Synthadotin (Tasidotin)

Tasidotin is a metabolically stable third-generation synthetic analog of dolastatin 15 with a terminal tertiary butyl moiety. It has demonstrated to have potent anticancer activity on cancer cell lines even at very low concentrations. Tasidotin C-carboxylate, a functionally active metabolite of tasidotin, is reported to be 10–30 times more effective with a reduced toxicity profile (Fanale et al. 2015). Its mechanism of action is more similar to that of taxol which binds along the length of microtubules and stabilizes microtubule plus ends at low concentrations and increases the catastrophe frequency at minus ends (Ray et al. 2007). Preclinical studies have suggested that tasidotin has a potent antitumor activity against pediatric sarcomas in xenograft models. This orally active microtubule inhibitor has completed three phase II trials for the treatment of metastatic melanoma, metastatic NSCLC, and hormone refractory prostate cancer which is previously treated with docetaxel. These trials suggested that the toxicity profiles were quite favorable (Simmons et al. 2005; Khazir et al. 2014).

3.4.3.2 Cemadotin

Cemadotin is another synthetic analog of dolastatin 15, which was developed with better chemical properties retaining the high cytotoxic profile of the lead compound with IC_{50} being 0.1 μM. It underwent various phase I clinical trials and phase II clinical trials against malignant melanoma, metastatic breast cancer, and NSCLC. However, the clinical trials were discontinued because of the severe cardiovascular toxicity, hypertension, acute myocardial infarction, and neutropenia caused by cemadotin in phase I clinical evaluation and inconsiderable cytotoxicity in phase II clinical trials (Simmons et al. 2005; Ray et al. 2007).

3.4.4 Monomethylauristatin E and Monomethylauristatin F

Monomethylauristatin E which is also known as vedotin and its phenylalanine variant monomethylauristatin F are the synthetic variants of soblidotin/auristatin E. Due to high cytotoxic properties, these two compounds have been used as warheads to link monoclonal antibodies and are currently in several clinical trials for the treatment of a wide variety of cancers. Currently, there are 68 clinical trials with the status “recruiting” of the total of 139 trials that involve these two potent warheads

listed (https://clinicaltrials.gov). Eventually many antibody-drug conjugates received FDA approval (Giddings and Newman 2013).

Vedotin linked with brentuximab has completed phase II clinical trials for the treatment of primary mediastinal large B-cell lymphoma and in combination with rituximab for the treatment of DLBCL. Patients are being recruited for the phase II clinical trials for treating Hodgkin's lymphoma, malignant mesothelioma, and non-Hodgkin's lymphoma. Many active clinical trials are listed in the US clinical trial registry involving brentuximab linked to vedotin in combination with other monoclonal antibodies for the treatment of lymphoma (NIH). Glembatumumab vedotin has completed phase II clinical trials for treating breast cancer, metastatic triple-negative breast cancer, and stage III/IV melanoma. Three phase II clinical trials in which patients are being recruited are for the treatment of SCC of lung, advanced melanoma, and triple-negative breast cancer. Polatuzumab vedotin in combination with other monoclonal antibodies is in three active phase I/II clinical trials for the treatment of non-Hodgkin's lymphoma and DLBCL, and recruitment is going on for phase I/II trials for treating DLBCL in combination with other cytotoxic drugs. Tisotumab vedotin is another antibody-drug conjugate, for which patients are being recruited for phase I/II trials for safety assessment during the treatment of solid tumors. GSK2857916 is the only antibody conjugate of MMEF under clinical trial. There is only one phase I clinical trial listed in which patients are being recruited for the treatment of relapsed/refractory multiple myeloma and other advanced hematologic malignancies expressing B-cell maturation antigen (BCMA) (Newman and Cragg 2016b).

3.4.5 Trabectedin (ET-743)

Trabectedin is a tetrahydroisoquinoline alkaloid, which was originally isolated from the Caribbean tunicate *Ecteinascidia turbinata*. Because of its stability and high natural availability, ET-743 was considered more suitable for clinical development than its analog ET-749, although it showed high cytotoxic potential *in vivo*. Trabectedin received FDA approval in 2015 for the treatment of metastatic liposarcoma and leiomyosarcoma, particularly which are previously treated with anthracyclines (Giddings and Newman 2013; Newman and Cragg 2016b). Trabectedin has completed phase II clinical trials for the treatment of metastatic breast cancer; ovarian, prostate, and pancreatic cancers; metastatic osteosarcoma; Ewing's family of sarcomas; and advanced malignancies. It has completed phase III trials for the treatment of advanced relapsed ovarian cancer in combination with DOXIL and dexamethasone, soft tissue sarcomas with dacarbazine, and advanced translocation related sarcomas with doxorubicin and ifosfamide. Currently, there are many phase I/II/III trials listed with the status "recruiting" for treating ovarian cancer, breast cancer, and soft tissue sarcomas as monotherapy and in combination with other therapies involving cytotoxic drugs/monoclonal antibodies/radiation.

The antitumor activity of ET-743 (IC_{50} of which is well below 1 nM) is attributed to its multifaceted mechanism of action in targeting cancer cells (Singh et al. 2008). It is reported that ET-743 binds to the minor groove of DNA and induces a unique bend in DNA toward the major groove. It inhibits active transcription by directly interacting with RNA polymerase II, causing its ubiquitination and proteasomal degradation. Further, it interferes with the transcription-coupled nucleotide excision repair and homologous recombination machineries and induces double-strand breaks in DNA. It causes cell cycle arrests in G2 phase and induces cell death. The cytotoxicity caused by trabectedin is independent of p53 status (Takebayashi et al. 2001; D'Incalci and Galmarini 2010). ET-743 has demonstrated antiproliferative properties against advanced ovarian and breast cancers, metastatic osteosarcoma, and mesenchymal tumors which have been previously treated with platinum drugs or taxanes in phase I clinical trials. In phase II clinical evaluation, soft tissue sarcoma and ovarian and breast cancer patients responded well to ET-743 treatment (Simmons et al. 2005; Giddings and Newman 2013; Newman and Cragg 2016b). Two structural variants and close relatives of trabectedin, namely, lurbinectedin (PM01183) and Zalypsis, underwent clinical development and entered clinical trials.

3.4.5.1 Lurbinectedin (PM01183)

Lurbinectedin is an isoquinoline alkaloid with the replacement of tetrahydroisoquinoline present in ring C of trabectedin with a tetrahydro- β-carboline moiety (Newman and Cragg 2016b). Like ET-743, PM01183 also binds to the minor groove of DNA that results in delayed progression through S-phase and eventually causes G_2/M phase arrest and cell death. Reports suggest that PM01183 has demonstrated to act in synergy with platinum drugs and is effective against platinum drug-resistant cell lines (Imperatore et al. 2014; Newman and Cragg 2016b). PM01183 has completed its phase II clinical trials for the treatment of NSCLC with docetaxel and gemcitabine and as a single agent against osteosarcoma. Currently, patients are being recruited for phase III clinical trials for the treatment of NSCLC and phase II trials for treating advanced solid tumors and metastatic breast cancer.

3.4.5.2 PM00104 (Zalypsis®)

Zalypsis is a synthetic tetrahydroisoquinoline alkaloid closely related to trabectedin and mimics many natural compounds of marine origin. It is structurally very similar to jorumycin which is isolated from the skin and mucin of the *nudibranch Jorunna funebris*, the family of renieramycins isolated from sponges, and the ecteinascidins, from a Caribbean tunicate (Romano et al. 2013; Petek and Jones 2014). Like ET-743 and PM01183, PM00104 also binds to the minor groove of the DNA through its reactive carbinolamine group. Surprisingly, according to some reports, although it binds to DNA and is cytotoxic, it does not activate DNA damage response. Zalypsis

initially demonstrated to be a manageable and reversible toxicity profile in preclinical trials (Romano et al. 2013). It also showed potent antiproliferative effects against a wide range of solid and hematological cancer cell lines *in vitro* (Romano et al. 2013) and human transplantable breast, renal, gastric, and prostate xenograft models in *in vitro* preclinical evaluation (Petek and Jones 2014). A phase II trial using Zalypsis for the treatment of metastatic Ewing's family of tumors has been completed. Currently, there is no active clinical trial listed in the registry.

3.4.6 Kahalalide F

Kahalalides are antitumor depsipeptides, originally isolated from Sacoglossan mollusk, *Elysia rufescens* (Newman and Cragg 2014). Among the kahalalides that were isolated, only kahalalide F demonstrated antitumor activity (Singh et al. 2008). Kahalalide F has a unique mechanism of action which is the induction of oncosis by which it causes cell death in cancer cells (Singh et al. 2008). Reports also suggest that it induces apoptotic mode of cell death by disrupting lysosomal membrane in tumor cells. Also, it targets the proteins involved in the Erb3 and PI3 kinase-Akt/PKB signaling pathways (Giddings and Newman 2013). It may also interfere with the gene expression of the proteins involved in DNA replication and cell proliferation (Mans 2016). Kahalalide F demonstrated to have potent antitumor activity *in vitro* and *in vivo* in various solid tumor models of breast cancer, colon cancer, NSCLC, and, exclusively, prostate cancer. It entered clinical trials for the treatment of prostate cancer, malignant melanoma, and NSCLC (Hussain et al. 2012). Currently, no clinical trials are listed that involve kahalalide in the US clinical trial registry. However, there is one ongoing phase II clinical trial in Europe to treat stage IIIB or stage IV NSCLC using kahalalide F after the first-line chemotherapy.

3.4.6.1 Elisidepsin (PM02734)

Elisidepsin (isokahalalide F, PM-02734) is a synthetic cyclic depsipeptide (Bhatnagar and Kim 2010), a derivative and an isomer of kahalalide F (Giddings and Newman 2013). Like the parent compound, elisidepsin appears to induce oncolytic rather than apoptotic cell death in cancer cells. It has completed phase I clinical trial for the treatment of advanced malignant solid tumors in combination with erlotinib. A study suggests that the trial was closed because of the inadequate antitumor activity of elisipepsin exhibited in combination with erlotinib (Giddings and Newman 2013).

3.4.7 Aplidine

Aplidine is a marine depsipeptide originally isolated from *Aplidium albicans*. It is structurally closely related to didemnin B, an antitumor compound isolated from a Caribbean tunicate *Tridemnum solidum.* Structurally, aplidine is dehydrodidemnin B, and this small structural difference is the reason for its stronger cytotoxicity and lower cardiotoxicity profile (Giddings and Newman 2013). It is reported that the antiproliferative property of aplidine is because of its ability to interfere with multiple regulatory pathways. Aplidine induces activation of Rac1 which leads to a strong activation of JNK pathway that rapidly induces apoptosis in breast, renal, and cervical cancer and melanoma cells (Munoz-Alonso et al. 2008).Currently, aplidine is in phase III clinical trial in combination with dexamethasone for the treatment of refractory/relapsed multiple myeloma (NIH). Aplidine is PharmaMar's second most advanced compound after trabectedin as it has completed phase II trials for the treatment of multiple myeloma and progressed to phase III trials (Khazir et al. 2014). It has completed phase II clinical trials for the treatment of leukemia, lymphoma, and phase I trial for advanced solid tumors treatment. Patients are being recruited for phase I/II trials for the treatment of multiple myeloma in combination with bortezomib and dexamethasone and as a monotherapy in phase II trial for treating lymphoma. The European clinical trial registry has one ongoing multicenter phase II clinical trial against relapsed or refractory indolent non-Hodgkin's lymphoid neoplasm using aplidine.

3.4.8 Epoxomicin and Derivatives/Carfilzomib

Epoxomicin is a tetrapeptide with an epoxy-b-aminoketone moiety isolated from an unidentified strain of actinomycetes. Its antitumor properties were first reported in 1992. Epoxomicin entered preclinical trials conducted by Bristol-Myers Squibb, but was withdrawn because of its unknown mechanism of action. Later, it was discovered that epoxomicin is a proteasome inhibitor by Crews' team at Yale University. With its mechanism of action discovered, several derivatives of epoxomicin were eventually developed (Giddings and Newman 2013).

Carfilzomib is a synthetic derivative of epoxomicin which was designed with a morpholino end group in order to increase its water solubility, oral availability, and other pharmaceutical properties (Giddings and Newman 2013). Carfilzomib is an irreversible proteasomal inhibitor and more selective in its action than bortezomib, which is a well-known proteasomal inhibitor (Crawford et al. 2011). Through the proteasomal inhibition, carfilzomib causes cell cycle arrest, promotes apoptosis, and thus inhibits the growth of the tumors. Unlike bortezomib, carfilzomib does not cause neurotoxicity which is a common side effect caused by bortezomib in multi-

ple myeloma patients. It also synergizes with dexamethasone to induce potent cytotoxicity against multiple myeloma (Orlowski and Kuhn 2008; Arastu-Kapur et al. 2011; Crawford et al. 2011). It received FDA approval in 2012 for the treatment of multiple myeloma and currently is in clinical investigations for the treatment of other cancers. Presently, there are active phase I–II clinical trials with carfilzomib for the treatment of NSCLC; ovarian, renal, and prostate cancers; and hematological malignancies in combination with other cytotoxic drugs. Carfilzomib is the second proteasomal inhibitor to be approved after bortezomib and the first not to cause adverse side effects like painful peripheral neuropathy (Arastu-Kapur et al. 2011).

3.4.9 Halichondrin B

Halichondrins are the complex structured polyether macrolides originally isolated from Japanese marine sponge *Halichondria okadai* in 1986. Halichondrin B exhibited robust antitumor activity with IC_{50} being 0.09 nM in B-16 melanoma cell lines. In 1991, halichondrin B was identified as tubulin-destabilizing agent found to strongly bind to tubulin at a site nearby but different from that of vinca. One of the derivatives which showed very high potency against tumor cells is the truncated analog of halichondrin B against human colon cancer cell lines, and that compound was named eribulin which was then progressed to further clinical evaluation (Singh et al. 2008; Giddings and Newman 2013). Halichondrins prevent microtubule assembly and cause delay in S-phase progression and G2/M phase cell cycle arrest which eventually results in apoptosis in cancer cells (Towle et al. 2001; Kinghorn et al. 2016).

3.4.9.1 Eribulin

Eribulin is one of the synthetic derivatives of the natural marine product halichondrin B, used in the treatment of metastatic breast cancer in patients who have previously been treated with an anthracycline or a taxane and metastasized liposarcoma. Eribulin received FDA approval for treating metastatic breast cancer in 2010 (Khazir et al. 2014). It effectively inhibits microtubule dynamics at low concentrations, causes prolonged cell cycle arrest, and eventually induces apoptosis in tumor cells, whereas at relatively much higher concentrations, it promotes tubulin depolymerization. Reports suggest that eribulin also hampers centromere dynamics (Newman and Cragg 2016b). As of now, clinical trials ranging from phase I to III are listed using eribulin as a monotherapy and/or in combination with other cytotoxic drugs for the treatment of different cancer types. Phase II trials for the treatment of NSCLC, advanced solid tumors, and prostate cancer using eribulin have been completed. Patients have been recruited currently for phase I–II trials for the treatment of soft tissue sarcomas, pediatric cancers, and cervical cancer, and a pilot study for the treatment of brain tumor has also been listed. There are several active clinical

trials ranging from phase I to III for the treatment of breast cancer using eribulin in combination with various other anticancer drugs.

3.4.10 Salinosporamide A (NPI-0052 and Marizomib)

Salinosporamide A or NPI-0052 was first isolated from *Salinispora tropica*, a marine streptomycete collected from the Bahamas. Salinosporamide A is structurally closely related to the terrestrial natural compound lactacystin, isolated from bacteria, and its derivative omuralide (Crawford et al. 2011; Giddings and Newman 2013). Salinosporamide A is a potent proteasome inhibitor binds to 20 S proteasome more selective than lactacystin, which is also a proteasome inhibitor (Singh et al. 2008). The antitumor activity of salinosporamide A is attributed to its irreversible binding to 20 S catalytic core subunit proteasome by covalently modifying its threonine residues in the active site (Moore et al. 2008). By inhibiting proteasome, salinosporamide also inhibits NF-kB pathway (Giddings and Newman 2013). Inhibition of proteasome results in an accumulation of poly-ubiquitinated proteins, which leads to cell cycle arrest, promotes apoptosis, and inhibits tumor growth and angiogenesis.

Marizomib has completed phase I clinical trials for the treatment of advanced malignancies including solid tumors, lymphomas, leukemias, and multiple myeloma as a monotherapy and NSCLC, pancreatic cancer, melanoma, or lymphoma in combination with vorinostat. It has completed a phase I trial for treating refractory/relapsed multiple myeloma with marizomib in combination with pomalidomide and low dose of dexamethasone. Currently, patients are being recruited for the multicenter phase I/II clinical trial for the treatment of grade IV malignant glioma using marizomib in combination with bevacizumab and another phase I trial using marizomib and an adjuvant temozolomide in combination with radiotherapy. The status of one more phase I/II clinical trial is unknown which is listed in the US clinical trial registry for the treatment of relapsed/refractory multiple myeloma for patients who have previously been treated with carfilzomib.

3.4.11 Cytarabine

Cytarabine is an FDA-approved anticancer drug for the treatment of AML, ALL, and CML in combination with other drugs (Priyankashukla 2014). It has also been approved as a monotherapy to treat and prevent meningeal leukemia (NCI). Although it is not available naturally in the form Ara-C from the marine environment, initially its development was inspired by a series of C-nucleoside-derived bioactive compounds which were originally isolated from the Caribbean sponge, *Cryptotheca crypta*/*Tethya crypta.* It is a pyrimidine nucleoside analog that contains arabinose rather than ribose or deoxyribose (Schwartsmann et al. 2001; Newman

and Cragg 2014, 2016b). Cytarabine is metabolized into cytosine arabinoside triphosphate which is its active triphosphate form and damages DNA by multiple mechanisms (Priyankashukla 2014). It inhibits α-DNA polymerase DNA replication and β-DNA polymerase, and cytosine arabinoside triphosphate incorporates into DNA. It inhibits not only DNA synthesis but even RNA polymerase and nucleotide reductase needed for the synthesis of DNA. It specifically causes S-phase cell cycle arrest (Miura and Izuta 2004).

As of now, there are a total of 1153 clinical trials ranging from phase I to IV have been listed on NIH clinical trial database for the treatment of various cancers using cytarabine as a single therapeutic agent and in combination with other cytotoxic drugs. Other than lymphoma and leukemia treatment, patient recruitment is going on for phase III clinical trial for the treatment of meningeal metastasis of breast cancer, phase II clinical trial for the treatment of medulloblastoma using liposomal cytarabine, and phase II clinical trial for the treatment of leptomeningeal metastasis from metastatic solid tumors using cytarabine, and several active trials have been listed in the registry which involve cytarabine or its derivatives.

3.4.11.1 Gemcitabine

Gemcitabine is a fluorinated derivative of cytarabine which is a broad-spectrum antimetabolite and deoxycytidine analog with anticancer property (Jimeno et al. 2004; Priyankashukla 2014). Upon administration, gemcitabine is converted into the active metabolites which get incorporated into DNA. Like cytarabine, gemcitabine also inhibits DNA polymerase and ribonucleotide reductase activity resulting in diminished deoxynucleotide pool available for DNA synthesis (Miura and Izuta 2004). It has been approved by FDA for the treatment of metastasized breast, ovarian, and pancreatic cancers and NSCLC (Schwartsmann et al. 2001) in combination with other cancer chemotherapeutics such as paclitaxel, cisplatin, carboplatin, and paclitaxel albumin-stabilized nanoparticle formulation (NCI). Currently, numerous active clinical trials involving treatment with gemcitabine as a monotherapy or in combination with other chemotherapeutics are going on. Some of them are a multicenter phase I trial for the treatment of solid tumors and bladder cancer; phase II trials for the treatment of T-cell lymphoma, biliary tract cancers, and metastatic colorectal cancer; and a phase III trial for the treatment of urothelial cancer for which patients are being recruited.

3.4.12 Plinabulin (NPI-2358)

Plinabulin (NPI-2358) is a potent orally active antineoplastic compound isolated from the cultures of a marine fungus *Aspergillus ustus* (Khazir et al. 2014). It is structurally a close relative of the fungal metabolite halimide/phenylahistin which exhibited potent antitumor activity against various *in vitro* and *in vivo* cancer

models (Newman and Cragg 2014). Plinabulin interferes with microtubule dynamics by selectively binding to colchicine domain of tubulin, disrupts mitotic spindle assembly, and brings about G_2/M phase cell cycle arrest. It also causes disruption of tumor vasculature and inhibits tumor cell proliferation. Plinabulin has demonstrated to have strong antitumor activity against several human cancer cell lines including multidrug-resistant cell lines *in vitro* (Gomes et al. 2015).Currently, patient recruitment is in progress for phase II/III clinical trials for the treatment of solid tumors by plinabulin in combination with docetaxel/pegfilgrastim, a phase III trial for the treatment of advanced NSCLC in combination with plinabulin and docetaxel, and phase I/II trials for the treatment of metastatic NSCLC with plinabulin plus nivolumab.

3.4.13 E7974

E7974 is a synthetic analog of hemiasterlin which was originally isolated from the marine sponge *Hemiasterella minor* in 1990 (Khazir et al. 2014). Like the parent compound, it also inhibits tubulin polymerization and has demonstrated similar IC_{50} values as those of vinblastine. It causes G_2/M phase cell cycle arrest followed by cell apoptosis (Khazir et al. 2014). A report suggests that it targets α-tubulin than β-tubulin unlike most of the β-tubulin interactive agents such as taxanes and vinca alkaloids although a slight binding to β-subunit of tubulin was also observed. E7974 has demonstrated potency against cancer cells with mutations in β-tubulin gene, which are resistant to the treatment with taxanes (Kuznetsov et al. 2009). E7974 seems to be a promising drug candidate to treat solid tumors. As of now, E7974 has completed three phase I clinical trials for the treatment of solid tumor malignancies.

3.5 Conclusions and Future Prospects

A huge number of natural compounds and their derivatives with anticancer properties have been used in the clinical trials for cancer therapy. The current status of natural compounds in clinical trials and their natural sources was documented in accordance with NIH or the corresponding EU clinical trial database. From the information presented in this chapter, researchers or clinicians get the knowledge of the natural compounds, their anticancer properties, their mechanisms of action, and their clinical trial status so that they can select the compounds of their interest for further study or clinical use. The major advantage of using natural compounds in anticancer therapy is that they are safe and less toxic unlike synthetic compounds which are rather unsafe and toxic to normal cells also, in addition to cancer cells. Selected natural compounds with anticancer properties of plant, microbial, and marine origin have been explored. The most potent natural anticancer drugs include

vinblastine, vincristine, and taxol from plants; dolastatin 10, cytarabine, and aplidine from marines; and bleomycin, doxorubicin, and dactinomycin from microorganisms, which have been used in cancer therapy. Plants have contributed numerous bioactive compounds which are clinically approved. Despite their contribution, for more than a decade, screening for bioactive lead molecules in plants was considerably reduced by the leading pharmaceutical companies, which were instead in favor of combinatorial chemistry by which libraries of millions of compounds can be synthesized. Moreover, characterization of the lead molecules in plant extractions was rather a time-consuming and laborious process. However, emerging biotechnology companies have taken up the drug discovery programs for identifying the lead molecules from the natural sources and developing clinically relevant drug molecules. Many of these natural compounds have been undergoing clinical trials. Previously, identification of the lead molecules used to take several months and, in some cases, years. But the remarkable progress made in the instrumentation and technology such as HPLC-MS (high-performance liquid chromatography-mass spectrometry), LC-MS (liquid chromatography-mass spectrometry), NMR (nuclear magnetic resonance spectrometry), and LC-SPE-NMR (liquid chromatography-solid-phase extraction (SPE)-nuclear magnetic resonance-mass spectrometry) coupled with high-throughput technology and also capillary NMR enables the rapid screening, identification, and characterization of the molecules which are available in the trace amounts in their source organisms. In the future, NMR coupled with HPLC and SPE techniques performed with high-throughput screening will be routinely used in anticancer drug discovery. Biotechnology and genetic engineering play a major role in bulk production of the lead molecules from microbes and marine sources with potent anticancer properties which are much needed to meet the increasing demand. Plant cell fermentation is another technology by which the plant-derived compounds can be mass produced, and the same technology has been employed to produce paclitaxel, one of the most successful anticancer drugs, which yields larger quantities of the desired compounds. Plant tissue culture/cell culture can also be used to produce the plant-derived bioactive compounds in bulk. Nature offers the scope for discovery of novel compounds from natural sources with new mechanisms of action, which is simpler now with the aid of advances made in the realm of drug discovery research. Discovery of novel anticancer compounds especially from natural origin has a constant demand as cancer poses new challenges such as detrimental adverse effects by existing synthetic drugs, development of multiple drug resistance, and emergence of new cancer variants. Future challenges in anticancer drug discovery from natural sources focus mainly on recompensing the shortcomings of the existing anticancer chemotherapeutic drugs with the natural compounds and their derivatives with the better understanding of their mechanisms of action. The best possible drug-target combinations are based on the understanding of the cancer-specific environment of mutated oncogenes, tumor suppressor genes, and their regulatory pathways. It would be ideal to design the targeted cancer therapeutic regimens with the natural compounds and their derivatives with relatively lower toxicity profiles in combination or as monotherapeutic agents. The clinical effectiveness of combined drugs has not been robustly demonstrated.

Therefore additional research using *in vivo* systems and better clinical trials is needed to determine the safety and clinical usefulness of nature-based drugs. The future success of anticancer drug discovery from natural source depends on the combinatorial chemistry based on the natural lead compounds and high-throughput drug screening technology. By cataloging the natural anticancer compounds in the clinical trials, we have attempted to create knowledge-based awareness to the scientific community for further exploring the natural sources to discover new lead anticancer molecules and, also, to clinically use existing molecules in cancer therapy.

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Chapter 4
Insight Approaches of Medicinal Plants for the Discovery of Anticancer Drugs

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Abstract Cancer is one of the most serious illnesses of our civilization. The International Agency for Research on Cancer estimated that 14.1 million new cancer cases were diagnosed and that 8.2 million patients died from this disease worldwide in 2012 alone. Therefore, there is an urgent need for novel anticancer agents as well as new treatment strategies. Natural products have been valuable sources of new therapeutic agents. Thus, the aim of present chapter is to provide an overview of the anticancer natural compounds highlighting the successful cases with clinical application such as Taxol, vincristine and omacetaxine mepesuccinate; those involved in clinical trials such as parthenolide, betulinic acid, ingenol mebutate and curcumin; and the ones with high degree of activity and safety profile such as resveratrol, ursolic acid, tetrandrine and triptolide. For each one of these compounds, it will be presented and discussed its natural origin, cell target, mechanism of action, pharmacologic aspects as well as the structural modifications that improve its anticancer properties in order to summarize the recent medicinal advances of natural anticancer compounds and their analogues. All these aspects aim to draw our readers' attention to the plants and/or their secondary metabolites therapeutic potential and in consequence value the plants' role in anticancer drug discovery.

Keywords Cancer · Clinical trials · Natural compounds · Medicinal plants · Secondary metabolites

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4.1 Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body. In fact, the types and subtypes of cancer that can be found in a specific organ are more than 100 (Liu et al. 2016a). Cancer is a multistage process resulted into an uncontrolled and abrupt division of body cells and is considered one of the leading causes of mortality. The reported numbers of cases per year as well as the predictions for the near future are unthinkable (Gali-Muhtasib et al. 2015; Turek et al. 2016). The fact that tumour development involves physiological capabilities such as limitless replicative potential, tissue invasion and metastasis (Liu et al. 2016a), joined with difficult diagnosis, infers that the therapeutic options are limited and involve mainly the use of synthetic anticancer molecules. Moreover, the approval of these molecule uses involves several stages of security that enhance the treatment budget; they are toxic to normal cells, that is, their side effects are huge, and usually their mechanism of action is through cell apoptosis. To cure this deadly disease, various treatment strategies and therapeutic targets are being developed (Ogbourne et al. 2004; Turek et al. 2016), but still we are so far from seeing the light at the end of the tunnel.

According to WHO (2017), the economic impact of cancer is significantly increasing, and approximately 70% of deaths from cancer occur in low- and middle-income countries. These facts, together with the undesired effects of some synthetic molecules and the knowledge that traditional medicine is the primary health care in several countries of the world, are used as an alternative to cure or improve the health of the countrymen. Thus, many researchers are focused to investigate the potential of medicinal plants or their derived natural products as an anticancer agent (Dias et al. 2012; Katz and Baltz 2016; Fabricant and Farnsworth 2001). The data available from FDA showed that 40% of the approved molecules for drug administration are pure natural compounds or inspired on them (Kinch et al. 2014). And from those, nearly 74% are used in cancer chemotherapy (Bhanot et al. 2011; Giddings and Newman 2013). Natural products have garnered increasing attention in cancer chemotherapy because they are viewed more biologically friendly and consequently more co-evolved with their target sites and less toxic to normal cells (Mishra and Tiwari 2011). Moreover, there are evidences that natural product-derived anticancer drugs have alternative modes of promoting cell death (Gali-Muhtasib et al. 2015; Khalid et al. 2016). Considering the current botanical nomenclature and according to the database "The Plant List" (www.theplantlist.org), several plant species mentioned in this chapter have an alternative name. Therefore, the name cited in the original paper is accepted. Further, taxonomic corrections regarding the author or correct names were rectified following the same database recommendations. Thus, the aim of this chapter is to provide an overview of the recent and most successful cases of medicinal plants and their secondary metabolites proven their anticancer potential in various clinical trials.

4.2 Medicinal Plants with Antitumoural Activity

According to the World Health Organization, around 80% of the global population still relies on botanical drugs, and in several countries, the herbal medicines are used with some level of integration into the traditional health-care system (Allam et al. 2014; Aziato and Odai 2016), while, in Western countries, the herbal medicine use is growing but following tight legislation and under surveillance (Enioutina et al. 2017). Regarding the antitumoural activity, plants constitute a common alternative for cancer treatment in many countries around the world, and more than 3000 plants worldwide have been reported to have anticancer properties (Graham et al. 2000; Alonso-Castro et al. 2011; Ochwangi et al. 2014; Tariq et al. 2017). However, many of the claims for the efficacy of such treatment should be viewed with some critical spirit once cancer, as a disease with specific mechanisms, was not known or was poorly defined in traditional medicine. In fact, in traditional medicine, the cancer term has been applied to describe general conditions such as inflammations, ulcers or dermatological conditions such as "hard swellings", abscesses, calluses, corns, warts and polyps, whereas currently it corresponds to a specific disease entity. The traditional medicine knowledge in the treatment of cancer has promoted investigations into natural sources mainly plants and marine life, resulting in the discovery of lead structures that are useful templates for the pharmaceutical industry.

4.2.1 Medicinal Plants Used in Traditional Medicine to Treat Cancer Diseases

An excellent work was published by Tariq et al. (2017), where the utmost information on ethnomedical, pharmacological and phytochemical aspects of anticancer plants from different countries was reviewed. However, some examples of plants used in traditional medicine to treat cancer, which are not cited by Tariq et al. (2017), are presented in tabular form (Table 4.1). These medicinal plants were chosen on the basis of their anticancer, cytotoxic and antitumour properties, for instance, *Annona crassiflora* (Formagio et al. 2015), *Clinacanthus nutans* (Teoh et al. 2017; Zulkipli et al. 2017), *Solanum lyratum* (Lin et al. 2013; Chiu et al. 2015), *Solanum aculeastrum* (Omosa et al. 2016), *Triumfetta rhomboidea* (Sivakumar et al. 2010), *Sesbania sesban* (Dianhar et al. 2014) and *Prunus africana* (Komakech et al. 2017). In addition, isolation of the anticancer and cytotoxic bioactive phytoconstituents has been also performed in some species. For example, ingenol mebutate isolated from *Euphorbia peplus* (Millsop et al. 2013), polysaccharide-peptide complex (Huang et al. 2016) and lupeol (Teoh et al. 2017) from *Clinacanthus nutans*, lyratol G and 1β-hydroxy-1,2-dihydro-α-santonin from *Solanum lyratum* (Nie et al. 2014), tomatidine and solasodine from *S. aculeastrum* (Koduru et al. 2007), 3-hydroxy-4′,7-dimethoxyflavone from *S. sesban* (Dianhar et al. 2014) and amyrin and β-sitosterol-3-*O*-glucoside from *P. africana* (Maiyo et al. 2016) are frequently used

Table 4.1 Medicinal plants used in traditional medicine for the cancer treatment

Species	Part of plant	Medicinal use	References
Rotheca serrata (*Clerodendrum serratum*)	A paste of leaves	Used by Siddis of Uttara Kannada, India, in their traditional medicine for tumour growth prevention	Bhandary et al. (1995)
Anthocleista djalonensis	Leaves	Used by traditional healers in Ogun State, Nigeria, for the management of cancer	Soladoye et al. (2010)
Olax subscorpioidea	Root		
Euphorbia peplus	Latex	As home remedy in Australia for the treatment of solar keratosis, skin cancer and basal cell carcinoma. Used by England native people to treat skin cancer	Drury (1991) and Butler et al. (2014)
Neonotonia wightii (*Glycine wightii*)	Leaves	Recommended by herbalists from Kakamega County, Kenya, to treat breast cancer	Ochwangi et al. (2014)
Prunus africana	Stem bark and roots	Recommended to treat colorectal, breast, skin and prostate cancer	
Sesbania sesban	Whole plant	Used to treat throat, uterine, skin cancer	
Solanum aculeastrum	Fruits and roots	Recommended to treat skin, breast and cervical cancer	
Triumfetta rhomboidea	Leaves	Used to treat gum cancer	
Urtica massaica	Leaves	Recommended to treat skin, uterine and breast cancer	
Zanthoxylum rubescens	Stem bark, leaves and root bark	Used to treat breast, colorectal, skin and oesophageal cancer	
Solanum lyratum	Not indicated	Used in Chinese traditional herbal medicine to treat liver, lung and oesophageal cancers	Chiu et al. (2015)
Annona crassiflora	Leaves and seeds infusion	Used by Cerrado (Brazil region) native people to treat tumours	Formagio et al. (2015)
Clinacanthus nutans	Leaves	Used in Malaysia and Singapore to treat liver, nasal cavity and general cancers	Zulkipli et al. (2017)

in the traditional medicine. However, several other species, for instance, *Anthocleista djalonensis*, *Glycine wightii*, *Olax subscorpioidea*, *T. rhomboidea*, *Urtica massaica* and *Zanthoxylum rubescens*, have not been evaluated for their anticancer activity or subjected to phytochemical and biological activities studies in order to identify the active secondary metabolites and/or determining its action mechanism. Moreover, despite the beneficial effects of plants in the cancer treatment reported and scientifically confirmed, it must be emphasized that their potential adverse effects, either through directly toxic effects or interactions with conventional drugs, should also be assessed. It should not be ignored that there are risks arising from unmonitored use of herbal medicinal with "miraculous effects" by the general population (Ben-Arye

et al. 2016). In addition, it must be considered that several of the plants reported in Table 4.1 have other traditional phytotherapy uses. For instance, *U. massaica* is used to treat diabetes (Kamau et al. 2016) and malaria (Muthaura et al. 2015); *Clinacanthus nutans* is used against dysentery and varicella zoster, herpes simplex and herpes genitals lesions (Zulkipli et al. 2017); *A. crassiflora* oil seeds is used against snake bites (Formagio et al. 2015); *O. subscorpioidea* is used to treat wounds (Adetutu et al. 2011); *T. rhomboidea* is used to treat anaemia (Das et al. 2015); *S. sesban* is used to cure digestive disorders (Dianhar et al. 2014); and *C. serratum* is popularly used in indigenous systems of medicine to treat respiratory disorders especially asthma (Patel et al. 2014). Proved or not, these uses may also interfere with the beneficial anticancer effect.

4.2.2 Plant Extracts with Cytotoxic/Antiproliferative Activities

The American National Cancer Institute, relatively to anticancer activity, assigns a significant cytotoxic effect of promising anticancer products for future bioguided studies if their IC_{50} values are lower than 30 μg/ml. Besides, in assessing the anticancer potential of any mixture and/or pure compound, the selectivity index (SI = IC_{50} non-tumour cells/IC_{50} tumour cells) and the use of a clinical drug as positive control are essential. The literature survey highlighted the most recent and promising results on the basis of their anticancer activity (Table 4.2). The *in vivo* assays were emphasized, even though the results show that the extracts are less active than the positive control (Viral et al. 2011) or even if the authors do not show the results of reference compounds (Ahmed et al. 2016), because they are less frequent and show the extract effect in conditions much closer to those found in clinical therapeutics. However, the plant extracts showed the antitumour activity both in *in vitro* and *in vivo* conditions failed to provide the comparative results of anticancer compound used in clinical trials. For example, Réthy et al. (2007) and Iweala et al. (2015) reported that the extracts are more active than positive control (cisplatin); nonetheless, they do not assay the extract selectivity. Conversely, Koňariková et al. (2015) report a very low IC_{50} value for the extract activity with a very high selectivity index (higher than 400), but they do not present the positive control results. The same, very promising extract activity values but no report about positive control activity can be noticed in Jain et al. (2016) work. On the other hand, Boutennoun et al. (2017) showed the percentage of cell death for the extract at 5 μg/ml, while the percentage of cell death to the positive control colchicine is given at 50 μg/ml; naturally, these different concentrations do not allow proper conclusions. We cannot fail to point out how much these aspects reduce the impact of such works and once again appeal to researchers that use the latest best practice guidelines on their anticancer assays. This research area is fundamental and usually the first step in a chain of methodologies that will allow the identification of new plants and/or their metabolites with potential use in cancer treatment.

Table 4.2 Plants whose extracts are cytotoxic towards cancer cell lines

Species	Extract tested	Anticancer model and activity level	References
Achillea collina	Chloroform extract from herbs	Tumour cell inhibitory activity against HeLa (cervical cancer cell line) (IC_{50} 1.74 μg/ml) (cisplatin IC_{50} 3.73 μg/ml; doxorubicin IC_{50} 0.089 μg/ml)	Réthy et al. (2007)
Xanthium orientale	Chloroform extracts from folium and from buds/flowers	Antiproliferative activity against A431 (epidermoid carcinoma cell line) (IC_{50} 0.71–0.75 μg/ml) (cisplatin IC_{50} 0.852 μg/ml; doxorubicin IC_{50} 0.086 μg/ml)	
Euphorbia peplus	Plant's sap	A phase I/II clinical trial showed 50–75% full remission in 15 months for the topical treatment of human nonmelanoma skin cancers	Ramsay et al. (2011)
Adiantum venustum	Ethanolic extract from leaves and stem	*In vivo* assay on Ehrlich ascites carcinoma bearing Swiss albino mice model. At 250 mg/kg (i.p.) increase 31.8% in life span (vincristine at 0.8 mg/kg increase life span 40.9%) without toxicity at 2000 mg/kg. Moreover, it reduces the packed cell volume and viable tumour cell count identical to vincristine at 0.8 mg/kg	Viral et al. (2011)
Annona sylvatica	Methanolic extract from leaves	Antitumour activity against UA251 (glioma cell line) (GI_{50} = 0.05 μg/ml; Vero cell line GI_{50} = 41.60 μg/ml) (doxorubicin GI_{50} = 5.13 μg/ml; Vero cell line GI_{50} = 1.38 μg/ml)	Formagio et al. (2015)
A. coriacea	Methanolic extract from floral capitulum	Antitumour activity against K562 (leukaemia cell line) (GI_{50} = 0.04 μg/ml; Vero cell line GI_{50} = 6.33 μg/ml) (doxorubicin GI_{50} = 0.89 μg/ml; Vero cell line GI_{50} = 1.38 μg/ml)	
Piper guineense	Dichloromethane extract from leaves	Cytotoxic activity against HL-60 (myeloid leukaemia cell line) and MCF-7 (breast cancer cell line) (IC_{50} = 3.62 and 6.54 μg/ml, respectively) (cisplatin IC_{50} = 3.06 and 17.48 μg/ml, respectively)	Iweala et al. (2015)
Camellia sinensis	Black tea extract	Antiproliferative activity against MCF-7 (breast cancer cell line) (IC_{50} = 0.0125 μg/ml; mouse healthy fibroblast cell line NIH-3 T3 IC_{50} > 5 μg/ml)	Koňariková et al. (2015)
Alcea rosea	Ethyl acetate from dried seeds	Extract inhibits tumour growth in HCT116 (colon cancer cell line) tumour xenografts in nude mice treated with 200 mg/kg body weight of extract (i.p.) for 21 days	Ahmed et al. (2016)

(continued)

Table 4.2 (continued)

Species	Extract tested	Anticancer model and activity level	References
Aphanamixis polystachya	Ethyl acetate extract from the stem bark	On mice inoculated with Dalton's lymphoma ascites cells, the extract at doses of 20 mg/kg/day (i.p.) prolonged the median survival time. *In vitro* activity against Dalton's lymphoma ascites cells (IC_{50} = 9 μg/ml)	Jain et al. (2016)
Vitis vinifera	Standardized grape seed extract (GSE) (90% procyanidins)	GSE significantly inhibited the 1198 bronchial premalignant cells (IC_{50} <10 μg/ml), while Leucoselect Phytosome (containing GSE at 56–112 mg/kg/day) administered via gavage inhibits the growth of A549 (human lung carcinoma) xenograft tumour in athymic nude mice model	Mao et al. (2016)
Solanum aculeastrum	Ethanol extract of the berries	Cytotoxic activity against human CCRF-CEM leukaemia cell line (0.8% of cell viability at 10 μg/ml, IC_{50} values of 1.36 μg/ml), more active than doxorubicin (6.5% of cell viability at 10 μg/ml)	Omosa et al. (2016)
A. odorata	Methanol extract from leaves	Cytotoxic activity against Wehi cell line (63.19% cell death at 5 μg/ml) (colchicine 81.01% of cell death at 50 μg/ml)	Boutennoun et al. (2017)
E. terracina	Chloroformic and hydromethanolic extracts from shoots	Cytotoxic activity against human leukaemia monocytes (THP-1) by induction of apoptosis (IC_{50} = 14.43 and 2.08 μg/ml, respectively) while is not toxic against normal cells (CD14+ and IEC6) (IC_{50}>100 μg/ml), both after 48 h of exposure	Jannet et al. (2017)
Sarcocephalus pobeguinii	Methanol extracts from leaves and bark	Cytotoxic activity against cervical cancer cell line (HeLa) (IC_{50} 10.19 and 15.26 μg/ml) with selectivity index of 18 and 14, respectively (doxorubicin IC_{50} 1.04 μg/ml)	Njoya et al. (2017)

4.3 Natural Compounds As Anticancer Agents

An important method of drug discovery is the isolation of natural and bioactive compounds from diverse life forms, medicinal plants being one of the most important sources. Thus, an increasing number of studies are being carried out on plants due to their anticancer ethnopharmacological use; these scientific investigations involved phytochemical studies to isolate and characterize the secondary metabolites and pharmacological evaluations to find the active principle. In these researches, both plant traditional applications are recognized, and new bioactive compounds are obtained. In cancer research, lead compound structures from natural sources can be used to design novel chemotherapies with enhanced biological properties.

Throughout history, plants have been a rich source of affordable natural compounds with diverse chemical structures and bioactivities (Chudzik et al. 2015; Das and Dhanjal 2015; Nwodo et al. 2016; Habli et al. 2017). In fact, several new cytotoxic compounds are isolated from plants each year constituting a source of new possibilities to explore in order to fight against cancer. The significant achievements of plant secondary metabolites for its anticancer potential are well explored. Some of them are used in the clinical or preclinical trials and are discussed below.

4.3.1 Natural Compounds with High Degree of Activity and Safety

The natural compounds have high degree of anticancer and cytotoxic activities. The mechanism of action and levels of safety of some selected natural compounds are well documented (Table 4.3 and Fig. 4.1), but still some of them are under clinical trials for fruitful results. The analysis of data showed that the various compounds from different families have some limitations that may prevent its use based on their low solubility and poor bioavailability. Having in mind the compound structure (Table 4.3 and Fig. 4.1), it is obvious that without chemical transformations just ursolic acid and tetrandrine could have their water solubility increased if transformed in the correspondent salt, especially in the case of tetrandrine, an alkaloid, for which there are few studies and could be easily transformed in a hydrochloric derivative. The rapid metabolization of polyphenols apigenin and resveratrol is certainly a drawback in their utilization, but it can be overcome using proper carriers. Deoxypodophyllotoxin and triptolide seem to be more complicated cases because chemical transformations will be necessary, being the first and obvious suggestions the methyl groups cleavage and the epoxy ring opening, respectively (Table 4.4).

4.3.2 Natural Compounds in Clinical Trials

4.3.2.1 Parthenolide

Parthenolide is the most noticeable germacranolide sesquiterpene lactone, due to its high cytotoxicity and the fact that it is currently being tested in clinical trials as anticancer agent (Orofino et al. 2012). Chemically, parthenolide has a 15-carbon (C_{15}) backbone derived from farnesyl diphosphate through cyclization and oxidative transformation (like all sesquiterpene) and is structurally characterized by a ten-membered ring with a five-membered-fused lactone moiety, being the presence of an α-methylene group and a C_4-C_5 epoxide ring are also characteristic in its structure (Fig. 4.2).

Parthenolide was first isolated from few leaves of *Tanacetum parthenium* (L.) Sch. Bip. an ornamental and medicinal plant used in the traditional medicine to

Table 4.3 Plant secondary metabolites with high antitumour activity and in preclinical trials

Compounds (type)	Activities/ actions	Remarks	References
Apigenin (flavonoid)	Natural sources	*Salvia officinalis*, *Lawsonia inermis*, *Turnera aphrodisiaca*, *Ocimum basilicum*, *Tamarindus indica* but also found in many other fruits and vegetables	Torquato et al. (2017)
	Cytotoxicity	*In vitro* and *in vivo* inhibition of some tumour cells of melanoma, lung, breast, colon, prostate, leukaemia and pancreatic cancers	Bridgeman et al. (2016), Sung et al. (2016), Erdogan et al. (2016), Zhou et al. 2017 and Zhao et al. (2017c)
	Other significant antitumour effects	Inhibitory effects of cisplatin on cell migration via downregulation of SNAIL expression. MAPK activation and subsequent p53 phosphorylation	Erdogan et al. (2017), and Liu et al. (2017)
	Mechanism	Targets leptin/leptin receptor pathway and induces caspase-dependent extrinsic apoptosis in HER-2. Induces apoptosis by downregulation of PI3K/Akt/NF-κB signalling. Induces autophagy and inhibition of UVB-induced mTOR signalling by a not Akt-dependent pathway	Seo et al. (2015), Bridgeman et al. (2016), Duan et al. (2016), Erdogan et al. (2016), and Sung et al. (2016)
	In vivo use limitations	Rapid metabolization in the intestine	Ali et al. (2017a) and Tang et al. (2017)
Deoxypodophyllotoxin (lignan)	Natural sources	*Anthriscus sylvestris*, *Podophyllum peltatum*; *Juniperus* spp.	Khaled et al. (2013), Seca et al. (2015), and Zhao et al. (2016a)
	Cytotoxicity	Inhibition of HL-60 cells (2 ng/ml), H460 cells, H460 xenografts, glioma cell xenograft and SGC-7901 cells *in vivo* and the cell lines H460/Bcl-xL *in vitro*. Inhibition of EphB2-EPD (IC50 1.93 nM), prostate DU-145, U-87 MG and SF126 cell lines	Muto et al. (2008), Wu et al. (2013), Yang et al. (2014a), Guerram et al. (2015), Wang et al. (2015a), Ma et al. (2016), and Hu et al. (2016)
	Other significant antitumour effects	Investigation on Heps tumour-xenografted mice and antitumour capacity. Exhibits higher anticancer activity than etoposide both at 15 mg/kg	Wang et al. (2016a)

(continued)

Table 4.3 (continued)

Compounds (type)	Activities/ actions	Remarks	References
	Mechanisms	Induces apoptosis by G_2/M cell cycle arrest in a caspase-dependent pathway and exerts potent antiangiogenic and vascular disrupting effects via stimulation of LKB1-AMPK. Induces apoptosis through Akt/p53/Bax/PTEN signalling pathway and stimulate upregulation of PARP-1, cytoplasmic accumulation of cytotoxic PAR polymer, nuclear translocation of AIF and overproduction of ROS	Muto et al. (2008, Wang et al. (2015b), Guerram et al. (2015), Hu et al. (2016), Jiang et al. (2013), and Ma et al. (2016)
	In vivo use limitations	Poor water solubility, acquired drug resistance and rapid elimination	Khaled et al. (2013), and Yang et al. (2014b)
Resveratrol (stilbene)	Natural sources	*Polygonum cuspidatum*, *Vitis vinifera*, *Arachis hypogaea*, *Vaccinium* spp., *Prunus* spp.	Burns et al. (2002), Sales and Resurreccion (2014), and Torquato et al. (2017)
	Cytotoxicity	Prevention and therapy of neuroblastomas, lymphoid, myeloma, breast, prostate, stomach, colon, pancreas, thyroid, ovarian and cervical carcinoma	Aggarwal et al. (2004), Peng et al. (2013), Gupta and Pramanik (2016), Sinha et al. (2016), Tsai et al. (2017), and Varoni et al. (2016)
	Other significant antitumour effects	Multidrug resistance reversion and synergetic effect with several approved anticancer drugs	Pavan et al. (2016), Sinha et al. (2016), Varoni et al. (2016), Alamolhodaei et al. (2017), and Redondo-Blanco et al. (2017)
	Mechanism	Acting in molecular targets associated with the modulation of transcription factors, protein kinases, cell cycle regulatory proteins and inhibition of angiogenesis and including signalling pathways involved in cancer metastasis	Pavan et al. (2016), Sinha et al. (2016), and Varoni et al. (2016)
	In vivo use limitations	Low aqueous solubility and rapid metabolization	Cottart et al. (2014)
Tetrandrine (alkaloid)	Natural sources	*Stephania tetrandra*, *Mahonia bealei*	Zhu et al. (2015), and Liu et al. (2016a)

	Cytotoxicity	Antitumour effects on oral, prostate, lung, gastric, breast, hepatic colon, renal, glioma, leukaemia, cervical and nasopharyngeal cancers cell lines at 1–30 μM concentration level	Liu et al. (2016a)
	Other significant antitumour effects	Synergistic cytotoxic effects with cisplatin on A549 and A549/DDP cell lines. Prevents MDR in U-2OS/Taxol by NF-κB inhibition. Nanoscale delivery systems using a Trojan horse strategy for the delivery of tetrandrine and modified liposomes delivery in combination therapy. Enhance a radiation sensitization effect on human glioma	Xu et al. (2014), Lu et al. (2017), Ma et al. (2017), Song et al. (2017a), and Ye et al. (2017)
	Mechanism	Promotes in oral cancer cells G_0/G_1 phase arrest, ROS intracellular accumulation, Ca^{2+} production, ER stress and finally apoptotic death through a caspase-dependent pathway and Beclin-1-induced autophagy. Also the inactivation of PI3K/Akt signalling through TGF-β upregulation to decrease the phosphorylation of PTEN. Triggers in DU145 cells the LC3-independent autophagy. Repress the nuclear translocation and expression of β-catenin	Chen et al. (2017), Lien et al. (2017), Qiu et al. (2017), and Zhang et al. (2017a)
	In vivo use limitations	Water insolubility	Liu et al. (2016a)
Triptolide (diterpene)	Natural sources	*Tripterygium hypoglaucum*, *T. regelii*, *T. forrestii*	Zhou et al. (2012)
	Cytotoxicity	Inhibits the proliferation of all 60 NCI cancer cell lines at IC_{50} values lower than 50 nM. *In vitro* and *in vivo* anti-invasion and anti-tumourigenesis activity against hepatocellular carcinoma cells. Supress tumour growth, invasion, migration and angiogenesis of head and neck squamous cell carcinoma. Inhibits human prostate cancer cells, CT26 and HCT116 colon and colorectal cancer cells and pancreatic cancer cells	Zhou et al. (2012), Gali-Muhtasib et al. (2015), Wang et al. (2016b), Yuan et al. (2016), Zhang et al. (2016a), Zhao et al. (2016b), Xi et al. (2017), and Yang et al. (2017a)
	Other significant antitumour effects	New delivery systems improve efficacy and reduce toxicity. *In vitro* and *in vivo* anti-MDR activity against: MCF-7/ADR. A549/Taxol via modulation of MAPK and PI3K/Akt signalling pathways; KB/VCR via CDK7/RPB1. Synergistic effect in the treatment of nasopharyngeal carcinoma as well as in thyroid cancer and liver cancer	Alsaied et al. (2014), Li et al. (2014, 2016), Kim et al. (2016), Xie et al. (2016), Yi et al. (2016), Xi et al. (2017), and Zhang et al. (2016b, 2017b)

(continued)

Table 4.3 (continued)

Compounds (type)	Activities/ actions	Remarks	References
	Mechanism	Induces apoptosis by extrinsic and intrinsic pathway, causing mitochondrial damage and then activating caspase-dependent pathways involving MAPK activation and NF-kB inhibition. Induces autophagy by inhibition of Akt/mTOR/p70S6 K pathway and activating the ERK1/2 pathway. In pancreatic cells activate the ER stress pathway; show ability to block NF-kB/MMP-9 activation in hepatocellular carcinoma cells. Inhibits the functional centres of Na+/K+-ATPase and Ca^{2+}/Mg^{2+}-ATPase. Inhibits the growth of orthotopically xenografted NSCLC cells by suppression of HA-CD44/RHAMM signalling pathway. Promote T and B cell populations	Meng et al. (2014), Mujumdar et al. (2010, 2014), Wang et al. (2016b), Chan et al. (2017), Xi et al. (2017), and Song et al. (2017b)
	In vivo use limitations	Short t½ values after oral administration. Poor water solubility, severe multi-organ toxicity	Li et al. (2014), Gong et al. (2015), and Xi et al. (2017)
Ursolic acid (triterpene)	Natural sources	*Oldenlandia diffusa*, *Vaccinium* spp. In many aromatic plants (*Thymus*, *Lavandula*, *Origanum* and *Eucalyptus*)	Kondo et al. (2011), Woźniak et al. (2015), Jain et al. (2016), and Torquato et al. (2017)
	Cytotoxicity	*In vitro* and *in vivo* activity against more than 60 cell lines belonging to 20 tumour types such as glioma, melanoma, colon, hepatic, lung, breast, gastric and prostate cancers without significant toxicity to normal cells	Kondo et al. (2011), Kim and Moon (2015), Woźniak et al. (2015), Jain et al. (2016), Pattnaik et al. (2017)
	Other significant antitumour effects	Growth inhibition of MDR cell line through apoptosis poly(lactic acid) nanoparticles load ursolic acid decrease cytotoxicity over normal cells, while chitosan-coated ursolic acid liposome exhibited better pharmacokinetic parameters	Jain et al. (2016), Wang et al. (2017a), and Antônio et al. (2017)
	Mechanism	Able to induce apoptosis; inhibit tumour promotion, metastasis and angiogenesis by suppressing the MAPK/ERK and PI3K/AKT/mTOR signalling cascades; decrease NF-B activity, modelling the Bcl-2, Bcl-xL BAX activity, reverse transcriptase inhibition, modelling matrix metalloproteinases activity, G_0/G_1 cell cycle arrest	Woźniak et al. (2015), Jain et al. (2016), Lewinska et al. (2017), and Pattnaik et al. (2017)
	In vivo use limitations	Low aqueous solubility, short plasma half-life and low oral bioavailability	Shanmugam et al. (2013)

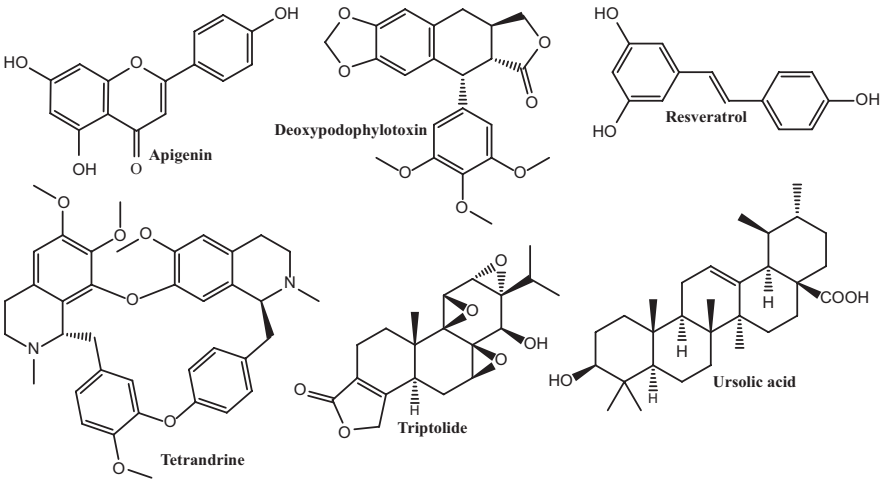

Fig. 4.1 Structures of natural compounds with high antitumour activity and in preclinical trials

relieve intermittent fever, arthritis pain and infant colic and as inducer of uterine contractions and also insect repellent (Ghantous et al. 2013). Although the concentration of parthenolide in native plants is usually quite low, the commercially available parthenolide is still nowadays obtained by extraction from its first-known natural source where it is found in the superficial leaf glands (0.2–0.5%) and comprises up to 85% of the total sesquiterpene content (Pareek et al. 2011). It is noteworthy that the *T. argenteum* (Lam.) Willd. leaves which possess higher parthenolide content (2.261 ± 0.002%) are not the main source of this compound (Orhan et al. 2015). Other new natural sources of parthenolide, including several *Tanacetum* species, were evaluated and subjected to new extraction procedures and to genetic engineering strategies (Seca et al. 2017), aiming to obtain this natural compound in higher yield and in an economically feasible manner. In the last years, there have been also an increasing number of attempts to uncover efficient methodologies towards its synthesis. Although two routes for total synthesis of parthenolide have been recently described (Long et al. 2014; Yang et al. 2015), the number of steps is huge, in some cases isomers were obtained, and the desired 6,7-*trans*-10-membered ring system is achieved with low selectivity. Besides the several steps needed, which contribute to lower yields, the methods are not green and altogether are the foundation for the economic failure of the procedures. One of the parthenolide strengths is its great ability to get involved in different mechanisms that induces apoptosis, and as more is known about its targets, more likely new applications will be exposed, and therefore its pharmacology continues to stimulate great interest in the scientific community. Therefore, in the last 3 years, a significant number of studies on parthenolide cytotoxic mechanism of action were published and showing that parthenolide can induce *in vitro* and *in vivo* inhibition of cell proliferation by distinct mechanisms and in various human cancer cells (Seca et al. 2017).

Table 4.4 Natural compound derivatives in clinical therapeutic and clinical development

Name	Lead compound (source)/ development phase	Major area of application	Other applications	References
Cabazitaxel	Paclitaxel (*Taxus brevifolia*)/in the market (Jevtana®) since 2010	Treatment of metastatic castration-resistant prostate cancer	In testicular germ cell tumours	Yao et al. (2017), and Oing et al. (2016)
Vinflunine	Vinblastine (*Catharanthus roseus*)/in the market (Javlor®) since 2009	Treatment of metastatic transitional-cell carcinoma of the urothelial tract	In non-small cell lung cancer	Genova et al. (2016)
Vintafolide	Vinblastine (*Catharanthus roseus*)/in clinical trial	Promise in the treatment of platinum-resistant ovarian and NSLC cancers (NCT01577654)	Phase II trial to evaluate its activity in higher FR expression solid tumours (NCT01002924)	Vergote and Leamon (2015)
Topotecan	Camptothecin (*Camptotheca acuminata*)/in the market (Hycamtin®) since 2009	To treat metastatic ovarian cancer and relapsed small cell lung cancer (EMA/248288/2015 EMEA/H/C/000123)	In glioma undergoing a clinically indicated surgical resection (NCT02500459)	Hanna et al. (2015)
Phenoxodiol	Daidzein (*Glycine max*)/in clinical trial development	Chemosensitizing agent for carboplatin in resistant ovarian cancer (NCT00382 811)	Effect on castrate and non-castrate prostate cancer cells (NCT005570 37)	Mahoney et al. (2014), and Xiao et al. (2016)
Alvocidib	Rohitukine (*Dysoxylum binectariferum*/ clinical trial development	To treat advanced, fludarabine refractory chronic lymphocytic leukaemia (NCT00464 633)	Biomarker-driven phase II acute myeloid leukaemia (NCT02520011)	Lanasa et al. (2015)

Parthenolide is a nuclear factor kappaB (NF-kB) cells inhibitor with great potential, since this family of proteins and their regulated genes have been correlated with cell proliferation, inflammation, angiogenesis, invasion and metastasis of the cancer cells (Vazquez-Santillan et al. 2015; Li et al. 2015). Parthenolide can prevent the DNA binding of p65/NF-kB subunits causing the alkylation of cysteine sulfhydryl groups in the p65 subunit, effect mainly observed at lower concentrations, while at higher concentrations, it preferentially inhibits the IkB kinase complex (Liu and Ou 2013; Gali-Muhtasib et al. 2015). Furthermore it can also act inhibiting tubulin carboxypeptidase by an independent NF-kB pathway (Fonrose et al. 2007), inhibiting the mitogen-activated protein kinase and causing impact on epigenetic mechanisms (Ghantous et al. 2013; Gali-Muhtasib et al. 2015). Besides it can induce the apoptosis of cancer stem cells in acute myelogenous leukaemia (AML) and blast

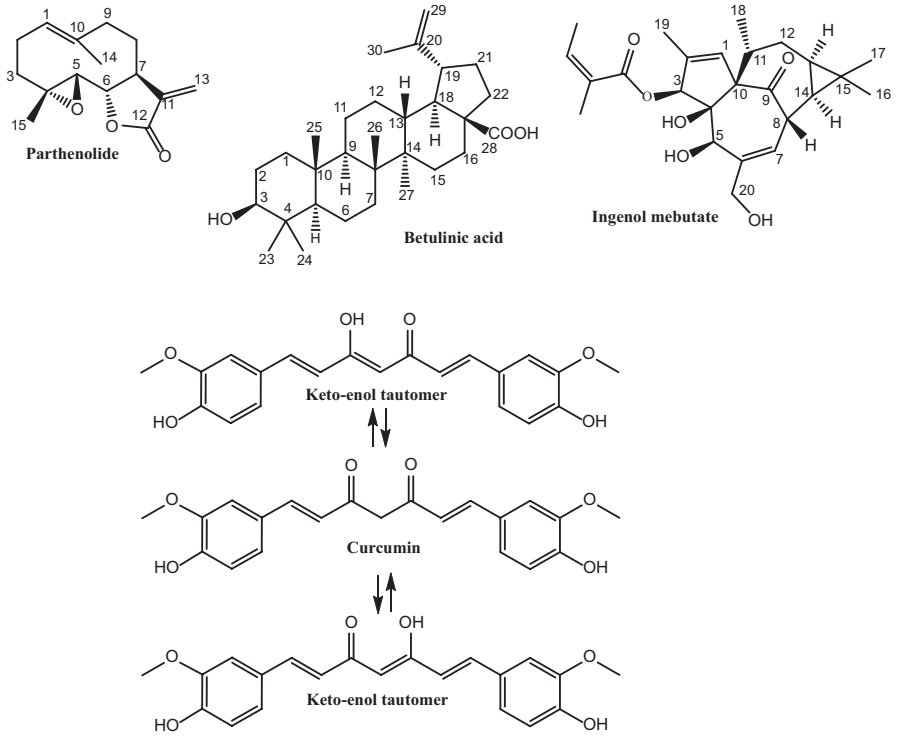

Fig. 4.2 Structures of natural compounds in clinical trials

crisis chronic myelogenous leukaemia not only through the inhibition of NF-kB but also through mechanisms involving proapoptotic activation of p53 and elevating ROS levels (Liu and Wang 2015). New evidence indicates that the antitumour action of parthenolide is also related to its ability to induce a caspase-independent form of death, stimulating oxidative stress and autophagic process (D'Anneo et al. 2013; Jeyamohan et al. 2016; Carlisi et al. 2016).

Parthenolide applied at concentrations lower than 25 μM exhibits a strong cytotoxic effect against several different types of tumour cell, such as non-small lung cancer cells, oral cancer cells, glioma radioresistant cells, cervical cancer cells, breast cancer cells, bone osteosarcoma cells and multiple myeloma cells (Seca et al. 2017). However, the most amazing cytotoxic effect of the parthenolide is to possess selective anticancer stem cell activity in multiple myeloma cells (MMSC) by inducing apoptosis (IC_{50} 1.5–3.5 μM) (Gunn et al. 2011). Cancer stem cells (CSCs), a subset of the heterogeneous cell population of a tumour, are self-renewable, can differentiate into all types of cancer cells and are rather resistant to active drugs which difficult its elimination (Khan et al. 2015; Moselhy et al. 2015). Currently, it is accepted that compounds that eliminate CSCs are expected to confer more durable and potentially curative therapies, and targeting CSCs seems to be a promising strategy for cancer therapy (Gupta and Pramanik 2016; Torquato et al. 2017).

Parthenolide was the first small molecule that selectively target leukaemia stem cells (LSCs) and AML cells, in addition to the aforementioned MMSC, while sparing normal haematopoietic cells (Pareek et al. 2011; Gunn et al. 2011; He et al. 2014). Based on the structure (Fig. 4.2) and anticancer activity results, some structure/activity relationship (SAR) can be drawn: (1) C-6 and C-7 configurations have more impact on the activities against some cancer cell lines than the C-1=C-10 double bond configuration (Long et al. 2014); (2) the replacement of the epoxide moiety by the potentially bioisostere cyclopropyl moiety might provide a more stable parthenolide derivative without changing the cytotoxicity level (Long et al. 2013); (3) replacement of the lactone moiety with lactam moiety greatly decreased the anticancer activity (Long et al. 2016); and (4) the C-14 methyl group of parthenolide might be important for its high anticancer activity (Long et al. 2016).

Parthenolide seems to reduce the frequency of ABCB5-positive cells (ABCB5 transporter is one of the recognized mediators of chemoresistance in melanoma and a marker for melanoma stemlike cells), and the melanoma cells that survive its treatment lose their self-renewing capacity (Czyz et al. 2013). Moreover, the cytotoxicity of parthenolide against stemlike cells derived from triple-negative breast cancers (TNBCs), an aggressive form of breast carcinoma associated with a high rate of recidivism, strongly suggests that it can be used as new therapeutic strategy against TNBCs (Carlisi et al. 2016). Regarding the selectivity demonstrated by parthenolide, it is expected that when used in combination with other drugs, it can significantly contribute to the complete tumour eradication.

The use of a single drug in the cancer treatment has proven to be limited because there are numerous types of cancer cells and resistance to chemotherapy is customary. Therefore, studies to assess synergistic effects involving parthenolide have given a new impulse to its use. This subject was reviewed recently (Wyrebska et al. 2014; Seca et al. 2017) and shows that combined effects of doxorubicin and parthenolide seem to be effective against melanoma cells highly resistant to chemotherapy (Wozniak et al. 2013) and enhance the apoptotic cytotoxicity of DOX in A549 DOX-resistant cells (Xin et al. 2013). The parthenolide cytotoxicity against AML Kasumi-1 cells is enhanced by the antifungal agent with antitumour properties ciclopirox (10–20 μM) (Sen et al. 2013), and the synergistic effect of parthenolide/dacarbazine and parthenolide/5-FU was established for metastatic melanoma or TRAIL-resistant human colorectal cancer cell lines (Koprowska et al. 2013; Kim et al. 2013). Recently it was reported that parthenolide potentiates the efficacy of balsalazide (a colon-specific prodrug used to treat ulcerative colitis) through synergistic inhibition of NF-kB activation and the combination of these dual agents prevents colitis-associated colon cancers (Kim et al. 2017).

Despite the positive effects of parthenolide as cytotoxic agent, in particular its great efficiency to selectively target CSCs and its safety profile established in phase I clinical trials, it exhibits a low bioavailability derived from low solubility in plasma blood, and because of this, it did not pass to the next phase. Moreover, the parthenolide stability is pH dependent, being unstable under both high acid and basic conditions (Adio 2009), and its half-life in mouse plasma is only 0.34 h (Zhang et al. 2012). To address these limitations to the parthenolide application as a clinical drug,

very interesting studies of new delivery systems have emerged. Recent advances have shown that nanoparticles used for drug therapy provide some advantages over conventional formulations, including increased solubility, enhanced storage stability, improved permeability and bioavailability, prolonged half-life, tissue targeting and minimal side effects, both *in vitro* and *in vivo* assays (Aljuffali et al. 2016) and some of them are approved by the Food and Drug Administration (Watkins et al. 2015). Also vitamin E-based nanomedicines already provide sufficient preclinical data, for example, to parthenolide delivery, to be applied as anticancer drug delivery system (Duhem et al. 2014). The synthetic $PSMA_{100}$-b-PS_{258} micelles encapsulating parthenolide have demonstrated excellent properties (increase the aqueous solubility, significant stability improvement and attractive physical dimensions and surface characteristics) giving them high likelihood for long circulation times *in vivo* (Baranello et al. 2015). Recently, EGF-conjugated mixed micelles of PEG2000-DSPE and vitamin E-TPGS were developed for targeted combination therapy with paclitaxel and parthenolide against paclitaxel-resistant and paclitaxel-sensitive non-small cell lung (Gill et al. 2016).

As a conclusion, parthenolide itself remains a very promising multiple target drug in terms of combating a wide range of human cancer types, showing a remarkable ability to beneficially interfere with almost all pathways that lead to activation of apoptosis and inhibition of cellular inflammation. Its low bioavailability entails limitations to its clinical use, but this seems to be overcome with innovative forms of parthenolide release. So if parthenolide presents higher bioavailability, its clinical application will be a reality and no doubt that it will be a great success and a stunning medical breakthrough for different cancer diseases.

4.3.2.2 Betulinic Acid

Betulinic acid (3*β*-hydroxy-lup-20(29)-en-28-oic acid), a lupane-type pentacyclic triterpene with a molecular formula of $C_{30}H_{48}O_3$ and biosynthesized from six different isoprene units (Fig. 4.2), was first identified and isolated from *Gratiola officinalis* L. and named "graciolon", then from the bark of *Platanus acerifolia* (Aiton) Willd. (named "platanolic acid") and after from *Cornus florida* L. and called "cornolic acid". Later on, after the confirmation that all have the same structure, was named betulinic acid (Sarek et al. 2011). This triterpene is extensively spread throughout the plant kingdom, and considerable amounts (up to 2.5%) are available in the outer bark of a wide range of plants, for instance, *Betula* spp., *Diospyros* spp., *Syzygium* spp., *Ziziphus* spp., *Paeonia* spp., *Sarracenia flava* L., *Anemone raddeana* Regel and *Lycopodium cernuum* L., among others (Ali-Seyed et al. 2016). However, these sources are not sufficient to meet the growing demand for betulinic acid. So it can be obtained through a selective oxidation of betulin (lup-20(29)-en-3,28-diol) (Pichette et al. 2004; Csuk et al. 2006), far more abundant (up to 30%) in birch bark than the acid (Holonec et al. 2012), a more profitable process. Pisha et al. (1995) were the first who reported the antitumour activity of betulinic acid and found that it killed melanoma cells in mice with low IC_{50} values (IC_{50} 0.5–1.5 μg/ml). Later on,

Singh et al. (2016) have also tested the anticancer activities of betulinic acid with respect to melanoma cells (Singh et al. 2016). However, recent evidence indicates that betulinic acid possesses a broader spectrum of activity against other cancer cell types, and because of that, betulinic acid has been selected by the National Cancer Institute to be added into the Rapid Access to Intervention in Development (RAID) program. Betulinic acid exhibits significant *in vitro* cytotoxicity in a variety of tumour cell lines and also inhibits the growth of solid tumours *in vivo*, comparable to some clinically used drugs and showing a good selectivity index for cancer over normal cells even at doses up to 500 mg/kg body weight (Lee et al. 2015; Gali-Muhtasib et al. 2015; Zhang et al. 2015; Luo et al. 2016). It shows anticancer proprieties against colorectal lung, colon, breast, prostate, hepatocellular, bladder, head and neck, stomach, pancreatic, ovarian and cervical carcinoma, glioblastoma, chronic myeloid leukaemia cells and human melanoma with IC_{50} values mainly between 1.0 and 13.0 μg/ml (Sarek et al. 2011; Gheorgheosu et al. 2014; Lee et al. 2015; Zhang et al. 2015; Ali-Seyed et al. 2016; Singh et al. 2016; Ali et al. 2017b).

Betulinic acid exhibits potent anticancer activity by multiple molecular targets (Luo et al. 2016), the induction of apoptosis by direct regulation of the mitochondrial apoptotic pathway being the best characterized mechanism. This pathway can be associated with mitochondrial collapse through direct opening of the permeability transition pore, decreases of mitochondrial outer membrane potential, downregulation of Bcl-2 family members, release of proapoptotic factors such as cytochrome c, increase of caspase activities, attenuation of both the constitutive and inducible STAT3 phosphorylation and nuclear translocation and its DNA binding (Zhang et al. 2015; Luo et al. 2016; Ali-Seyed et al. 2016). Betulinic acid also may induce apoptosis by stabilizing p53 and downregulating NF-kB-mediated signalling (Ali-Seyed et al. 2016; Shankar et al. 2017), although this does not appear to be a target common to all cells (Zhang et al. 2015). The anti-metastatic effect of betulinic acid seems to be through the prevention of the epithelial-to-mesenchymal transition in highly aggressive melanoma cells (Gheorgheosu et al. 2014), while in breast cancer cells acts by downregulating the matrix metalloproteinases expression (Luo et al. 2016). It also induces an antiangiogenic response (Ali-Seyed et al. 2016) under hypoxia mediated by STAT3/HIF-1α/VEGF signalling pathway (Zhang et al. 2015), can block the cell cycle in the G1 phase through inhibition of Cyclin B1 and Hiwi in mRNA and potently induces autophagy as a survival mechanism in response to permeability transition pore opening and mitochondrial damage (Gali-Muhtasib et al. 2015; Luo et al. 2016). Recently, it was described as a new cell death pathway that depends on cardiolipin modification, whereby betulinic acid selectively kills tumour cells (Potze et al. 2016a, b). It was demonstrated that betulinic acid is able to affect the cancer stem cells directly, inducing cell death via loss of clonogenic capacity by inhibition of the stearoyl-CoA-desaturase (SCD-1), an enzyme that is overexpressed in tumour cells and rapidly impacts on the saturation level of cardiolipin, a mitochondrial lipid that, among other functions, regulates mitochondria-dependent cell death (Potze et al. 2016a, b). Proteasome inhibition assays suggest the proteasome is the main target for betulinic acid (Waechter et al. 2017). However

the regulatory effects of betulinic acid on NF-κB pathway and on Bax or Bak expression are not well clarified (Zhang et al. 2015).

Betulinic acid seems to be a very effective chemosensitizer for anticancer drug treatment in chemoresistant cell lines once it promotes the inhibition of multidrug resistance proteins *in vivo* and *in vitro* (Luo et al. 2016). For example, the combination of betulinic acid with 5-FU reverts apoptosis induction in the 5-FU-resistant cells (Jung et al. 2007; Luo et al. 2016). These results clearly demonstrate that in some cases, it is possible to circumvent acquired chemoresistance by combination therapy of anticancer drugs with chemosensitizers as betulinic acid. Moreover, betulinic acid has strong synergy with mithramycin A by inhibition migration and invasion on pancreatic cancer cells at nontoxic concentrations by suppressing Sp1 and uPAR level (Gao et al. 2011). Also a synergistic effect of betulinic acid with tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) to inhibit liver cancer progression *in vitro* and *in vivo* through targeting p53 signalling pathway was reported by Xu et al. (2017). Betulinic acid is slightly soluble in water, and therefore its water solubility is a drawback that should be overcome to improve its absorption and bioavailability. The main targets for SAR studies were the 3-hydroxy, C-20 alkene and 28-carboxylic acid moieties (Fig. 4.2). The 3-OH oxidation increased cytotoxic activity but decreased selectivity, introduction of groups, such as amine or hydroxy, at the C-28 increased activity, while modifications at C-20 did not enhance cytotoxicity (Yogeeswari and Sriram 2005; Gali-Muhtasib et al. 2015; Ali-Seyed et al. 2016). It can be concluded that modifications may improve the cytotoxicity and/or the water solubility but not the selectivity. It seems that the presence of the free 3-hydroxy group and the 28-carboxyl group is the most important feature. Recently, a clearer and more realistic method, 3D-QSAR by CoMFA and CoMSIA, shows the structure-cytotoxicity relationship of betulinic acid derivatives against human ovarian cancer cell A2780, and the main conclusions were an electropositive group at C-2 α-site; an electronegative and hydrogen bond acceptor group at C-2 β-site; bulky groups at the C-3 β-site; bulky and electronegative groups at C-3 α-site; and bulky, electronegative and hydrogen bond donor or acceptor groups at C-28 side chain would be beneficial for its antitumour potency (Zhang et al. 2015). Due to its extraordinary potential as antitumour agent, betulinic acid was involved in phase I/II clinical trials to evaluate its safety and effectiveness. The study involved the topical application of 20% betulinic acid ointment to treat dysplastic nevi that can be transformed in melanoma.

4.3.2.3 Ingenol Mebutate

The phytochemical study of *Euphorbia peplus* latex sap yielded several macrocyclic diterpenes (Rizk et al. 1985), including ingenol mebutate (Fig. 4.2). It was identified as the most active antitumour component (Ogbourne et al. 2004). In fact, the *E. peplus* sap, as referred in Table 4.2, has been shown to be effective against human nonmelanoma skin cancer in a phase I/II clinical study (Ramsay et al. 2011). This ingenene-type diterpene can also be isolated from other *Euphorbia* species

such as *E. paralias*, *E. antiquorum*, *E. palustris*, *E. helioscopia* and *E. quadrialata* (Gotta et al. 1984). Ingenol mebutate has been prepared by semisynthesis in three steps, involving the selective esterification of the ingenol 3-OH (Liang et al. 2012), which is isolated from the seeds of *E. lathyris* (yield ~100 mg/kg). A recent report for fairly short and efficient route of the ingenol synthesis was published (Jørgensen et al. 2013), but the total synthesis of ingenol is still unaccomplished. Ingenol mebutate, also known as ingenol angelate and PEP005, is a 3-monoester of the ingenol diterpene with the (*Z*)-2-methylbut-2-enoic acid having the chemical formula $C_{25}H_{34}O_6$ (Fig. 4.2). Ingenol mebutate chemical stability is pH dependent and can undergo facile acyl migration involving the hydroxy groups, mainly the 5- and 20-OH (Fig. 4.2), which are also required for the biological activity. In this regard, the ester moiety at the three-position is also important (Liang et al. 2013).

Ingenol mebutate showed potent antiproliferative effects on different cell lines in a dose- and time-dependent manner (Serova et al. 2008; Benhadji et al. 2008), especially against colon 205 cell lines with IC_{50} 0.01 μM that means more active than staurosporine (IC_{50} 0.029 μM) or doxorubicin (IC_{50} 1.5 μM) (Serova et al. 2008). This compound is also very effective against a wide range of established subcutaneous tumours in mice after three daily topical applications of 42 nmol (Ogbourne et al. 2004). Its effectiveness to damages in the tumour vasculature is related with the fact that ingenol mebutate is transported through the epidermis into the deep dermis via a P-glycoprotein (Collier et al. 2014).

Treatment with this compound, both *in vitro* (230 μM) and *in vivo* (42 nmol), rapidly caused swelling of mitochondria probably by loss of their membrane potential and cell death by primary necrosis and is, therefore, unlikely to have its activity compromised by the development of apoptosis resistance in tumour cells (Ogbourne et al. 2004). There are evidences that this rapid action of ingenol mebutate is due to its dual action combining cytotoxic and immunomodulatory effects in which rapid lesion necrosis and antibody-dependent cellular cytotoxicity mediated by neutrophils occur (Rosen et al. 2012). The mechanism of action of ingenol mebutate is also partially related to the modulation of protein kinase C (PKC) to which it has a potent binding affinity by activating PKCδ and inhibiting PKCα (Benhadji et al. 2008; Matias et al. 2017). *In vitro* low isozyme selectivity was verified with a Ki ranging from 0.105 to 0.376 nM (Kedei 2004). The immunomodulatory effects seem to be at least partially mediated by PKC (Serova et al. 2008; Doan et al. 2012; Liang et al. 2013). All these results support the potential of ingenol mebutate for further development of cancer immunotherapy. In fact, the cutaneous treatment of non-hyperkeratotic, non-hypertrophic actinic keratosis (a premalignant precursor of squamous cell carcinoma) with a gel formulation of ingenol mebutate (formerly PEP005 produced by LEO Pharma and marketed as Picato®) was recently approved by both FDA and EMA agencies (Tzogani et al. 2014). Unfortunately, the current gel based on isopropanol gives rise to some local skin responses, being the majority resolved within 2 weeks after treatment with no scarring observed (Collier et al. 2014). The most frequently reported adverse reactions associated with this application are lower to moderate "local skin responses" and included erythema, flaking/scaling, swelling, crusting, erosion/ulceration and vesiculation/postulation (Tzogani

et al. 2014; Berman 2015). Ingenol mebutate shows a favourable safety and tolerability profile exhibiting lack of systemic absorption and photosensitivity, and there is no evidence of drug interactions (Collier et al. 2014).

4.3.2.4 Curcumin

Curcumin or diferuloylmethane is a polyphenol compound that has been extracted from the rhizome of turmeric (*Curcuma longa* L.; Fig. 4.2), a tropical Southeast Asian plant mainly known due to its use as spice. Curcumin constitutes 2–5% of turmeric powder (Chainani-Wu 2003) and has been used for a long time in countries such as China and India as traditional medicines (Hatcher et al. 2008; Kocaadam and Sanlier 2017). This ancient remedy has brought the attention of scientific community for a wide range of curcumin beneficial properties including anti-inflammatory, antioxidant, chemopreventive, chemotherapeutic and chemosensitizing activity (Hatcher et al. 2008; Kocaadam and Sanlier 2017). Research interest on curcumin anticancer properties has been developed based on low rate occurrence of gastrointestinal mucosal cancers in Southeast Asian populations and its association with the regular turmeric use in their dietary (Mohandas and Desai 1999; Sinha et al. 2003). Curcumin (a bis-α,β-unsaturated β-diketone) has a molecular formula of $C_{21}H_{20}O_6$ and is an orange-yellow crystalline lipophilic phenolic substance that, in solution, exists in equilibrium with its keto-enol tautomeric forms (Fig. 4.2). It is not very soluble in water and also not very stable, although its degradation increases in basic medium (Wang et al. 1997).

A large volume of experimental data establishing the therapeutic efficacy of curcumin in *in vitro* cellular level as well as in some ex vivo tumour-derived cancer cells/solid tumours like brain tumours; pancreatic, colorectal, lung, breast, prostate and skin cancers; leukaemia; hepatocellular carcinoma; and head and neck carcinoma and including cytotoxic effects on cancer stem cells and anti-metastatic activity were reviewed recently (Chinembiri et al. 2014; Perrone et al. 2015; Osterman and Wall 2015; Mock et al. 2015; Gupta and Pramanik 2016; Pavan et al. 2016; Chen et al. 2016; Jordan et al. 2016; Niedzwiecki et al. 2016; Deng et al. 2016; Klinger and Mittal 2016; Imran et al. 2016; Redondo-Blanco et al. 2017; Borges et al. 2017). Equally important, free curcumin was shown not to be cytotoxic to normal cells, including hepatocytes, mammary epithelial cells, kidney epithelial cells, lymphocytes, neural myelin sheaths and fibroblasts, at the dosages required for therapeutic efficacy against cancer cell lines (Bisht et al. 2010; Buyuklu et al. 2014; Soliman et al. 2014; Sordillo and Helson 2015; Yu et al. 2016). The scientific interest and pharmacological potential of curcumin anticancer effects become also evident from the number of patents on curcumin-based therapeutics registered in the last 5 years. Several studies have shown that curcumin can modulate a variety of cancer-related targets or pathways (Hatcher et al. 2008; Strimpakos and Sharma 2008; Deogade and Ghate 2015; Osterman and Wall 2015; Shanmugam et al. 2015; Kumar et al. 2016; Chen et al. 2016; Kocaadam and Sanlier 2017; Imran et al. 2016; Pavan et al. 2016; Allegra et al. 2017), which may be responsible for its effective-

ness in combating cancer diseases. The mechanism of action includes modulation of CYP enzymes by elevation of transcription factor Nrf2 level via mitogen-activated protein kinase (MAPK) signalling pathway and Akt pathway (Schwertheim et al. 2017); mitotic catastrophe induction due to caspase activation and mitochondrial membrane polarization (Gali-Muhtasib et al. 2015); and promotion of autophagic cell death, an important death inducer in apoptosis-resistant cancer cells by beclin-1-dependent and beclin-1-independent pathways (Gali-Muhtasib et al. 2015; Yang et al. 2017b). Additionally, curcumin-mediated arrest of the cell cycle at the checkpoints G1, S-phase and G2/M phase, modulating the cell cycle regulators including upregulation of cyclin-dependent kinase inhibitors (CDKIs) (Dasiram et al. 2017), promotes the inhibition of transcription factor NF-κB by preventing nuclear translocation of NF-κB and attenuates DNA-binding ability of NF-κB contouring the problem of chemoresistance (Uwagawa and Yanaga 2015) and promotes the inhibition the crucial steps to angiogenesis by downregulation of the PGDF, VEGF and FGF expression and downregulation of MMPs via NF-κB, ERKs, MAPKs, PKC and PI3K inhibition (Fu et al. 2015). Moreover, several *in vitro* studies disclose the ability of curcumin to bind and inhibit tubulin polymerization, to bind DNA, to mediate modulation in expression of sirtuins like SIRT1, to promote the downregulation of the pro-oncogenic and upregulation of anti-oncogenic miRNAs expression, to mediate suppression of HMGB1 followed by protection from inflammation and oxidative stress and to act as a potent immune modulator (Ramasamy et al. 2015; Kumar et al. 2016; Haris et al. 2017; Sri Ramya et al. 2017). Despite the existing knowledge about the multiple action mechanisms of curcumin, it is far from a full understanding of their biological properties. For example, alongside the antiproliferative ability, curcumin may have a proliferative effect on some specific cells. Probably, curcumin survival and proliferative effects depend on its concentration, treatment period and the type of cells.

On the other hand, although curcumin is a natural product used in the diet (granted an acceptable daily intake level of 0.1–3 mg/kg-BW by the Joint FAO/WHO Expert Committee on Food Additives), the doses administered in clinical trials exceed those normally consumed in the diet. An average intake of curcumin of 60–100 mg per day (typical value to Indian diet) and even the systematic *in vivo* (at doses of curcumin up to 300 mg–3.5 g/kg-BW administered for up to 14–90 days) or clinical studies (1.2–12 g daily of oral intake for 6 weeks to 4 months) did not demonstrate any adverse effects at the population, animal and patient level (Strimpakos and Sharma 2008; Gupta et al. 2013). Moreover, curcumin has been reported to act as chemosensitizer to some clinical anticancer drugs (e.g., gemcitabine, paclitaxel and 5-fluorouracil, doxorubicin) and exhibits synergistic effect in combination with other natural products (e.g., resveratrol, honokiol, epigallocatechin-3-gallate, licochalcone and omega-3), aspects that could be used as an effective strategy to overcome tumour resistance and reduce recurrence (Deng et al. 2016; Klippstein et al. 2016; Pimentel-Gutiérrez et al. 2016; Allegra et al. 2017). These observations therefore suggest that a superior therapeutic index may be achieved with curcumin when used in combination and could be advantageous in the treatment of some tumours. Anyway, extensive studies are still needed to assess

the exact mechanism of curcumin synergic effect. Nevertheless, the clinical translation of curcumin has been significantly hampered once it is poorly absorbed, improperly metabolized and shows poor systemic bioavailability. Consequently patients would have to consume up to 8–10 g of the free curcumin orally each day, in order for detectable levels in the circulation (Gupta et al. 2013; Kumar et al. 2016). Thus several strategies have been proposed to counter the bioavailability issue of curcumin involving (1) the use of adjuvants like piperine, which interfere with curcumin metabolism by glucuronidation; (2) curcumin formulations based on the nanotechnology with liposomes, micelles and phospholipid, among others; and (3) the use of curcumin analogues (Yallapu et al. 2015; Rahimi et al. 2016; Chen et al. 2016; Liu et al. 2016b; Puneeth et al. 2016; Sri Ramya et al. 2017). As a result of the anticancer potential of curcumin and despite the limitations to its application in clinical therapeutic, there are currently 17 open clinical studies involving curcumin, mainly studies of combined curcumin therapy with other substances for the treatment of several types of cancer.

4.3.3 Natural Compounds in Clinical Use

In addition to the compounds extracted from plants with high anticancer potential described above, several other compounds have successfully run the entire long, selective, expensive and bureaucratic process from their chemical identification to their effectiveness in the cancer treatment, being approved and available in the market. Each of these compounds has its history of success and limitations, which has explained in respective to their historical, chemical, pharmaceutical and clinical trials. The paclitaxel (trademark Taxol™; Fig. 4.3), the diterpene alkaloid, is the focus of several past studies during the last decades because of its complex structure and unique therapeutic mechanism (promoting tubulin assembly and stabilizing microtubules). It is isolated from the bark of *Taxus brevifolia* Nutt., which has become one of the most active cancer chemotherapeutic drugs that is marketed since 1992. It was first used to treat advanced ovarian cancer and then metastatic breast cancer, and from there paclitaxel has been found to be active in a wide variety of cancers in humans and also with veterinary use (Khanna et al. 2015; Bernabeu et al. 2017). Besides its anticancer activity, paclitaxel can exert a variety of positive influences on the immune system (Pawar et al. 2014) and play a potential role against neurodegenerative diseases (King et al. 2013) as well as inhibiting botulinum neurotoxin (Dadgar et al. 2013).

Despite its clinical success, research on this powerful natural anticancer agent does not slow down, looking for new solutions to some of the weaknesses of this drug like found new sources to obtained paclitaxel since it is not abundant in nature and its synthesis is very difficult (Liu et al. 2016c; Gallego et al. 2017; Wang et al. 2017b). Nowadays, the supply of paclitaxel for the production of drugs is met by semisynthesis from the precursor baccatin III (Fig. 4.3), which is present in much higher quantities and readily available from the needles of *T. brevifolia* and can be

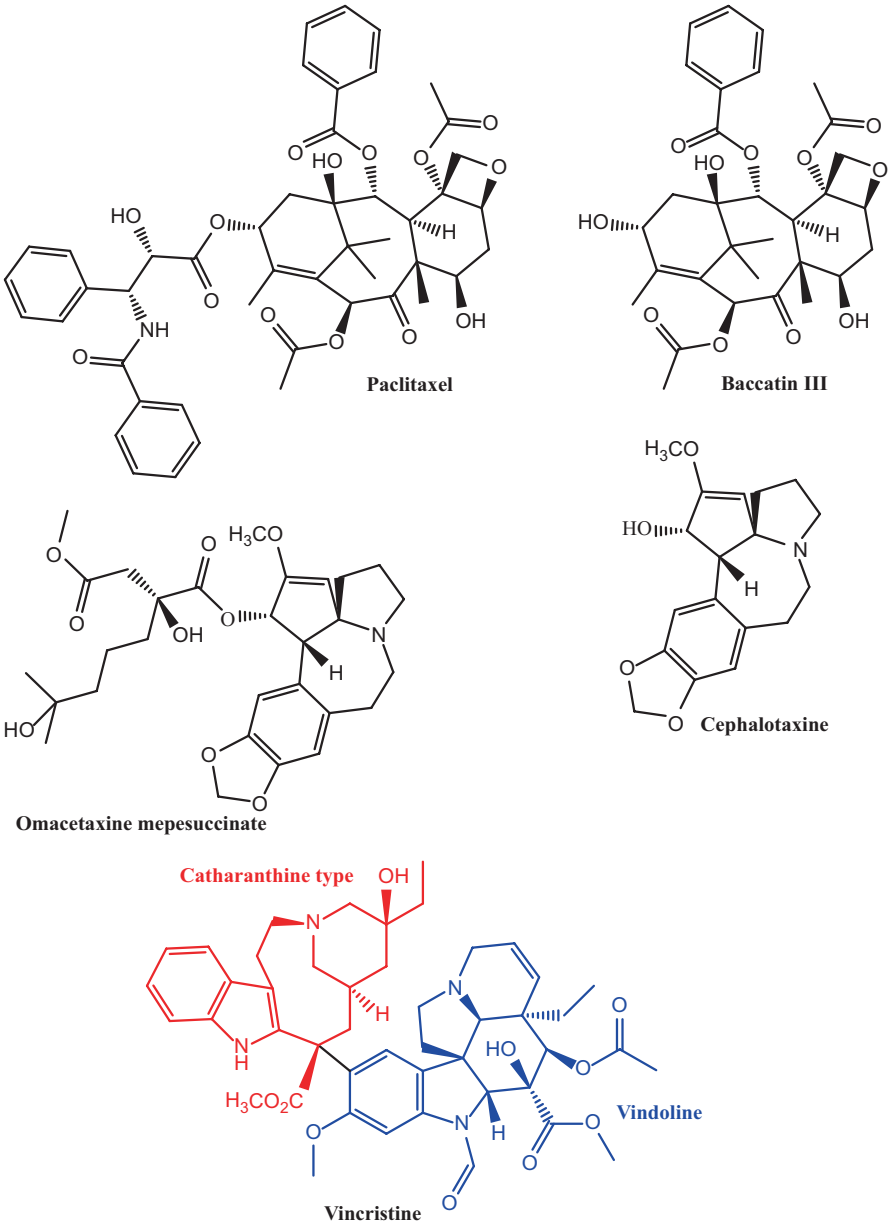

Fig. 4.3 Structure of natural compounds in clinical use and their precursors

efficiently transformed into paclitaxel. Fermentation technology with microbes or plant cell culture followed by extraction, chromatographic purification and crystallization gives paclitaxel (Zhang et al. 2016c; Gallego et al. 2017; Wang et al. 2017b). Recent research on paclitaxel also promotes the development of new delivery systems, new formulations and new derivatives to overcome its poor aqueous solubility, clinical neurotoxicity, neutropenia and multidrug resistance (Khanna et al. 2015; Barbuti and Chen 2015; Chen et al. 2015; Wen et al. 2016; Meng et al. 2016; Kim 2017; Brufsky 2017; Soliman 2017; Zong et al. 2017; Bernabeu et al. 2017).

In 2012, Abraxane®, an injectable nanoparticle of paclitaxel attached to a human protein albumin, was approved by EMA (EMA/99258/2015, EMEA/H/C/000778) as being more effective than conventional paclitaxel-containing medicines. Abraxane® is recommended to treat metastatic breast cancer. All the same, paclitaxel is already a blockbuster of pharmacy industry, and the most recent developments suggest that it will continue to be a success story for many more years. Other example of successful anticancer compound isolated from plants is omacetaxine mepesuccinate (Fig. 4.3), firstly known as homoharringtonine. Omacetaxine mepesuccinate story is one of the ups and downs; in fact it is the compound that has the record for the longest time spent before its approval by the US FDA (40 years). Before the acceptance of the name omacetaxine mepesuccinate, a cephalotaxine alkaloid had already been isolated from the evergreen tree bark *C. harringtonii* Koch and identified as homoharringtonine. This compound aroused great interest in the scientific community because of its potent antiproliferative activity against murine P-388 leukaemia cells with IC_{50} values of 17 nM (Chang et al. 2017). Scientific Chinese community was the first to demonstrate its role as an antileukaemic substance in animal models and also its effectiveness in the treatment of patients with acute myeloid leukaemia, chronic myeloid leukaemia, myelodysplastic syndrome, acute promyelocytic leukaemia and intrathecal therapy for central nervous system leukaemia, but not acute lymphoblastic leukaemia and solid tumours (Lü and Wang 2014; Isah 2016).

The main mechanism of action of homoharringtonine involves inhibition of protein translation and consequently inhibition of elongation step of protein synthesis. Homoharringtonine interacts with the ribosomal A-site, especially of the short-lived oncoproteins (BCR-ABL1) and of the antiapoptotic proteins that are upregulated in leukemic cells, leading to apoptosis (Lü and Wang 2014; Rosshandler et al. 2016). Unfortunately, its development into a useful anticancer drug was hindered by its difficult production. Actually the required *C. harringtonii* trees to isolate this alkaloid in the necessary quantity are unaccomplished. Additionally, the discovery and popularization of the tyrosine kinase inhibitor (TKI), like imatinib (synthetic compound sold under the brand names Gleevec® and Glivec®), to treat chronic myeloid leukaemia have drastically reduced the interest in homoharringtonine. The first semisynthetic homoharringtonine, currently known as omacetaxine mepesuccinate, Ceflatonin, CGX-653 or Myelostat, was obtained by direct esterification of cephalotaxine (Fig. 4.3), an alkaloid extracted from dry leaves of *Cephalotaxus* sp. (instead bark where it exists in lower percentage). This semisynthetic methodology is a supply guaranty and can be used in industrial production (Lü and Wang 2014).

Reports about patients in which the TKI therapy failed, due to resistance or intolerance, prompted the omacetaxine mepesuccinate reappearance. Positive results with these patients, as well as with those carrying the T315I mutation (Chung 2014), associated with the omacetaxine mepesuccinate ability to downregulation of Mcl-1 and effectively kill stem/progenitor cells (Nazha et al. 2013; Damlaj et al. 2016; Rosshandler et al. 2016), increased significantly the interest in omacetaxine mepesuccinate and its approval as commercial drug to treat chronic myeloid leukaemia. After complete clinical development, it was demonstrated that omacetaxine mepesuccinate produced durable haematologic and cytogenetic responses in patients with chronic myeloid leukaemia in chronic and accelerated phases, regardless of mutational status (Lü and Wang 2014; Cortes et al. 2015; Rosshandler et al. 2016; Damlaj et al. 2016). Omacetaxine mepesuccinate was approved in 2012 by FDA to US market under the trademark Synribo® to be used in the treatment of advanced chronic myeloid leukaemia resistant to two or more tyrosine kinase inhibitors (FDA database; Rosshandler et al. 2016). During clinical development, it was also confirmed that omacetaxine mepesuccinate exhibits bioavailability after subcutaneous administration similar to those observed following intravenous administration (Heiblig et al. 2014), and in February 2014, after demonstrating that reconstituted omacetaxine mepesuccinate can remain stable and sterile if refrigerated for up to 6 days, the FDA granted full approval of omacetaxine mepesuccinate for subcutaneous injection. This was indeed an improvement because patients have the opportunity to self-administer their therapy (Shen et al. 2014; Rosshandler et al. 2016; Damlaj et al. 2016).

Myelosuppression (mainly thrombocytopenia, neutropenia and anaemia) is the primary haematologic toxicity observed with omacetaxine mepesuccinate treatment. However, its damage can be limited with frequent parameter control, adequate dose modifications and patient training for signs and symptoms and of appropriate medical support (Chung 2014; Akard et al. 2016). Thus, the myelosuppression events should not prevent omacetaxine mepesuccinate administration. Recent studies showed new improvement for omacetaxine mepesuccinate use (Lam et al. 2016). It is a good candidate to work synergistically with FLT3 inhibitors in the treatment of FLT3-ITD acute myeloid leukaemia subtype and can be used in phase II/III clinical trials. The success of omacetaxine mepesuccinate continues, being involved in 23 clinical studies, 6 (NCT03135054, NCT03170895, NCT02440568, NCT02078960, NCT01873495, NCT02159872) of them are still open, and developments in the near future regarding therapies combined with other drugs in therapeutic use and the application in therapy of other haematologic malignancies.

Similarly, vincristine has also proven success in cancer therapy (Fig. 4.3). It is a natural alkaloid extracted from the leaves of *C. roseus* (L.) G. Don and is one of the first natural compounds to be identified as having oncologic properties although initially it has been studied, along with vinblastine (Figs. 4.4 and 4.5), for their antidiabetic properties (Gidding et al. 1999; Vipasha et al. 2016). In chemical terms, vincristine is asymmetrical and dimeric, composed of a dihydroindole nucleus, vindoline, linked by a carbon-carbon bond to an indole nucleus, catharanthine-type portion (Fig. 4.3). It has been extensively used in chemotherapy for more than

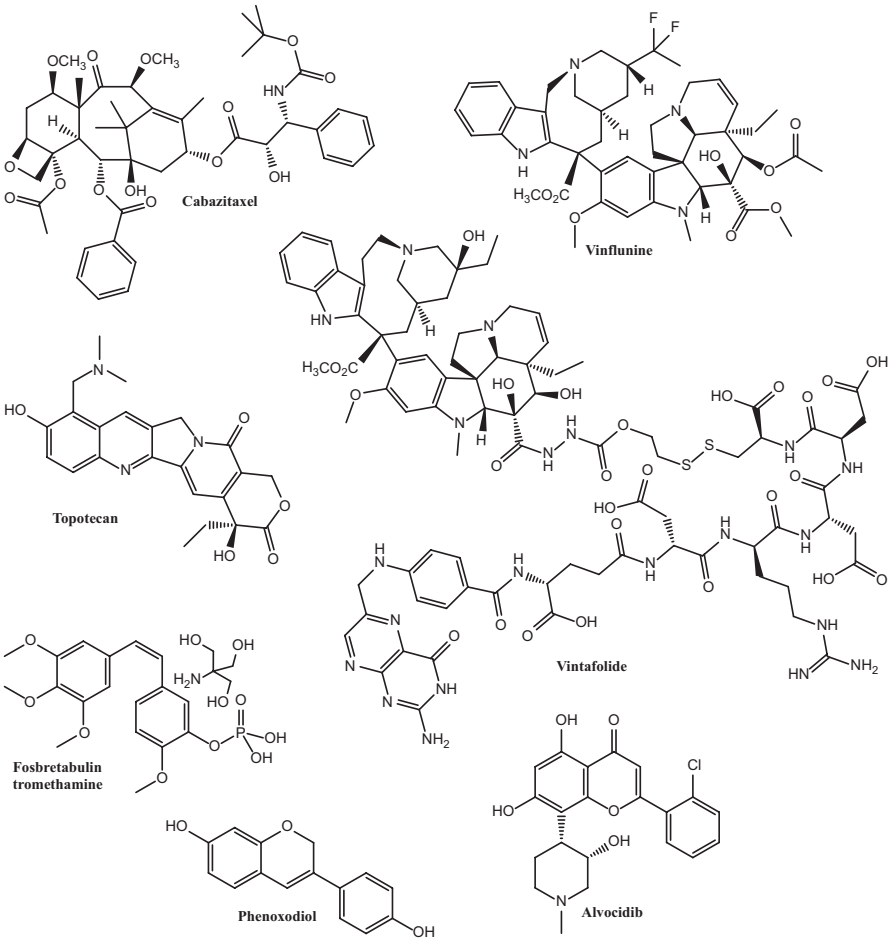

Fig. 4.4 Structure of the derivatives of natural compounds

50 years (mainly under trade name Oncovin®) in both adult and paediatric oncology practice against acute lymphoblastic leukaemia, rhabdomyosarcoma, neuroblastoma, lymphomas and nephroblastoma (Moore and Pinkerton 2009; Basmadjian et al. 2014).

Vincristine exerts its cytotoxic effects via stabilization of the microtubule structure by blocking the polymerization of tubulin molecules into microtubules and thus preventing the formation of the mitotic spindle, disrupt of intracellular transport and decrease of tumour blood flow, with the latter probably as a consequence of anti-angiogenesis (Wang et al. 2016c). At the end of vincristine-mediated cellular disruption, apoptosis occurs and consequently inhibition of cancer cell propagation. Although the applications of vincristine are extensive and with success, the side effects of vincristine cannot be ignored, and the major effect is neurotoxicity, a dose-

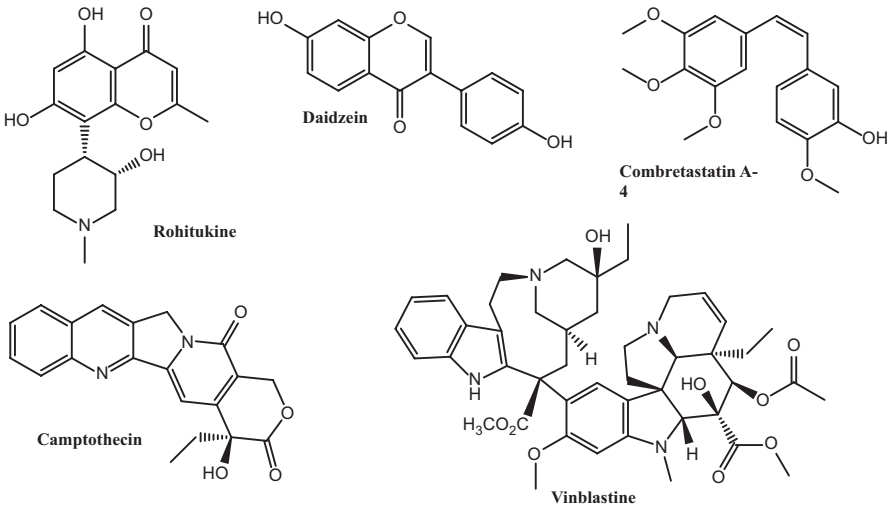

Fig. 4.5 Structure of the lead compounds

dependent side effect causing peripheral neuropathy (Moore and Pinkerton 2009; Wang et al. 2016c). The symptoms appear after a few vincristine administrations and normally disappear a few days after the treatment, although several cases of permanent sequels were reported (Jain et al. 2014; Mora et al. 2016; Velde et al. 2017).

Despite the long history of the clinical application of vincristine, there are three factors that diminish its impact in the fighting cancer: (1) its antitumour mechanism which is cell cycle-specific and the duration of its exposure to tumour cells that can significantly affect its activity, (2) its rapid clearance rate and wide biodistribution (Shah et al. 2016a; Wang et al. 2016c) and (3) vincristine-related neurotoxicity. One way to mitigate these three weaknesses is the encapsulation of vincristine into liposomes, and in 2012 the sphingomyelin/cholesterol liposomal vincristine (Marqibo®) was approved by FDA to treat adults with relapsed acute lymphoblastic leukaemia. Clinical trials involving Marqibo® are underway to paediatric patients with relapsed or chemotherapy-refractory solid tumours and leukaemia (NCT01222780; Shah et al. 2016b). Combined therapy has been the direction followed in the research involving vincristine. In fact, combination chemotherapy cannot only enhance the destruction of tumour cells but also decrease toxicity and drug resistance with drugs exhibiting different mechanisms of action. Therefore, there are in-progress open clinical trials involving combined vincristine therapy (NCT02879643; NCT01527149). Very recently, the treatment of infantile fibrosarcoma by adjuvant therapy after excision, using vincristine and dactinomycin, where the duration of chemotherapy was determined according to tumour response, was reported. At the end, there was no functional impairment and no evidence of recurrence at 18 months after therapy (Yoshihara et al. 2017). Vincristine and some of its encapsulated

formulations are involved in clinical studies in which they are tested against other types of cancer such as small cell lung cancer (NCT02566993), advanced cervical cancer (NCT02471027) or liver cancer (NCT00980460). Thus, vincristine alone or combined, encapsulated or not, continues its long history of therapeutic application, increasingly effective and each time with fewer side effects.

4.4 Natural Compound Derivatives with Anticancer Applications

Several natural compounds with unique anticancer effects are not used in clinical practice due to their physico-chemical properties and/or their toxicity. On the other hand, plant occurring secondary metabolites often can be excellent lead compounds for drug development despite sometimes having complex structures and limited oral bioavailability. Thus, modifying the chemical structure of these more promising compounds is one strategic way to increase their anticancer action and selectivity, improve their ADME (absorption, distribution, metabolism and excretion) properties and decrease their toxicity and side effects. The most recent anticancer natural compound-derived drugs as well as their structure and area of application are listed and depicted in Fig. 4.4. Comparing these structures with their precursors, it can be noticed that in some cases, the modifications were slight, such as protection of hydroxy groups (e.g., fosbretabulin tromethamine), addition (e.g., topotecan) or elimination (e.g., phenoxodiol) of a functional group and introduction of bulky groups (e.g., alvocidib), among others. The most noticeable one is the vinblastine transformation into vintafolide (Fig. 4.4) that involves the introduction of a peptide-type chain. The mentioned transformations do not reveal a typical change, quite the opposite. So each case is unique, and it would be beneficial if more SAR studies were available as well as action mechanisms were disclosed. The above-mentioned strategies for the development of new anticancer drugs were explained in detail by using the examples of dimethylaminoparthenolide (DMAPT), an important bioactive compound in preclinical development, and minnelide, a prodrug in clinic trial development.

Dimethylaminoparthenolide (DMAPT) (Fig. 4.6) is a parthenolide derivative obtained by the introduction of an amino group, which can improve the hydrophilic character if a salt form is used. DMAPT showed the ability to selectively eradicate LSCs and thus led to clinical trials for the treatment of AML, ALL and CLL in the United Kingdom (Peese 2010), but unfortunately the conclusions of this study are not known. It is, however, recognized that DMAPT exhibits a LD_{50} of 1.7 μM against AML cell lines and *in vivo* studies, using rats, demonstrated an oral bioavailability of 70% (Peese 2010). *In vitro* and *in vivo* studies with DMAP, alone or in combination with other drugs, have evidenced its beneficial effect on chemoprevention and treatment of pancreatic cancer, mainly by inhibition of NF-κB target genes (Yip-Schneider et al. 2013). It induces cytotoxic effects on hormone-insensitive

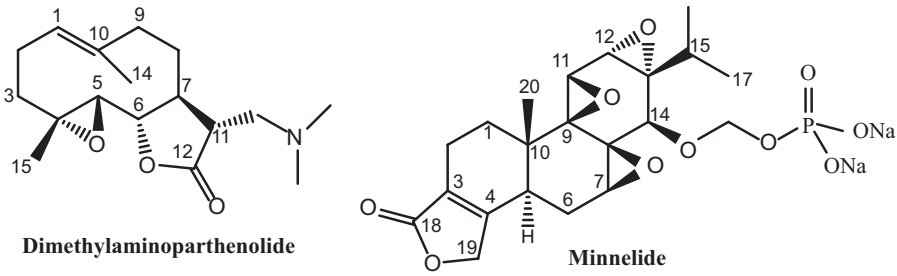

Fig. 4.6 Structures of dimethylaminoparthenolide and minnelide

human breast cancer MDA-MB-231 cells, through a similar mechanism of parthenolide (D'Anneo et al. 2013), being also highly promising for the treatment of glioma. In fact it was tested for antiproliferative activity against glioblastoma multiform cell lines, the most aggressive form of glioma (Ortensi et al. 2013; Ostrom et al. 2015), and the obtained results (Hexum et al. 2015) demonstrate that (a) DMAPT and parthenolide were found to be similarly active against human and murine glioma cell lines; (b) DMAPT can be transported through the blood-brain barrier; (c) DMAPT accumulates in brain tissue with biologically relevant concentrations; (d) DMAPT is nontoxic even following continuous dosing for several weeks (100 mg/kg, 30 days); and finally (e) a statistically significant delay in tumour growth kinetics *in vivo* is observed after treatments with DMAPT. All these results showed that DMAPT is highly promising for the treatment of glioma and may become one of the next-generation multiform drugs for glioblastoma.

An *in vivo* study demonstrates that DMAPT significantly reduce the number and size of lung tumours, mediated, at least in part, via inhibition of STAT3 signalling pathway, suppressing levels of pSTAT3 and MCL1 in lung tissues of NNK-treated mice (Song et al. 2014). Nakshatri et al. (2015) demonstrated that DMAPT is a potent pharmacological agent that can reverse NF-κB-dependent and NF-κB-independent cancer-specific epigenetic abnormalities. DMAPT showed ability to upregulate NSD1 and SETD2, two tumour suppressors, upon NF-κB inhibition. Additionally DMAPT increased KMT5C and H4K20me3 independent of its NF-κB inhibition attribute and adding KMT5C to the list of NF-κB-independent epigenetic targets of parthenolide (Nakshatri et al. 2015). The current knowledge and mechanisms of action of DMAPT are insufficient and its potential application in a variety of biological situations yet to be explored by future investigators.

Similarly, minnelide is a triptolide with low water solubility and serious toxicity. Thus, the researchers have tried to find ways to enhance its bioavailability and reduce its toxicity, by systematic structural modifications, mainly protecting the 14-hydroxy group of triptolide (Fig. 4.6). The details of its synthesis are under patent protection (WO/2010/129918), but it is known that it involves a four-step pathway: (1) react triptolide with acetic acid and acetic anhydride at room temperature for 5 days, (2) transform into a dibenzyl ester derivative by reaction with dibenzylphosphate, (3) obtain the corresponding dihydrogen phosphate by reductive

removal of dibenzyl group and (4) obtain the disodium salt by subsequent reaction with sodium carbonate (Chugh et al. 2012). In fact, minnelide or 14-*O*-phosphonooxymethyltriptolide disodium salt (Fig. 4.6) is ineffective against tumour or non-tumour cells, but in the presence of alkaline phosphatase, which is common in all tissues in the body, the phosphate ester group is cleaved, and the unstable *O*-hydroxymethyl intermediate spontaneously will release formaldehyde and triptolide (Rivard et al. 2014). Because of this behaviour in biological systems, minnelide is classified as a prodrug. It is much more water soluble than triptolide (it is a sodium salt), and no overt signs of toxicity were observed when animals received minnelide for 385 days at a concentration of 0.42 mg/kg (Chugh et al. 2012). However, two cases of patients within a phase I multicentre trial exhibit symptoms of minnelide acute toxicity involving the cerebellar cortex that fade with discontinuation of therapy (Roshan et al. 2017).

Minnelide is more effective in preclinical studies in multiple animal models of pancreatic cancer (0.28 mg/kg daily) than gemcitabine (100 mg/kg twice a week), which is one of the first-line chemotherapeutic agents for pancreatic cancer (Chugh et al. 2012). After this first report about the potent minnelide activity against several *in vivo* models of pancreatic cancer (Chugh et al. 2012), the efficacy of minnelide has been increasingly documented. Minnelide exhibited antiproliferative effects and induced apoptosis in non-small cell lung carcinoma and tumours partially mediated by inhibition of NF-κB pathway (Rousalova et al. 2013). Alsaied et al. (2014) report a strong *in vivo* hepatocellular carcinoma tumour growth inhibition rates after 2 weeks of treatment with minnelide [84% at 0.21 mg/kg (i.p.), while sorafenib 10 mg/kg orally exhibits only 59%]. In the same year, the effectiveness of minnelide, in monotherapy or combination therapy, to inhibit *in vitro* and *in vivo* the epithelial ovarian cancer was also demonstrated (Rivard et al. 2014). Banerjee and Saluja (2015) also showed that minnelide was a more effective drug reducing tumour burden and tumour-related morbidity in different and complementary *in vivo* pancreatic cancer models. Moreover, they confirmed that the combination of minnelide with sorafenib is indeed a successful mixture to treat hepatocellular carcinoma (Banerjee and Saluja 2015). Minnelide, at a low dose of 12.5 nM, showed not only significant pancreatic ductal adenocarcinoma tumour regression but also decreases significantly the pancreatic cancer stem cell population (Nomura et al. 2015). Modi et al. (2016) show that combination of low doses of minnelide and oxaliplatin does not have potential serious adverse effects, but this combined therapy is very effective to potentiated apoptotic cell death by suppressing oxaliplatin-induced DNA damage repair pathway in pancreatic cancer. Recently, it was demonstrated that minnelide (at a dose of 0.21 mg/kg) regressed subcutaneous tumours derived from castration-resistant prostate cancer cells more efficacious than the standard docetaxel and enzalutamide and induces apoptosis by downregulation of androgen receptor and its splice variants (Isharwal et al. 2017). Minnelide has been the subject of a phase I clinical trial in the treatment of pancreatic cancer and other gastrointestinal tumours (NCT01927965). Moreover phase I trials to study Minnelide™ capsules in patients with advanced solid tumours (gastric, breast, pancreatic, colon-rectal, stomach and prostate metastatic cancer) (NCT03129139) and phase II trials in patients with refractory pancreatic cancer (NCT03117920)

were recently initiated. All these studies show that minnelide, a water-soluble derivative of natural compound extracted from a Chinese herb, is very effective against a number of malignant diseases and points out to its high pharmaceutical potential.

4.5 Conclusions and Future Prospects

Medicinal plants and their anticancer secondary metabolites can serve as an important tool in chemotherapy. Naturally, a long way from the isolation to the treatment utilization is necessary, but the successful cases herein presented are, surely, a motivation to find new one. It is clear that plants, in particular those known as medicinal ones, are an important source of biologically active molecules that may be used as drugs or inspire the synthesis of new and safer compounds. In this regard several bioactive metabolites should be subjected to in-depth studies to fully understand their mode of action and consequently mimic their structures to develop new anticancer drugs. But no less important is the phytochemical study of several plant extracts aiming the isolation and characterization of the active metabolites. New active compounds can be obtained as well as new sources of inspiration to develop anticancer drugs.

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Chapter 5
Designing of Natural Anticancerous Drugs and Their Delivery System

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Abstract Chemoprevention of cancer with conventional therapeutic approach has shown severe side effects along with high possibility of recurrence of the same. This results in decreased therapeutic efficacy of the chemotherapeutic agents used currently. Therefore, to reduce their ill effects, an alternative gateway for cancer treatment and prevention needs to be explored. The natural, plant-based anticancer compounds are proving to be a substantial target in replacing the common approaches which employs chemical drugs. The natural compounds derived from various medicinal plants like vinca alkaloids, cyanidin, curcumin, fisetin, rosmarinic acid, etc. have already been documented with a remarkable antineoplastic property. The aim of the chapter is to focus on the various phytocompounds and their mechanism of action to treat the tumorigenic growth accompanied with the drug delivery system. Furthermore, the limitations and barriers associated with the formulation of phytocompounds and their possible enhanced therapeutic efficiency by nanoparticle-based drug delivery mechanics are also discussed.

Keywords Chemoprevention · Nanoparticles · Medicinal plants · Phytocompounds · Therapeutics

5.1 Introduction

Natural products have been used for over 40 years to treat cancer. The important sources of the compounds are plants and microbes from marine and terrestrial environments. Plant alkaloids and podophyllotoxins have the antitumor property (Verdine 1996), while the other major contribution on natural compounds is shown by microbes. There is always an increasing demand for the discovery of potential drugs to treat cancer, because of the development of resistance to chemotherapeutic drugs. Additionally, undesirable side effects and high toxicity are also associated

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with the drugs, which increase the surge for the development of new anticancer drugs with high therapeutic index or lesser side effects (Holland 1997; Manju et al. 2017). Natural products have brought about a great success in the society. The use of microbial and plant secondary metabolites has helped in increasing the life span in the twentieth century (Bandyopadhyay et al. 2017). They have reduced suffering and pain and helped in revolutionizing medicines by assisting the transplantation of genes or organs. The chemical diversity is based on the geographical and biological diversity. Thus, the researchers are exploring the entire globe for natural product-based therapeutics because of their easy access to terrestrial life and pharmaceutically active compounds (Henkel et al. 1999; Gouvea and Kassicieh 2005; Skinnider et al. 2017). There are approximately one million natural products known till now. There are about 500,000–600,000 plants known to produce therapeutically active products. The chemical structures of 160,000 natural products have been known, and the number is increasing by 10,000 per year (Knight et al. 2003). Out of these, 100,000 are from plants. So, the need of the hour is to identify and isolate plant products with anticancer property with high therapeutic index and fewer side effects (Fenical and Jensen 1993; Desai et al. 2008).

Cancer is a group of diseases involving the growth of unwanted cells and having the ability to spread to different parts of the body. There were approximately 1.5 million new cases in the USA in 2008, and the number is increasing exponentially. About US$ 60 million worth of antitumor compounds have been traded in the year 2007 alone. Natural products are the most significant anticancer agents (Roessner and Scott 1996). The natural products account for three quarters of antitumor agents used in cancer treatment (Cragg et al. 1997). The current treatment of cancer includes radiotherapy, chemotherapy, and surgery (Harvey 2008). They have proven to be useful in different types of cancer such as colorectal, breast, ovarian, pancreatic, and lung cancer (McCaskill and Croteau 1997). But, the effectiveness of chemotherapy is restrained by the toxic effects produced on the nontargeted cells. Hence, alternative and complementary therapies like herbal therapies are used increasingly (Raskin et al. 2002). Such intrusions have not been studied or tested rigorously. The use of plants for the treatment of cancer is not new; indeed, they are one of the most important sources of bioactive compounds for treatment of many ailments. Industrialized societies rely on plants for development of bioactive molecules from rural herbal plants (Cragg and Newman 2005). Plant extracts either cause the target cells to undergo cell death or target dividing cells by impeding mitosis. The aim of the chapter is to focus on the various phytocompounds and their mechanism of action to treat the tumorigenic growth accompanied with the drug delivery system. Furthermore, the limitations and barriers associated with the formulation of phytocompounds and their possible enhanced therapeutic efficiency by nanoparticle-based drug delivery mechanics are also discussed.

5.2 Limitations of Synthetic Chemotherapeutic Drugs

Various approaches have been generated for treating different types of cancers, out of which some main approaches are surgical excision, irradiation, and chemotherapy which are used effectively depending on the type of tumors, their location, and their stage of development. Chemotherapy is one of the efficient therapeutic approaches applicable for treatment of various localized and metastasized tumors which can be used alone or with a combination of other therapies (Jemal et al. 2008, 2010). Different series of the chemically synthesized drugs are used to treat tumors by delivering them in the form of a beam to kill cancerous cells in the patient's body. However, being a potential therapeutic approach, there are some limitations to it like lack of aqueous selectivity and solubility along with development of multidrug resistance (Li 2002). Many chemotherapeutics derived either from natural or synthetic source have hydrophobic characteristics and need solvents to prepare the dosage form which leads to severe toxicity. Lack of selectivity or targeting of anticancer drugs toward tumor cells also contributes in causing significant damage to normal proliferating cells (Kwon 2003). Also, another limitation like multidrug resistance (MDR) caused due to efflux pumps like P-glycoprotein (Pgp) in the membrane of the cells, diverting various anticancer drugs out of the cells and restricting cell damage, causes negative impact (Callaghan et al. 2014). Secondly, nontargeted delivery of these anticancer drugs in the human body also affects the availability of dosage within the tumorous cells and results in suboptimal treatment caused by excessive toxicity (Agostinis et al. 2011). Therefore, an efficient natural product-based drug delivery system with targeted approach can help in culminating all the barriers and limitations (Das and Khuda-Bukhsh 2016).

5.3 Need for Natural Product-Based Drug for Cancer Therapy

The established synthetic chemotherapeutic drugs sometimes show the toxicity that creates a significant barrier in the cancer treatment. Many approaches have been proposed for treating cancers using plant-derived or natural products, and a large number of natural products have been explored for their potential against cancerous cells. It was largely observed that natural products show encouraging characteristics in terms of their easy absorption and metabolism in the human body with a lesser toxicity in comparison with synthetic drugs. Plants are the major source of bioactive compounds with numerous health benefits and anticancer activities. Natural compounds obtained from plant resources are well-utilized since ancient history for treating different kinds of human diseases in India, China, Egypt, and Greece, and several chemo-drugs have been developed from the traditionally utilized plants

(Arumugam et al. 2016; Swamy et al. 2016, 2017; Swamy and Sinniah 2016; Mohanty et al. 2017). Several classes of phytocompounds such as phenolics, alkaloids, flavonoids, etc., isolated from various medicinal plant species, have shown a noteworthy antiproliferative efficacy and can be used as a source to develop novel anticancer agents against different cancers (Mohanty et al. 2014, 2017; Swamy and Sinniah 2015). Approximately, 50–60% of patients diagnosed with cancer in the United States are utilizing plant-derived agents or nutrients (alternative medicines) for their treatment outstating the traditional therapeutics like chemotherapy (Gutheil et al. 2012). These plant-derived bioactive compounds include genistein (soybean), curcumin (turmeric), sulforaphane (broccoli), tea polyphenols (green tea), isothiocyanates (cruciferous vegetables), resveratrol (grapes), diallyl sulfide (garlic), apigenin (parsley), and the list continues.

5.4 Drug Discovery from Natural Products for Cancer Therapy

From 1981 to 2002, the United States Food and Drug Administration (US FDA) had approved over 62% natural source-based cancer drugs (Newman et al. 2003), and thereafter, again till 2006, the approvals and inclusions of phytocompounds reach to 47% (Newman and Cragg 2007), and the reassessment in 2010 exhibited the rise of 50%, showing the existence of efficacious phytocompounds equally potent to treat cancer (Newman and Cragg 2012). These natural products exhibit various approaches to combat the tumor growth like angiogenesis inhibition, blocking signaling pathway, induce apoptosis, etc.

Apigenin, a flavonoid found in parsley, has been reported to exhibit potential cytotoxic properties, comparable to doxorubicin to counter breast cancer and colon cancer (Hoensch and Oertel 2011; El-Alfy et al. 2011). Also, it has been extensively discussed by Chung et al. (2007) and later by Turktekin et al. (2011) that apigenin showed cell death in human colon cancer cell lines in male Sprague-Dawley rats, by decreasing azoxymethane (AOM)-induced aberrant crypt foci (ACF) synthesis which increases apoptosis and contributes in preventing cancer of the colon (Chung et al. 2007; Leonardi et al. 2010; Turktekin et al. 2011). Apigenin also alters leptin receptor pathway and causes cellular death in lung adenocarcinoma cell line (Bruno et al. 2011). The other most explored phytocompound is curcumin, showing an immense non-proliferative effect on colon, breast, brain, and lung cancer in various studies (Bachmeier et al. 2010; Senft et al. 2010). Curcumin has a potential of inducing apoptosis in cancer cells without leading to toxicity to the healthier cells and can affect many cancerous compounds or pathways like inhibiting NF-κB which cause inflammatory diseases inducing cancer, dispersion of raptor from mTOR, preventing mTOR complex I, etc. (Aggarwal and Shishodia 2004; Bachmeier et al. 2007; Beevers et al. 2009). Ravindran et al. (2009) stated that curcumin can regulate the proliferation of cancerous cells by controlling various cell

signaling pathways such as cell growth pathway (cyclin D1, c-myc), cell survival pathway (Bcl-x, Bcl-2, XIAP, cFLIP, c-IAP1), caspase activation pathway (caspase-3, caspase-8, and caspase-9), death receptor pathway (DR4 and 5), protein kinase pathway (AMPK, JNK, and Akt), tumor suppressor pathway (p53, p21), and mitochondrial pathways (Ravindran et al. 2009).

Saffron, a food coloring component extracted from dry stigma of *Saffron crocus* flower, is marked as an efficient compound for its novel anticancer properties against hepatocellular and pancreatic carcinoma (Abdullaev and Espinosa-Aguirre 2004; Amin et al. 2011), skin carcinoma (Das et al. 2010), and colorectal and breast cancer (Chryssanthi et al. 2011). Crocetin (an active compound of saffron) disrupts the cancerous cell growth by preventing synthesis of nucleic acid, decreasing the free radical formation process, causing cell death and disturbing signal-mediated pathway of growth factors (Aung et al. 2007). In a study by Nam et al. (2010), crocetin showed its potential in inhibiting LPS-induced nitric oxide release, reducing the synthesis of IL-1β, TNF-α, and ROS and causing the suppression of NF-κB pathway and LPS effect on hippocampal cell death (Chryssanthi et al. 2011) (Table 5.1).

Cyanidin is a pigment extracted from grapes or red berries which contain antioxidant and radical scavenging properties that reduces the risk of cancer by limiting the proliferation of cells and COX-2 and iNOS gene expression in cells with colonic cancer (Bathaie and Mousavi 2010). In the study conducted by Lim et al. 2011, C3G disabled benzo[a]pyrene-7,8-diol-9, 10 epoxide-induced NF-κB and AP-1 activation and MKK4, MEK, MAPK, and AKt phosphorylation, preventing Fyn kinase signaling pathway activation, which makes it suitable for cancer treatment (Nam et al. 2010). NF-C3G also resists the activation of ethanol-induced ErbB2/cSrc/FAK pathway in the metastasis of breast cancer (Kim et al. 2008). Cyanidin significantly inhibits UVB-induced COX-2 expression and PGE2 secretion in epithelial skin cells by repressing NF-κB and AP-1 which are controlled by MAPK. It also targets MKK-4, MEK1, and Raf-1 to suppress UVB-induced COX-2 expression (Lim et al. 2011).

Brassica vegetables like cauliflower, broccoli, collard greens, etc. contain indole-3-carbinol (I3C) which converts into a digestive form called diindolylmethane (DIM) when exposed to the acidic condition of the stomach (Xu et al. 2010). Both derivatives showed anti-carcinogenic potential in different cancer types. I3C showed effective signs in preventing lung adenocarcinoma induced by tobacco smoke by modulating receptor tyrosine kinase/PI3K/Akt signaling pathway partially (Kim et al. 2010). I3C and DIM also showed remarkable anticancerous effect in different hormone-responsive cancers such as ovarian, breast, and prostate cancers (Kim and Milner 2005). Gingerol, a potential compound derived from different spices of ginger, demonstrated effective anticancer properties in treatment of tumors present in pancreas, breast, ovarian, and colon (Jeong et al. 2009; Acharya et al. 2010; Qian et al. 2011). It has antioxidant, antitumor, and anti-inflammation characteristics and reduces the expression of iNOS and TNF-alpha by inhibiting phosphorylation of IκBα and NF-κB nuclear translocation (Park et al. 2006a, b; Rhode et al. 2007; Oyagbemi et al. 2010).

Table 5.1 Bioactive compounds derived from natural resources eliciting anticancer activities

Plant	Botanical name	Bioactive compound	Type of cancer targeting	References
Saffron crocus	*Crocus sativus*	Saffron	Lung cancer, pancreatic cancer, skin carcinoma, colorectal cancer, and breast cancer	Abdullaev and Espinosa-Aguirre (2004) and Amin et al. (2011)
Cruciferous vegetables	*Brassica* spp.	Indole-3-carbinol (I3C), diindolylmethane (DIM)	Lung, breast, prostate, and ovarian cancers	Kim and Milner (2005) and Xu et al. (2010)
Tea	*Camellia sinensis*	Kaempferol	Pancreatic cancer and lung cancer	Cui et al. (2008) and Gacche et al. (2011)
Broccoli	*Brassica oleracea*			
Grapefruit	*Citrus paradisi*			
Red grapes skin	*Vitis vinifera*	Resveratrol	Skin and gastro-intestinal tract tumor, colon cancer	Kim et al. (2008)
Peanuts	*Arachis hypogaea*			
Ginger	*Zingiber officinale*	Gingerol	Colon cancer, breast and ovarian cancer, and pancreatic cancer	Jeong et al. (2009), Acharya et al. (2010), and Qian et al. (2011)
Catclaw acacia	*Acacia greggii*	Fisetin	Colon cancer	Acharya et al. (2010)
Guajillo	*Acacia berlandieri*			
Strawberries	*Fragaria ananassa*			
Apple	*Malus*			
Turmeric	*Curcuma longa*	Curcumin	Colon cancer, breast cancer, lung metastases, and brain tumor	Bachmeier et al. (2010) and Senft et al. (2010)
Grapes	*Vitis vinifera*	Cyanidin-3-glucoside (C3G)	Brest cancer, colon cancer	Bathaie and Mousavi (2010) and Lim et al. (2011)
		Cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside		
Green tea	*Camellia sinensis*	Epigallocatechin gallate (EGCG)	Brain, prostate, cervical, and bladder cancers	Das et al. (2010)
Rosemary	*Rosmarinus officinalis*	Rosmarinic acid	Colon cancer, breast cancer, leukemia	Xu et al. (2010)

(continued)

Table 5.1 (continued)

Plant	Botanical name	Bioactive compound	Type of cancer targeting	References
Parsley	*Petroselinum crispum*	Apigenin	Breast cancer, colon cancer, lung cancer	El-Alfy et al. (2011) and Hoensch and Oertel (2011)
Celery				
Chamomile	*Apium graveolens*			
Egyptian plant	*Matricaria chamomilla*			
Moringa peregrine	*Moringa peregrina*			

A natural flavonol extracted from tea, broccoli, grapefruit, etc. known as kaempferol was explored for its anticancerous, antiangiogenic, and radical scavenging effect (Park et al. 2006a; Gacche et al. 2011; Calderon-Montano et al. 2011). Kaempferol was also inspected for treating lung and pancreatic cancer (Nöthlings et al. 2007; Cui et al. 2008). The studies revealed that kaempferol plays a role of aryl hydrocarbon receptor (AhR) antagonist to restrict the upregulation of ABCG2 which alters the resistance of ABCG2-mediated multi-drug and effectively treat esophageal cancer (To et al. 2012). Another study also disclosed that kaempferol can cause cell death of ovarian cancerous cells by activating the p53 in intrinsic pathway (Luo et al. 2011).

5.5 Designing Plant-Based Drugs to Encounter Cancerous Growth

Plants are used as a source of medicine and for health benefits since the ancient history. Approximately, 80–85% of the population depends on conventional therapy for their routine health-related issues. The traditional medicines involve the use of plant extracts or bioactive components (Elujoba et al. 2005). Much development has been done for treatment and control of cancer, but the scope of improvement still remains. The main limitation of synthetic drugs is associated with their negative impacts as mentioned above. Therefore, natural therapies involving plants or drugs derived from plants are used to treat cancer. The investigation of anticancer agents derived from plants commenced in the 1950s wherein vinca alkaloid (vincristine and vinblastin) was discovered and developed and cytotoxic podophyllotoxin was isolated (Cragg and Newman 2005). The following are some plant-derived antitumor agents which are in clinical use.

5.5.1 Vinca Alkaloids

Vinca alkaloids are a class of organic compounds and are made of hydrogen, carbon, oxygen, and nitrogen. These are a part of therapeutics which are obtained from the Madagascar periwinkle plant (Moudi et al. 2013). The alkaloids which are naturally extracted from *Catharanthus roseus* have cytotoxic and hypoglycemic effects (Tiong et al. 2015). They are used to treat diabetes and high blood pressure and can be used as disinfectants. They are also used to treat cancer. The vinca alkaloids in clinical use are vinblastine (VBN), vincristine (VCR), vindesine (VDS), and vinorelbine (VRL). VRL, VBL (vinblastine), and VCR have been approved for their use in USA. The mechanism of action of cytotoxicity of vinca alkaloid is the interaction with tubulin and, thus, disruption of the function of microtubules, particularly those microtubules which comprise the mitotic spindle apparatus, leading to metaphase arrest (Moudi et al. 2013). The alkaloids can have other effects rather than the disruption of microtubule. Many other biochemical activities that do not include the effect on microtubule occur only after treating the cells with therapeutically irrelevant dose of alkaloid. Both the vinca alkaloids and anti-microtubule agents have their influence on malignant and nonmalignant cells in non-mitotic cell functions, because microtubules are involved in many non-mitotic cell cycles.

The alkaloids connect to the binding sites of tubulin, and the connection between them occurs rapidly and it can reverse too. The evidence demonstrates the existence of two vinca alkaloid binding sites per one mole of tubulin dimer (Correia and Lobert 2001). This leads to interruption of microtubule congregation. One of the most crucial aspects of low drug concentration is shortening at the end of microtubule leading to production of kinetic cap thereby suppressing its function and decrease in the rate of bone growth (Stanton et al. 2011). The disturbing effect of vinca alkaloid especially at the end of spindles leads to metaphase arrest and occurs at drug concentrations lower than those that decrease the microtubule mass. It has been observed that microtubule-disrupting agents and vinca alkaloids have the capability to inhibit proliferation of malignant cells *in vitro*. For example, VBL within a range between 0.1 and 1.0 pmol/L leads to the blockage of chemotaxis, malignant angiogenesis, and the spreading of fibronectin which are all the important steps of angiogenesis. Also, other lymphoid tumors and normal fibroblasts were unaffected at the minute concentrations. VBL in combination with Ab against VEGF (vascular endothelial growth factor) leads to increased antitumor response; this is also observed even in tumors which are resistant to the cytotoxic effect of the drug at low concentrations of VBL. The cell proliferation is inhibited by vinca alkaloids by binding to microtubules, which leads to mitotic blockage and apoptosis. The destabilization of microtubules is caused by binding of VCR and related compounds to tubulin and, thus, blocking the polymerization.

Vinca alkaloids are included in chemotherapy regimens for medicinal therapy. VBL is used to treat Hodgkin's and non-Hodgkin's lymphomas and testicular carcinoma. VBL is same to VRL. They have antitumor activity against bone and breast

cancer and osteosarcoma. VRL helps to decrease the stability of lipid bilayer membranes. VRL has been approved in the USA for managing and treating lung cancer. VCR is approved for curing Wilms' tumor, leukemia, neuroblastoma, Hodgkin's disease, rhabdomyosarcoma, and several other lymphomas (Rowinsky and Donehower 1995). VCR is used for to treat various nonmalignant hematologic disorders, for example, hemolytic uremic syndrome, thrombotic thrombocytopenia purpura, and refractory autoimmune thrombocytopenia (Rowinsky and Donehower 1995). VDS has similar effects as VBL. Antineoplastic activity of VDS is mentioned in pediatric solid tumors, malignant melanoma, blast crisis of chronic myeloid leukemia, acute lymphocytic leukemia, metastatic breast, and colorectal, renal, and esophageal tumors (Joel 1996). Vinflunine was developed recently adding two fluor molecules by the superacidic chemistry and is the first fluorinated microtubule inhibitor that belongs to the family of vinca alkaloids. It is used to treat second-line TCCU (transitional cell carcinoma of the urothelium), carcinoma of the breast, and lung carcinoma (Bennouna et al. 2008).

5.5.2 Podophyllotoxin Derivatives

The two species of Podophyllaceae family, *Podophyllum peltatum* and *P. emodi*, are reported to be used in the treatment of warts and skin cancer (Kelly and Hartwell 1954). *P. peltatum* is a perennial and native herb found in North America. *P. emodi* grows over the range of the Himalayas and contains white flowers growing below the leaves and has paprika-like fruits. And the parts are highly noxious. Thus, the utilization of podophyllin formulations provides an example of how an extract with ethnopharmacological properties despite of causing toxicity can be utilized for drug development. In 1980, Podwissotzki isolated podophyllotoxin from the crude mixture of podophyllin and established the structural formula. But, the correct structural formula was elucidated in the 1950s after the discovery and advancement in spectroscopic techniques. Lignan (closely related podotoxin) was also isolated during the time and was introduced in clinical trials, but due to unacceptable toxicity and lack of efficacy, it was dropped. In the 1960s, etoposide and teniposide were developed at Sandoz Laboratory in Switzerland which can be used for treating testicular and bronchial cancers (Gordaliza et al. 2004) (Table 5.2).

5.5.2.1 Pharmacological Activity

Podophyllotoxin derivatives bind reversibly to solid or soluble tubulin and inhibit the binding of colchicine to tubulin by competitively binding to it. Tubulin regulates the assembly of microtubules.

Table 5.2 Phytochemicals used for cancer therapy

Phytochemicals	Source	Drug delivery system	Medicinal properties	References
Podophyllotoxin	*Podophyllum peltatum*	Lipid nanoparticles	Brain tumors, small cell lung cancer, testicular cancer, lymphoblastic cancer	Kelly et al. (1954)
Taxol	*Taxus brevifolia*	Polymeric nanoparticles	Breast cancer, ovarian cancer, and lung cancer	Rowinsky and Donehower (1995) and Jennewein and Croteau (2001)
Vinblastine	*Vinca rosea*	Vinblastine-loaded folic acid-functionalized meso-porous silica nanoparticles	Hodgkin's lymphoma, non-Hodgkin's lymphoma	Correia and Lobert (2001) and Moudi et al. (2013)
Etoposide	*Podophyllum peltatum*	Polymeric nanoparticles	Ewing's sarcoma, testicular cancer, glioblastoma multiforme, Kaposi's sarcoma, lung cancer	Gordaliza et al. (2004)
Teniposide	*Podophyllum peltatum*	Albumin nanoparticles	Hodgkin's lymphoma, acute lymphocytic leukemia, brain tumors	
Homoharringtonine	*Cephalotaxus*	Lipid nanoparticles	Chronic myeloid leukemia	Cragg and Newman (2005)
Elliptinium	*Bleekeria vitiensis*	Polymeric nanoparticles	Breast cancer	Kaur et al. (2011)
Camptothecin	*Camptotheca acuminata*	Liposomal nanoparticles	Lung cancer, ovarian cancer, and cervical cancer	Nirmala et al. (2011)
Docetaxel	*Taxus baccata*	Nanoliposomes, activated nanoparticles	Breast cancer, stomach, head, neck, non-small cell lung cancer	Lawania and Mishra (2013)
Topotecan	*Camptotheca acuminata*	Liposomal nanoparticles	Ovarian, small cell lung cancer	Qin et al. (2017)
Irinotecan	*Camptotheca acuminata*	Silica nanoparticles	Colorectal cancer	

Microtubule Inhibition

Tubulin (a 6S dimeric protein of 110,000 M_1) is the building block of microtubules. Tubulin consists of two nonidentical chains designated as α and β of approximately 50,000 M_2. The tubulin dimers polymerize to form a prototype, which further organizes to produce microtubules. Microtubules undergo changes rapidly; the equilibrium between free tubulin and microtubules leads to constant assembly/disassembly at both the ends of microtubule (Margolis and Wilson 1981; Bohlin and Rosen 1996). Microtubule inhibitor causes arrestation of malignant cells, mitotic spindle, and acceleration/inhibition of release of hormones and enzymes. They affect the plasma membrane directly. Also, it is observed that the microtubules inhibit the release of chemotactic substances from phagocytic leukocytes in dot-like attractant assay (Xu et al. 2005). As low as 5 μM concentration of podophyllotoxin causes complete inhibition of tubulin polymerization.

Neutrophil Chemotaxis

It was observed that neutrophil chemotaxis was inhibited by podophyllotoxin (by 15%); inhibition was maximum at podophyllotoxin concentration of 0.01 μg/ml. A different concentration of CPH 82 (podophyllotoxin glycosides) was chosen by Rantapaa-Dahlqvist et al. (1994) to mimic the clinical conditions when they studied the cell cycle *in vitro*. Out of nine cell lines, eight presented cell accumulation in the G_2 phase of the cell cycle. It was observed that the progression through G phase slowed down in various cell lines. Further, the blockage was seen in G_1 and G_2 phase in all the three cell lines. For some cell lines, the development of M cells appeared considerably hindered. A dose-dependent and different pattern of cell cycle arrest was seen in podophyllotoxin CPH 82 and taxol (Dahlqvist et al. 1994).

Cell-Mediated Lympholysis

Podophyllotoxins inhibit the division of mouse spleen cells against stimulator cells and induce cytolytic activity. A sudden fall in cell-mediated lympholysis was caused by podophyllotoxins at the concentration of 1.5–3.0 ng/ml. The effect of drug was effective when added within 72 h. Large doses of podophyllotoxin had an impact on *in vivo* and *in vitro* immune responses. Because of many adverse events which were caused by contaminants in crude extracts, therefore, it is available in highly pure form. CPH 82 is a therapeutically active compound which is made up of two similar benzylidenated podophyllotoxin glucosides (a derivative of podophyllotoxin) and has been formulated to increase the absorption of the intestine. Various anti-inflammatory screening tests are done on CPH 82 (Bohlin and Rosen 1996).

5.5.3 Taxanes

It is also effective against Kaposi's sarcoma. Paclitaxel was first isolated from the bark of the *Taxus brevifolia* Nutt. (Taxaceae) (Nirmala et al. 2011). Several species of *Taxus* (*T. baccata* L. and *T. canadensis* Marshall.) and different parts of *T. brevifolia* are used by the tribes of America for the treatment of cancer, and this has been documented (Prakash et al. 2013). In Ayurveda, the leaves of *T. baccata* are used for treating cancer. The biosynthesis of paclitaxel takes place in the leaf region of different *Taxus* species, wherein baccatins are converted to paclitaxel and analogues of paclitaxel. For example, docetaxel (Taxotere) is an important natural renewable source of this class of drug (Jennewein and Croteau 2001). It has shown efficacy against rheumatoid arthritis, multiple sclerosis, and psoriasis. Docetaxel is used for treating breast cancer. Approximately 12 taxane analogues are in preclinical and clinical trials. Out of 2069 clinical trials of cancer by NCI (National Cancer Institute), 248 have been recorded as drugs derived from taxanes, which includes 134 with paclitaxel (Taxol), 105 with docetaxel (Taxotere), and 10 with miscellaneous taxanes (which includes single agent or combination with other anticancer agents). Twenty-three taxanes are in preclinical development (Lawania and Mishra 2013). Taxanes bind to β-tubulin in the microtubules and promote α,β-tubulin assembly. The binding mimics with a nucleotide "GTP" with some significant variations. The main cause of cytotoxic effect of paclitaxel is mitotic arrest because of the inhibition of microtubule dynamics. The inductions of apoptosis by biochemical events which may bind the paclitaxel to microtubules are still not known. The apoptosis might occur following a typical exit from mitosis to G_1 multinucleated state or directly after mitotic arrest. However, evidences indicate that arrestation of G_2/M phase of the cell cycle is not the only mechanism for apoptosis stimulated by paclitaxel. At high paclitaxel concentrations, formation of multipolar or monopolar spindles corresponded to a drop in fraction of HeLa cell line, whereas, at low concentration, the inhibition of spindle fibers was correlated to altered bipolar spindles, thereby proposing that spindle dynamics are also compromised (Abal et al. 2003).

5.5.4 Camptothecin Derivatives

Camptothecin (CPT) is the class of anticancer drugs which was isolated from the bark and stem of Chinese ornamental tree *Camptotheca acuminata* Decne (Nyssaceae) (Nirmala et al. 2011). This tree is used for treating cancer in traditional Chinese medicine. CPT is a quinolone alkaloid which is toxic to cells and inhibits topoisomerase 1 (DNA enzyme). CPT possesses an excellent anticancer property, but the solubility is low and produces an adverse drug reaction in the body. Because of these drawbacks of CPT, medicinal and synthetic chemists have developed different derivatives of CPT, i.e., irinotecan and topotecan. It consists of a planar

pentacyclic ring which includes pyrrolo [3,4-β]-quinoline moiety (rings A, B, and C), pyridine (ring D), and a chiral center (position 20) within α-hydroxy-lactone ring (S configuration, E-ring) (Qin et al. 2017). It is observed that, because of its planar structure, it inhibits topoisomerase.

CPT binds to DNA and topo 1 (via hydrogen bond) thus producing a ternary and a stable complex. It averts religation of DNA and consequently begins damage of nucleic acid resulting in cell death. E-ring of CPT interacts with three varied locations of the enzyme. The hydroxyl group at the location 20 forms hydrogen bonding to a side chain present on aspartic acid (Asp533) of topo 1. It is worth to be noted that the configuration of chiral carbon is (S) because of the fact that (R) is inactive. Further, lactone ring interacts with amino groups on arginine 364 (Arg364) by forming a hydrogen bond. The pyridine ring interacts with +1 cytosine present on the noncleaved strand and helps to stabilize DNA-topo 1 by making a hydrogen bond. The hydrogen bonding is between the amino group in the ring D of +1 cytosine and carbonyl group in position 17. The cytotoxicity of the complex is due to the alteration of single-stranded breaks to double-stranded breaks in the course of S phase (synthesis phase) (Liu et al. 2000).

5.5.4.1 Structure-Activity Relationship (SAR)

Some modifications can be done to improve the activity of CPT. It has been noted that the substitution at position 7, 9, 10, and 11 improved the physical properties (metabolic stability and potency) and activity of CPT. On the other hand, replacement at position 12 and 14 results in inactive derivative. Addition of an alkyl group increases its efficacy. Substitution of alkyl group (chloromethyl and ethyl) at position 7 exhibited increase in cytotoxicity. It is because of the reason that these groups react with DNA in the presence of topo 1 resulting in increase in tumorigenic activity (Liu et al. 2000). Also, increasing the number of carbon atoms at position 7 results in enhanced hydrophobicity and, ultimately, higher stability and potency in plasma. Examples of analogues of CPT that have been modified at position 7 are karenitecins and silatecans (Liu et al. 2000). They are inhibitors of topo 1 and have alkylsilyl group at position 7 thereby making them more stable and lipophilic. Silyl camptothecins or silatecans lead to reduction in drug-HAS interactions resulting in plasma stability, and also, they are able to cause transient opening in the blood-brain barrier (BBB). The most active silatecan is DB-67 (a 10-hydroxy derivative) (Baker Jr. et al. 2014). BNP1350 belongs to the class of karenitecins and is capable of overcoming drug resistance and is toxic to cells. Lipophilic moieties such as oxyiminomethyl or iminomethyl can make CPT lipohillic. The activity of CPT can be considerably improved by adding a functional group "hydroxyl" at position 11 or 10 and the groups which are able to withdraw electrons (bromo, chloro, and nitro) at position 10 and 9. But the problem with them is that they are insoluble in aqueous solutions, thus causing difficulty in administrating them (Van Hattum et al. 2000).

5.5.5 *Homoharringtonine*

Homoharringtonine (omacetaxine mepesuccinate or HTT) is a semisynthetic preparation which is isolated from the Chinese plant, *Cephalotaxus harringtonia* var. drupacea (*Cephalotaxus*) which has a potential anticancerous activity (Cragg and Newman 2006). HTT (a cephalotaxine ester) inhibits the synthesis of protein and is a well-known agent to treat hematological cancer. It is synthesized from the extract of *Cephalotaxus* species of plant (mainly from leaves). Elliptinium was extracted from species of various genera of the Apocynaceae family which includes *Bleekeria vitiensis* (Fijian plant) with anticancer property (Kaur et al. 2011). For treating chronic myeloid leukemia (CML), HTT received orphan drug designation from EMEA in October 2005. Further, in March 2006, HTT obtained orphan drug status from the Food and Drug Administration (FDA) for curing CML. CML was approved as fast track designation by the FDA in November 2006. HTT was marketed under the brand name Synribo (TM) in October 2012 and has been accepted by the FDA for patients who are resistant or intolerant to tyrosine kinase inhibitors. The purified HTT is known to show efficiency against various cancers. Elliptinium is a therapeutically active compound which is sold in France and is used for treating breast cancer. The pharmacodynamics of omacetaxine is not clearly understood. HTT is involved in inhibition of protein synthesis by binding to 80S ribosomes in eukaryotic cells and interfering with the chain elongation which leads to its antineoplastic activity. This molecule also causes initiation of apoptosis and differentiation in cancerous cells (Chan et al. 2004).

5.6 Types of Drug Delivery Routes for Distribution of Anticancer Drugs

At present, the cancer therapy, especially in regard to drug delivery, has undergone an enormous change from the conventional approach. This evolution is based on the need to increase the therapeutic index of the bioactive compounds. Even though cancerous cells are more susceptible to chemotherapy drugs as compared to normal cells, the drugs can cause injury to normal cells and are therefore nonselective. Some other issues associated with conventional approach are low therapeutic index, low solubility, nonspecific distribution, lack of targeting ability, and systemic toxicity. Efforts are being made to kill the cancerous cells and restrict the death of normal cells. To attain this goal, there is a need for development of a carrier system for existing and new drugs and improved therapeutic targets in correspondence to molecular changes in cancerous cells. Nanotechnology, an emerging field, unravels the various possibilities associated with it. Nanoparticles (NPs) are 1–100 nm in size. The size of the particle is made in this range to bypass the fenestrations of epithelium of cancer cells causing the high concentration of NPs at the site of tumor. The nanoparticles are used for targeted drug delivery. Further, NPs have the ability

of crossing various biological barriers (blood-brain barrier, cerebrospinal fluid, etc.). They have a large surface area, due to which a large amount of drugs can be loaded onto them, and have modifiable electronic, optical, biological, and magnetic properties. After the binding of nanoparticles to the receptor of the cell, the NPs undergo phagocytosis or receptor-mediated endocytosis and release the drug in the cytoplasm. NPs were developed in the late 1960s. The first nanoparticle conjugated with drug lipid vesicle was developed during the 1970s. Sandimmune® was the first micellar drug to be approved by the FDA for systemic administration of NPs in humans in 1983 (Siddiqui et al. 2015). Further, in the 1990s, the first polymeric nanoparticle-drug conjugate Adagen® was clinically approved for use in humans (Allen and Cullis 2004).

5.7 Mechanism of Targeting of Nanoparticles

Nanoparticles target tumor cells in two ways: active and passive targeting. Active targeting occurs by specific interaction between the target cell and the drug/drug carrier by receptor ligand interaction, antigen (Ag), and antibody (Ab) recognition for localization of drug intracellularly. On the other hand, passive targeting of drugs refers to localization of drugs in the area surrounded by tumor cells with leaky vasculature. The phenomenon is known as enhanced permeation and retention (EPR). It enables targeted delivery of nanoparticles to the tumor site. It is based on the size of NPs. The surface of NPs can be modified with various ligands that enables it to interact with specific receptors present on the tumor cells, thereby, imparting active targeting (Danhier et al. 2010). Nanoparticles for delivery of drugs include various designs in terms of shape, size, and materials. Each particle differs in terms of release kinetics, capacity of drug loading, stability of drugs, drug and particle stability, and the ability of targeted delivery. So, the following are examples of nanoparticles used for targeted delivery:

5.7.1 Polymeric Nanoparticles

The polymeric nanoparticles are generally produced from organic polymers of either natural or synthetic origin. They are biocompatible and biodegradable and are accepted for drug delivery of nanomaterials (Soppimath et al. 2001). Their surface can be modified by chemical transformations; they have an outstanding pharmacokinetic control and are appropriate for encapsulation and delivery of a wide variety of drugs. The polymers used in the formulation of polymeric nanoparticles are poly(butyl)cyanoacrylate, polylactic acid, gelatins, poly(lactic-co-glycolic acid) copolymer, poly(alkylcyanoacrylate), polyglycolic acid, chitosan, and poly(methyl methacrylate) (Kumari et al. 2010). Additionally, the coatings of polymeric-based nanoparticles can be functionalized onto different types of NPs so as to vary and

enhance their biodistribution properties. PLGA and PLA have been approved by the FDA for human use. Various classes of drugs such as low-molecular-weight compounds can be encapsulated in the polymeric nanoparticles. Furthermore, these polymeric NPs have been used to treat breast cancer, thereby reducing the proliferation of cancer cells. The matrix which is made up of polymers prevents the degradation of drugs and also provides a system for controlled release of the drug. The level and extent of drug release are based on the drug-to-polymer ratio, the composition and molecular weight of the drug, and the polymer used (Alexis et al. 2008). The process of internalization and localization of NPs intracellularly is governed by the surface properties of the NPs. The advantages of polymeric nanoparticles are as follows: biodegradable polymers are used for the preparation of NPs, there is sustained and controlled release of the drug at the targeted site, the degradation of particles can be controlled by the constituents of the matrix, the encapsulation efficiency is high, bioactive compounds can be integrated into the delivery vehicle without any chemical reaction, and the vehicle can be used for different routes of administration including nasal, oral, parenteral, intraocular, etc.

In recent decades, various drug-linked polymeric nanoformulations have been fabricated for the treatment of cancer. PLA/PLGA and PCL are the most common polymers used as a carrier system. They are nontoxic, biodegradable, and biocompatible and have been used in medical sciences for more than 20 years. MTZ (mitoxantrone) is an antineoplastic agent which is shown to have activity against malignant glioma cell line but is unable to cross the BBB and causes adverse systemic side effects. Doxorubicin (DOX)-loaded PLGA microspheres (Yoo and Park 2001), cisplatin-loaded PLGA-mPEG nanoparticles (Gryparis et al. 2007), TX-loaded poly(lactic)-tocopheryl PEG succinate NPs (PLA-TPGS NPs), and poly(ethylene oxide)-modified polycaprolactone nanoparticles (PEO-PCL NPs) are some examples of polymeric nanoparticles used for cancer therapy. Yemisci prepared PLGA microspheres loaded with MTZ and studied on rat models. It was observed that MTZ-loaded microspheres efficiently delivered the bioactive compound to the site of the tumor, thereby preventing the proliferation of glioma cells without producing any side effect (Yemisci et al. 2006). Another polymeric nanocarrier, chitosan, is shown to be toxic against numerous tumorigenic cell lines both *in vivo* and *in vitro*. Chitosan possesses many remarkable properties like nontoxicity, biodegradability, and biocompatibility. Qi et al. formulated chitosan nanoparticles (CNPs), and the effect of CNPs was detected on BEL7402 (human hepatocellular carcinoma cell line). The results revealed that CNP acts as a promising candidate for treating hepatocellular carcinoma.

5.7.2 Gold Nanoparticles

Gold nanoparticles (AuNPs) enjoy a long history dating back to the Roman times, where they were used for staining glasses for decorative purposes. They have interesting physicochemical properties which make them ideal candidates for countless

biomedical applications ranging from biodiagnostics, cellular imaging, immunostaining, drug/DNA delivery, and biosensing (Khlebtsov and Dykman 2011; Zeng et al. 2011; Rudramurthy et al. 2016). Hans Heinrich Helcher published a comprehensive treatise on AuNP in 1718. It was indicated that the utilization of boiled starch in drinkable gold preparation prominently increased the stability. Faraday did pioneering work in terms of the synthesis of stable aqueous dispersions of AuNP. The preparation of colloidal AuNP was first described by him in 1857 (Link and El-Sayed 1999). He basically defined the formation of varied colored solutions that were prepared by bonding $AuCl_3$with sodium citrate. Unknown to him, the reaction produced AuNPs ranging from a size of 12–60 nm. In the 1950s, it was discovered that AuNPs could be attached to protein biologics without affecting the activity leading to their usage in histopathology and immunodiagnostics. It is not surprising that there are countless methods of gold nanoparticle synthesis of controllable size, monodispersity, and shape in the literature, which include synthesis both in the form of aqueous and organic solutions. The modification of gold nanoparticle surface has also garnered a lot of interest lately. The strong affinity of gold for thiol and amine groups has enabled the fabrication of gold nanoparticles with biomolecules like amino acids and proteins. AuNPs, when used in conjunction with specific surface moieties, can be taken up by cells. Their unique properties make them potential candidates for transporting and unloading pharmaceuticals, essentially because they are inert and nontoxic and can be easily synthesized.

AuNP can be functionalized with a bioactive compound and a targeting ligand that particularly identifies a specific receptor (active targeting) (Ghosh et al. 2008). For example, transferrin can be functionalized onto AuNP and can be used for targeting tumor cells that overexpress the transferrin receptor (Wang et al. 2013). They have been employed for the delivery of antitumor agents like tumor necrosis factor (TNF), through the enhanced permeation and retention (EPR) effect which is characteristic of tumor cells. On exposure to laser photoradiation, AuNPs with specific aspect ratios or compositions have the potential to produce local heat that can destroy the diseased tissues in case of solid tumors. AuNP was covalently bound to gemcitabine and cetuximab (active agents to treat pancreatic cancer). EGFR is overexpressed in 60% of pancreatic cancers, and the nanocomplex is under investigation under phase II clinical trial. The AuNP-gemcitabine-cetuximab combination was superior to any of the agents used alone. Thirty percent inhibition of tumor was observed when nonconjugated agents were used in combination, whereas >80% of inhibition was noticed when a low dose of complex gemcitabine was used (2 mg/kg). However, Jiang et al. (2008) reported that citrate-coated AuNPs were synthesized with numerous trastuzumab Ab to allow cross linking and targeting with human epidermal growth factor receptor (HER-2) in breast cancerous cells (SK-BR-3).

5.7.3 Liposomes

Artificial phospholipid vesicles ranging from 500 to 1000 nm in size can be used not only for the loading of a number of water-soluble drugs but also for water-insoluble drugs. The pH-sensitive liposomes are now being utilized to achieve the release of active compounds. Liposomes need to be derived from pH-sensitive compounds in order to become pH-sensitive. They get endocytosed in the intact form by fusing with the membrane because of the acidic pH of the endosome and then discharge their components in the cytoplasm. New findings are focused upon the construction of liposomes with lipid compositions that render them pH-sensitive and usage of various pH-sensitive polymers. The class of pH-sensitive liposomes that have been extensively studied offer a number of advantages like the prevention of damaging action of external media on the drugs because of being enclosed within the liposomes, biocompatibility, biological inertness, low toxicity, less antigenic reactions, selective accumulation in the target organ or tissue, increase in efficiency of the bioactive compound, and decrease in liposome loss in the reticuloendothelial system (RES).

Currently, there are many products which have been marketed, and some are undergoing preclinical and clinical trials for treating cancer. Doxil, a PEGylated liposome, was the foremost liposomal formulation accepted by the FDA for treating Kaposi's sarcoma in AIDS patients. It encapsulates doxorubicin, an anticancerous drug, and is commercialized by Johnson & Johnson. It is approved for treating ovarian and breast cancer. DaunoXome is another liposomal formulation of daunorubicin which is accepted by the FDA for treating Kaposi's sarcoma related to AIDS. CPX-351 is another liposomal formulation developed by Celator Pharmaceuticals which is made with a combination of daunorubicin and cytarabine (Lasic 1996). It presented favorable results in phase III clinical trial on subjects suffering from acute myeloid leukemia (AML). Lipoplatin is another formulation of cisplatin which is developed by Regulon Inc. and is in phase III clinical trial and is used for treating lung cancer.

5.7.4 Iron Oxide Nanoparticles

Magnetic nanoparticles comprise of a core of magnetic iron oxide (usually magnetite-[Fe_3O_4] or maghemite [γ-Fe_2O_3]) and a shell of polymers such as dextran, silica, or PVA or metals like gold onto which attachment of functional groups can be done using various cross-linkers (Sun et al. 2008). Recently cobalt/gold nanoparticles with the same core-shell strategy have been synthesized with the advantage of having a higher magnetic moment than magnetite or maghemite (Peng et al. 2008; Rahman et al. 2011; Espinosa et al. 2016; Kanagesan et al. 2016a, b).

Methotrexate (MTX) has both therapeutic effect in cancerous cells that overexpress folate receptor on their surface and targeting role. Kohler and colleagues synthesized iron oxide nanoparticles conjugated with MTX (MTX-IO NP) by amidation between amine group on the NP and carboxylic acid on MTX. The results presented that cancer cells internalized a high level of MTX-IO NP than the control cells (negative) (Kohler et al. 2006). Multifunctional polymeric micelle was developed by Nasongkla and colleagues and loaded with superparamagnetic iron oxide nanoparticles (SPIO NP) in the micelle at 6.7 w/w %. Doxorubicin was loaded inside the micelle at 2.7 w/w % which would release in a pH-dependent manner. The advantage of a multifunctional nanoparticle is the encapsulation of SPIO and DOXO NP in the micelle core that avoids the direct contact of hydrophobic SPIO surfaces and absorption of blood proteins, thereby reducing nonspecific uptake by the reticuloendothelial system (RES). Additionally, cRGD ligand (Arg-Glyc-Asp) was linked onto the surface of micelle by thiol-maleimide linkage so that it can target $\alpha_v\beta_3$integrins which are expressed on tumor epithelial cells. The targeted cells internalize the complex, and high concentration of DOXO is released in the cell nuclei (Nasongkla et al. 2006).

5.7.5 Dendrimers

Dendrimers are macromolecular compounds with an inner core with a series of branches and the shape of which can be altered as and when required. Convergent or divergent growth polymerization is used to fabricate these from AB-type monomers. The number of functional groups doubles or triples with every layer. The surface groups can be functionalized with the drug molecules, or they can be loaded in the interior of the core. Various types of polymers can be used like polyamidoamine (PAMAM), polyethyleneimine, polypropyleneimine, poly(L-glutamic acid) (PLGA), and polyethylene glycol (PEG). They have a nanometric size, their surface can be modified easily, and they can be easily prepared and cause amplification of response due the easy functionalization of their surface with different functional groups (Caminade and Turrin 2014). Moreover, a therapeutic nanodevice like PAMAM dendrimer is used as a drug delivery carrier system. Fractional acetylation of G_5 (generation 5) PAMAM dendrimer was done so as to neutralize a portion of primary amino functional groups, improve the solubility of PAMAM during the conjugation reaction of FITC (fluorescein isothiocyanate), and stop nonspecific targeting interactions. The remaining non-acetylated amino group was used for conjugating FITC, and folic acid targets overexpressed folate receptors on cancer cells and MTX (methotrexate, a chemotherapeutic drug). The nanodevice was utilized for targeted drug delivery to treat carcinoma (Baker 2009; Madaan et al. 2014).

5.7.6 Polymeric Micelle

These are constructed by the spontaneous compilation of block copolymers that have an amphiphilic character in hydrophilic media, and these micelles have a diameter of some nanometers. These consist of a structure called core-shell with the inner core which assists as a storehouse of hydrophobic drugs which is bounded by an outer shell of hydrophilic carriers. Their advantages include controlled drug release, easy preparation, and effective modification without affecting the drug (Nishiyama and Kataoka 2006). Kabanov et al. (1998) synthesized micelle-based carrier system by forming micelles which consisted of Pluronic P85 and murine polyclonal Ab which are targeted against α_2-glycoprotein for delivery of haloperidol (a neuroleptic drug). The polyclonal Ab was attached to the micelle using analog butylpoly(25)(oxypropylene)poly(20)(oxyethylene) ether of 2-hydroxyacetaldehyde (BPEA). For cancer therapy, monoclonal Ab can be conjugated to the micelle. EGFR, a transmembrane glycoprotein, is overexpressed on the cells of solid tumors, and it was targeted with mAb C225. The external domain of EGFR binds to Ab and is appropriate for micelles to target various cancer cells. Folate can also be conjugated to the micelle because the folate receptor is overexpressed in different cancers including breast, ovary, kidney, lung, and myeloid cell (Oerlemans et al. 2010; Alexander-Bryant et al. 2013).

5.7.7 Graphene Oxide Nanosheets

Graphene oxide is a sheet made by oxidation of graphite. The carbon layers in graphite are interspersed with oxygen molecules, and then reduced, to separate each of the carbon layers or a few layers of graphene. So, the oxidation and reduction processes lead to formation of graphene oxide. This leads to increase in interplanar spacing between the carbon layers. And then, the completely oxidized product is dispersed in a base solution to make graphene oxide. Graphene oxide is considered as a starting material to make various processable materials (superconductor, composite for antibacterial activity, supercapacitors, carrier system to deliver drugs, base material for future application of water purification). Graphene oxide, a carbon nanomaterial (CNT), has an enormous potential in medical (Depan et al. 2011; Premkumar and Geckeler 2012) and material (Lian et al. 2011; Shahil and Balandin 2012) science because of its unique physicochemical, mechanical, and optical property. The popularity of graphene oxide as a drug carrier (Liu et al. 2011), imaging probe (Markovic et al. 2011), and biomedical device is increasing nowadays. The properties that can be exploited for loading of poorly soluble drugs are π–π stacking, high surface area, and hydrophobic or electrostatic interactions of graphene without compromising efficiency and potency. Graphene and GO have many unique and advantageous properties and have turned out to be potential candidates for targeted, local, and systemic drug delivery systems (Liu et al. 2013).

In an experiment by Chen et al. (2011), GO was functionalized with PEI (cationic polymer), and then, it was utilized for drug delivery. PEI is a non-viral gene vector and has an affinity for phosphates present in DNA or RNA, and this polymer can be modified chemically. The PEI modification resulted in enhanced cell selectivity, transfection efficacy, and decrease in cell toxicity. But, the limitation of biocompatibility still persists in its biomedical application (Li et al. 2013). GO functionalized with PEI (PEI-GO) was used for the delivery of gene, and it was noticed that cytotoxicity of PEI-GO was less than PEI alone. PEI-GO facilitated in achieving high transfection of drug gene (Chen et al. 2011). In a recent study, graphene foams are used as 3D porous scaffolds in neural stem cell cultures (NSCs). The porous scaffolds have an ability of stimulating NSCs electrically and help in proliferation and differentiation of neural stem cells. Graphene oxide can be utilized as a nanocarrier for delivery of anticancer drugs to a target site. For example, doxorubicin, an anticancer drug, is adsorbed on the surface of GO sheet by hydrogen bonding and π–π stacking, thus suggesting that graphene oxide can be used as a nanocarrier. There is an urgent need to assess the toxicity of graphene oxide, since the risk evaluation of graphene oxide is lacking. Recently, it was found that graphene causes low toxicity in macrophages (Li et al. 2012), stem cells (Akhavan et al. 2012), and lung cells (Vallabani et al. 2011). Toxicity in adenocarcinoma cells was observed, using A549 cell line (Chang et al. 2011). Toxicity in fibrosarcoma cells was also observed using L929 cells. *Ginkgo biloba*-graphene oxide nanocomposite (Gb-rGO) was synthesized and tested for the biocompatibility of GO, and Gb-rGO was evaluated on human breast cancer cells by various assays like apoptosis, cell viability, and alkaline phosphatase assay. The results exhibited that the synthesized Gb-rGO is cytocompatible to the cells. Further, the nanocomplex can be used in different biomedical applications like drug delivery, imaging, biosensing, and tissue engineering (Gurunathan et al. 2014).

5.8 Conclusions and Future Prospects

Synthetically derived anticancer drugs used for the conventional approaches such as chemotherapy have many limitations because they exhibit a toxic effect on the noncancerous cells in the human body which causes health-related problems. Thus, there is a need for other therapeutic approaches that can treat cancer effectively with minimal side effects. The anticancer agents derived from natural products are the desired approach for nontoxic treatment of cancerous cells. The secondary derivatives of plant extracts like flavonoids, polyphenols, etc. were broadly explored for their potential characteristics to treat cancer. Some of them showed valuable anticancer properties like antioxidant activity, induction of apoptosis, cytotoxicity to cancer cells, anti-inflammatory properties, target specificity, etc. Naturally derived drugs were generated and progressed to clinical examination for further studies about their secondary metabolites, stability, and long-run procedures. The natural products derived from plants have the ability to decrease cancer. These may show

anticancer activity in various animal models of skin cancer, leukemia, and sarcomas. The uses of nanoparticles have increased exponentially over the past few years for cancer therapy and drug delivery. In the future, more researches are desired for the development of sophisticated and novel applications that may result in regression of tumor, early detection, and effective response to chemotherapy. Moreover, it also has new perspectives for efficient and conventional formulations of drugs because of instability or poor bioavailability.

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Chapter 6
Applications of Nano-based Novel Drug Delivery Systems in Herbal Medicine-Mediated Cancer Therapy

Saumya Srivastava and Anjana Pandey

Abstract Nanotechnology is a fast-growing field with numerous applications in the field of medical science. One such application comprises nanoparticle biosynthesis from plant extracts and their compounds with their potential applications in cancer therapy. These plant-based nanoparticles have been observed to be effective against various types of cancerous cells both *in vitro* and animal models. Another application of nanotechnology is the herbal therapeutics comprising the use of novel nano-based drug delivery systems in the treatment of cancer. These novel drug delivery systems aid in increasing the therapeutic value and bioavailability of the herbal medicine. The application of nanoherbal formulations as novel drug delivery systems (NDDS) is more valuable as compared to other therapies. These novel drug delivery systems include phytosomes, liposomes, microsphere, nanocapsules, ethosomes, transfersomes, nanoemulsions, and polymeric nanoparticles. The effectiveness of these different plant-based nanodrug delivery systems has been studied against various cancer types. These alternative drug delivery systems help in increasing the efficiency of a drug delivery and safeguard the drug from metabolic processes alongside its sustained delivery, proper distribution, and protection from physical and chemical deterioration. In addition, they reduce the possible side effects of the drugs. In spite of the advancements, cancer endures to be a predominant and fatal disease. This has led to the increased use of nano-based anticancer drugs and their delivery systems, also known as nanotherapies against tumors due to their ability of site-specific targeting and multifunctionality. In this chapter, recent advancements in application of plant-based nanomaterials in cancer therapy and impending strategies are discussed.

Keywords Drug delivery systems · Ethosomes · Nanocapsules · Nanoemulsions · Nanotechnology

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6.1 Introduction

Cancer is characterized by a condition when there is an uncontrolled growth of cells, having the capacity to invade and spread to distant body parts. The World Health Organization (WHO) has reported that among the main determinants of morbidity and mortality throughout the world, cancer is a major cause and it is projected to increase by 70% in the next two decades. The worldwide deaths estimated due to cancer till 2012 are 8.2 million with lung cancer topping the list, followed by liver, stomach, colorectal, and breast cancers (WHO 2015). Natural products from plant resources are being used in the treatment of different disorders in Egypt, China, India, and Greece from ancient times, and numerous drugs have been developed from medicinal plants (Arumugam et al. 2016; Swamy et al. 2016, 2017; Swamy and Sinniah 2016; Mohanty et al. 2017). The first documented proofs on the uses of medicinal plants date back to 2600 BC. Researchers throughout the world are focusing on use of herbal medicines to modulate immune system against cancer. Thorough understanding of the synergistic interactions of different constituents of herbs possessing anticancer activity and novel formulations can be useful to kill the cancerous cells without any other cellular toxicity. The very first phytochemicals that are being used clinically include vinca alkaloids, vinblastine, and vincristine from the Madagascar periwinkle, *Catharanthus roseus* (Apocynaceae). The isolation and identification of paclitaxel from the bark of Pacific yew, *Taxus brevifolia* Nutt. (Taxaceae), also represent the successful application of a natural product in the field of drug discovery. Epipodophyllotoxin, an isomer of podophyllotoxin, has been isolated and is reported to have antitumor activity. Etoposide and teniposide are another two semisynthetic derivatives of epipodophyllotoxin which are being utilized in the treatment of lymphomas and bronchial and testicular cancers. *Bleekeri avitensis* A.C. Sm., a medicinal plant of Fijian origin, is being used in France for treatment of breast cancer (Cragg and Newman 2005). Similarly *Allium sativum* consists of more than 100 biologically active secondary metabolites including alliinase, alliin, S-allyl cysteine (SAC), allicin, diallyl trisulfide (DATS), diallyl disulfide (DADS), and methyl allyl trisulfide. *Aloe vera* contains aloe emodin, a compound reported to activate the macrophages against cancer. Silvestrol isolated from the fruits of *Aglaia silvestris* (M. Roemer) Merrill. (Meliaceae) is reported to exhibit cytotoxicity against lung and breast cancer cell lines (Cragg and Newman 2005). Acetogenins isolated from *Annona* species possess significant anticancer activity against leukemia and sarcoma. *Arctium lappa* consists of various potent anticancer agents and is being used in the treatment of malignant melanoma, lymphoma, and cancers of the breast, pancreas, esophagus, ovary, bile duct bladder, and bone (Shabani 2016).

Different alkaloids and flavonoids isolated from numerous medicinal plants exhibit cytotoxic efficacy against different types of cancerous cells both *in vitro* and *in vivo* (Mohanty et al. 2014, 2017; Swamy and Sinniah 2015). One of the modes of

action of these compounds includes inhibiting cancerous cell growth. Nanotechnology has helped in the development of new dosage forms in medicine. Till now, researchers have majorly concentrated on the use of metallic nanoparticles due to their rapid actions. Metallic nanoparticles possess unique physical and chemical properties due to their size leading to diverse biomedical applications. Silver, gold, iron oxide, zinc oxide, aluminum oxide, and copper oxide are some of the major metallic nanoparticles synthesized (Rudramurthy et al. 2016; Kanagesan et al. 2016a, b).

AgNPs synthesized using *Premna serratifolia* leaves exhibited noteworthy anticancer activity in carbon tetrachloride (CCl_4)-induced liver cancer in a study performed on Swiss albino (BALB/c) mice. In another study by Sre et al. (2015), the cytotoxic activity of biologically synthesized AgNPs using *Erythrina indica* on MCF-7 (breast cancer) cells and HepG2 (hepatocellular carcinoma) cells was reported. The *in vivo* and *in vitro* cytotoxic effects of AuNPs have also been stated in numerous studies, exhibiting the anticancer properties of AuNPs by the induction of oxidative stress. AuNPs synthesized using *Gymnema sylvestre* leaf extracts have also been investigated for their anticancer activity against hepatocellular carcinoma (HepG2) cell lines at concentrations of 250 μg/ml (Rao et al. 2016). Iron oxide nanoparticles have been observed for their antitumor activity both directly and indirectly via nontoxic wavelength radiation, which gets readily absorbed by toxic stimuli of reactive oxygen species production. The synthesis of iron oxide (Fe_3O_4) nanoparticles from seaweed (*Sargassum muticum*) has also been stated recently. Titanium oxide is another inorganic nanoparticle which can be surface-modified for inhibition of tumor cell growth. The advancements in nanotechnological approaches have the capability to deliver a solution to limitations faced by other dosage forms. It can be surface-engineered to inhibit tumor growth. The innovative nanotechnological approaches may provide a solution to limitations faced by many of phytochemical, physicochemical, and pharmacokinetic properties (Rao et al. 2016). This chapter discusses on the role of nano-based formulations and their applications in the field of herbal medicine and applications of nanotechnology in designing novel drug delivery systems containing bioactive compounds from the plant origin.

6.2 Hallmarks of Cancer

Cancer reconceptualization has come due to research advancements that have been made in the past decades at the molecular, cellular, and biochemical levels. Some of the specific traits which are acquired by the cancer cells result in the complexity of treating this disease. These features include self-reliance in growth signals, antigrowth signal insensitivity, unlimited replication capacity, persistent angiogenesis, avoidance to apoptosis, invasion, and metastatic potential (Fig. 6.1). These characters are shared by almost all types of cancer (Flavahan et al. 2017). Several growth

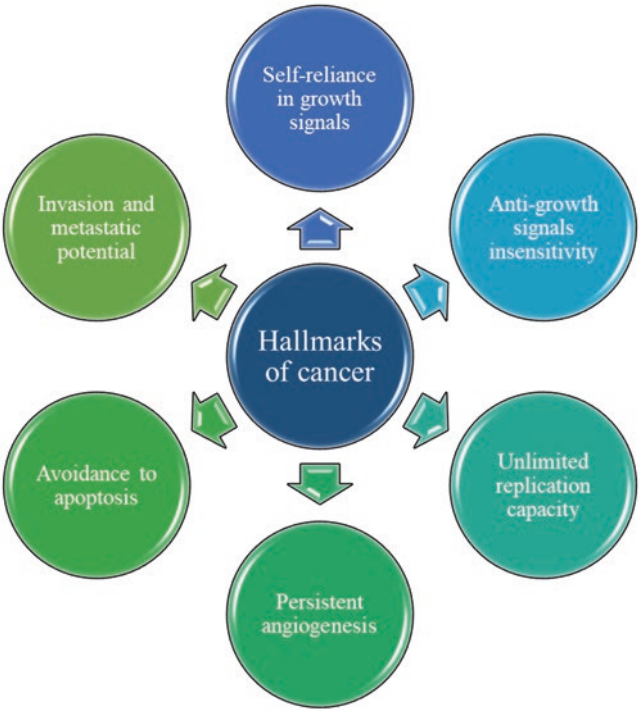

Fig. 6.1 Diagrammatic representation of various cancer hallmarks

and anti-growth signals regulate the cell cycle carefully in normal cells. Cancer cells disrupt these regulatory mechanisms and impart their own proliferative signals. Growth factors are supplied to the cancer cells via either growth factor ligands produced by themselves or by stimulation of the normal tumor-associated stromal cells which supply growth factors necessary for the cancer cells in return (Bhowmick et al. 2004). The cancer cells have the property to evade the growth suppressors in order to have prolonged growth-promoting factors. Various tumor suppressor genes have been investigated and certified, out of which two proteins, namely, RB (retinoblastoma-associated) and TP53 (tumor protein p53), play crucial roles in cell senescence and apoptosis. The cellular signals from varied extracellular ligands incorporated by RB protein decides the fate of a cell, i.e., whether a cell should proceed in the cell cycle or not (Burkhart and Sage 2008; Hanahan and Weinberg 2011; Flavahan et al. 2017). TP53 gene can stop the cell cycle in the presence of abnormal signals until the reappearance of normal conditions or otherwise in conditions of a permanent damage to DNA and apoptosis induction (Evan and Littlewood 1998; Xia et al. 2017).

Cancer cells have the ability to escape the natural cell barrier to cancer growth, i.e., apoptosis. Different members of Bcl-2 family molecules regulate apoptosis.

Cancer cells have different mechanisms to avoid apoptosis including damage to TP53 function, enhancement in anti-apoptotic factor expression, and decreased expression of pro-apoptotic factors (Adams and Cory 2007; Dzutsev et al. 2017). Due to senescence and crisis phase, normal cells undergo a limited number of cell cycle, but these obstacles can be bypassed by cancer cells resulting in the immortalization and infinite potential of replication. At the end of the chromosomes, telomeres are present that prevent the end-to-end fusion of normal cell DNA which can frighten the cell viability. The length of telomeric DNA is also regulated, but in the cancer cells, telomerase (a DNA polymerase enzyme) responsible for addition of the telomere at the DNA ends and prevents the telomere erosion (Dagg et al. 2017).

Cancer cells are capable of developing new blood vessels by activating an "angiogenic switch." Pro- and anti-angiogenic members regulate this process (Folkman and Shing 1992). Angiogenesis can be inhibited by TSP-1 (thrombospondin-1), endostatin, and angiostatin; these are natural barriers. Tumor progression in mice has been seen after deleting the genes encoding these inhibitors (Nyberg et al. 2005). Tumor cells invade and metastasize by establishing the communication between tumor cells, tumor microenvironment, and stromal cells (Ost et al. 2015). Cancer cells also require the nutrients for growth. Recent discoveries have investigated that the cancer cells also have the capability to reprogram the cell's metabolism in order to get the fuel (van der Heiden and De Berardinis 2017). Potential of cancer cells to evade the immune system has also been reported (Flavahan et al. 2017). These features provide the basic stage for most of the cancer research advancement and are helpful in revealing the cancer concepts.

The worldwide load of cancer augmented to approximately 14 million new cases per year in 2012, which is further expected to increase at a rate of 22 million annually in the next two decades. It is also projected that cancer deaths are going to increase from 8.2 to 13 million annually. Worldwide, most common types of cancers diagnosed in 2012 were lung (1.8 million cases, 13.0% of the total), breast (1.7 million, 11.9%), and large bowel (1.4 million, 9.7%). The cancers most commonly responsible for deaths include lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%) (GLOBOCAN 2012). In this regard, phytomedicines have been playing as important source of pharmacologically active compounds since ancient times, and their usage has increased owing to their therapeutic activity and lower effects as compared to other drugs. Nanotechnology can be employed to improve targeted delivery of drugs, delivery of poorly water-soluble herbal drugs, co-delivery of two or more drugs, release of large herbal molecules, and observation of sites of drug delivery by incorporating herbal drugs with imaging modalities (Liong et al. 2008; Lambert 2010; Gunasekaran et al. 2014). Researchers in the fields of herbal medicine and nanomedicine have observed that therapeutic nanoparticles (NPs) are more effective drug delivery system as compared to traditional drug delivery systems.

6.3 Role of Plant-Based Remedies and Herbal Medicine in Cancer Treatment

Herbal products, specifically medicinal plants, are being utilized in the treatment of various diseases since thousands of years. Terrestrial plants are being employed as medicines in India, China, Egypt, and Greece from thousands of years, and a large number of modern chemo drugs against cancers have been discovered and synthesized from them. The first compounds/agents taken into clinical use for different types of cancer treatment including lymphomas, leukemia, lung and breast cancers, advanced testicular cancer, etc. include vinca alkaloids (vinblastine and vincristine) isolated from *C. roseus* which ascertained a new era for utilization of plant materials as anticancer drugs. The revelation of the compound paclitaxel isolated from barks of the Pacific yew, *T. brevifolia* (Taxaceae), also narrates a success story in natural product drug discovery (Cragg and Newman 2005; Stathis et al. 2017). *Taxus baccata* is also reported for its utilization in the Indian system Ayurvedic medicine for treatment of cancer. Paclitaxel is expressively active against advanced breast and small and non-small cell lung cancer. Irinotecan and topotecan are two semisynthetic derivatives synthesized from camptothecin, which are being used in the treatment of ovarian and small cell lung cancer and colorectal cancer, respectively (Bhokare et al. 2016; Stathis et al. 2017). Epipodophyllotoxin, an isomer of podophyllotoxin, is isolated as an antitumor compound from the roots of *Podophyllum peltatum* and *P. emodi* (Berberidaceae). Etoposide and teniposide, two semisynthetic derivatives of epipodophyllotoxin, are being utilized in the treatment of bronchial and testicular cancers and lymphomas (Shabani 2016). Homoharringtonine, isolated from the Chinese tree *Cephalotaxus harringtonia* (Cephalotaxaceae), is also a plant-derived agent take into clinical use (Itokaw and Wang 2005). Combretastatins, isolated from the bark of *Combretum caffrum* (Combretaceae), is active against leukemia and colon and lung cancers (Ohsumi et al. 1998; Sherbet 2017).

6.4 Nanotechnology and Nanomedicine

Technology at the nanoscale is playing a crucial role to unfold solutions for many biological problems that were rigid a few years ago. In recent times, silicon chip technology has helped to manipulate the atoms and molecules resulting in revolution in the field of life sciences. Nanotechnology and nanomedicine are regarded as two complementary disciplines, targeting for betterment of life. Applications of nanotechnology in medicine consist of both materials and devices, which can be designed to interact with living systems at molecular scale with high specificity. This eventually results in their potential applications in targeted cellular- and tissue-specific uses and thus helps in acquiring higher therapeutic efficacy (Gunasekaran et al. 2014; Gortzi et al. 2008). Hence, in the existing situation, nanotechnology is

beneficial in solving problems in different fields of medical science (Bhati-Kushwaha and Malik 2017). Different types of nanomaterials used in therapeutic applications are explained below.

6.4.1 Polymer Nanoparticles

Polymer nanoparticles are colloidal and solid particles having their size in the range of 10–1000 nm. These are also called as nanocapsules and nanospheres. Polymeric nanoparticles are synthesized by using preformed polymers or by polymerization of monomer units. For this purpose, different methods like salting out, solvent evaporation, supercritical fluid evaporation, rapid expansion of supercritical solution, and dialysis are being employed (Ma et al. 2017). The selection of preparation method is done on the basis of numerous factors including the area of application, type of polymeric system, size obligation, etc. The polymeric nanoparticles, prepared by these techniques, have been observed for their efficient therapeutic activities.

Lectins are known to bind carbohydrate moieties. This lectin-carbohydrate interaction is highly specific and can be utilized in development of NPs directed to certain lectins (De Mejia and Prisecaru 2005; Sharon 2007). Various lectins have been observed to exhibit anticancer activities *in vitro*, *in vivo*, and also in human case studies and are being used as therapeutic agents, for inhibition of tumor growth (Parveen and Sahoo 2008). Likewise, Tsutsui et al. (2007) developed a drug delivery system capable of targeting brain tumors using bionanocapsules (BNCs). In their study, they substituted the pre-S1 peptide with the antibody affinity motif of protein A and prepared hybrid BNCs conjugated with antihuman EGFR antibody recognizing EGFRVIII. The results exhibited that the hybrid BNCs were efficiently delivered to glioma cells and not to the normal glial cells. PLG [Poly(lactide-co-glycolide)] and its monomers have been found to have potential in wide applications for encapsulation and delivery of various anticancer drugs. In another study, Zhang and Feng (2006) analyzed the drug encapsulation efficiency, *in vitro* drug release, cellular uptake, and cytotoxicity of TX-loaded poly(lactide)-tocopheryl PEG succinate NPs. The drug encapsulation efficiency and *in vitro* drug release profile of this drug were analyzed using HPLC and the cancer cell lines HT-29 and Caco-2 (Zhang and Feng 2006; Xiong et al. 2017).

6.4.2 Metallic Nanoparticles

Metallic nanoparticles are nanosized metals with size ranging from 1 to 100 nm. There are numerous liquid phase approaches for synthesis of metallic nanoparticles, including chemical reduction, reverse micelle and sol gel, etc. Novel metallic nanoparticles having spherical shape and size were synthesized by different chemical reduction methods (Schwarz et al. 2004; Wang et al. 2008). MNPs have a wide

range of applications owing to distinctive features including large surface area and process a large number of low coordination sites and electronic structure between molecular and metallic states. MNPs are being used in drug delivery, magnetic separation of labeled cells and other biological entities, and also as agents for contrast enhancement in magnetic resonance imaging.

Gold-silver (Au-Ag) nanorods conjugated with molecular aptamers were shown to need up to six times lower laser power irradiation to induce cell death compared to Au nanoshells or Au nanorods. These aptamer Scg8-AuAg nanorod conjugates exhibited exceptional hyperthermia efficiency and selectivity toward CEM cells, larger than the affinity of aptamer probes alone. Bimetallic Au-Ag nanostructures having dendrite morphology have been examined to destroy A549 lung cancer cells. The photothermal activity of these dendrites needed lower NP concentrations and laser power for efficient damage to the cancer cells. AuNPs have been reported for their application as vehicles for the delivery of different anticancer agents, including paclitaxel. The hydrophobic drug administration necessitates molecular encapsulation, and the efficiency of nanosized particles in evading the reticuloendothelial system is well studied (Conde et al. 2012; Hnawate and Deore 2017).

6.4.3 Magnetic Nanoparticles

Magnetic nanoparticles have been produced with numerous different phases and compositions including both pure metals and metal alloys (Schwarz et al. 2004). A number of methods have been reported including sonochemistry, coprecipitation, solvothermal, combustion synthesis, colloidal method, hydrothermal method, thermal decomposition, and microemulsion methods (Iwaki et al. 2003). The key applications of magnetic NPs are in the field of bioseparation processes, in which conjugation of the target biomolecules with magnetic nanoparticles, functionalized with specific receptors, produces complexes and hence can be easily separated using applied magnetic field, thus providing a suitable and time-saving method for bioseparation (Kharisov et al. 2014).

6.4.4 Nanomedicine in Therapeutics

During the past decades, much consideration has been paid to the enhancement of new novel drug delivery systems (NDDS) for herbal medicines. Plant-based unique drug delivery system has advantages over traditional drug delivery systems for delivery of herbal drug at optimal rate and targeted drug delivery, thus minimizing toxicity and enhancing bioavailability of the drug. In these drug delivery systems, the dissemination control of drug is attained by loading the drug in a carrier (Biju

et al. 2006). Herbal compounds have become more popular with time due to their extensive applications in the treatment of a variety of diseases with lower toxicity and enhanced therapeutic effects (Atmakuri and Dathi 2010). But herbal medicines possess some short comings including, instability at acidic pH and metabolism occurring in lever resulting in lower levels of drug in blood. These constrains can be overcome by utilization of nano based novel drug delivery systems (Uhumwangho and Okor 2005). Amalgamation of novel drug delivery technology with herbal compounds reduces the drug deterioration rates, metabolism, and also side effects due to drug accumulation. Numerous NDDS including microspheres, liposomes, and phytosomes are being studied for the delivery of herbal drugs. Combination of herbal compounds in these delivery systems also assists in the upsurge in solubility, improved stability, reduced toxicity, greater biological activity, enhanced distribution, sustained drug delivery, and also guarding from degradation in metabolic processes. One such example is the use of liposomes in delivery of anticancer compounds by increasing the drug concentration in tumor-affected area and lowering the drug exposure in normal cells and tissues, hence checking toxicity effects (Yadav et al. 2011).

Nanotechnology has a wide range of applications in the development of new drug delivery systems which can overcome the drawbacks of conventional drug delivery systems including immediate requirement for drugs with improved therapeutic efficacy against CSCs (cancer stem cells). Nanoparticle (NP)-based carriers (nanocarriers) including micelles, liposomes, polymeric nanoparticles, dendrimers, etc. are being preferred as drug delivery systems. Though various anticancer agents have been identified, most of these have limitations when rendered to clinical studies such as off-target effects, low pharmacokinetics, a hydrophobic nature, inconsistent stability, and lower distribution of the drug. Nanotechnology can help to overcome these limitations. In the past years, researchers have concentrated on developing a single nanoformulation capable to carry dual drugs (one specific against CSCs and a second against bulk tumor cells) for their delivery to the target site through active or passive targeting (Singh et al. 2017).

6.5 Novel Drug Delivery Systems for Herbal Drugs

Before entering in the blood stream, the phytoconstituents of the herbal drugs face highly acidic pH of the stomach, and some of them also get metabolized in the liver, due to which the optimum quantity of the biologically active compounds does not reach the blood leading to lower or negligible therapeutic effects of the drug. Use of nanocarriers with herbal drugs carry optimal quantity of the drug to the action site, detouring barriers including acidic pH and metabolism in the liver, and upsurge the sustained drug circulation in the blood (Kuntal et al. 2005). Some of the types of novel herbal drug delivery systems are discussed below.

6.5.1 Phytosome

Phytosomes are phospholipid-based NDDS, which have been observed to be favorable for herbal drug delivery. Mixing the phytoconstituents at specific molar ratios with phosphatidylcholine leads to synthesis of phytosomes. It is the phytolipid-based drug delivery system which bridges the conventional and novel delivery systems. The word phytosome narrates to phyto (plant), whereas some represents cell-like. Phytosomes are unconventional types of herbal preparations with better absorption characteristics, as compared to conventional herbal compounds. Phytosomes also exhibit better therapeutic and pharmacokinetic profiles as compared to conventional extracts obtained from medicinal plants. Phytosomes can be synthesized by mixing the polyphenolic phytoconstituents at specific ratios with phosphatidylcholine. The phytosomal analysis has been focused on *Silybum marianum*, which consists of liver-protective flavonoids. The fruit of the *S. marianum* plant (family, Asteraceae) consists of flavonoids exhibiting hepatoprotective activities (Atmakuri and Dathi 2010). The phytosome plays a role in protecting phytoconstituents from degradation in digestive systems and gut microorganisms by synthesizing little compartment capable of being transferred to the lipid-friendly surroundings of the enterocyte cell membranes and then entering the blood stream (Sharma 2014).

Phytosomes can be identified as complex between a natural product and natural phospholipids. This complex can be prepared by reactions of stoichiometric quantities of substrate and phospholipids in a suitable solvent. It has been observed that the phospholipid substrate interaction can be attributed to the formation of hydrogen bonds between the polar head of phospholipids and the polar functional groups present on substrate using spectroscopic techniques. In aqueous environments, phytosomes acquire micellar liposomal-like structures. In phytosomes the active compound is attached to the polar head of phospholipids, thus becoming an integral part of the membrane (Kumar et al. 2017).

6.5.2 Liposomes

Liposomes are regarded as concentric and bilayered compartments where the aqueous phase is surrounded by a lipid bilayer membrane majorly consisting natural or/and synthetic phospholipids. The liposomes are sphere-shaped particles which encapsulate the drugs in the interior. Liposomes are synthesized using phospholipids, having hydrophilic polar head and hydrophobic tail (Ju Qun and Guo 2007). The polar end comprises of phosphoric atom bound to a hydrophilic molecule. Liposomes have the capability to encapsulate both hydrophilic and lipophilic compounds. Liposome exhibits properties which make them capable to augment the bioavailability, bio-distribution, ingredient solubility, altered pharmacokinetics, and both *in vitro* and *in vivo* stability. Liposome-based NDDS can increase the therapeutic efficacy of herbal compounds (Saraf 2010).

Paulis et al. (2012) observed that liposomes having size of 100 nm were capable to move out of the vasculature (extravasation) slowly and were slower to go to the tissue and release the active constituents over a period of time resulting in controlled and sustained release of the drug and also helped in maintaining the higher drug concentrations over a longer period of time being suitable in treatment of myocardial infarction and also to enhance the cardioprotection in a rat model of ischemia/reperfusion (IR) injury. The lipophilic interior of the liposome helps in the inclusion of drugs having lipophilic nature. Liposomes also help in improving the bioavailability of the drug as compared to conventional drug delivery systems (Geldenhuys et al. 2017).

6.5.3 Nanoparticles

Nanoparticles are nanosized structures comprising of semisynthetic or polymers. Recently, nanoparticles for herbal compounds have engrossed much consideration. Nanoparticles represent colloidal systems having particles of size distribution from 10 to 1000 nm. It is regarded as an efficient system as the compound is encapsulated in the nanoparticle and gets delivered at site of action easily. The nanospheres are spherical particulates with solid core having dimensions in nanosize. Nanospheres consist of herbal compounds implanted in the matrix or engrossed onto the surface, whereas nanocapsules exhibit vesicular system, where the herbal drug is encompassed inside (Vyas and Khar 2002). The nanoparticulate system is advantageous due to its increased solubility as compared to traditional drug delivery systems. Microencapsulation of herbal drugs in nanoparticles is an efficient way employed for the protection of drug or food ingredients from degradation, premature interaction, and volatile losses. The advantages of the nanoparticle include improvement in absorption of the herbal compounds, reduction in dosage formulation, and increase in solubility (Prabhu et al. 2010).

The nanoparticles can be classified into two categories, viz., hard and soft, dependent on their physicochemical characteristics and the method by which the drug is loaded into the nanoparticles. Hard NPs can be classified as materials on which the drug can be loaded only on the surface through passive adsorption or covalent attachment. Examples of hard nanoparticles include quantum dots, silver (Ag) NPs, gold (Au) NPs, and metal oxide NPs. Soft nanoparticles are the materials in which the drug can be loaded into the central core. The examples of soft NPs include liposomes, micelles, and polymeric NPs such as poly(D,L lactic-co-glycolic acid) (PLGA) (Sangtani et al. 2017).

6.5.4 Emulsions

Emulsions are biphasic systems where one phase is dispersed into the other as minute droplets with diameter ranging between 0.1 and 100 μm. In emulsion-based drug delivery system, the drug is distributed properly due to lymph affinity.

Microemulsions can be defined as solutions consisting of nanosized droplets of an insoluble liquid disseminated in an aqueous buffer where the droplets are covered with a surfactant for reducing the surface tension among both the phases. Emulsions result in targeted sustained release and also enhance the permeability of drugs into the mucous and skin (Cui et al. 2009).

Microemulsions have vast range of applications both in drug targeting and controlled release of the drug (Garg and Goyal 2012). The major problem faced in the delivery of oil-soluble drugs through oral route is its low aqueous solubility. Microemulsions exhibit imitable capability to evade problems related to solubility (Goyal et al. 2013). Microemulsions can encapsulate the drugs having variable solubility due to the presence of polar, nonpolar, and interfacial domains present in them. Microemulsions safeguard the amalgamated drugs from both oxidative and enzymatic degradation. Microemulsion formulations consisting of cyclosporin A, ritonavir, and saquinavir are available commercially (Fricker et al. 2010). Microemulsions help in reducing drug dosage and also enhance the bioavailability (Goyal et al. 2014). Betamethasone dipropionate exhibits antiproliferative, immunomodulatory, and anti-inflammatory activity (Kaur et al. 2016a, b). The addition of corticosteroids and keratolytic agents in microemulsions results in an improved and sustained corticosteroid delivery rate, resulting in superior antipsoriatic activity. Use of hydrogels as immunotherapeutic agents has abundant potential for refining the efficacy of vaccines and immunotherapeutics in disease treatment (Kaur et al. 2016a, b).

6.5.5 Microsphere

Microspheres consist of fine particles, having diameters ranging between few micrometers. Microspheres are also regarded as microparticles. Microspheres can be prepared using numerous synthetic and natural materials. Microspheres can be either biodegradable or non-biodegradable. Examples of biodegradable microspheres include altered starch microspheres, albumin microspheres, gelatin microspheres, polypropylene dextran microspheres, and polylactic acid microspheres. Solid and hollow microspheres have large variations in their densities and hence different applications (Scarfato et al. 2008). The polymers utilized in synthesis of microspheres can be either biodegradable or non-biodegradable. Different polymers are being used in fabrication of these carriers, including gelatin, albumin, polypropylene, modified starch, polylactic acid, dextran, polylactide, etc. (Burgess and Hickey 2009). The drug release in this type of delivery system is regulated by the dissolution and degradation of encapsulating matrix.

Numerous methods, including evaporation and ionic cross-linking techniques, are being used (Das and Senapati 2008) in preparation of mucoadhesive, buoyant microspheres. These microparticle systems have advantages as compared to other delivery systems as they can be ingested or injected and help in sustained release

action and site-specific delivery. Gastroretentive floating microspheres of silymarin have been studied for sustained drug delivery (Garg and Gupta 2010). Microspheres containing turmeric oleoresin have been prepared using a spray-drying technique. The stable emulsion safeguarded the resin from degradation by different conditions and chemical agent, and it also enhanced the therapeutic effect of the drug (Kshirsagar et al. 2009). Targeted delivery of rutin (Xiao et al. 2008) from formulated microspheres (rutin alginate-chitosan) was observed through its targeting on cardiovascular and cerebrovascular regions. Oxidized cellulose microspheres incorporated with camptothecin (Chao et al. 2010) were synthesized by a spray-drying process and have been effectively utilized to enhance the solubility and cytotoxicity of camptothecin.

6.5.6 Ethosome

Ethosomes are phospholipid-based pliable nanovesicles with higher content of ethanol (20–45%). Ethanol is observed to be an effectual permeation enhancer. Ethosomes are prepared as unique lipid carriers comprising of phospholipids, ethanol, and water. Ethosomes enable the herbal drugs to reach deeper skin layers and/or systemic circulation. Owing to higher amounts of ethanol, the lipid membrane is packed less compactly as compared to conventional vesicles, with comparable stability (Touitou and Godin 2000). Transdermal drug delivery systems assist the movement of therapeutic drugs through the skin and into the general circulation for systemic effects (Shaik et al. 2011). It is observed that herbal drugs can be employed with superior efficacy by incorporating them into transdermal drug patches alongside penetration enhancers such as terpenes (Rathva et al. 2012). The commonly available transdermal drug delivery patches are the nicotine patches to help people quit smoking (Jatav et al. 2011).

6.5.7 Solid Lipid Nanoparticles

Solid lipid nanoparticles were first developed in the 1990s. These are specifically used in the delivery of lipophilic herbal drugs. Solid lipid nanoparticles can be prepared by various methods such as homogenization and warm microemulsion. The typical size of solid lipid nanoparticles lies between 50 and 1000 nm. Solid lipid nanoparticles comprise lipid matrix, which solidifies at room and body temperatures (Pople and Singh 2006). The main advantages of solid lipid nanoparticles (SLNs) as compared to conventional drugs include exceptional physical stability and protection of assimilated drugs from deterioration (Kakkar et al. 2010). Various types of nanodrug delivery systems that have been synthesized for delivering plant-based anticancer drugs are tabulated in the Table 6.1.

Table 6.1 Different types of nanodrug delivery systems synthesized for delivering plant-based anticancer compounds

Drug carrier	Active ingredients	Biological activity	References
Phytosome	Quercetin	Quercetin is a dietary flavonol which shows poor absorption after oral administration. To increase its bioavailabilty, quercetin-loaded phytosome nanoparticles (QP) have been prepared, and its encapsulation efficiency was found to increase. Treatment of ovariectomized model with QP has shown increased level of serum calcium and glutathione as well as improved lipid profile. Therefore, QP emerged as a promising therapy for hormonal replacement	El-Fattah et al. (2017)
	Silybin	In this study, the effect of silybin on the accumulation and metabolism of lipid and oxidative stress was examined on liver steatotic cells by incubating it with phytosome and silybin complex with vitamin E. And, it has been shown that fat accumulation as well as oxidative imbalance was decreased	Vecchione et al. (2016)
	Naringenin	Complex of naringenin-phospholipid has been investigated for its antioxidant activity in carbon tetrachloride-induced rats. After performing the liver function test, naringenin-phospholipid complex was found to have better antioxidant capacity than free form of naringenin; it also enhanced the antioxidant activity of biomolecules for liver protection	Semalty et al. (2010)
Liposome	*Myrtus communis*	In this study, antibacterial activity of *Myrtus communis* has been investigated against several bacterial strains as food preservatives	Bouyahya et al. (2016)
	Diospyrin	Diospyrin is a plant product, i.e., bisnaphthoquinonoid. It shows antitumor activity *in vivo* and cancer cell lines of human *in vitro*. But due to some toxicity, it was encapsulated in lysosome to reduce the toxicity	Hazra et al. (2005)

(continued)

Table 6.1 (continued)

Drug carrier	Active ingredients	Biological activity	References
Nanoparticles	Berberine	Berberine is a plant alkaloid that reduces the *H. pylori* proliferation. This study has developed a unique nanoparticle berberine carrier with a heparin shell for localization of berberine at the infection site of *H. pylori*, and it has been observed that the *in vitro* drug carrier method controlled the berberine release, which interacted to intercellular space at the infection site precisely	Chang et al. (2011)
	Piperine	In this study, the piperine liposome complex formulation has been prepared and investigated by spectroscopic methods, and the formulation was found to be stable for 3 weeks after storing it at 4 °C	Pentak (2016)
	Ginseng	Ginseng, a phytochemical-mediated synthesis of gold nanoparticles have exhibited shieling effect from aggregation alongside leading to the nontoxic nature of ginseng-AuNP conjugates analyzed against normal cervical cell lines	Leonard et al. (2011)
Emulsions	Docetaxel	Microemulsions containing docetaxel as bioactive constituent were prepared, and the increased bioavailability of the drug was observed. Also the increased solubility of the hydrophobic drug was observed	Cui et al. (2009)
Microspheres	Quercetin	The study focused on development of a delivery system which can exhibit controlled release of the drug in treatment of rheumatoid arthritis along with increased biocompatibility of the delivery system	Natrajan et al. (2010)
	Zedoary oil	Zedoary turmeric oil (ZTO) shows broad pharmacological action spectrum including antitumor, antibacterial activity, etc. The ZTO-microsphere formulation increases the bioavailability of drugs in comparison to conventional methods	Ghule et al. (2016)
Ethosomes	Matrine	Ethosome-matrine complex has been found to increase per cutaneous permeation, and it also helps in the improvement of anti-inflammatory effect	Bhokare et al. (2016)

6.6 Advantages of Plant-Based Nanodrug Delivery Systems

Nanosized delivery systems have numerous advantages when compared to conventional drug delivery systems (Kuntal et al. 2005). Some of them are given below:

(1) Capable to deliver high drug concentrations to infected sites due to their distinctive size and high loading capacities.
(2) Deliver the drug in the smaller particle size thus increasing the surface area of the drugs resulting in better dissolution in the blood.
(3) The drug persists at active sites for longer periods, resulting in enhanced permeation and retention effect, which include greater permeation through barriers due to smaller size and better retention owing to poor lymphatic drainage in tumor-affected tissues.
(4) Novel drug delivery systems exhibit passive targeting to the site of action without the need of any particular ligand moiety.
(5) Use of novel drug delivery systems results in lowering of the side effects.
(6) Decrease in the dose of the drug formulation.

6.7 Conclusions and Future Prospects

Herbal medicine is accepted throughout the world as an alternative system of medicine. Yet, the available drug delivery systems for herbal compounds are traditional and outdated. A widespread research is happening in the field of NDDS for herbal medicine. Though, research in the mentioned fields is still at the initial stages. Numerous plant constituents including tannins, flavonoids, terpenoids, etc. exhibited improved therapeutic efficacies after incorporation into NDDS as compared to conventional plant extracts. Henceforth, great potential is available in the development of NDDS. Nanomaterials in drug delivery systems have been incorporated into formulations having specific characteristics both *in vivo* and *in vitro*. Small number of clinical studies have exhibited greater efficacy in animal models, but translation of these results into clinical success is very limited. Successful conversion needs to reconsider the role of nanomaterials in drug delivery, their limitations, and facing inconvenient facts. Nano-based drug delivery systems for herbal drugs exhibit potential to augment the biological activity. Though, noteworthy challenges persist for execution of clinically sustainable therapies. Trials of novel approaches to regulate nanomaterial- biological system interactions signify recent challenges in rendering these technologies into therapies. The challenges in the development of nano-based drug delivery systems include the feasibility of scale-up and the possibility of attaining multifunctional systems to accomplish numerous biological and therapeutic requirements. For the mentioned approach to be fruitful, fine-tuning of the procedures to increase the usefulness of the nanoparticle in novel drug delivery systems is required. Herbal remedies are thriving resources for valuable compounds including antioxidants and other phytoconstituents. This type of two-way approach

among the traditional herbal remedies and novel approaches of contemporary drug delivery system such as nanotechnology has established the therapies to the pharmaceutical sciences in the near future that will augment human health. It is estimated that the effective significance of the natural products and herbal remedies being combined with the nanocarriers will improve the importance of existing drug delivery systems.

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Chapter 7
Toxicological and Pharmacological Use of Anticancer Compounds

Charles Gnanaraj, Sivapragasam Gothai, Katyakyini Muniandy, Rengarajan Thamaraiselvan, and Palanisamy Arulselvan

Abstract Knowledge of medicinal plants from traditional medicinal drug therapy has brought limitless implications towards the discovery of novel compounds for modern therapeutic applications particularly for anticancer research. Research approaches have been taken for the development of traditional medicinal products into effective, non-toxic and clinically proven pharmaceuticals. Plant-derived anticancer compounds might exert adverse toxic effects in humans, depending on the species of plant, drug administration route, dose regimens and drug receptiveness. Due to this possibility, it is important to assess the toxicity profiles of new potential anticancer compounds through proper toxicological tests. The importance of preclinical toxicology studies is continually drifting from the use of conventional methods to assess the impending adverse effects of new anticancer agents over the past 10 years. Recent practice encompasses designing and carrying out more personalized agent-directed research within the framework of clinical pharmacology guidelines. Distinctive standpoints have been brought into view by clinical application of anticancer drugs that are apparent in their discovery and development. Evolving

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standards in estimating novel drug candidates through clinical and preclinical trials manifest the cytotoxic compound enhancements over time, with substantial aid from circumstances, and the current interest in discovering drug candidates with specifications on reaching targeted cells. This chapter highlights the relevance of plant-derived anticancer compounds through their pharmacological and toxicological uses.

Keywords Anticancer compounds · Clinical pharmacology · Drug discovery · Toxicity · Polyphenols

7.1 Introduction

Knowledge of medicinal plants from traditional medicinal drug therapy has brought limitless implications towards the discovery of novel compounds for modern therapeutic applications particularly for anticancer research. Plant-derived metabolic compounds have been used in modern medicinal practices for cancer prevention and as a possible curative treatment in current clinical trials (Arulselvan et al. 2016a; Mut-Salud et al. 2016; Redondo-Blanco et al. 2017). Almost 35,000 plant species have been reported to possess medicinal properties that have ethnobotanical values for various ailments (Bao and Fenwick 2004). This number may not be accurate, since most of the ethnobotanical identifications were orally passed on through decades and not all of them have been recorded. It was stated that more than 3000 plant species are involved directly in anticancer drug development process. Over the past 70 years, nearly 40% of anticancer drugs were derivatives of plants, and an additional 8% were analogues of natural products, which might be indirectly attributed to the known medicinal properties of the host plant (Van et al. 2004). There is an improvement in the research areas of anticancer therapeutics as a result of increasing demand among the public for natural products with minimal side effects. Plant-derived anticancer compounds might exert adverse toxic effects in humans, depending on the species of plant, drug administration route, dose regimens and drug receptiveness (Ndhlala et al. 2013). Due to this possibility, it is important to assess the toxicity profiles of new potential anticancer compounds through proper toxicological tests. Recently, naturally derived chemotherapeutic agents such as vincristine (vinca alkaloids) and vinblastine, teniposide (podophyllotoxin analogues) and etoposide, camptothecin-derived topotecan and paclitaxel (Taxol) were included in the medical treatment methods (Jantan et al. 2015). Formerly, these renowned drugs were in the form of tea, tinctures, powders, poultices and other crude drugs and herbal formulations. Evolving standards in estimating novel drug candidates through clinical and preclinical trials manifest the cytotoxic compound enhancements over time, with substantial aid from serendipity, and the current interest in discovering drug candidates with specifications on reaching targeted cells (Mishra and Tiwari 2011).

Pathophysiology of cancer is implemented through oxidative stress, which manipulates each stage of cancer mutagenesis beginning from damage incurred to DNA to the final stage of metastasis (Cook et al. 2004). Free radicals are known to

target cellular contents such as carbohydrates, lipids, proteins and most importantly DNA (Bishayee et al. 2011). Free radicals or reactive oxygen species (ROS) are capable of damaging the DNA or other constituents of the cell by abruptly producing massive numbers of pro-oxidants. ROS are able to modify the cell signalling pathways through a cascade of events, causing cancer proliferation and promoting tumour formation. Apoptosis- and cell proliferation-inducing transcription factors and pathways such as NF-kB, MAP-kinase and AP-1 are notably influenced by the ROS causing detrimental effect (Rehman et al. 2013; Ponmari et al. 2014). It is suggested that prevention of DNA damage could be the key to chemoprevention of ROS-related genetic disorders. Plant-derived secondary metabolite compounds with antioxidative properties have been proven to possess efficient ability to scavenge free radicals.

The recent broadening of the drug discovery horizons along with the applications of advanced computational methods including instrumentations and bioinformatics tools has further added to new avenues of drug development (Cragg and Newman 2005). Medicinal plants have provided an opening point for new synthetic compounds. Plant-derived compounds with anticancer properties could serve as a drug lead to be optimized into synthetic drug or as a potential drug candidate by itself. *Podophyllum peltatum* (Berberidaceae)-derived compound, podophyllotoxin, was terminated from clinical trials as a result of inefficiency and intolerable toxicity in mice (Gordaliza et al. 2004). However, the pioneer clinically approved drug etoposide was made out of this compound as a chemical derivative. At times, there might be certain obstacles in finding for novel anticancer compounds from plants, but current research trend allows vital drug leads to be derived from commonly known compounds with prominent biological activity. Camptothecin derived from *Camptotheca acuminata* Decne. (Nyssaceae) tree initially exhibited intolerable myelosuppression. But later, this compound was found to be interesting and revitalized as it was able to cause a selective inhibition of topoisomerase I, which is involved in the cleavage and reorganization of DNA (Cragg and Newman 2004). Thereafter in the year 2002, camptothecin was reckoned for one-third of the worldwide anticancer market valued at over 2.75 billion dollars.

Some plants have medicinal properties and can be used as natural therapeutics; besides this, they could also cause lethal toxicity to humans because of their self-protection mechanism (Wink and van Wyk 2008). Few plant-derived compounds are known to cause mortality to humans even at a small dose. Compounds with known toxic effects were later isolated and utilized for beneficial purposes by humans (Ndhlala et al. 2013). Certain toxic compounds have been reported to have medicinal values as well. For instance, cardiac glycosides which are normally used to poison arrows for hunting by indigenous people have also shown antimicrobial activity (Botha and Penrith 2008). Similarly, plants containing lectins are safely being used in traditional medicine as treatment for bacterial and fungal infections, but lectins are proven to cause cellular necrosis to the gastrointestinal tract cell linings (Gonzalo 2011).

In the past few decades, compounds with cytotoxic properties were identified and developed for anticancer therapeutics. Thousands of cytotoxic compounds are being identified, but only a few of them are used clinically (Narang and Desai 2009)

because the discovery of anticancer drug process is unique and has to pass through several challenges at every stage of drug development. Severe toxic effects might be caused by anticancer agents; hence, it should be carefully estimated and examined. Proliferation of normal cells such as hair follicles and cells in the digestive tract as well as blood cell formation in the bone marrow is affected due to therapeutic treatments targeting cell cycle, resulting in gastrointestinal toxicity, neutropenia and hair loss (Shoemaker 2006). These obvious side effects are generally reversible and can be recovered in the posttreatment period depending on the type of therapy. Mild or visible side effects will deteriorate with time; however, certain anticancer agents may cause a permanent toxicity or major dysfunction to vital organs such as the liver, kidney and heart and the nervous system (Narang and Desai 2009). In general, the consequences of therapeutic treatment by anticancer agents will develop immediately or shortly upon treatment, but sometimes they become apparent only after a longer period of time past treatment. If this period of delay is long enough, it may affect the benefit-risk equilibrium, and this interval might correspond to the time of progression-free survival.

Cancer is generally known as the disease of overwhelming cell division; hence all initiatives to overcome this problem are focused towards identifying novel compounds with anti-proliferative properties. Hence, efficiency in reducing the tumour size is focused as the main aim of preclinical and clinical studies. As part of drug discovery programmes, screening of novel compounds is done by testing them on rapidly growing tumour models. Although this screening model is able to identify several clinically important anticancer compounds, the success has been prominent in rapidly proliferating malignant tumours such as germ cell tumours, lymphoma and childhood leukaemia. Adversely, these compounds are not successful for common slowly proliferating epithelial tumours found in adults such as colorectal, breast and lung cancers (Samoylenko et al. 2013). Due to this drawback, researchers are encouraged to upgrade the prescreening and screening methods by including diverse tumour types and cell lines (Sznarkowska et al. 2017).

The global success ratio of assessments and anticancer drug development demonstrate difficulties and challenges involved in the process. Previous studies and analysis show that the percentage of failure for anticancer compounds in clinical trials was at its worst at the time period of 18–27 years ago (Mathijssen et al. 2014). It was recorded that only 1 out of 20 anticancer compounds would proceed from clinical trials to the market upon regulatory approval, and the rest failed. Candidate compounds in other therapeutic areas demonstrated double the percentage of success in reaching the market as compared to anticancer drugs. An analysis carried out in the USA showed similar approval success ratio for anticancer drugs in the past decade. Similarly, studies on the economic advancements of novel anticancer drugs and the priority review ratings given by the Food and Drug Administration have shown that anticancer drugs gain maximum profit (Workman and Collins 2014). It was also found in the study that clinical endorsement scales were comparable for anticancer drugs and non-cancer therapeutic drugs but huge volume of anticancer drug candidates were neglected in advanced stages of clinical tests where failures would be costly (Zhang et al. 2012). Reports from the past decade show that the time frame for anticancer drug development was usually lengthier as compared to

non-cancer therapeutics, estimated to be around 7 years. except for central nervous system drugs which averagely take longer time, due to the need for multiple assessments over a wide range of indications. The report also says that the number of endorsed novel anticancer compounds improved significantly and gradually from the past three decades (Alqahtani et al. 2013).

On the whole, it is our vision to watch the success of anticancer drugs to increase in the market and a rapid drug development process to be imposed with a minimal failure. It is important to understand the deteriorating factors of anticancer compounds in the clinical use as it is the only way to focus and restructure problematic areas. Earlier statistics exhibited that in the early 1990s, bioavailability and pharmacokinetic limitations of anticancer compounds are the major known problems (Van et al. 2004). Thus, analytical assays for the absorption, distribution, metabolism and excretion properties were developed to overcome the problem of bioavailability and pharmacokinetics of anticancer compounds. But as time changes, new problems have aroused. The success ratio of clinical evaluation of anticancer drugs can be enhanced through testing them in different animal models shaving a closer physiology with humans. For instance, molecularly labelled human tumour xenografts in immunosuppressed mice are persistently beneficial to discover novel drugs, with elevated prominence on early channel patient-derived xenograft and orthotopic implantation (Guo et al. 2006).

It is clearly seen that by focusing on developments in medicinal chemistry, the eminence of the chemical leads being recognized for the optimization against novel targets has enhanced drastically with the identification of lead-like and drug-like chemical properties. The practice of high-performance, multi-parameter outlining currently permits us to anticipate impending problems such as toxicity and metabolism, which could be highlighted during the early stages of the optimization process (Workman and Collins 2014). Remarkably, natural products still remain to be of great importance to be used as therapeutic agents and possess several biological functions characterized with various inhibitory mechanisms and novel targets regardless of their possible cytotoxic adverse effects. Furthermore, the diverse assortments of natural compound structures relentlessly impress and instigate the medicinal chemistry research. In several circumstances, synthetic alterations of natural products are essential to improve the understanding of structure-activity relationships as well as to progress on physicochemical structures and biological characteristics. This chapter aims to highlight the pharmacological and toxicological uses of plant-derived anticancer compounds and the importance of natural products as potential therapeutic agents against cancer.

7.2 Discovery of Plant-Derived Anticancer Compounds

Plants are rich in antioxidant compounds which are well-known for their free radical scavenging effects and oxidative stress prevention, thus providing chemopreventive measures against chronic ailments (Arzamastseva et al. 2007). Antioxidants are able to restrict free radicals and restore cellular and organ functions by healing the

injury done by free radicals. Polyphenols are a major group of antioxidants which are most abundantly found in plants and possess a unique chemical structure having the potential of scavenging free radicals. High reactivity and the potential to donate electron or hydrogen to stabilize the unpaired electron of free radicals attribute to the antioxidative property of polyphenols (Chanda and Dave 2009). Antioxidant compounds are well-known to possess anticancer properties. Natural antioxidant deficiency in humans is the major cause for many clinical conditions. The onset progression of pathological conditions due to oxidative stress could be delayed, reduced or reversed significantly through additional distribution of antioxidant compounds (Gnanaraj et al. 2016). Therefore, scientific measures are currently being carried out to explore and identify plant-derived antioxidant compounds with anticancer potentials along with clinical efficiency (Mut-Salud et al. 2016; Redondo-Blanco et al. 2017). Though microbes and marine products are natural sources of antioxidants, plants are always prioritized, because they are easily accessible and are rich in secondary metabolite compound diversity.

Polyphenols have tremendous medicinal properties, but the metabolism of these compounds in the human body has certain limitations. The absorption and distribution of polyphenols in the human body usually occur during the consumption of fruits and vegetables in the diet. But for a specific antioxidant compound to reach the target cell or organ through diet is a challenging process; hence bioavailability of the specific compound is solely accountable. Upon drug administration into the human body, it is recognized as a xenobiotic and subsequently undergoes biotransformation in the liver. During this process, there are high possibilities for the pure compound drug to lose its structure, thus losing its medicinal properties. This is why most of bioactive compounds isolated or derived from plants do not pass the clinical trials. Drug discovery process is thriving each year with hundreds of compounds being reported with therapeutic properties (Alsarhan et al. 2014). Most of the reported compounds exhibit potential as chemotherapeutic agents in preclinical evaluations but eventually fail in the clinical trials (Androutsopoulos et al. 2010). Scientists came with a solution to overcome this problem by developing drug transporters that will be co-administered along with the original drug. These drug transporters are supposed to protect the drug from losing its medicinal properties by maintaining its chemical structure till it reaches the target cell or organ. The primary technique for structural damage prevention of drug is through modifying the liver phase II metabolizing enzymes associated with biotransformation and excretion and xenobiotics from the body. Though it seems to be successful, it raises certain doubts on the long-term effect of liver enzyme modification which might lead to unwanted mutations or adverse effects and loss of activity of certain classes of enzymes. If the condition prolongs, it might cause the organ to be exposed to greater harms as it will become defenceless against the toxic organic compounds. This debate is opened in a constructive way in order to achieve a winning solution for this problem. Androutsopoulos et al. (2010) reported in a review on bioactive compounds as prospective anticancer agents that methoxylated flavonoids have a better bioavailability compared to hydroxylated flavonoids and could serve as therapeutic cancer drug for the chemoprevention of cancer cells. The metabolic pathways of methoxylated

flavonoids need to be studied in depth to ascertain its clinical prospects for safety pharmacological insights for human diet consumption.

Compounds evaluated for possible anticancer properties might be of synthetic or natural origin. Natural products often deliver fresh indications in the discovery of unique chemical structure with potential anticancer properties. A recent study found that 80% of the available small-molecule anticancer drugs are natural products, the compound-related analogues or imitations of natural product compounds (Newman and Cragg 2012). Moreover, this percentage has not significantly changed in the past 70 years. It is noted that most of anticancer drugs approved around the world contain naturally inspired agents. Several naturally derived compounds such as etoposide, a derivative of mandrake plant *P. peltatum*; Taxol, a derivative of the Pacific yew, *Taxus brevifolia*; and vincristine, a derivative from *Vinca rosea*, the periwinkle plant, are known for their anticancer properties. There are also several microbe-derived compounds that are used as anticancer agents such as bleomycin and doxorubicin which are *Streptomyces* bacteria fermentation products, rhizoxin which is a derivative of the fungus *Rhizopus chinensis* and L-asparaginase which is a derivative isolated from *Escherichia coli* or *Erwinia carotovora* broths. Anticancer compounds isolated from natural products other than plants and microbes include bryostatin, a derivative of the sea moss *Bugula neritina*, and cytarabine, derived from the marine sponge, *Cryptotethya crypta* (Sultana and Ata 2008; Narang and Desai 2009; Aung et al. 2017).

7.2.1 Notable Types of Antioxidants

Natural compounds with potent anticancer properties are extensively present in various plant groups. Studies have been conducted in depth on the phytochemical constituents of plants for their therapeutic potentials. There are several methods and techniques to extract phytochemicals or pure compounds from plants, predominantly using chromatography such as high-performance liquid chromatography, microemulsion electrokinetic chromatography and micellar electrokinetic chromatography, and their structures will be elucidated based on nuclear magnetic resonance spectroscopy analysis. In the late 1980s, major successful anticancer compounds of plant origin were discovered, including doxorubicin, vinblastine, irinotecan, paclitaxel, etoposide, methotrexate and gemcitabine, but due to lack of popularity in the 1990s, anticancer compounds from natural products were not given priority. Recent researches have gained interest in using plant derivative anticancer compounds as potent curatives of cancer (Arulselvan et al. 2016b). Interestingly, natural product compounds such as flavonoids, alkaloid, lactones, sesquiterpenes, diterpenoids and polyphenols have been widely studied and are found to exhibit a diverse range of chemopreventive mechanisms against numerous cancer forms in both *in vitro* cell culture and *in vivo* animal models (Gothai et al. 2016). Some of these compound's chemical structures are depicted in Fig. 7.1.

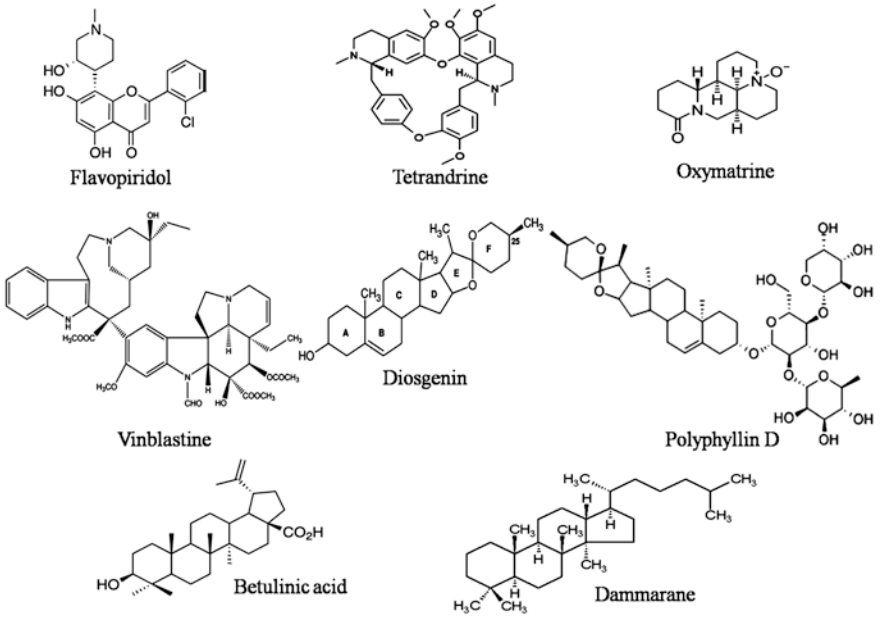

Fig. 7.1 Chemical structures of some of the natural plant-derived anticancer compounds

7.2.1.1 Flavonoids

Flavonoids are the common antioxidants found in plants under six subgroups, namely, anthocyanins, flavones, isoflavonoids, flavonols, flavanones and chalcones. They provide support to plants as secondary metabolites through chemical signalling, pigmentations, microbial and insect repellent, promoting or inhibiting growth and many more. Flavonoids have been reported to have therapeutic potentials; however, at the same time, they could cause detrimental side effects (Kyselova 2011). Previous study has shown that flavonoids impede cell proliferation and angiogenesis, induce cell apoptosis, cause cell cycle arrest and reverse multidrug resistance or a mixture of the previously described mechanisms. Flavonoids with various structures and methods of action are currently under clinical evaluation (Aung et al. 2017). Genistein has been examined on many patients, for preventive as well as curative treatment for prostate, breast and bladder cancers. Flavopiridol, a semisynthetic flavonoid, with anticancer properties is credited to downregulation of cyclins D1 and D3, inhibition of cyclin-dependent kinases (CDKs) and inhibiting phosphorylation of CDKs. Flavopiridol induces apoptosis, cell mortality and cell cycle checkpoint arrest, but the specific pathway mechanisms for anticancer properties remain uncertain. Clinical trials have shown that combination effects of few flavonoids are more encouraging as compared to a single compound effect. Therapeutic

effects of flavonoid compounds in combination are assumed to have a promising outcome as a potent anticancer drug for cancer treatment and prevention, with concerns to their safety and accessibility.

7.2.1.2 Alkaloids

Naturally present toxic compounds are known as alkaloids and commonly found in both plants and animals. These compounds have been traditionally used for medicinal purposes as well as other activities like hunting. They function as protective and supportive metabolites as well as neurotransmitters in plants. Though alkaloids are poisonous and could cause lethality, they are still being structurally modified to be used as synthetic drugs in modern medication systems. Some notable alkaloids that are currently in use as medicine are quinidine, codeine, colchicine, quinine, vincamine, emetine and reserpine (Qiu et al. 2014). Certain well-synthesized semisynthetic alkaloid derivative anticancer compounds are vinorelbine, vindesine, vincristine and vinblastine. There are other alkaloid compounds with anticancer properties such as oxymatrine, a major quinolizidine alkaloid found in Aiton plant *Sophora flavescens*, and tetrandrine, a natural alkaloid found in the medicinal plant *Stephania tetrandra*. Oxymatrine is reported to possess cytotoxic effect on human colon cancer cells through downregulation of human telomerase reverse transcriptase (hTERT) gene and upregulation of mad1 gene as well as p53 gene in a dose-dependent manner. Oxymatrine also inhibits the growth of gallbladder cancer cells by activating apoptotic signal Bax along with caspase-3 and suppressing Bcl-2 and NF-kB. Oxymatrine is also reported to have anticancer properties for human lung cancer cells and Hela cells by inducing apoptosis. Therefore, this compound could be useful for cancer therapeutic treatments. Tetrandrine also has anticancer properties such as anti-proliferative and apoptosis-inducing effect on human oesophageal cancer cell lines, ECa109 and ECa109-C3, and human monoblastic leukaemia cells U937. It is also reported to be effective in reversing multidrug resistance in human nasopharyngeal cancer cells (KBv200) and Adriamycin-resistant human breast cancer cells (MCF-7/Adr). Tetrandrine compound's molecular mechanisms of action in cancer cells include upregulating cell death promoters such as Bak, Bax, Bad and apaf-1 and downregulating apoptotic regulators such as Bcl-xl and Bcl-2, discharging cytochrome c and activating caspase-3 and caspase-9 in the apoptotic mitochondrial pathway (Aung et al. 2017). Activation of intrinsic apoptosis pathway highlights the effectiveness of tetrandrine as a potent therapeutic anticancer compound. A semisynthetic analogue of alkaloid compound isolated originally from the periwinkle plant *Catharanthus roseus* known as vinblastine is an antimitotic drug. It was reported that this compound disrupts microtubules resulting in disappearance of mitotic spindles, thus inhibiting cell proliferation.

7.2.1.3 Saponins

Saponins are a type of secondary metabolite compounds of plants and marine organisms. It is easy to detect saponins in plants as they have foaming features. These compounds are usually used in the industry as biochemical reagents. The information regarding the functions of saponins in plants is scarce except for the protection against microbes. They have been reported to possess cytotoxicity against human cancer cell lines as well as being used as nutraceuticals and adjuvants in vaccines due to their membrane-penetrating mechanism (Francis et al. 2002; Nassiri and Hosseinzadeh 2008). Chikusetsusaponin IVa butyl ester (CS-IVa-Be) is an apoptotic triterpenoid saponin extracted from the medicinal plant, *Acanthopanax gracilistylus*. The herbal derivative has been found to cause cell cycle arrest at G0/G1 stage in multiple cancer cell lines. This compound is reported to promote apoptosis in MDA-MB-231 cells through inhibition of IL-6-induced STAT3 activity via IL-6/JAK/STAT3 signalling pathway. It also triggers the tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a particular inducer of cancer cell apoptosis, in TRAIL-resistant MDA-MB-231 cells by upregulating death receptor 5 (DR5) (Aung et al. 2017). Polyphyllin D is a steroidal saponin compound with promising anti-proliferative property, extracted from the medicinal plant, *Paris polyphylla*. The anticancer activity of polyphyllin D was detected through the degeneration of mitochondrial membrane and fragmentation of DNA, leading to mitochondrial dysfunction and loss of membrane integrity in MDA-MB-231 and MCF-7 cells. It has been documented that administration of polyphyllin D for 10 consecutive days reduced up to 50% of tumour growth in mice. Another steroidal saponin compound with anticancer properties is diosgenin which has been reported to promote apoptosis by reducing protein expression and mRNA of 3-hydroxy-3-methylglutaryl-CoA reductase in HCT-116 human colon cancer cells. Similarly, macranthoside B, a triterpenoid saponin extracted from *Lonicera macranthoids*, has proven to induce mitochondrial-mediated apoptosis in numerous tumour cells via increased Bax/Bcl-2 ratio (Millimouno et al. 2014; Aung et al. 2017). A wide range of saponins have been evaluated for their cytotoxic properties, and a number of compounds have shown to be a potent inducer of apoptosis. However, the prospective of this type of compound remains to be explored thoroughly as they might be efficient in combination with other classes of anticancer compounds or drugs to synergistically boost their therapeutic action in cancer.

7.2.1.4 Terpenoids

Terpenoids are large multicyclic lipid compounds which are almost the same as terpenes and are found mostly in all living organisms. Terpenoid derivatives of plants have a strong aromatic quality and have been documented in traditional medicinal practices for their pharmacological benefits (Ayoola 2008). Derivatives of triterpene molecules, isopentenyl pyrophosphate oligomers, are known as triterpenoids (Bishayee et al. 2011). These compounds have been reported to possess

much therapeutic potential and, most importantly, exhibit cytotoxic effects in cancer cells without causing toxicity to healthy cells (Rabi and Bishayee 2009). Preclinical evaluation of triterpenoids has proven antitumour effect in animal models (Petronelli et al. 2009). Till date, many triterpenoids have been derived and modified from natural compounds to optimize their anticancer properties and anti-inflammatory effects for clinical trials (Mullauer et al. 2010). Triterpenes such as betulinic acid, dammarane, squalene, lupine, ursane, ursolic acid and oleanolic acid have been reported to possess cytotoxic and antineoplastic activities against cancer cell lines (Chudzik et al. 2015). A recent report by Borkova et al. (2017) analysed the cytotoxic activity of triterpenoid thiazoles against eight cancer cell lines and two non-cancer fibroblasts and found that they possessed effective anti-proliferative properties.

7.2.2 Advances in Plant-Derived Anticancer Compounds

Plant-derived anticancer compounds often target tumour cells through the regulation of cell death pathways such as intrinsic and extrinsic apoptosis and autophagic pathways. Furthermore, it has been reported that cell signalling pathways activated by natural anticancer compounds are diverse and distinct for different targets. It was found that different signalling pathways could be activated by the same compound depending on the target cell types. Many studies have been conducted to proof the pathways, and one of them is the *in vitro* and *in vivo* test for prostate cancer treatment using isoflavones and phytoestrogens from soy which exhibited deactivation of NF-kB signalling, inhibition of angiogenesis and induced apoptosis. NF-kB pathway is known to initiate cancer cell proliferation and also causes inflammation (Nair et al. 2006). Induction of NF-kB activation has been reported to cause resistance to chemotherapy. Constitutively activated NF-kB stimulates the proliferation of cancer cells thus saving them from apoptosis or autophagy. Fundamentally activated NF-kB can often be seen in various cell lines, cancer cells, xenograft animal models or clinical sites (Hassanzadeh 2011). In recent studies, NF-kB signalling is being deeply analysed as a favourite target of cancer cell proliferation. It seems to be an encouraging target and is worth for further research to find out the possible therapeutic mechanisms for cancer prevention (Arulselvan et al. 2016b). Though there is a clinical use of NF-kB inhibitors, they seem to be distant from model drugs due to their detrimental side effects. Therefore, it is recommended to perform extensive research on natural product-derived anticancer compounds for their optimal therapeutic mechanism on NF-kB signalling pathway and preventive measures of cancer cell proliferation.

Analogues of anticancer compounds derived from natural products have often been synthesized as a step to increase anticancer efficiency or reduce toxicity (Aung et al. 2017). For instance, daunomycin was found to be causing cardiotoxicity; therefore, an analogue doxorubicin was created. Similarly, an analogue of cisplatin was synthetized, carboplatin was able to reduce renal toxicity, and an analogue of camptothecin, topotecan, also has reduced toxic effect. Analogues of prevailing

anticancer agents have also been developed to increase the pharmacokinetic availability and improve tumour targeting accuracy of compounds. Examples of such anticancer agents are 9-alkylmorpholinyl, an analogue of doxorubicin, synthesized to reduce drug affinity to P-glycoprotein which is the cellular efflux protein, and another agent is tauromustine, a nitrosourea anticancer compound joined to the brain targeting peptide taurine to target central nervous system tumours. Anticancer compounds of natural origin sometimes could not compensate the excessive demand for drug supply (Sultana and Ata 2008). To overcome this problem, the original compound-related analogues have to be developed to continuously supply therapeutic drugs. An example of such drug is taxotere, an analogue of Taxol which is a plant-derived anticancer compound with extremely low yields. Synthetic compounds have been developed in recent years to be used as analogues of natural anticancer compounds with novel structures (Redondo-Blanco et al. 2017). Selection process for a suitable synthetic drug candidate is developed through combinational chemistry and computer modelling of drug-receptor interactions. Laboratory synthesis-based development of potential anticancer compounds has advanced from analogues, and improvisations have led to coherent design established for drug-enzyme or drug-receptor relations. Prominent models of synthetic anticancer drugs developed from analogues of natural products are 5-fluorouracil, fluorinated pyrimidine base and methotrexate, an analogue of folic acid. Other examples of anticancer drugs discovered via coherent designing method by exploring drug-target interactions and molecular mechanisms include mitomycin C-related indolequinone EO9 which is actively used against hypoxic tumours and edelfosine, an ether lipid which targets cellular membranes (Mathijssen et al. 2014; Aung et al. 2017). Developments in anticancer drug discovery process have found numerous compounds subsequently requiring a thorough screening process to shortlist potential compounds to be subjected to preclinical tests. The selected compounds will undergo tests such as *in vitro* cell line and *in vivo* animal assays for toxicological classifications, efficiency quantification and bioavailability through pharmacokinetics and pharmacological potentials prior to clinical trials.

7.3 Toxicological Measures of Anticancer Compounds

In the early years of the 1950s, almost all cancer drugs were known to be cytotoxic agents. They commonly react by damaging and inhibiting DNA synthesis pathways or by disrupting the mechanism of cell proliferation, for instance, via binding to microtubules or blocking topoisomerases. Most anticancer compounds were found via screening for possible compounds with the ability to destroy cancer cells, such as paclitaxel, a plant-derived compound with microtubule inhibition property. Based on nitrogen and sulphur mustards, DNA-alkylating agents were structurally reformed to control their chemical reactivity rates thus prompting the formation of drugs like ifosfamide and cyclophosphamide. It was later found that these drugs could cause adverse effects to other vital organs and some effects were irreversible

and could cause mortality (Workman and Collins 2014). Therefore, toxicity assessment for anticancer compounds is an important process. An approximation of the clinical schedule of anticancer drugs should be assessed by toxicology tests as the toxicity of the drug could be highly influenced by its administration schedule (Philomena 2011). General toxicities encountered during cancer treatments are skin and hair follicle toxicity; gastrointestinal, haematological and urinary tract toxicity; cardiac toxicity; pulmonary toxicity; metabolic abnormalities; nervous system toxicity; gonadal toxicity; and hepatic toxicity.

Estimation of the possibility to recover from the toxicity caused by therapeutic agents must be given in order to determine the seriousness of contrary effects and to understand whether it is reversible or not. Whenever there is severe toxicity occurrence during a clinical exposure of cancer treatment and the period of recovery could not be determined by scientific measures, a terminal non-dosing period study will be imposed. The degree and severity of pathologic injury and the recovering capability of the target organ system could be included in this study to show the effect of the drug (Remesh 2012). When a study on recovery from toxicity has been initiated, it should be presentable to assist the clinical development of therapeutic drugs. However, it is not necessary to exhibit the complete recovery process as time is crucial for cancer treatments.

7.3.1 Approaches in Toxicological Assessments

Toxicity and therapeutic effects caused by cytotoxic compounds are the results from similar molecular mechanisms, and they are often correlated directly to the dose regimens. In clinical studies, patients are usually dosed to a maximum concentration of drug compounds to achieve maximal medical benefits, provided the dosage does not cause serious side effects (Mathijssen et al. 2014). The serious ill situation of the patients, disparity in individual drug reaction and harmfulness along with the limitations of the therapeutic anticancer compounds constitute the major challenge for their clinical practice and development. Considering these factors, novel approaches have been made for the development of instruments for specifying a drug therapy for individual patients and to monitor the drug response and toxicity by means of alternate indicators. As an example, tumour treatment has been specialized for individual patients depending on the nature of tumour, state of disease and histology of tumour cells.

Toxicological studies of anticancer compounds involving animal models are targeted to assess the toxicities of the drug, possibilities for reversibility and severity of the compound toxicities prior to calculating a safe entry dose and dosage schedule for clinical trials on humans. Toxicity is estimated at a regular and therapeutically active dose for most of the cytotoxic anticancer compounds (Shoemaker 2006). This is due to the same mechanism attributing to toxicity and therapeutic effect. The common harmfulness of cytotoxic compounds includes the suppression of the bone marrow which can be clearly witnessed in rodent species, such as mice

and rat, and therefore toxicological evaluation is mostly conducted on smaller animals under this principle. Considering the cost and ethical reasons, higher animals such as apes have been avoided in the routine toxicological tests. The severity and occurrence of acute toxicities are determined in the targeted organs through biochemical, histopathological and haematological evaluations soon after the dosage administration, while the long-term or chronic toxicities are determined by means of sacrificing and investigating the animals few weeks upon the dosage administration.

7.3.2 Effective Dosage Estimation of Anticancer Compounds

The drug to dosage correlation varies among species and is normally determined based on the body surface area, though other factors such as body weight and age have also been considered. Dosage levels that cause mortality to 10%, 50% and 90% of the animals (LD_{10}, LD_{50} and LD_{90}; LD, lethal dose) will be subjected through the same route of administration to determine the phase I starting dosage of a cytotoxic compound. These dosage parameters could also be referred to the terms of dosages that cause severe lethal toxicity or severe toxic dose (STD) instead of counting mortality as an end point (Narang and Desai 2009). The proposed phase I starting dosage is normally 1/10th of the LD_{10} or STD_{10}.The proposed phase I starting dosage is usually examined in other animal models, usually dogs or rats, to determine the presence of substantial toxicity as well as to reduce the risk related with human trial of a new anticancer compound (Samoylenko et al. 2013). Therefore, the preclinical animal toxicology practice normally comprises of single-dose and multiple-dose fatality or lethal toxicity tests in mice and subsequently single-dose and multiple-dose toxicity verification tests in rats or dogs. In case of severity, permanent toxicities are demonstrated in the animal model at the proposed phase I entry dosage, then the human entry dosage will be decreased to 1/6th of the highest dosage given to the animal model, provided the dosage did not cause lethal or permanent injury. The steps involved in the toxicity assessment for potential anticancer compounds are represented in Fig. 7.2.

Assessment of anticancer compound toxicities in animals has certain restrictions, since anticancer compounds are fundamentally harmful with normally a dose-dependent expression of symptoms. The assessment of dosage regimens related to the clinical doses for human using animal model toxicology is difficult to attain within a therapeutic range, causing the risk of lower or higher approximation of the compound's toxicological outline. This situation could be attributed to the genomic variations in the pharmacodynamic and pharmacokinetic reactions of the compounds which might be due to dissimilarities in protein binding, the metabolic and excretion pathways and the sensitivity of target cells. Moreover, uncommon toxicities such as those of neuromuscular or cardiovascular basis are challenging to be detected in animal models. Previous studies have witnessed about 0.5% of mortality due to toxicity in the phase I clinical tests of anticancer drugs among approximately

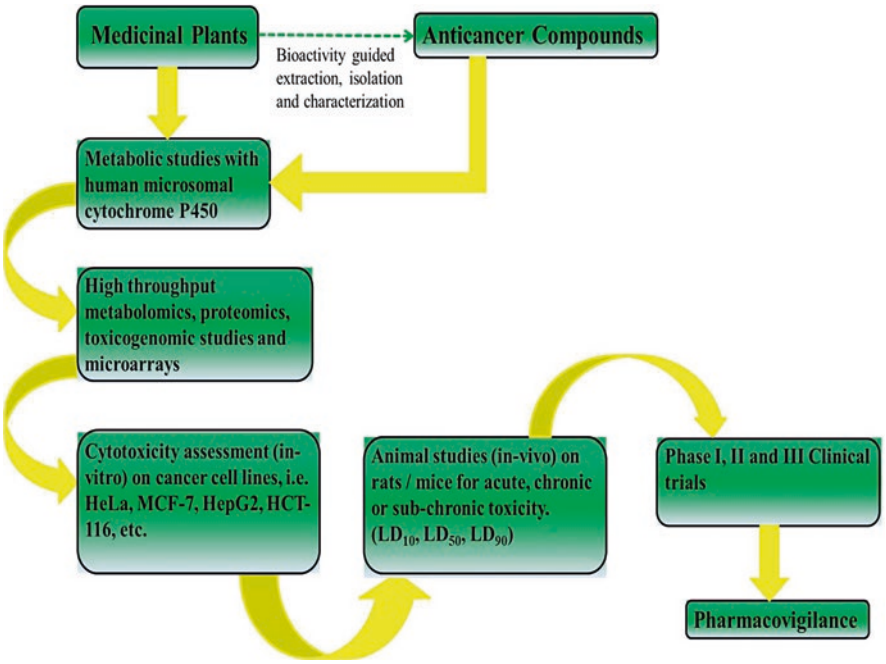

Fig. 7.2 Schematic diagram of toxicity assessment for potential anticancer compounds

6000 volunteers (Grunwald 2007).Underestimation of starting dosage could increase the time and procedures in the dosage acceleration experiments, hence raising the costs and time for development and causing unethical administration of unproductive dosages to a big number of extremely sick patients. On the other hand, higher estimation of starting dosage is very much unwanted due to safety precautions. Both under- and higher estimations could cause a novel compound to be rejected from further development due to these reasons. Higher estimation example was observed in the anticancer drug fludarabine where it caused substantial bone marrow suppression at the 1/10th mice LD_{10} dosage at the phase I studies but did not cause any substantial bone marrow suppression in dogs at dosage levels up to 20 times higher than the LD_{10} dosage (Narang and Desai 2009). This discrepancy has been related to the differences in species towards the drug pharmacodynamics, where the efficacy of the drug phosphorylation is higher in human bone marrow cells as compared to dog bone marrow cells. Underprediction of dosage is demonstrated by brequinar sodium through increased clinical trial period, which required 19 dose acceleration steps over a time of approximately 3 years or more to attain the maximum tolerated dose (MTD), since the MTD was 40-folds increased than that of the mice LD_{10} dosage (Eisenhauer et al. 2000). Another notable example is the flavone acetic acid, a synthetic flavonoid, for which the single dosage LD_{10} in mice was showing similar results to that in rats, but testing on dogs and humans showed tolerance level up to fourfold increased dosages. The reason for this is rapid drug excretion in the higher species, hence causing underestimation of the clinical start-

ing dose. These cases prove the importance of a better estimation of cytotoxic compound toxicities in humans to avoid prolonged phase I trials other than lethal drug toxicities. Currently, pharmacokinetic experiments are often being added in the toxicology assessment methods. Microdosing of drug has been recommended for human trials to comprehend the drug pharmacokinetic properties before the proposed dosages could be administered (Narang and Desai 2009). Microdosing is a new method to learn the behaviour of drugs in humans through administration of very low doses but high enough to study cellular response without producing whole body effects.

7.4 The Evolving Role of Natural Products as a Renewable Source of New Drug Leads

A very common method to identify bioactive compounds in plants is through pharmacological testing of crude extracts from the potential medicinal plants followed by isolation and characterization of the bioactive compounds attributable for the therapeutic effect of the extract. The extraction method of plant extracts is rather easy and low in time investment and cost. However, to ensure reproducible data, it is critical to cautiously record the processing of the plant material beginning from the collection, identification and extraction, since the technique of extraction can highly impact on the chemical composition and consequently influence the bioactivity of the plant extract (Atanas et al. 2015). Therefore, selecting an appropriate extraction method involving different solvents is very crucial and should be carefully well-thought-out. To maximize the yield, the extraction and fractionation are needed to be optimized for medicinal plants with identified target compound classes and chemical structures. In cases with unknown bioactive compounds in the investigated plant, extracting diverse classes of compounds is valuable. Commonly used solvents in extraction method are 70% ethanol or methanol, as these solvents have been found to extract a maximum yield of bioactive molecules.

There are two principal approaches in the isolation of new lead compounds which are bioactivity-guided isolation and metabolic profiling (Halket et al. 2005). Plant crude extracts demonstrating bioactivity are usually subjected to the identification of pure bioactive compounds. To define the chemical traits of plant-derived compounds, isolation of the pure compounds is mandatory using stepwise separation methods and spectral analysis to inaugurate the exact chemical structure of the compound. Novelty of structure and new action methods are the normal characteristics of plant drugs. Bioassay-guided isolation involves the assessment of crude extracts biological activity, followed by fractionation and biological testing until pure active compounds are obtained. Technologies used in the fractionation process are liquid-liquid extraction, using organic solvents of opposing polarity such as ethyl acetate, n-butanol and chloroform. Following each fractionation step, bioactive fractions are guided to deeper separation, generally by column chromatography,

flash chromatography, vacuum liquid chromatography and thin-layer chromatography (Luc and Arnold 2005). However, the traditional bioactivity-guided isolation is time-consuming, costly and exhausting. Furthermore, plant crude extracts represent complex mixtures interactions of active and inactive compounds which may result in strong bioactivity in a crude extract from additively or synergistically weak active compound action. Thus, fractionations may break this collective bioactivity, resulting in a trustable bioactivity (Park et al. 2007).

Metabolic profiling is an evolving substitute to bioactivity-guided fractionation. As for metabolic profiling, the metabolites present in the natural sources provide information on the chemical composition, thus allowing for the detection and isolation. This type of profiling can be obtained by using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) (Berkov et al. 2008). Pattern of the compound bioactivity and chemical profile of plant extracts correlate in guiding the isolation and identification of compounds at an earlier stage. Quantification and characterization of plant-derived secondary metabolites require extremely sensitive and reproducible analytical techniques such as the nuclear magnetic resonance (NMR) spectroscopy or high-performance liquid chromatography (HPLC). This method permits pointed isolation of specific bioactive compounds and dereplication of already known compounds. It is recommended to use bioactive fractions with fewer compounds for screening and quantification purposes (Kawaguchi et al. 2010).

7.4.1 Primary Screening of Anticancer Drug Discovery

The assortment of plants based on molecule entities serves as the initial biological screening in a drug discovery programme. The discovery of drug from plant necessitates a multidisciplinary field in which the accomplishment is mostly related to a relevant set of *in vitro* and *in vivo* assays. However, the choice of the bioassay largely depends on the objectives of a study (Lee et al. 2012). The examination of plant derivative compounds starts with the screening of numerous bioactive compounds using *in vivo* organ, tissue or animal models followed by *in vitro* investigation of mechanistic underpinnings (forward pharmacology approach). Although screening of random compounds in animal models is still a useful method, the disadvantages are apparent. Further, it is laborious and necessitates the involvement of large amount of compounds with low sensitivity. In most situations, it is impractical to supply adequate amounts of pure compound isolates for animal trialling, since the amount of bioactive compounds present in plants is relatively low (Ming-Wei et al. 2007).

Remarkable improvements in the pharmacological research have led to modern approaches involving various cellular and molecular bioassays known as the reverse pharmacology approaches (Alqahtani et al. 2013). It begins with screening of numerous compounds isolated from plants against predesignated ailment-relevant targeted protein, with the objective to recognize promising hit bioactive constituents

with the preferred activity that will be studied in detail using relevant *in vivo* models with the purpose to validate them. Animal testing could be reduced through this method, but it must be understood that promising hits might accidentally get rejected upon exerting toxic effects on cell-based screening, which in fact might be successful in animal models due to biotransformation in the liver. It generally needs great time and effort during the primary phases with complex and huge library of unique bioactive chemical constituents. In this sense, optimization of the lead is needed in order to guarantee *in vivo* efficacy. Screening of thousands of compounds within a short time period was made possible through the development of combinatorial chemistry (high-throughput technologies), thus accelerating the pace of isolating target molecules which could be developed further to potential drug candidates with preferred therapeutic activities.

Molecular target assays depend on the evaluation of the physical interaction of the investigated target protein with the desired compound. The biggest group of target proteins approved by the FDA is receptors (e.g. G-protein-coupled receptors (GPCRs), followed by enzymes (e.g. hydrolases) and transporter proteins (e.g. voltage-gated ion channels) (Alkhalfioui et al. 2009). These assays reflect the protein-protein interaction essential for the practical activity or protein initiation upon binding with the test compound and provide important preliminary data about the mechanism of action binding test compound to the active centre of the target protein early in the discovery process. This preliminary information on how a compound may affect the progress of a disease process can be valuable asset in determining the status of the compound for further development. Thus, rapid biochemical screening test of large libraries of pure compounds is being employed as targets for plant-derived lead discovery (Klekota et al. 2006; Swinney and Anthony 2011).

Assays with biological systems such as mammalian cell cultures (cell-based assay) are an alternate to *in vivo* assays for the primary evaluation of pharmacological activity due to the ease of the assay design. These assays deliver cellular level information on bioactivity compounds depending on the objective of the research and the expected outcome (Cragg and Newman 2004). For example, studies of angiogenesis most appropriately require endothelial cells and primary mammalian cells, or immortalized cell lines can be used for this purpose. However, immortalized cell line results are often less relevant because prolonged *in vitro* maintenance permits build-up of phenotypic changes and mutations. Cell-based assays can be phenotypic or phenotypic oriented. Phenotypic models provide results on an altered cellular phenotype with entangled regulation, for instance, cell migration, while target-oriented assays aim on the inter-reaction of test compounds with the role of a particular protein or pathway such as caspase activity (Atanasov et al. 2013). The relevance of *in vitro* assays is however restricted as compared to *in vivo*, where they are unable to deliver knowledge on the regulating aspects of the compound pharmacokinetic activities (absorption, distribution, metabolism and excretion). Yet, cell-based assay signifies imperative tools to isolate and characterize active constituents.

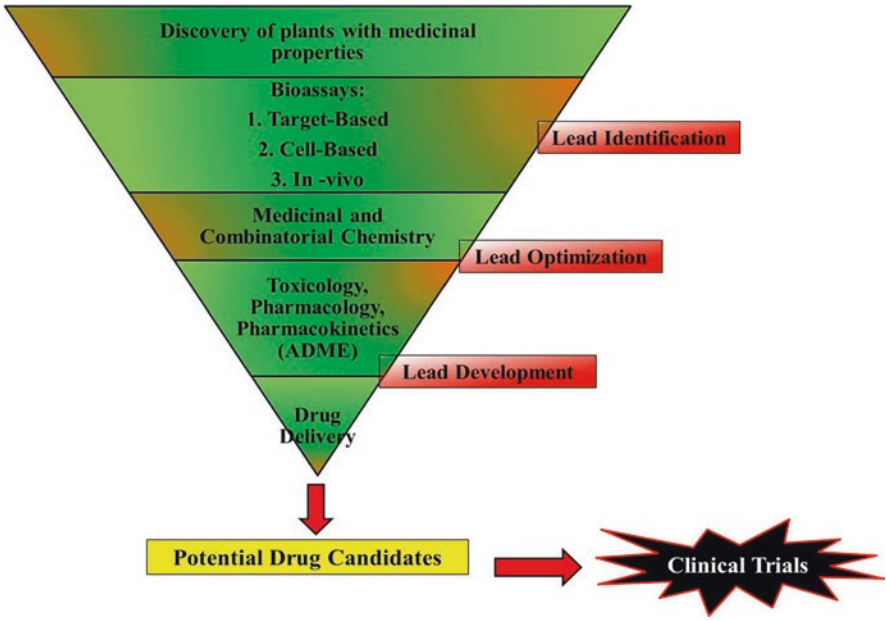

Fig. 7.3 Schematic diagram of plant-derived drug lead development

According to the reverse pharmacology method, isolated bioactive compounds from traditional cell-based assays or molecular target are further evaluated for their anticancer potential using *in vivo* assays using likely animal models to provide toxicological and pharmacological results prior to clinical trials on humans. Animal models such as mice and rat are crucial for drug validation and evaluation due to the sensibly high similarity and homology among mammalian physiology and genomes. Moreover, information on the bioavailability, efficacy, toxicity and side effects of the drug could be gathered through animal models that will be basically used for safety and pharmacokinetic studies that are imperative for clinical trials (Zhang et al. 2012; Alqahtani et al. 2013). It is a common pharmacological practice to assess candidate drug compounds on larger animals such as dogs and swine. Due to high cost and ethical issues, drug testing on larger animals is only carried out at the final stage prior to human tests. Indeed, such regulations require the pharmaceutical industry to employ both rodent and non-rodent models. And so, preclinical animal models will continue to be essential for assessing efficacy and side effects, and their predictive values will be revealed when more compounds enter human clinical trials (Leonard and Randall 2005; Parasuram 2011). Figure 7.3 represents the scheme of developing plant-derived drug lead molecules.

7.4.2 The Notion of Synergy and Mechanisms of Actions

At present, 62% of the newly developed anticancer agents have been from natural products or based on natural product models. All of these natural products brought substantial biological findings associated with their distinctive mechanisms of action with most cases evidenced in synergistic role (Marcy and Douglas 2005). Plants develop a great complexity of phytochemicals resulting from plant's defences to produce chemical defence to predatory micro- and macro-organisms. These defence chemicals exist as small molecules that apt into predator's enzymes or receptor, thus interfering with its immune system. However, the predators over time habitually acquire resistance to plant's chemical defence, and ergo, plants co-evolve to produce resistance inhibitors, and these plant defences serve as a great source of therapeutic molecules. In contrary to pharmaceutical models which act on the basis of single compound, often culminating in resistance and failure in drug response synergistic effect of multiple bioactive compounds is considered to have better therapeutic potentials (Rasoanaivo et al. 2011). However, scientific efforts to understand the complexity of interactions among the constituents in medicine plants with clinical benefit can be problematic because of the lack of sufficient information, documentation and standardization. Herbalists justifiably point out most medicinal plants are categorized as an idiosyncratic herb which has been alleged to specific kinds of toxicity. The best known are dryopteris, viscum, and corynanthe with potential hepatotoxicity (Hamid and Hedayatollah 2013).

Though validation of traditional remedies can be challenging, a great prospective exists to investigate the possible therapeutic values of medicinal plants. For reaping such benefit, the medical and pharmaceutical communities have adopted the concept and philosophy of the "silver bullet" approach therapy, where pharmaceutical industries have been very much geared to the "single-ingredient" and "one-drug-one-target" paradigm (Chun et al. 2011). Therefore, new approaches like metabolomics aid to formulate novel strategies for recognizing valid targets and discovering leads from medicinal plants against them. Thousands of novel molecular targets have been identified as implications of various ailments, since human genome was sequenced. Plant-derived compounds with known bioactivity might have targeted and promising effect in the modern screening assay directed towards the disease-causing molecular targets. Several reports have been made on the reactivity of compounds isolated from traditional medicinal plants on validated molecular targets, hence resurrecting the interest in regularly isolated compound classes in plants (Jeffrey and Michal 2009; Philomena 2011).

Yet a relatively new-fangled field, medicinal plant-based drug discovery, has played a crucial part in most new clinical applications for the past 500 years towards battling cancer. Cancer chemoprevention is a strategy to control cancer by administration of synthetic or natural compounds to inverse or suppress the process of carcinogenesis (Butler 2004). Since cancer therapy is intended to befall preceding the onset of cancer diagnosis, toxicity is mostly unexpected. In the case of prophylactic treatment and inhibiting cancer reappearance, tolerable toxicity levels are acceptable. As such, edible plants, dietary supplements, botanicals and all medicinal con-

stituents were recommended as potential chemopreventive agents for cancer. This is attributable to well-documented ethnopharmacological information about possible drug candidate compounds which were used traditionally with therapeutic potentials for humans (Park and Pezzuto 2002). Currently, plant-derived anticancer agents in clinical use can be grouped into four main classes: vinca (or *Catharanthus*) alkaloids, epipodophyllotoxins, taxanes and camptothecins. These compounds have been found to act on two important biochemical targets, tubulin and topoisomerase (Marcy and Douglas 2005).

Vinca alkaloids such as vinblastine and vincristine were isolated from *Catharanthus roseus* (Madagascar periwinkle plant). Medicinal applications of this plant lead to the discovery of major vinca alkaloids in clinical use: vinblastine (VBL), vinorelbine (VRL), vincristine (VCL) and vindesine (VDS). Only VBL and VCL are approved for clinical use. The main mechanisms of vinca alkaloid cytotoxicity are their interactions with tubulin and disruption of microtubule "vinca domain" function, disrupting the assembly of microtubules (essential to cell division) in mitosis and leading to cell apoptosis (Moudi et al. 2013). Epipodophyllotoxins are substances naturally occurring in the root of American Mayapple plant (*P. peltatum*). Etoposide and teniposide are semisynthetic derivatives of epipodophyllotoxins. These are topoisomerase II (enzyme essential for cell growth by regulating levels of DNA supercoiling) inhibitors which bind tubulin, causing DNA strand breaks during the G2 phase of the cell cycle by irreversibly inhibiting DNA topoisomerase II (Demain and Vaishnav 2011).

Taxanes are a class of diterpenes. They were originally identified from *T. Brevifolia* Nutt. The taxanes, including paclitaxel derivatives, act as a microtubule stabilizer, thereby blocking cell cycle progression as depolymerization is prevented or the tubulin assembly is interfered (Jordan and Wilson 2004). Camptothecin is isolated from *C. Acuminate* Decne. Camptothecin and its clinically used analogues, irinotecan and topotecan, arrest the cell cycle at the S-phase by affecting the activity of topoisomerase I, leading to the inhibition of DNA replication and transcription (Kingston 2009).

7.4.3 Bioavailability of Plant-Derived Anticancer Agent in Drug Discovery and Development: The Barrier of Oral Administration

Chemo drugs are mainly designed in a way it can disrupt the cell cycle of the cells, so it may not be able to distinguish between the malignant and the healthy cells in the body, i.e. interfere with both of them. However, there is a gap in between them to achieve anticancer effects or life-threatening cytotoxicity, known as the therapeutic index. Unfortunately, most of the anticancer drugs are listed under the narrow therapeutic index (NTI) drugs. Therefore, they are either prescribed closely or even at the maximum tolerated dose (Mathijssen et al. 2014). Despite optimizing the dosage according to the NTIs, another major problem faced by the drug development

company is to overcome the low bioavailability (Godugu et al. 2014). Bioavailability is the relative absorption of drug to the targeted site of action from the total amount administrated dosage of drug into the system. This is important to ensure an adequate amount of drug reaches the action site, essential for an efficient pharmacodynamics to establish an effective therapeutic achievement.

In cancer chemotherapies especially, a preparation of a wide range of dosage to cater for a better bioavailability is almost impossible as it may cause adverse drug reactions resulting in more DNA damage and more cell killing including the normal fast-growing healthy cells. Blood cells, digestive and reproductive system-associated cells and hair follicles are likely to be affected by chemotherapy causing side effects such as fatigue, nausea, indigestion, diarrhoea, hair loss and anaemia (Nonnekens and Hoeijmakers 2017). Therefore, to reduce the likelihood and the severity of the side effects caused, it is important to study the whole mechanism of the drug as well as its therapeutic actions in order to optimize the bioavailability of the drug.

Orally administered drugs remain as one of the patients' preferences for chemo as they are cheaper and involve low technical maintenance compared to the injection-related therapy (Arber et al. 2017). Anticancer drugs via oral administration shows three-quarters of all the cancer therapies available and being developed (Colomer et al. 2010). However, limited bioavailability from this route of administration is the chief challenge faced, and they are mainly due to the activity of the cytochrome P450 (CYP) enzyme such as CYP 34 and the drug transporters, such as P-glycoprotein (P-gp), deposited in the intestinal wall and the liver (Leeuwen et al. 2013). Upon oral delivery, the drug undergo first-pass metabolism in the liver by the enzyme CYP 34 before being released to the systemic circulation. Moreover, the efflux pump P-gp that is also present in the gut lining plays its role in the drug expulsion back to the intestine rather than releasing to the systemic blood circulation. These factors affect the bioavailability of the drug negatively by influencing the achievement of desired plasma drug concentration for an efficient therapeutic effect. Due to the high susceptibility of metabolism in the gastrointestinal tract, the dosage needed to cause a response will be significantly higher than the intravenous dose, but this may cause untoward effects. However, the functionality of the enzyme as well as the transporters varies among patients which further contribute to the limitation for oral drugs. On the other hand, intravenous route assured 100% bioavailability compared to other routes of administration. Yet, this venipuncture method is less preferred compared to oral route due to its inconveniences as it is exposed to many risks including the development of cannula-related infections and thrombophlebitis and a frequent visit to the clinic (Cyriac and James 2014).

As each and every patient is made up of uniquely different set of a genome, the susceptibility of therapy will be slightly different from one individual to another. Besides genomic variance, other inter- and/or intra-individual varying factors such as the age, gender, body mass and health conditions influence the drug bioavailability level (Almeida et al. 2009). Drug prescriptions should be incorporated with the mentioned factors in order to enhance the patients' compliance with the medication. An individual's body composition, blood flow and plasma protein concentration also affect drug distribution. As ageing proceeds, human physiological functions undergo changes which usually reduces the absorption rate of the drug thereby

affecting the drug distribution process (He et al. 2011). Furthermore, older patients tend to have comorbid medical conditions such as diabetes, hypertension, arthritis and heart complication that affect the overall rate of drug delivery mechanism. Prior knowledge about a patient's medical background is vital, because certain drug, for example, fluoropyrimidines, increases the risk of occurrence of cardiotoxicity in coronary artery disease patients compared to patients without the disease (Albini et al. 2010). Dose reduction or intensification is usually recommended to cater for different conditions of the patients, but it may reduce the therapeutic effect or cause lethal cytotoxicity, respectively. Therefore, to tailor for inter- and/or intra-individual variances, individualized chemotherapy involving the study of the individual's cellular, molecular or genetic contents in selecting an optimized chemotherapy provides a better chemotherapy outcome and survival.

Another approach to increase the drug bioavailability is the modification in the galenic formulation of the drug. Adapting prodrugs into drug design is one of the versatile approaches to increase its bioavailability. Upon administration, the prodrugs undergo some biological or chemical modification by the CYP450 enzyme, before being pharmacologically active (Tsume et al. 2014). Therefore, the pharmacodynamic structures of the parent drug are preserved from being altered by the enzymes. Prodrug-derived ideas have been amended into the development of anticancer drugs, such as cyclophosphamide, dacarbazine, tegafur, flutamide and duocarmycin (Montellano 2013).

7.5 Obstacles in Medicinal Plant-Based Drug Discovery: Renewal of the Interest in Drug Discovery

Compounds derived from medicinal plants have unceasingly fascinated researchers globally for being biocompatible and are regarded as potentially safe and effective. Although medicinal plant-based drug discovery has been proven successful, future undertakings certainly have constant challenges as the drug discovery from plants is a lengthy and complicated process. Natural product chemists need to prove their quality and results consistently in drug discovery and development in order to keep them in level or ahead of other drug discovery efforts (Marcy and Douglas 2005). Lead identification, optimization and development and clinical trials take significantly a long time period of an average 10 years upwards and require a lot of money (>800 million US$) to be spent in obtaining numerous leads that are cast-off during the drug discovery process. Indeed, a ratio of only 1 in 5000 lead drug candidates will effectively progress through clinical trials and be allowed pharmaceutically. Although the trend towards a loss of the development of new bioactive agents from the medicinal plants is evident, several recent positive strategies can be adopted towards reversing this trend (Veeresham 2012).

It is apparent that at present, drug-based therapeutic strategies will predominate in the twenty-first century. Acceptance of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects. But, their safety and efficacy always remain questionable. The grow-

ing capability of cancer cells to resist the chemotherapeutic drug is one of the main drawbacks of conventional chemotherapy. Besides, the effort to maximize the drug efficacy and minimize cytotoxic effects has been a constant struggle in chemotherapy as overdosage can produce many harmful effects in the patients (Aung et al. 2017).The side effects arise typically when the chemotherapy damages the healthy cells that maintain the body's function and appearance depending on the nature of the drug. Cardiotoxicity, hepatotoxicity, nephrotoxicity and ototoxicity are some of the leading problems generating from the non-specific cytotoxic nature of these chemotherapeutic drugs. Although chemotherapy is the key way to control cancer, the dreadful effects of conventional chemotherapeutics result in the dependence on alternative perspective that sets the stage for a future platform of natural products (Mondal et al. 2014).

The advent of conventional cancer chemotherapy limitations has led to a booming of searching for anticancer drugs from natural products, where people began to pay consideration to the herbal ingredients. Furthermore, high selectivity, strong activity, low side effects and a wide range of antitumour drugs from natural resources have led people to focus on cancer prevention research (Butler 2004).Compounds extracted from natural resources can enhance immune cells or body immunity and antitumour effects and inhibit the proliferation of cancer cells. Not just biologically toxic compounds have attracted people's attention but also more non-toxic ingredients that have healthcare effects. Disease prevention is more important than treatment, so is cancer. With the progress of science and technology, people now have deep understanding of tumour, making the prevention of cancer possible. The development of cancer prevention and healthcare products from natural products has broader prospects and greater economic and social benefits when compared with chemically derived anticancer drugs (Veeresham 2012).

7.6 Conclusions and Future Prospects

The importance of natural products for therapeutic purposes has gained public attention in recent years. Plant-derived antioxidant compounds with known anticancer properties are potential candidates for clinical trials and to be developed as possible therapeutic drugs. This is due to minimal side effect of plant products as compared to synthetic drugs. Although plant-derived anticancer compounds might exert toxicity to humans, they are mostly less harmful than other drugs. The toxicological properties of plant-derived compounds are screened with current methods prior to clinical trials. This procedure ensures the safety of the anticancer compounds for human consumption. Ethnobotanical claims and antioxidant activities of several medicinal plants can be taken into consideration for anticancer drug development purposes, implementing reactive oxygen species as the etiological factor for cancer. Preventive measures are always better than curative treatments; hence, it is emphasized to increase the intake of exogenous natural antioxidants in our diet. Additional intake of natural antioxidants will not cause any detrimental effect to the

human body system as the antioxidants will be biotransformed and balanced by the liver. Plant-derived anticancer compounds might exert toxic effects if administered at extreme doses; therefore, it is important to standardize the effective dose of each compound as well as the formulated drug. There is a rise in problems related to drug resistance in cancer patients who are undergoing chemotherapy, due to the unsuccessful drug formulations in chemically synthesized anticancer drugs. We believe that plant-derived anticancer compounds will be able to overcome the problem of drug resistance among cancer patients. Drug delivery methods should be enhanced in a way to use natural products alone as drug vessels instead of using synthetic vessels. Pharmacological evidences of anticancer compounds have risen in a steady pace, but yet to attain success. This is due to the limitations of plant-derived pure compounds described earlier in this chapter. We strongly support the use of multiple compounds in chemoprevention for better pharmacological results. Drug discovery and formulation process should include multiple antioxidant compounds from the same plant to provide a synergistic effect towards treating cancer. Through this procedure, the bioavailability and other setbacks of anticancer compounds could be breached, and at the same time, pharmacological values of plant-derived compounds and pharmaceutical needs for novel anticancer drugs could be compensated.

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Chapter 8
Recent Advancements in the Clinical Evaluation of Plant-Derived Anticancer Compounds

Jayabalan Shilpha, Lakkakula Satish, and Manikandan Ramesh

Abstract Cancer is a chief global health burden and the leading cause of human death worldwide next to heart diseases. The alarming rise in the mortality rate owing to cancer has driven the chase for anticancer agents to effectively combat this disease. Searching for novel and efficient compounds of natural origin has been a major aspect of concerns because they exhibit less toxic side effects. Numerous secondary metabolites from plants and their semisynthetic analogs have been identified as an excellent, novel lead structures in developing promising anticancer agents. In the current scenario, several successful anticancer drugs and their derivatives have been obtained from plant sources, and many of them are in clinical trials. Phytocompounds such as vinca alkaloids, taxanes, podophyllotoxin, camptothecin, homoharringtonine, and their derivatives have appreciably influenced cancer research on many facets. Likewise, some of the other plant-derived anticancer agents including omacetaxine mepesuccinate, ingenol mebutate, β-lapachone, flavopiridol, curcumin, etc. are currently being under phase I and II clinical trials, either individually or in concert with other anticancer agents for the treatment of a broad range of tumors like lymphomas, leukemias, and solid tumors. Customary anticancer drug discovery has targeted mainly on the cytotoxic agents that hamper metabolic pathways critical to cell division. However, during recent years, several molecular target-based compounds have been emerged concentrating on other cellular process of cancer cells such as apoptosis, metastasis, angiogenesis, etc. Hence,

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the present anticancer drug discovery involves high-throughput screening of phytocompounds against a series of such molecular targets. The present chapter discusses the clinical evidences of some important phytocompounds of anticancer plants, overview of their current clinical status, and recent advances in their molecular mechanism of action.

Keywords Anticancer agents · Cytotoxic agents · Clinical trials · Natural compounds · Secondary metabolites

8.1 Introduction

Cancer is the foremost public health problem that represents the world's primary causes of death with an episode rate of 2.6 million and more cases per annum (May 2014; Siegel et al. 2015). Cancer, a set of devastating diseases and major cause of mortality in the United States of America, next only to heart diseases, is responsible for nearly one of every four deaths. The World Health Organization (WHO) has speculated that without immediate measures, the worldwide deaths due to cancer will elevate to nearly 80% by 2030, with more frequent occurrence in the middle- and low-income countries. Given the morbidity and mortality rate linked to the disease, along with the considerable economic burden, there persists to be a vital necessitate for more effective approaches. Despite the remarkable efforts over the past few decades to develop the existing therapeutic choices, and a vast number of efficient chemotherapeutic anticancer agents have been successfully identified and exploited in clinical practice, cancer still appears to be the major cause of fatality in most countries. The raise in the frequency of cancer, as well as the detrimental side effects of cancer chemotherapy, demands the discovery of novel therapeutic agents from natural sources.

Throughout the history, nature has provided an infinite resource of medicinal plants that have occupied a dominant role in treating human ailments and are used as their crude forms including infusions, syrups, and ointments (Ansari and Inamdar 2010). Plant-derived secondary metabolites significantly represent a boundless source of chemicals for new drug discovery. The compounds obtained from plants as such may not serve as the drug but may form the basis for screening, selecting, and developing many potential novel therapeutic agents (Dias et al. 2012). In reality, the idea of chemoprevention involving chemical drugs is commonly employed for treating cancers, however, with a limited therapeutic success due to toxicity issues (Pan et al. 2013). The chemoprevention of cancer using phytochemicals has revealed effective outcomes against a range of malignancies (Shukla et al. 2014). Thus, natural products as cancer therapeutic agents are of a particular interest among therapist due to their low toxicity profiles and potential effectiveness. Nearly 60% of the existing anticancer drugs are mainly the plant-derived pure compounds or their

chemical analogs (Newman and Cragg 2012). Nature exists as an abundant source of biologically diverse and active chemotypes, though relatively a few of the originally isolated natural compounds are clinically tested as effective drugs. Researchers and clinicians are now focusing on search of natural products with multi-targeted potentials for effective chemoprevention (Millimouno et al. 2014; Cragg and Pezzuto 2016). Moreover, it offers a platform for the development of novel and safe drugs by means of appropriate understanding of the intricate synergistic interaction between varieties of constituents of anticancer herbs (Khazir et al. 2014).

Today's cancer therapy involves majorly of natural products as anticancer agents from various sources such as plants, animals, and microorganisms. With the advent of new technologies like high-throughput screening and combinatorial synthesis, plant-based anticancer drug discovery has been now expanding as evidenced by substantial increase of natural anticancer agents under clinical trials. A detailed collection of natural anticancer compounds from plants can be observed at Naturally Occurring Plant-Based Anticancer Compound-Activity-Target database (http://crdd.osdd.net/raghava/npact/) which comprises of about 1980 experimentally demonstrated compound-target interactions. Some of the major classes of plant-derived anticancer compounds that have drawn attention in current clinical trials include vinca alkaloids, taxanes, podophyllotoxin, camptothecin, homoharringtonine, and their derivatives. The present chapter discusses the clinical evidences of some important phytocompounds of anticancer plants, overview of their current clinical status, and recent advances in their molecular mechanism of action.

8.2 Clinical Evidences of Anticancer Potential from Traditional Medicinal Plants

For millennia, plant products have served as a thriving source of therapeutically valued compounds and presently gained significant applications in the fields of pharmacy, medicine, and biology (Swamy and Sinniah 2016; Arumugam et al. 2016). Nature has been proven to be the eternal reservoir of bioactive molecules for drug discovery, offering structural diversity and a broad-spectrum novel and exciting medical activities (Mohanty et al. 2017; Swamy et al. 2017). Besides phytocompounds have a long history of treating several human illnesses and occupy a major fraction of the current pharmacopeia. According to the World Health Organization (WHO), plant-based medicine is still the mainstay of about ~80% of populations in many developing countries (Swamy et al. 2011, 2016; Akhtar et al. 2014; Gezahegn et al. 2015). For the past two decades, almost 50% of new drugs introduced in the market had a natural product origin, and this rate is even high for anticancer drugs, wherein almost two thirds of such drugs were of natural origin (Newman and Cragg 2007). Nearly more than 3000 plant species have been reported to possess anticancer activity and utilized in cancer therapeutics. In addition to the presence of unique pharmacophores and a maximum degree of stereochemistry, phytocompounds are

better than synthetic compounds due to their efficient delivery to the intracellular site of action as they commonly belong to the category of active biological metabolites and the real substrates for membrane transport mechanisms (Hert et al. 2009). Furthermore, when compared to synthetic ones, natural products have superior bioavailability which gives more advantage in the early stage of drug discovery by preventing false positives. The intricacy in the development of anticancer drug is very well understood from the fact that merely 5% of the candidates reaching clinical trials get the approval for marketing (Choi et al. 2017). The extent of clinical potency and toxicity of several anticancer agents obtained from traditional medicinal plants is indefinite and elusive. For instance, research on greater part of traditional anticancer plants is in the preclinical stage or is not being actively practiced. Many of the ayurvedic anticancer plants such as *Allium sativum*, *Aloe vera*, *Andrographis paniculata*, *Annona atemoya/muricata*, *Asparagus racemosus*, *Berberis aristata*, *Curcuma longa*, *Calotropis gigantea*, *Datura metel*, *Euphorbia hirta*, *Moringa oleifera*, *Nigella sativa*, *Phyllanthus niruri/amarus*, *Picrorhiza kurroa*, *Piper longum*, *Plumbago zeylanica*, *Podophyllum hexandrum*, *Rubia cordifolia*, *Tinospora cordifolia*, *Semecarpus anacardium*, *Taxus baccata*, *Vinca rosea*, and *Withania somnifera* have scientifically demonstrated anticancer properties and are now actively exploited for the treatment of different cancer types (Balachandran and Govindarajan 2005). In saffron (stigmata of *Crocus sativus*) selective and assuring anticancer properties have been observed at both *in vitro* and *in vivo* conditions but not hitherto in clinical trials (Schmidt et al. 2007). The various forms of pomegranate such as juice peel and oil were found to exert potent anticancer activity, by causing hindrance to cell cycle, tumor cell proliferation, invasion, and angiogenesis (Lansky and Newman 2007). Thus, future research on these anticancer plants would aid in identifying safe and competent anticancer drugs and in furtherance the exploration of their mode of action.

8.3 Clinical Uses of Major Plant-Derived Anticancer Compounds

There are four main structural classifications of anticancer compounds derived from plants, namely, alkaloids, terpenoids, polyphenols, and lignans. These groups of compounds include some of the most active novel chemotherapeutic agents for current use in a clinical setting. One of the major setbacks associated with the usage of plant-based compounds is poor bioavailability or meager solubility that poses complications in manufacturing of bulk amounts required to serve as medicines (Fridlender et al. 2015). The major strategy adopted for diverse plant-derived substances is the utilization of semisynthetic or synthetic analogs. Some of the major plant-derived anticancer compounds in clinical use and under different phases of clinical trials are discussed below.

8.3.1 Alkaloids

8.3.1.1 Vinca alkaloids

The epoch of using plant source as anticancer agents began with the identification of two important alkaloids from the Madagascar periwinkle, *Catharanthus roseus* G. Don (Apocynaceae), such as vinblastine and vincristine (Verma and Singh 2010). For almost a half century, these two compounds have been employed in clinical oncology, which act by inhibiting the polymerization of tubulin molecules and the formation of the mitotic spindle resulting in metaphase arrest and apoptosis (Khazir et al. 2014). Later, a number of semisynthetic analogs of these two important drug molecules have been developed. The first semisynthetic vinca alkaloid to enter into human clinical trials was vindesine (Jordan and Wilson 2004), which is primarily used in the treatment of acute lymphocytic leukemia (ALL). Occasionally, it is used in the treatment of breast cancer, colorectal cancer, renal cell cancer, blast crisis of chronic myelocytic leukemia (CML), and non-small-cell lung cancer (NSCLC). Vinorelbine is another semisynthetic derivative of vinblastine which gained approval to treat metastatic breast cancer (MBC) in 1991. Vinflunine, a dihydro-fluoro derivative of vinorelbine was approved as second-line chemotherapy in metastatic urothelial cancer (Bachner and De Santis 2008; Mamtani and Vaughn 2011). Vinflunine is actively used in phase II/III clinical trials to treat advanced stage of cancers like MBC and NSCLC. In addition, many other structural analogs of vindesine are undergoing clinical trials including anhydrovinblastine (Hydravin), vinglycinate, and vintripol.

8.3.1.2 Homoharringtonine

Cephalotaxus alkaloids including harringtonine, isoharringtonine, and homoharringtonine (HHT) were acquired from *Cephalotaxus harringtonia* var. *drupacea* (Cephalotaxaceae). Cephalotaxus alkaloids are inhibitors of protein synthesis. Homoharringtonine in its purified form has been found effective against a range of leukemias, especially those which are resistant to standard treatment, and has been observed with complete hematologic remission capacity in late chronic-phase chronic myelogenous leukemia (CML) patients. Homoharringtonine (Omacetaxine mepesuccinate) has been clinically tested with CML in myeloid blast crisis (CML-MBC) patients (Cragg and Pezzuto 2016).

8.3.1.3 Colchicine

Colchicine, a nitrogenous alkaloid isolated from *Colchicum autumnale* is one of the oldest known antimitotic drugs, available till now due to its potent antitumor properties. It attaches irreversibly to tubulin and thereby blocks microtubule formation,

inhibits cell cycle progression, and subsequently induces apoptosis. The major setback of colchicine in clinical usage is its nonspecific action on both actively dividing tumor cells and normal cells. However, derivatives of colchicine, namely, colchicinamide and deacetylcolchicine were made with minimum toxicity and greater specificity to tumor cells combined with effective drug delivery approaches (Sivakumar 2013). Colchicinamide has been targeted toward the treatment of breast cancer and various other solid tumors. Deacetylcolchicine has been now subjected to phase II clinical trials for its effectiveness against melanoma, Hodgkin's lymphoma, and chronic granulocytic leukemia. Recently, a series of novel deacetamidothiocolchicine-7-ols and their ester exhibited potent inhibitory activity against tubulin polymerization and selective cytotoxicities against colon and CNS cancers as well as melanoma *in vitro* (Muhtasib et al. 2015).

8.3.1.4 Ellipticine

Ellipticine, an indole alkaloid isolated from *Ochrosia elliptica* is a topoisomerase II inhibitor and showed efficient anticancer effect in many humans and animal tumor systems (Marie et al. 2012). The analogs of ellipticine such as DHE (N-2-(diethylaminoethyl)-9-hydroxy-ellipticinium chloride, NMHE (2-N-methyl 9-hydroxy-ellipticine), and retelliptine dihydrochloride (SR 95325 B) were at phase II and phase I clinical trials on metastatic breast cancer and murine solid tumor models, respectively (Iveta et al. 2013).

8.3.1.5 Camptothecin Alkaloids

Camptothecin (CPT) was isolated from *Camptotheca acuminata* which is a quinoline alkaloid, with topoisomerase-I inhibitor activity and stimulates cell death by DNA damage (Khazir et al. 2014). Nevertheless, it was withdrawn from clinical trials owing to its low aqueous solubility and severe toxicity. To overcome these problems, a sequence of novel CPT analogs were developed and approved for use in clinical setting such as topotecan, irinotecan, and belatecan which are effective inhibitors of DNA topoisomerase-I, a crucial component in DNA replication and transcription (Oberlies and Kroll 2004). Irinotecan is used in the treatment of colorectal cancers, while topotecan is actively used for treating ovarian and small-cell lung cancers. In addition, there are other camptothecin derivatives which are in preclinical development stage (Rahier et al. 2005; Khazir et al. 2014) (Table 8.1).

Table 8.1 Semisynthetic analogs and prodrugs of camptothecin under clinical trials

CPT semisynthetic analogs	Clinical status	Target
9-Aminocamptothecin (9-AC)	Phase II	Ovarian and malignant lymphoma
Karenitecin (BNP-1350)	Phase II	Malignant melanoma and brain tumors
Diflomotecan (BN-80915)	Phase I	Solid tumors
Gimatecan (ST-1481)	Phase I and phase II	Advanced solid tumors, epithelial ovarian, and fallopian tube cancers
Elomotecan (BN-80927)	Phase I	Advanced solid tumors
Exatecan mesylate	Phase II	Gastric cancers
Rubitecan	Phase III	Pancreatic cancer
CZ-48	Phase I	Solid tumors
XMT-1001	Phase I b	Lung cancers
EZN-2208	Phase I	Advanced malignancies

8.3.2 Terpenoids

8.3.2.1 Taxol and Its Semisynthetic Analogs

Taxol, (paclitaxel) a highly effective chemotherapeutic diterpenoid, isolated from the bark of *Taxus brevifolia* (Pacific yew tree) is active against ovarian and breast cancers (Newman et al. 2003). Paclitaxel was the first compound discovered to support microtubule formation and has been administered in the treatment of different types of cancers such as ovarian, breast, NSCLC, and Kaposi sarcoma (Newman and Cragg 2012). A number of its semisynthetic derivatives such as docetaxel, cabazitaxel, and abraxane (ABI-007) have been developed and proficiently utilized in the treatment of broad range of tumors, prostate cancer, and breast cancer, respectively. There are several other novel semisynthetic analogs in development which are being designed to improve the pharmacology and therapeutic index (Saklani and Kutty 2008; Khazir et al. 2014) (Table 8.2).

8.3.2.2 Betulinic Acid

Betulinic acid, a pentacyclic triterpenoid and potent inhibitor of topoisomerase, is commonly occurring in Betula species (Betulaceae) (Cichewicz and Kouzi 2004; Fulda 2009; Mullauer et al. 2010). Betulinic acid has been reported to induce apoptosis in human melanoma cell lines by selectively arresting their growth and to exhibit *in vivo* activity in athymic mice possessing human melanoma cells (Pan et al. 2010). Betulinic acid has also shown significant cytotoxic effect against neuroectodermal and malignant brain tumor cell lines (Cichewicz and Kouzi 2004). So far, a greater number of betulinic acid derivatives have been synthesized to enhance its therapeutic activity. NVX-207 is one of the novels and well-tolerated Beta derivatives that has shown prominent antitumor activity in treatment-resistant canine

Table 8.2 Semisynthetic analogs of paclitaxel in clinical trials

Paclitaxel semisynthetic analogs	Clinical status	Target
Paclitaxel poliglumex (CT-2103, Xyotav)	Phase III	Non-small-cell lung carcinoma (NSCLC)
7-DHA-Taxol (Taxoprexin 13)	Phase II	Advanced NSCLC, eye and skin melanoma
	Phase III	Advanced lung cancers
Larotaxel (XPR9881)	Phase II	Breast cancer
	Phase III	Metastatic urothelial tract or bladder cancer
Ortataxel (BAY-59-862)	Phase II	Breast cancer
TPI-287	Phase I/II	Melanoma, neuroblastoma, and medulloblastoma
Milataxel (MAC-321)	Phase II	Colorectal cancer
Tesetaxel (DJ-927)	Phase I/II	Advanced solid tumors
BMS-184476	Phase I	Human tumor xenografts and advanced solid tumors
RPR 109881A	Phase III	Breast cancer

cancer patients. NVX-207 has also shown excellent responses in phase I/II study of dogs afflicted with naturally occurring cancer undergoing local treatment of NVX-207 (Willmann et al. 2009). The topical application of 20% of betulinic acid ointment has been evaluated under phase I/II clinical trials for the treatment of dysplastic melanocytic nevi (NIH 2013). Recently, Kutkowska et al. (2017) have showed the synergistic capacity of betulinic acid with sorafenib on induction of apoptosis and inhibition of clonogenic activity in various non-small-cell lung cancer cell lines.

8.3.3 Polyphenols

8.3.3.1 Resveratrol

Resveratrol (trans-3,5,4′-trihydroxystilbene) is a phytoalexin that occurs in various plant species such as mulberries, peanuts, and grapes. Resveratrol has been recognized as a useful candidate for cancer chemoprevention owing to its remarkable inhibitory effects on sequential cellular actions such as tumor initiation, promotion, and progression (Zhu 2011). In all three stages of carcinogenesis (initiation, promotion, and progression), resveratrol has exhibited chemotherapeutic and chemopreventive activities, as demonstrated in both UVB and chemically induced skin carcinogenesis in mice and various other murine models of human cancers (Nobili et al. 2009). Several *in vivo* and *in vitro* studies have confirmed its capacity to modulate and control diverse signaling pathways and various targets (Athar et al. 2007). Being a chemopreventive agent, resveratrol is now undergoing phase I studies in patients with colorectal cancer as well as in healthy subjects who are highly susceptible to the development of melanoma (Nobili et al. 2009). Recently, Singh et al.

(2016) have summarized the various ongoing clinical trials of resveratrol against human cancer. Resveratrol has been evaluated for its role in treating colorectal cancer, gastrointestinal tumors, and in follicular lymphoma.

8.3.3.2 Combretastatin and Its Analogs

Combretastatin, a stilbenoid separated from the bark of *Combretum caffrum* Kuntze (Combretaceae) is a vascular disrupting agent. It restrains the growth of blood vessel in tumors, resulting in necrosis and cell death of tumors. The combretastatin A series are well known for their tubulin assemblage inhibitory and cytotoxicity activities against *in vitro* human cancer cell lines. Combretastatin A4 is active against colon and lung cancers and leukemia which are now at phase I/II clinical trials (Banerjee et al. 2008). Combretastatin A4 phosphate (CA4P) is the phosphate-bearing prodrug of combretastatin A4, which is capable of inducing morphological alterations within endothelial cells and cause selective and prompt vascular dysfunction of tumors (Delmonte and Sessa 2009; Pan et al. 2010). CA4P is currently examined in phase II clinical studies for treating anaplastic thyroid carcinoma. The other phosphate prodrug of combretastatin A-1, OXi4503 has been found outstanding in preclinical studies and has been investigated in phase IIb clinical trial to study its safety and efficacy against solid tumor in the liver (Patterson et al. 2012). Moreover, combretastatin CA4P has been actively investigated for several types of thyroid cancer including anaplastic, medullary, and stage four follicular thyroid cancer (Jaroch et al. 2016; Xie and Zhou 2017).

8.3.4 Lignans

8.3.4.1 Podophyllotoxins

Podophyllotoxin is an important anticancer lignan obtained from *Podophyllum emodi* or *P. peltatum* (Berberidaceae). The two most important and successful analogs of podophyllotoxin are etoposide (VP16 or VP16-213) and teniposide (VM26). Etoposide is one of the most dynamic anticancer agents administered in the treatment of Hodgkin's and non-Hodgkin's lymphomas, testicular teratoma, small-cell lung cancer, and a variety of other malignancies. Due to limitations, such as reduced water solubility, the development of resistance, and toxicity by tumor cells, investigators are exploring new synthetic analogs for new lead compounds with superior therapeutic index and extensive therapeutic scope. As a result of these several semi-synthetic analogs such as tafluposide, etoposide phosphate, NKH-611, GL331, azatoxin, and Top-53 have gone through clinical trials for treating a variety of cancers (Liu et al. 2002; Sargent et al. 2003; Lee and Xiao 2012). Very recently, deoxypodophyllotoxin (DPT) has been reported to have strong antitumor effect on lung cancer, breast cancer, and gastric cancer (Khaled et al. 2017).

8.4 Other Anticancer Compounds in Clinical Trials

Curcumin, a yellow polyphenol obtained from *Curcuma longa* L. (Zingiberaceae), is the most important dietary supplement and chemopreventive agent (Singh 2007). Curcumin arrests multiplication of a broad range of malignant cells via modulating several intracellular signaling pathways, by exerting its action on various targets, such as growth factor receptors, cell surface adhesion molecules, transcription factors, and protein kinases (Aggarwal et al. 2007). Curcumin is now undergoing phase II clinical trials to treat pancreatic cancer at advanced stage as well as colorectal cancer (Johnson and Mukhtar 2007; Dhillon et al. 2008). Recently, Panda et al. (2017) have extensively reviewed the promising role of curcumin as an adjuvant in combinatorial therapy for treating several cancer types. Currently, many clinical trials involving curcumin as an anticancer agent have been ongoing, and few have been completed including breast cancer, lung cancer, colon cancer, oral cancer, prostate cancer, and cervical and gastric cancers.

Indirubin, an isomer of indigo is an alkaloid present in "Qingdai," a Chinese traditional medicine that has been reported to possess broad-spectrum anticancer activity (Eisenbrand et al. 2004). A derivative of indirubin, namely, meisoindigo, has significant ability to hinder cyclin-dependent kinases, arrest cell cycle at G0/G1 phase, promote cell differentiation and apoptosis and has also been found to inhibit growth of tumor in xenografts of HT-29 colon cancer (Zuo et al. 2008). Meisoindigo is currently under phase III stage of clinical trial as a chemotherapeutic agent against chronic myelogenous leukemia (CML) (Pan et al. 2010). Perillyl alcohol, a limonene type of monoterpenoid, occurring in essential oils of aromatic plants like lavender (*Lavandula intermedia*), peppermint (*Mentha piperita*), etc., has been reported to stimulate apoptosis, cellular differentiation, and inhibition of cell cycle at G1 phase (Yeruva et al. 2007). Recently, perillyl alcohol has been involved in phase II clinical trial for treating metastatic breast cancer patients (Bailey et al. 2008).

Protopanaxadiol (20S-protopanaxadiol) is a dammarane group of triterpene aglycone obtained by hydrolysis of ginseng (*Panax ginseng* C.A. Mey.; Araliaceae) saponins. The mode of action of protopanaxadiol was found to be inducing apoptosis in cancer cells and arresting cell cycle by means of regulating different signaling pathways involving caspases. It has also been observed to possess cytotoxic activity in multidrug-resistant tumors by exhibiting P-glycoprotein blocking activity (Liu et al. 2007). Protopanaxadiol has now been taken to phase I clinical trial for the chemotherapy of lung cancer and other solid tumors (Saklani and Kutty 2008). Flavopiridol is a synthetic compound whose chemical structure is similar to rohitukine a compound isolated from *Dysoxylum binectariferum*, an indigenous plant of India (Khazir et al. 2014). Flavopiridol being a potent inhibitor of cyclin-dependent kinases (cdks) was the first compound to reach clinical trial (Shapiro 2004). It is currently undergoing a number of phase I and phase II clinical trials for leukemias, lymphomas, and solid tumors.

Ingenol mebutate (ingenol-3-angelate 44) is a substance identified from the sap of *Euphorbia peplus* (Fallen and Gooderham 2012). It plays a prominent role in skin

Table 8.3 Other anticancer agents in clinical trials

Name	Source	Functions	Clinical status	References
Bruceantin	*Brucea antidysenterica*	Inhibitor of peptidyl transferase in elongation reaction	Phase II	Da Rocha et al. (2001)
4-Ipomeanol	*Ipomoea batatas*	DNA binder	Phase II	Lakhanpal et al. (2001)
Pervilleine A	*Erythroxylum pervillei*	P-glycoprotein inhibitor	Lead molecule	Mi et al. (2003)
Dimethyl xanthene-9-one-4-acetic acid	Flavone-8-acetic acid analog	TNF-α inducer	Phase II	Westwell (2003)
NM-3	Isocoumarin derivative	Inhibitor of angiogenesis by VEGF expression inhibition	Phase I complete	Quesada et al. (2006)
Silvestrol	*Aglaia foveolata*	Mitochondrial apoptotic pathway promoter	Lead molecule	Kim et al. (2007)
Salvicine	*Salvia prionitis*	Non-intercalative topoisomerase II inhibitor	Phase II	Cai et al. (2008)
Triptolide	*Tripterygium wilfordii*	Inducer of apoptosis by modulating p53	Phase I	Saklani and Kutty (2008)
Phenoxodiol	Genistein derivative	Topoisomerase II inhibitor and apoptosis promoter	Lead molecule	Silasi et al. (2009)
Quercetin	Onions, apples, red wine, and tea	Inducer of apoptosis, androgen receptor inhibitor, and angiogenesis inhibitor	Phase I	
Berbamine	*Berberis amurensis*	Caspase-3-dependent apoptosis	Lead molecule	Kapoor (2012)
Roscovitine (CYC 202)	(*Raphanus sativus*)	CDK inhibitor	Phase II	Slovackova et al. (2012)
Kanglaite	*Coix lacryma-jobi*	Inhibitor of mitosis of tumor cells during G2/M phase	Lead molecule	Zhan et al. (2012)
Santonin	*Artemisia maritima*	NF-Kb inhibitor	Lead molecule	Khazir et al. (2013)

chemotherapy; particularly, a gel form of the compound has been used for the treatment of acid keratosis. Beta-lapachone a quinone obtained from lapacho tree (*Tabebuia avellanedae*) bark is a powerful DNA topoisomerase I inhibitor that acts by causing delay in cell cycle at G1 or S phase prior to inducing either apoptosis or necrotic cell death of human carcinoma cells, including colon, lung, ovary, breast, and prostate (Nobili et al. 2009). At present, it is investigated in a phase I and II study (Anonymous 2014). Moreover, a large number of other anticancer agents in clinical trials are listed in Table 8.3.

8.5 Recent Advancements in Mechanism of Action of Natural Anticancer Drugs

The conventional screening of plant-based anticancer agents was principally based on examining the cytotoxic activity either *in vitro* using cancer cell lines or *in vivo* using animal models. Several natural anticancer agents discovered actually through such assays have been found to exhibit their cytotoxic activity by interacting with tubulin. But the major limitations of cytotoxic agents are attributed to their toxicity and tumor regression effects. As recent researches have revealed a number of molecular targets associated with specific cancers, anticancer drug discovery is currently focused on high-throughput screening of phytocompounds against a series of such targets. The major advantage of using molecularly targeted agents is that their mechanism of action may not exhibit significant or direct toxicity. Their actions are mainly targeted toward transmembrane, nonnuclear intracellular or extracellular processes and are illustrated by matrix metalloproteinase (MMP) inhibitors, farnesyl transferase inhibitors, receptor tyrosine kinase inhibitors, and angiogenesis inhibitors. There are various modes of action by which phyto-anticancer compounds exert their action on tumor cells such as facilitation of oxidative stress, inflammation, apoptosis, autophagy, and inhibition of tumor cell survival, cell division and differentiation, metastasis, and angiogenesis (Fig. 8.1). So far, the major molecular mechanisms such as facilitation of apoptosis, inhibition of metastasis, inhibition of angiogenesis, and using antibodies against tumor-specific antigens have been investigated for the development of molecular target-based anticancer agents (Narang and Desai 2009; Singh et al. 2016).

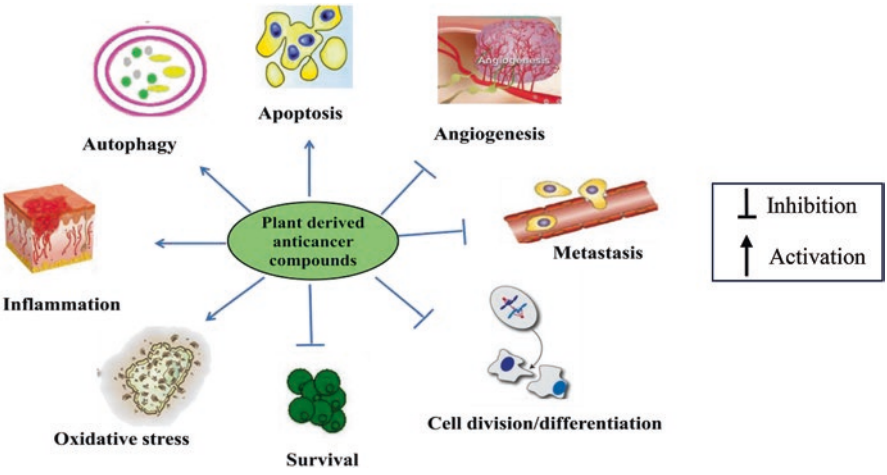

Fig. 8.1 Mechanism of action of plant-derived anticancer compounds on tumor cells. ⊥ = Inhibition; ↑ = Activation

8.5.1 Facilitation of Apoptosis

Apoptosis is a physiological intracellular process mediated by a network of organized signaling pathways ultimately leading to cell death and removal of dead cells by means of phagocytosis. DNA damage caused by cytotoxic drugs usually triggers apoptosis in the cell via two signaling mechanisms – Bcl-2 family proteins mediated activation and release of mitochondrial pro-apoptotic proteins known as caspases or upregulation of pro-apoptotic receptors on cancer cells, which further activates apoptotic signaling pathway by consequent interaction with their ligands. These pro-apoptotic receptors comprise the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors Fas (CD95 or APO-1). Moreover, anticancer drugs may also activate lipid-based signaling pathways resulting in reduced apoptosis threshold or alter other cytoprotective pathways involving heat shock proteins, nuclear factor-kB, and cell cycle regulatory pathways. Muhtasib et al. (2015) have briefly reviewed the apoptosis-mediated cell death mechanisms of some important plant-derived anticancer drugs such as resveratrol, genistein, berberine, triptolide, and betulinic acid. Some brassinosteroids such as homocatasterone and epibrassinolide were also found to exert anticancer effects through apoptosis. Also, apigenin was observed to induce apoptosis in lung adenocarcinoma cells by targeting leptin receptor pathway (Singh et al. 2016). Harmine, a -carboline alkaloid isolated from the seeds of *Peganum harmala* was found to exhibit *in vitro* and *in vivo* anticancer activity against thyroid cancer by means of inducing apoptosis of TPC-1 (thyroid cancer cell line) cells via regulating the ratio of Bcl-2/Bax in a dose-dependent manner (Ruan et al. 2017). Similarly, curcumin was found to induce an endoplasmic reticulum stress-mediated apoptosis in prostate cancer (Rivera et al. 2017).

8.5.2 Inhibition of Metastasis

Metastasis is the process where the tumor cells spread from region of the body to another which is facilitated by cellular movement and remodeling of the extracellular matrix (ECM), which gives shape to the tissue. Several extracellular enzymes such as plasminogen activators, matrix metalloproteinases, and proteases have been involved in the remodeling of extracellular matrix resulting in metastasis of cancer. Thus, compounds targeting these proteases and matrix metalloproteinases have been now developed as potential anticancer agents. Nimbolide from *Azadirachta indica* functions as a potent anticancer agent by inhibiting proliferation and metastasis of tumor cells in pancreatic cancer (Subramani et al. 2016). Chanvorachote et al. (2016) have reviewed a number of potential anti-metastatic plant compounds against lung cancer including imperatorin, artonin E, oroxylin A, gigantol, moscatilin, baicalein, etc.

8.5.3 Inhibition of Angiogenesis

Recent strategies for cancer therapy, other than just tumor cytotoxicity, have been under intensive development, such as anti-angiogenic therapeutic approach. The process of formation of new blood vessels called angiogenesis is an indispensable prerequisite for the growth and metastasis of tumor cells. Thus, inhibition of angiogenesis is considered as the ideal way to block oxygen and nutrient supply to the tumors, causing tumor deterioration. Tumor angiogenesis involves three major events such as switch, proliferation, followed by endothelial cell migration to initiate the formation of new blood vessels as well as remodeling of extracellular matrix. There are a number of signal transducers that take part in angiogenesis including the vascular endothelial growth factor (VEGF), the integrins, plasminogen activation system, and the matrix metalloproteinases. Hence, nowadays, drug targets have been developed to prohibit one or many aspects of these pathways, e.g., VEGF antibodies and VEGF receptor antagonists. Zhang et al. (2014) have elaborated the various anticarcinogenic actions of ellagic acid including angiogenesis via VEGFR-2 signaling pathway in breast cancer and Akt, Shh, and Notch pathways in pancreatic cancer. Epigallocatechin-3-gallate (EGCG) from green tea was found to exhibit anti-angiogenesis effect by downregulating the expression of VEGF in various cancer types (Min and Kwon 2014). On contrary, ferulic acid has been reported to inhibit fibroblast growth factor receptor 1(FGFR1) in a non-VEGF angiogenic pathway (Yang et al. 2015). Platycodin D from *Platycodon grandiflorum*, a Chinese medicinal plant, has been shown to exhibit angio-suppressive activity by regulating VEGF expression (Khan et al. 2016).

8.5.4 Targeting of Tumor-Specific Antigens by Antibodies

Induction of immune responses by employing antigens that are specific to each type of tumor is a much appreciated objective in cancer therapy, due to its exception from dose-limiting toxicity. On the other hand, usage of antibodies against antigens of specific tumor can also be done to target tumors by involving radio isotopes, prodrug-converting enzymes, and toxins. Tumor regression through antibodies involves complement fixation or antibody-dependent cellular toxicity (ADCC) mediated via monocytes, natural killer cells, and granulocytes. In addition, some novel strategies including the expression of target antigens on the antigen-presenting cells (APCs) or dendritic cells have also been exploited to activate T-cell immune response. In recent times, anticancer immunotherapy involving monoclonal antibodies targeting a number of antigens such as PD-1, VEGF, EGFR, PAP, etc. has gained much clinical significance. Currently, the monoclonal antibodies like ipilimumab (metastatic melanoma), nivolumab (NSCLC), pembrolizumab (metastatic breast cancer), trastuzumab (breast cancer), etc. are in phase III clinical trials (Wurz et al. 2016).

8.6 Conclusions and Future Prospects

Natural anticancer drug discovery is a dominant strategy to identify not only new classes of anticancer agents but also their novel mechanisms of action. Having known the bottomless diversity of nature, it is worth mentioning that chemical lead molecules can be created which are capable of interacting with the majority or nearly all therapeutic targets. Moreover, perhaps only less than 20% of the plants identified on earth have been taken up for investigating their therapeutic potential, thus indicating that relatively a smaller percentage is still available for screening anticancer activity. Recently, with the arrival of high-throughput screening, a huge number of effective experimental agents can be readily screened and examined, and choice can be made for discovering prototype ligands valuable of future development as therapeutic substances. While drug-associated toxicity continues to be a considerable obstacle for presently available chemopreventive and chemotherapeutic drugs, the usage of natural compounds in combination with current chemotherapeutic agents as adjuvant therapy may aid to alleviate drug-associated toxicities. Hence, it is apparent that efficient drug discovery and development will entail multidisciplinary collaborative approaches including the discovery of natural product lead, integrated with combinatorial and medicinal chemistry.

However, one of the major hurdles faced in the isolation of anticancer compounds from plants is the limited accessibility of the starting material. Even though many plant-derived compounds have previously been isolated and characterized, often the available compound quantities are inadequate for evaluating a wide range of anticancer activities. Hence, supply constraints remain a major hindrance for the successful clinical research and commercialization of plant anticancer compounds. Also, correct identification of anticancer plants is yet another challenge in anticancer drug discovery. Hence, the usage of combination of methods like genetic and biochemical analysis along with morphological and anatomical characterization is essential for an unambiguous identification of anticancer plants. *In vitro* production of potent anticancer compounds through various biotechnological approaches such as *in vitro* propagation, plant cell cultures, hairy root cultures, elicitation, metabolic engineering, and heterologous expression would be a promising strategy to meet the pharmaceutical demand of anticancer plants. The conduct of meticulous clinical trials desirable for approval of anticancer drugs signifies another major intricacy. High-throughput screening of phytochemicals for identifying anticancer activity in turn requires high robustness, reliability, reproducibility, and accuracy, as upholding the stability of plant extracts might be difficult, owing to their complexity. Therefore, a highly sophisticated experimental design is indispensable for the anticancer drug discovery. Moreover, computational approaches are powerful knowledge-based method that acts as an efficient filter tool in the search of anticancer activities from plants. Hence, the above-described challenges have reduced interest in plant-based anticancer drug discovery nowadays, which can be rejuvenated through the use of synergistic approaches including in silico methods, combinatorial synthesis, ethnopharmacological knowledge, plant biotechnology, and a wide range of cell-based, *in vitro* and *in vivo* models for testing bioactivity.

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Chapter 9
Organosulfur Compounds of Garlic as Potent Chemotherapeutic Agents Against Cancer

Irfan Ahmad Ansari, Imran Khan, Mohd Salman Khan, and Mohd Sayeed Akhtar

Abstract Experimental and epidemiological studies over the past few decades have provided ample evidences with regard to the association amid plant food consumption and decreased cancer risk. Many phytochemicals have proved their anticancer potential to be used as therapeutics against cancer. Among them garlic (*Allium sativum*) has been of much interest, mostly due to the epidemiological reports, which linked the increased garlic consumption with reduced prevalence of many human diseases. Garlic and their constituent organosulfur compounds (OSCs) have been attributed to several medicinal properties like hypocholesterolemic, fibrinolytic, immunostimulatory, antimicrobial, antiviral, antifungal, and anticancer effects. The OSCs have been shown to have strong anticarcinogenic property against a variety of chemical carcinogens in different preclinical animal model studies. Extensive researches are still being carried out to elucidate the molecular mechanism of action of OSCs. The main focus of the present chapter is to give an overview of the past and present studies undergoing on organosulfur compounds for exploring their potential as an adjunct in cancer chemotherapeutics and research.

Keywords Allicin · Carcinogenesis · Chemoprevention · Organosulfur compounds · Phytochemicals

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9.1 Introduction

The experimental and epidemiological studies over the past few decades have provided ample evidences for the association amid plant food consumptions and decreased cancer risk. Many phytochemicals have proven their anticancer activities and now been used as an effective therapeutics against the cancer. Among them garlic (*Allium sativum* L.) has received much attention because of the epidemiological reports, which may linked the increased garlic consumption with reduced prevalence of several human diseases. The protective and beneficial role of garlic is known from ancient times since 1400 BC (Rivlin 2001). Garlic and their organosulfur compounds (OSCs) have been attributed to have several medicinally important properties like hypocholesterolemic (Yeh and Liu 2001; Mikaili et al. 2013), hypoglycemic (Sheela et al. 1995; Augusti and Sheela 1996), immunostimulatory (Lau et al. 1991; Lamm and Riggs 2000), antimicrobial (Lu et al. 2011), antiviral (Shojai et al. 2016), antifungal (Cellini et al. 1996; Avato et al. 2000; Goncagul and Ayaz 2010), and anticancer effects (Thomson and Ali 2003). Early population-based studies have provided strong proof in support of the anticancer property of *Allium* vegetables (You et al. 1989; Steinmetz et al. 1994; Hsing et al. 2002). The OSCs have been shown to have strong anticarcinogenic property against a variety of chemical carcinogens in different preclinical animal model studies (Reddy et al. 1993; Suzui et al. 1997).

The epidemiological investigations from past researchers have shown an inverse correlation between garlic intake and cancer incidences (Kim and Kwon 2009; Salem et al. 2011). For example, a lower incidence of esophageal and stomach cancers was found to be inversely correlated with garlic consumption (Munoz et al. 2001; Pelucchi et al. 2009). Similarly, the reduced risk of colorectal and prostate cancer has been well documented and linked with increased garlic intake (Hsing et al. 2002; Tanaka et al. 2004, 2006). Similarly, Satia et al. (2009) have also reported an inverse correlation between regular garlic intake and incidence of breast, endometrium, and lung cancer. Despite of extensive researches in the past two decades, the scientists are still working to elucidate the molecular mechanism of anticancer property of OSCs. Thus, the main focus of the present chapter is to give an overview of the past and present studies undergoing on organosulfur compounds for exploring their potential chemotherapeutic agents against cancer.

9.2 Chemical Constituents of Garlic

Chemical analyses have indicated that garlic bulbs, leaf, shoot, flower, and roots are the source of many compounds having medicinal properties with varied pharmacological functions (Ross 1999). Fresh garlic has been shown to have 65% water, 28% carbohydrates, 2% proteins (mainly alliinase), 1.5% fiber, 1.2% essential amino acids, vitamins (β-carotene, ascorbic acid, biotin, thiamin, riboflavin, niacin, nicotinic acid, etc.), minerals (Al, Fe, Mg, Co, Cu, Mn, Ni, P, K, Na, Sn, Zn, Se, Cr. etc.), organosulfur compounds (alliin, allicin, Z-ajoene, diallylsulfide (DAS),

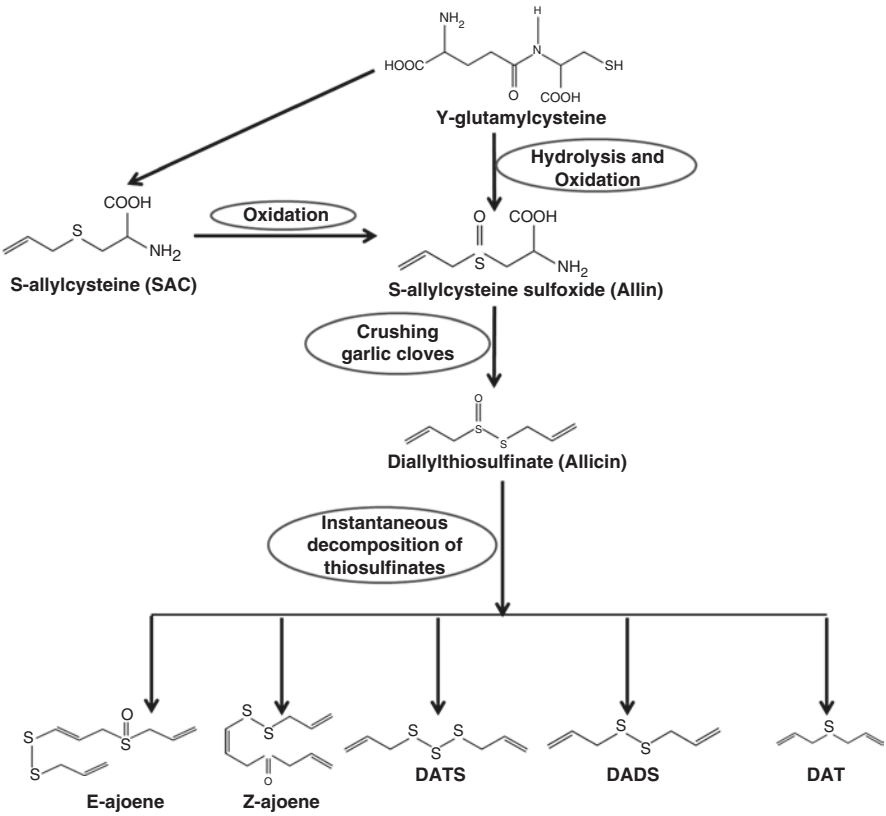

Fig. 9.1 Chemical reactions in processed garlic and generation of some important organosulfur compounds

diallyldisulfide (DADS), diallyltrisulfide (DATS), methyl-allyldisulfide (MADS), methylallylsulfide (MAS), methylallyltrisulfide (MATS), methyl-propyldisulfide (MPDS), dimethyltrisulfide (DMTS), dimethyldisulfide (DMDS), allyl-propyldisulfide (APDS), S-(2-carboxy-propyl)-glutathione, S-allylmercaptocysteine (SAMC), S-allylcysteine (SAC), and many other chemical compounds like p-coumaric acid, caffeic acid, ferulic acid, quercetin, sativosides, scordines, scordinines, degalactotigonin, gitonin, dithiins, dithiane thione, thiazole, etc. (Abdullah et al. 1988; Li 2000; Ejaz et al. 2003).

Block (1985) has nicely explored the chemistry of organosulfur compounds of garlic. γ-glutamyl-S-alk(en)yl-L-cysteine is the main sulfur compound of garlic, and it is hydrolyzed and oxidized into alliin (Fig. 9.1). Alliin is an important precursor of the wide array of organosulfur compounds of garlic, which have now been attributed for their anticancer effect (Reddy et al. 1993; Suzui et al. 1997). An enzyme alliinase is released when the garlic cloves are processed which acts on alliin to give rise to very unstable compounds called thiosulfinates. Allicin is one of the major thiosulfinates generated during the processing of garlic. These thiosulfinates, including allicin, which may be degraded or oxidized to oil-soluble OSCs

like DAS, DADS, DATS, dithiins, and ajoene (Munchberg et al. 2007) (Fig. 9.1). These OSCs can undergo further chemical transformations after reacting with free sulfhydryl groups (-SH) of cysteine, glutathione, or proteins producing S-allylcysteine, S-allylmercapto-cysteine, S-(2-carboxy-propyl)-glutathione, etc. (Lanzotti 2006; Corzo-Martinez and Villamiel 2007; Verma et al. 2008).

9.3 Mode of Action of OSCs in Cancer Prevention

OSCs of garlic have been shown to exhibit anticancer activity against many cancer cell lines via various mechanisms like inhibition of mutagen/carcinogen activation via modulation of cytochrome P450 (CYP)-dependent enzymes (phase I detoxification enzyme) and phase II detoxification enzymes, neutralization of free radicals, inhibition of the formation of DNA adducts and effects on cancer cell proliferation and tumor growth like disruption of the cell cycle progression, induction of apoptosis, modification of histone proteins, inhibition of metastasis and angiogenesis, and reversal of drug resistance (Fig. 9.2). The mechanism of action of OSCs has been established by extensive research on several types of cancer cell lines and various experimental animal models (Thomson and Ali 2003; Sengupta et al. 2004a, b; Velmurugan et al. 2005; Kalra et al. 2006; Prasad et al. 2008; Howard et al. 2008).

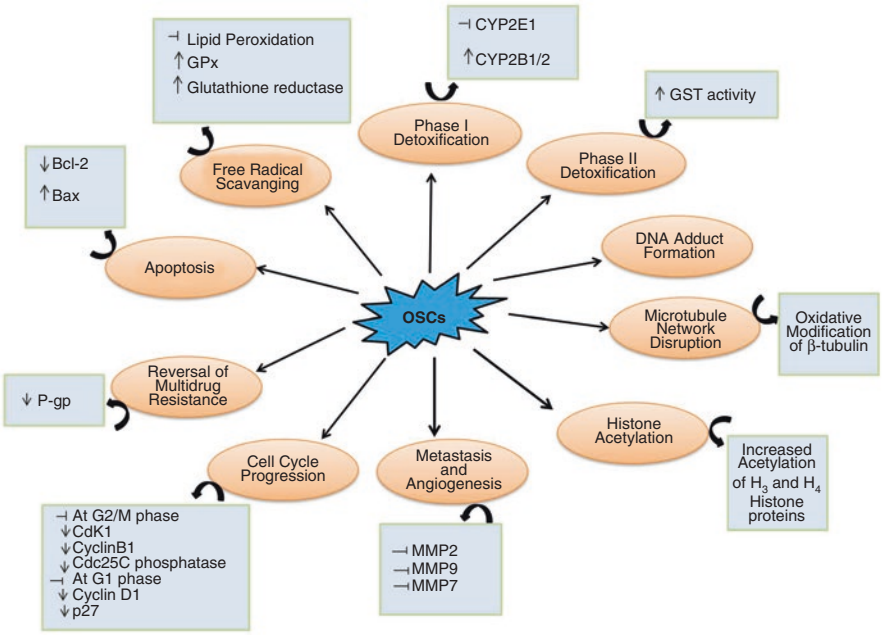

Fig. 9.2 Diagrammatic representation of mechanism of action of organosulfur compounds (OSCs) of garlic. ⊥ indicates inhibition at that step; ↑ indicates upregulation; ↓ indicates downregulation

9.3.1 Alterations in the Gene Expression of Phase I Detoxification Enzymes

The cytochrome P450-dependent monooxygenase has been considered to be responsible for the carcinogenic activation of many environmental pollutants. Garlic-derived OSCs have been extensively studied for their potential to reduce tumor growth in many animal models, primarily through alteration of expression of cytochrome P450 (CYP) enzymes (Ho et al. 2010; Melino et al. 2011; Wanwimolruk and Prachayasittikul 2014). For example, DAS-induced suppression of mutagenesis, caused by vinyl carbamate and N-nitrosodimethyl amine in S *almonella typhimurium* TA100, was found to be correlated with the inhibition of CYP2E1 enzyme-mediated phenol hydroxylation and N-nitrosodimethylamine demethylation (Surh et al. 1995). N-dimethylnitrosamine demethylase activity was also competitively inhibited by DAS (Brady et al. 1988). In another study, intake of garlic powder or oil has been reported to inhibit the hepatic microsomal CYP2E1 activity in mouse and rat (Park et al. 2002a; Zeng et al. 2009). A time- and dose-dependent decrease in the activity of CYP2E1 and increase in CYP2B1 by DAS were observed in hepatic microsomes (Brady et al. 1991; Jin and Baillie 1997). A significant decrease in the CYP2E1 activity by DAS, DADS, and AMS in rat liver was also observed (Reicks and Crankshaw 1996; Davenport and Wargovich 2005; Wargovich 2006). Expression and activities of several monooxygenases and transferases including epoxide hydrolase and CYP2B1/2 in rat liver and intestine were found to be increased by DADS (Haber et al. 1995; Wu et al. 2002; Davenport and Wargovich 2005). An increase in the CYP2B1 expression and decrease in the CYP2E1 expression were also observed by using garlic oil (Sheen et al. 1999). An increase in the activities of ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, and pentoxyresorufin O-dependentylase and decrease in the activities of nitrosodimethylamine N-demethylase and erythromycin N-demethylase were observed in rats treated with dimethyl sulfide (DMS), methylpropyl disulfide (MPDS), dipropyl sulfide (DPS), dipropyl disulfide (DPDS), and DADS. The above observation was accompanied by an increase in the expression of CYP2B1/2 and decrease in the expression of CYP2E1 (Siess et al. 1997). The above observation clearly indicated that inhibition of the carcinogen activation might be one of the mechanisms by which OSCs provided protection against chemically induced cancers.

9.3.2 Induction of Phase II Detoxification Enzymes

The OSCs of garlic have been shown to behave as a double-edged sword in the chemoprevention of chemically induced cancers by suppressing carcinogen activation and augmenting degradation of activated carcinogenic species through the activation of phase II detoxification enzymes like glutathione-S-transferase and quinone reductase, etc. (Sparnins et al. 1988; Hu and Singh 1997; Hu et al. 1997; Ho et al.

2010; Wanwimolruk and Prachayasittikul 2014). The OSCs of garlic have been shown to prevent B(a)P-induced forestomach and lung cancer in mice, and this was found to be correlated with an increase in the hepatic GST activity (Sparnins et al. 1988). In another study feeding of garlic powder increased the mammary and liver GST in rats (Liu et al. 1992). Expression of different classes of GST like alpha (mGSTA3-3, mGSTA1-2, mGSTA4-4), mu (mGSTM1-1), and pi class GST (mGSTP1-1) in the liver, lung, and forestomach was found to be induced by DAS, DADS, and DATS treatment to A/J mice (Hu et al. 1997; Bose et al. 2002; Andorfer et al. 2004). However, chemoprevention of B(a)P-induced forestomach cancer in A/J mice by OSCs was found to be correlated with the induction of mGSTP1-1 (Hu and Singh 1997; Andorfer et al. 2004). In other interesting studies, OSCs of garlic like DAS, DADS, DPS, and DPDS have been shown to induce the expression of NAD(P)H: quinone oxidoreductase enzyme, implicated in the degradation of activated quinone metabolite of B(a)P, in the lung and forestomach of A/J mice (Singh et al. 1998; Fukao et al. 2004). DADS has also been shown to augment the expression of quinone reductase, GST, and uridine diphosphate (UDP)-glucuronosyl transferase in different organs of rats, and induction of these enzymes was attributed to the protective activity of OSCs against gastrointestinal tract (GIT) cancers (Munday and Munday 1999). In general, all the above studies suggested that the activation of phase II detoxification enzymes represents another mechanism to explain OSC-induced chemoprevention of experimental cancers.

9.3.3 Inhibition of the Formation of DNA Adducts

Formation of DNA adducts is supposed to be a preliminary step in carcinogenesis. The anticarcinogenic action of garlic can be attributed to its role in preventing DNA-carcinogen adduct formation and activation of carcinogen (Liu et al. 1992; Lin et al. 2002). Formation of dimethylbenz[a]anthracene (DMBA)-DNA adduct has been found to be decreased by different garlic preparations in rat mammary gland, and these adducts were positively correlated with mammary tumor incidence (Amagase and Milner 1993). N-acetyl-2-aminofluorene (2-AF)-induced DNA adduct formation was found to be inhibited by DAS and DADS in human bladder tumor cells (Chung et al. 2004). Further, benzo(a)pyrene[B(a)P]-DNA adduct formation in human peripheral blood lymphocytes was significantly reduced by a water extract of raw garlic and SAC (Hageman et al. 1997). The *in vitro* nitrosation reactions were inhibited by onion and garlic juices in a dose-dependent manner (Shenoy and Choughuley 1992). The water extract of garlic and deodorized garlic powder have been reported to reduce N-nitrosomorpholine (NMOR) production, a liver carcinogen (Dion et al. 1997). The garlic powder has also been shown to decrease the formation of 7-methyldeoxyguanosine (7-MedG) and O6-methyldeoxyguanosine (O6-MedG) in rat liver (Lin et al. 1994).

9.3.4 Scavenging of Free Radicals

Free radicals have been implicated in several age-related diseases, including cancer (Borek 2001; Chung 2006; Iciek et al. 2009). Garlic possesses strong antioxidant activity due to its organosulfur compounds (Shobana and Naidu 2000). The free radical scavenging property of garlic has been attributed to allicin, a major thiosulfinate of crushed garlic (Siegers et al. 1999). Allicin has also been shown to prevent lipid peroxidation in liver homogenates (Prasad et al. 1995). N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)-induced genotoxicity and oxidative stress were also reduced by garlic preparations in Swiss mice (Kumaraguruparan et al. 2005). In an interesting study, glutathione peroxidase (GPx) activity was increased by garlic and onion oils, and ratio of reduced/oxidized GSH was inhibited (Perchellet et al. 1986). DAS treatment also leads to increased GPx activity in mouse forestomach tumor induced by B(a)P. Glutathione reductase activity was also increased by DAS and DADS administration, while garlic oil augmented SOD activity (Gudi and Singh 1991). Similarly, catalase activity was decreased by DAS and crushed garlic preparations in the liver of rat and mice (Sheen et al. 1996). Aged garlic extract (AGE), SAC, and SAMC have been shown to have free radical scavenging property against t-butyl hydroperoxide-induced ROS in microsomal fraction of rat (Imai et al. 1994). DAS, DADS, and AMS have also been shown to neutralize free radicals generated by CCl_4 (Fanelli et al. 1998). AGE, containing both water- and lipid-soluble organosulfur components including S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), has been reported to have antioxidant property by augmenting cellular antioxidant enzyme like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Borek 2001). SAC and SAMC have been attributed for the increased biosynthesis of glutathione in human prostate cancer cells (Pinto et al. 1997).

9.3.5 Disruption of Cell Cycle Progression

Cell cycle is a complex control system which ensures precise sequence of events like growth, replication, and division of a cell (Molinari 2000; Murray 2004). Cell cycle arrest is induced due to cellular stresses like DNA damage and microtubule network disruption via activation of the cell cycle checkpoints (Molinari 2000; Murray 2004). The G_1/M checkpoint prevents replication of damaged DNA, and G_2/M checkpoint blocks mitosis until the replication is accomplished correctly. Several studies have shown that disruption of cell cycle progression is the primary mechanism of action of many anticancer agents. OSCs of garlic have also been shown to inhibit the growth of many cancer cell lines *in vitro* through inducing cell cycle arrest, mainly in G_2/M phase of the cell cycle (Arunkumar et al. 2006; Omar and Al-Wabel 2010; Wang et al. 2012; Scherer et al. 2009). For the first time, DADS was shown to cause dose- and time-dependent cell cycle arrest in G_2/M phase of

human colon cancer cells (Knowles and Milner 1998, 2000). The DADS-induced G_2/M arrest in colon cancer cells was accompanied with decreased kinase activity of Cdk1/cyclinB1 complex, reduced complex formation between Cdk1 and cyclinB1, and decreased Cdc25C phosphatase level (Knowles and Milner 2000). Later on, other OSCs of garlic were also shown to exert similar effects on different *in vitro* and *in vivo* cancer models (Tan et al. 2004; Wu et al. 2004; Xiao et al. 2005; Herman-Antosiewicz and Singh 2005; Arunkumar et al. 2006; Herman-Antosiewicz et al. 2007). For example, DADS was shown to inhibit Cdk1 activity by inducing its phosphorylation in HL-60 cells and also reduced Cdk1 expression in human prostate cancer PC-3 cells in a dose-dependent manner (Tan et al. 2004; Arunkumar et al. 2006). DATS was found to be more potent inducer of cell cycle arrest at G_2/M phase in comparison to DAS or DADS (Xiao et al. 2005). This finding supports the fact that a change in the oligosulfide chain length of OSCs could alter its biological property. Induction of G_2/M phase arrest in PC-3 cells by DATS was shown to be due to increased inhibitory phosphorylation of Cdk1, reduction in Cdk1/cyclinB1 activity, augmented inhibitory phosphorylation of Cdc25C, and reduced expression of Cdc25C (Xiao et al. 2005). Garlic OSCs have also been known to cause cell cycle arrest in phases other than G_2/M. For example, DADS was shown to induce S-phase arrest in human nasopharyngeal carcinoma cells (Zhang et al. 2006). Similarly, allitridi was shown to cause G_1 phase arrest in human gastric cancer cells BGC823 via downregulation of cyclin D1 and upregulation of p27 protein (Lan and Lu 2003). Therefore, the above evidences indicate that disruption of cell cycle progression is the major and common mechanism of action of many structurally different OSCs of garlic.

9.3.6 Disruption of Microtubule Network

OSCs of garlic have been shown to affect the microtubule network in different cancer cells that could lead to mitotic arrest or apoptosis (Xiao et al. 2005; Hosono et al. 2008). For instance, SAMC was shown to cause microtubule depolymerization and cytoskeleton disruption in human colon cancer SW480 cells and NIH3T3 mouse fibroblasts (Xiao et al. 2003). DATS was also shown to disrupt microtubule network and inhibit spindle formation in human colon cancer DLD-1 and HCT-15 cells causing mitotic arrest (Hosono et al. 2005). Interestingly, DATS caused oxidative modification of tubulin-β at Cys12 and Cys354 residues (Hosono et al. 2005). Similarly, Z-ajoene was shown to cause inhibition of tubulin polymerization *in vitro* and disruption of microtubule network leading to G_2/M phase arrest in normal marsupial kidney cells (Li et al. 2002).

9.3.7 *Induction of Apoptosis*

Apoptosis is an evolutionary conserved process of cell death vital for normal embryonic development and maintenance of tissue homeostasis. A number of pathological conditions including cancer have been attributed to dysregulated programmed cell death, and therefore, apoptosis is considered as a potential target in cancer prevention and therapy (Kaufmann and Gores 2000; Ghobrial et al. 2005). Numerous studies have shown that OSCs of garlic could affect various cellular signaling pathways linked to apoptosis. Majority of these OSCs induce mitochondrial pathway of apoptosis which involves disruption of mitochondrial membrane potential and release of apoptotic factors from the mitochondria to the cytosol (Thornberry and Lazebnick 1998; Hengartner 2000; Xiao et al. 2005; Caro et al. 2012; Wang et al. 2012). Mitochondrial apoptotic pathway is tightly regulated by the Bcl-2 family of anti-apoptotic proteins (Bcl-2, Bcl-xL) and pro-apoptotic proteins (Bax and Bak) (Chao and Korsmeyer 1998). OSCs of garlic have been shown to induce apoptosis by altering the cellular levels of the Bcl-2 family proteins. For example, the ratio of Bax/Bcl-2 was found to be increased in neuroblastoma cells SH-SY5Y and lung cancer cells H460 and H1299 by DAS and DADS treatment (Hong et al. 2000; Karmakar et al. 2007). Likewise, DADS was also shown to induce a time-dependent increase in Bax level and simultaneous reduction in Bcl-xL level in breast cancer cell line MDA-MB-231 (Nakagawa et al. 2001). Z-ajoene was also shown to induce programmed cell death in HL-60 cells in association with caspase-mediated degradation of Bcl-2 protein (Li et al. 2002). DATS was also shown to cause a decrease in Bcl-2 expression with a simultaneous hyperphosphorylation of this protein resulting in an increased Bax/Bcl-2 ratio ultimately leading to induction of mitochondrial pathway of apoptosis (Xiao et al. 2004). The hyperphosphorylation of Bcl-2 in DU145 and PC-3 cells was shown to occur due to activation of JNK and ERK1/2 kinases. Moreover, DATS was shown to be more potent inducer of apoptosis in comparison to DAS and DADS in human prostate cancer PC-3 and DU145 cells (Xiao et al. 2004). Similarly, a significant increase in the Bax and Bak along with apoptosis was observed in prostate cancer LNCaP cells treated with DATS (Kim et al. 2007). In an *in vivo* study, DATS was shown to inhibit PC-3 xenograft tumor growth in nude mice which was correlated with increased apoptosis along with the augmentation of Bax and Bak proteins in tumor tissue (Xiao et al. 2006). In an earlier study, Akt-Bad pathway was attributed, up to some extent, to regulate apoptosis induced by DATS treatment in human prostate cancer PC-3 and DU145 cells (Xiao et al. 2006). A marked reduction in Akt activity was observed in DU145 and PC-3 cells due to DATS treatment, and consequently the phosphorylation of Bad at Ser155 and Ser136 was inhibited, which reduced interaction of Bad with 14–3-3β protein (Xiao et al. 2006).

In several previous studies, reactive oxygen species (ROS) have been shown to play critical role in OSC-induced apoptosis in cancer cells. For example, DADS was shown to induce apoptosis in HL-60 cells which was correlated with augmented intracellular ROS production (Kwon et al. 2002). Moreover, DADS was also shown

to induce intracellular ROS generation in neuroblastoma cells SH-SY5Y which was apparent as early as 15 min of treatment and leads to oxidation of cellular lipids and proteins (Filomeni et al. 2003). Interestingly, production of ROS in DADS-treated neuroblastoma cells was found to be due to activation of JNK pathway (Filomeni et al. 2003). Additionally, an increase in the free intracellular Ca^{2++} ion has been attributed to the apoptosis-inducing property of OSCs (Sakamoto et al. 1997; Karmakar et al. 2007; Sundaram and Milner 1996a, b; Park et al. 2002a, b). For example, DADS was shown to augment intracellular calcium ion level with concomitant activation of capase-3 (Park et al. 2002b). Likewise, DAS and DADS were also shown to raise intracellular calcium ion level in SH-SY5Y neuroblastoma cells followed by activation of a calpain, a non-caspase cysteine protease, which triggers caspase-independent pathway of apoptosis (Karmakar et al. 2007).

Interestingly, OSCs have been observed to exert differential effect on normal and cancer cells. Surprisingly, malignant cells were found to be more sensitive to apoptosis induced by OSCs in comparison to normal cells. For instance, DAS and DADS, at the dose of 50 or 100 μM, minimally affected the viability of primary neurons but exerted marked reduction in the viability of neuroblastoma cells at the same concentration (Karmakar et al. 2007). Likewise, DATS did not affect the viability of normal prostate epithelial cells PrEC event at a dose that was cytotoxic to prostate cancer cells (Xiao et al. 2005; Kim et al. 2007). Z-ajoene was also shown to exhibit insignificant effect on peripheral mononuclear blood cells from healthy individuals but induced apoptosis in human leukemia cells (Dirsch et al. 1998). The exact mechanism regarding the differential effect of OSCs on cancer cells and normal cells still remains to be elaborated.

9.3.8 Modification of Histone Acetylation

Modification of histone acetylation has been suggested as one of the mechanisms attributed to the anticancer property of garlic OSCs (Druesne-Pecollo et al. 2004a, 2006; Nian et al. 2009). For the first time, DADS and its metabolite allyl mercaptan were shown to increase acetylation of H_3 and H_4 histone proteins in DS19 mouse erythroleukemia and K562 human leukemia cells (Lea et al. 1999). Similar effect of DADS and allyl mercaptan was shown *in vivo* by the same group in rats (Lea and Randolph 2001). Moreover, increased histone acetylation was also correlated with antiproliferative property of allicin, SAMC, and SAC on human colon cancer cell line Caco-2 and human breast cancer cell line T47D (Lea et al. 2002). DADS was also shown to induce hyperacetylation of histone proteins H3 and H4 in human colon cancer cell line Caco-2 and HT-29 via inhibiting histone deacetylase (Druesne-Pecollo et al. 2004a, b). Moreover, histone deacetylase inhibition was also shown to be responsible for the hyperacetylation of the promoter of p21 gene (Nian et al. 2008, 2009).

9.3.9 Inhibition of Metastasis and Angiogenesis

Metastasis, a primary cause of morbidity in patients with cancer, is a multistep process involving the detachment of cancer cells from the primary tumor site, entry into systemic circulation, and invasion at new sites. The whole metastatic process requires multiple molecular events including angiogenesis (Folkman 2003). Several *in vitro* and *in vivo* studies have shown that garlic and its OSCs can affect metastatic and angiogenic processes (Powolny and Singh 2008; Ng et al. 2012). It was reported for the first time that AGE could suppress migration of rat sarcoma cells *in vitro* (Hu et al. 2002). On the basis of this finding, various OSCs of garlic were then studied for their antimetastatic property. In a recent study, DADS was shown to reduce the migration of prostate cancer cell line LNCaP by inhibiting the expression of MMP2 and MMP9 (Shin et al. 2010). SAC was also shown to inhibit the invasion of breast cancer cell line MDA-MB 231 via augmentation of E-cadherin expression and reduction of MMP2 expression. Recently, DAS, DADS, and DATS were also shown to reduce the expression of MMP2, MMP7, and MMP9 in colon cancer colo 2005 cells (Lai et al. 2011). Furthermore, OSCs have also been reported to suppress angiogenic process which leads to development of new blood vessels from already established vascular network. For example, AGE was shown to suppress proliferation and invasiveness of transformed human and rat endothelial cell line (Matsuura et al. 2006). DAS, DADS, and DATS were shown to inhibit the proliferation of human umbilical vein endothelial cells, and DATS was found to be most potent among them (Xiao et al. 2006). Alliin was also shown to inhibit the angiogenesis, and VEGF and FGF-2 induced capillary tube formation in human umbilical vein endothelial cells (Mousa and Mousa 2005). DAS and DADS were also shown to reduce proliferation and migration of endothelial cells along with the downregulation of MMP2 and MMP9 expressions (Thejass and Kuttan 2007a, b). *In vitro* studies, supporting the antimetastatic potential of OSCs of garlic, have also been confirmed by *in vivo* studies. For example, SAMC has been shown to reduce the pulmonary metastasis by 85% in mice with prostate cancer when administered orally (Howard et al. 2007). Oral administration of DATS also reduced the pulmonary metastasis by around 50% in TRAMP mice (Singh et al. 1995). Thus, it can be concluded from the above evidences that garlic and its OSCs have great potential as inhibitors of metastasis and angiogenesis.

9.3.10 Reversal of the Multidrug Resistance

One of the major hurdles in cancer chemotherapy is the multidrug resistance caused due to overexpression of P-glycoprotein (P-gp), a membrane transporter to efflux xenobiotic compounds from a cell. OSCs have been shown to modulate the P-gp-mediated multidrug resistance (Arora et al. 2004; Demeule et al. 2004). DAS has been shown to enhance the cytotoxic action of vinblastine and other *Vinca* alkaloids

in K562 leukemic cells resistant to vinblastine. Interestingly, in this study, DAS treatment lead to reduced P-gp protein expression in K562 cells up to a normal level (Arora et al. 2004).

9.4 Clinical Trials Conducted with Garlic

Clinical evidences from cell culture and animal models have been reported in population-based studies and suggested the protective effects of garlic intake. To date, most of the clinical trials were conducted to observe the chemopreventive property of garlic on various types of cancer. A double-blind randomized clinical trial was conducted on 37 patients to study the effect of high-dose aged garlic extract (AGE) on colorectal adenomas by Tanaka et al. (2006). They found a significant reduction in size and number of adenomas in patients who received high dose of AGE for 1 year. Similarly, a factorial double-blind, placebo-controlled clinical trial, conducted with aged garlic extract and oil and/or vitamin supplement, showed a nonstatistically significant reduction in gastric cancer incidence and mortality (Ma et al. 2012). However, in another clinical trial, a borderline significant negative association between garlic and onion intake and gastric cancer risk was also observed (Millen et al. 2007). Similarly, the clinical trial in women having high consumption of onion and garlic showed a significant reduction in primary invasive epithelial ovarian cancer risk (Schulz et al. 2005), while a clinical study on Chinese men and women showed high intake of garlic significantly reduced the risk of liver cancer (Zhang et al. 2013). An Italian case-control study showed a significant association between high levels of garlic consumption and reduced risk of endometrial cancer (Galeone et al. 2009). Moreover, the hospital-based study, conducted by Wang et al. (2012a, b) showed that high amount of shallot and garlic intake was significantly associated with decreased risk of multiple myeloma, while Salem et al. (2011) found that the high consumption of garlic was linked with reduction of prostate cancer (Salem et al. 2011).

9.5 Conclusions and Future Prospects

During the past few decades, extensive research has been done on garlic and its organosulfur derivatives for its potential to control the growth and proliferation of various cancer cells under *in vitro* and *in vivo* conditions. The outcome of these researches suggested that the organosulfur compounds target many signaling pathways such as apoptosis, cell cycle, metastasis, angiogenesis, and drug resistance. This may be attributed to the anticancer property of garlic and its organosulfur compound derivatives. Moreover, the clinical trials conducted so far have mainly used aged garlic extract or garlic oil or total garlic, rather than the isolated OSCs in animal studies. To facilitate confident and meaningful decisions in the future, this gap

of knowledge should be filled by conducting clinical trials with OSCs. Despite the availability of extensive information on OSCs and their mechanism of action on different cancers, more in-depth future investigations on garlic and its organosulfur derivatives are desired to unravel many unsolved issues related to their target genes regulating various cellular signaling pathways involved in cell survival, growth, proliferation, and maintenance of tissue architecture.

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Chapter 10
Relevance of Traditional Unani (Greco-Arab) System of Medicine in Cancer: An Update

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Abstract Cancer is one of the most dreadful diseases worldwide. The incidence of cancer has drastically increased in low- and middle-income countries and is a major cause of morbidity and mortality in the developed countries. Use of alternative medicines along with conventional ones against cancer managed the symptoms, control side effects, and improve the quality of life and mental state. Indian systems of medicine (ISM) including Unani (Greco-Arab medicine) have been used for healing and preventative health care all over the world since decades. Cancer (Arabic-*Sartan*) has been well described in Unani classical texts as a hard inflammation (*Warm-e-Salabat*) with lesion. The cancer management in Unani system includes regimenal therapy (*Ilaj bi'l-Tadbir*), dietotherapy (*Ilaj bi'l-Ghidhā*), pharmacotherapy (*Ilāj bi'l-Dawā*), and surgery (*Ilāj bi'l-Yad*). In pharmacotherapy, many Unani drugs have been evaluated preclinically (*in vitro* and *in vivo* studies) for its anticancer activities and found to be very effective, but unfortunately, no attention has been paid to explicate the efficacy of compound formulations of these Unani medicines in cancer management, till date. The aim of this chapter is to exploit and bring together the Unani system of medicine with conventional cancer therapies at interdisciplinary and integrative levels and also to discuss the future strategies for the prevention and management of cancer.

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10.1 Introduction

Cancer, a fatal noncommunicable disease (NCD), opened the eyes of the world. It is a cluster of disorders involving transformation, dysregulated apoptosis, cell proliferation, angiogenesis, and metastasis. An extensive research during the past century has unfolded various facts about the biomechanics of the cancer, and most of the anticancer drugs are employed to withhold signaling of cell cycle and growth factors, inflammation, and angiogenesis. The pathways linked to this process lead to chronic inflammation, a major mediator of tumor progression (Gupta et al. 2010). Cancer continues as a scourge of humankind and is considered as a burden on the populations of low- and middle-income countries. The occurrence of cancer has increased from 12.7 million in 2008 to 14.1 million in 2012, and this trend is projected to persist, with the number of new cases expected to go up further by 75%. This will take the number of cancer-related cases up to 25 million over the next two decades. Cancer is a major cause of mortality across the world with deaths projected to rise over 13.1 million in 2030. Cancer is the second largest NCD after ischemic heart disease with only 20% is getting cured and 20% get a prolonged life after treatment. The findings of GLOBOCAN showed that high-resource countries have the highest incidence of cancer (Ferlay et al. 2015) and also provide the finest services for detection, diagnosis, and treatment, as may be deduced from mortality and survival data. The results of the above finding showed approximately 14.1 million new cases of cancer were diagnosed worldwide (excluding non-melanoma skin cancer) and 8.2 million estimated deaths from cancer, affecting the entire global populations. These estimates correspond to age-standardized incidence and mortality rates of 182 and 102 per 100,000, respectively, but there were slightly more incident cases (53% of the total) and deaths (57%) among men than among women.

According to World Cancer Report (2014), more than 60% of the world's cancer cases occur in Africa, Asia, and Central and South America, which may account for about 70% of the cancer deaths. Among all types of cancers (excluding non-melanoma skin cancer), the highest incidence rates were reported from high-income countries such as North America and Western Europe (together with Japan, the Republic of Korea, Australia, and New Zealand). In case of men, the five most prevalent sites of cancer were the lung (16.7% of the total), prostate (15.0%), colorectum (10.0%), stomach (8.5%), and liver (7.5%), while, in case of women, the incident sites were the breast (25.2% of the total), colorectum (9.2%), lung (8.7%), cervix (7.9%), and stomach (4.8%). Among men, lung cancer had the highest incidence rate (34.2 per 100,000) followed by prostate cancer (31.1 per 100,000), while in women the breast cancer had a higher incidence rate (43.3 per 100,000) followed by colorectal cancer (14.3 per 100,000). In India, during the year 2011, it was estimated that 0.44 million died owing to cancer, and it is predicted to increase up to 0.60 and 0.70 million by year 2021–2026, respectively, as a result of change in size

and composition of population (D'Souza et al. 2013). Murthy et al. (2008) reported that lung, esophagus, prostate, stomach, oral, and pharyngeal cancers are predominant in men, while cervix and breast cancers are common in women followed by oral cavity, stomach, and esophagus. The new cases of cancer were more prone in women's compared to men's, and most of the deaths occur at home (Dikshit et al. 2012). In India, the breast cancer has ranked first among Indian females with age-adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women (Malvia et al. 2017).

The Unani system of medicine originated in Greece, with its more than twenty-five centuries of legacy established by Hippocrates (Father of Medicine) and put forward by other eminent Unani physicians of well-known civilizations (Roman Egypt, Arabia, and India). The Unani stalwarts such as Galen, Rhazes (Razi), and Avicenna (Ibn Sina) have noticed and mentioned different types of cancer prevalence known to them at that period of time. They recommended myriad of treatment modalities in management, prevention, and prognosis of cancer. The earliest "humoral theory" given by Hippocrates on the basis of four humors was the first theory which explained the cause of cancer. This theory of cancer was passed on by the Romans and was embraced by the influential doctor Galen's medical teaching, which remained the unchallenged standard through the Middle Ages for over 1300 years (ACS 2014). The aim of this chapter is to exploit and bring together the Unani system of medicine with conventional cancer therapies at interdisciplinary and integrative levels and also to discuss the future strategies for the prevention and management of cancer.

10.2 Traditional Medicines and Their Significance

Traditional medicines have been used since thousands of years to cure health-related issues. The World Health Organization (WHO) defined the traditional medicine as "the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observations handed down from generation to generation, whether verbally or in writing." Access to "modern" health-care services and medicine may be limited in developing countries. TM becomes the only affordable treatment available to poor people and in remote communities. Health-care providers worldwide including major pharmaceutical giants are turning to incorporate many of these into their mainstream activities.

Across the world, traditional medicine is either the mainstay of health-care delivery or serves as a complement to it. In some countries, traditional medicine or non-conventional medicine may be termed complementary medicine. Traditional medicine and complementary medicine are an important and often underestimated part of health care in almost every country in the world, and the demand for its services is increasing. Traditional medicine, of proven quality, safety, and efficacy, contributes to the goal of ensuring that all people have access to care. Many countries

now recognize the need to develop a cohesive and integrative approach to health care that allows governmental health-care practitioners and, most importantly, those who use health-care services to access traditional medicines and complementary medicine in a safe, respectful, cost-efficient, and effective manner (WHO 2013). There are two well-defined terms which are related to traditional medicines: complementary treatment and alternative treatment. Complementary treatments are used in combination with mainstream therapy (conventional therapy) and are usually intended to treat side effects. On the other hand, alternative treatments are used in place of conventional therapy and usually have their own therapeutic potential. Half of the world's cancer patients use complementary medicine for treatment. Approximately 50–60% cancer patients in the USA utilize agents derived from different parts of plants or nutrients, either as stand-alone or concurrently with conventional therapeutic regime of chemotherapy or radiation therapy (Gutheil et al. 2012). The systematic review and meta-analysis done worldwide to estimate the prevalence of use of complementary medicine in treatment of cancer was 40% that has increased from 25% in 1970 to 49% after 2000 (Horneber et al. 2012). Though many research works are under progress, no literature highlights that traditional medicine can be used as an alternative to the mainstream therapies in curing cancer (Deng and Cassileth 2014). But, they can be considered complimentary to the regular therapies in reducing the side effects and improving their mental well-being.

Indian systems of medicine (Ayurveda, Unani, and Siddha) and traditional Chinese medicine (TCM) have been used for hundreds of years for healing and preventative health care all over the world. Among Indian systems, Ayurveda is the oldest system with documented history of its practice since more than 5000 years, whereas the Unani (Greco-Arab) system of medicine originated in Greece and passed through many countries before establishing itself in India during the medieval period. It is based on well-established knowledge and practices relating to the promotion of positive health and prevention of disease. With the fusion of traditional knowledge of ancient civilizations like Egypt, Arabia, Iran, China, Syria, and India, these systems are being practiced in the country with diverse preferences of people and infrastructural facilities. TCM has increasingly become popular in the West including in cancer patients (Boon et al. 2007) and plays an important role in minimizing disability, protecting patients suffering from complications, and helping patients to live well (Yoder 2005). Chinese medicine assisted in supportive and palliative care by reducing side effects of conventional treatment of cancer or improving quality of life (Ernst 2009). It is estimated that the US National Cancer Institute (NCI) spends around $120 million each year on complementary and alternative medicine (CAM)-related research projects (Jia 2012).

10.3 Origin of Unani (Greco-Arab) System of Medicine

The Unani system of medicine (*Yūnānī Niẓām-i Ṭibb or Ṭibb*) owes its immediate origin to ancient Greece (*Yūnān*) and was further adopted by the Arabs into a specialized medical science based on the principles given by Buqrat (Hippocrates)

and Jalinoos (Galen). Greco-Arab refers to medicine developed during the Golden Age of the Arab-Islamic Empire, which extended from Andalusia (Spain) and Maghreb states (North Africa) in the west to Central Asia and India in the east, with the central lands of Egypt, Bilad al-Sham (Greater Syria), and Iraq. It spanned a period of roughly nine centuries, from the middle of the seventh century to the end of the fifteenth century, by which time it had broken up into three distinct empires, the Ottoman, the Safavid, and the Mughal (Saad and Said 2011). The herbal basis of Unani therapy can be traced back to its earliest originators in ancient Egypt, which gave primary place to plants in disease treatment. They had also initiated the use of surgery as a method of treatment. The studies of Papyri show the ability of Egyptians in the field of medicine. Imhotep (2800 BC) and Amenhotep (1550 BC) are some noted physicians of Egyptian period. Due to their great contributions in the field of medicine, Mesopotamia also occupied an important place in history. They had used urine sample as a diagnostic tool. The Greek period of Unani medicine began with Asclepius (*Asqalībūs*-1200 BC), who was a great scholar of medicine. During Asclepian period, the Greeks developed the art of medicine in the light of medical knowledge of Egyptians and Babylonians.

The theoretical framework of Unani medicine is based on the teachings of Hippocrates (460–370 BC), the overarching physician of the classical period of Unani medical history. He emphasized the natural causes of disease and recorded the existing medical knowledge to set the grounds for medicine to develop as a systematic science. The three fundamentals of Hippocratic medicine were observation, experience, and rational principles, which still hold valid in the field of medicine and science. The closing years of the creative age of Greece were graced by the great Roman scholar Galen (129–200 AD). He made valuable addition to medicine by conducting experiments and elevated the status of an art to the rank of a scientific discipline of medicine. Galen, one of the most illustrious scholars in the history of medicine, gathered most of the medical knowledge of his time and arranged it systematically in a way that continued to be authoritative for the next centuries. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates and Galen, formed the roots for later Greco-Arab and Islamic medicine and modern Western medicine. Theophrastus (about 300 BC), in his book *History of Plants*, dealt with the medicinal qualities of herbs and noted the ability to change their characteristics through cultivation. Dioscorides (100 AD) mentioned the collection, storage, and use of medicinal herbs, and Galen wrote 30 books on these subjects and is well known for his complex prescriptions and formulas used in compounding drugs, sometimes containing dozens of ingredients. Taken together, a vast medical literature explored the synthesis and practice of medicinal plants was developed.

The Arabs introduced the Unani system of medicine to India in the eighth century, and soon it caught the attention of the masses. During the period between the thirteenth and seventeenth century, the Unani system of medicine established its roots in India. The Delhi Sultans, the Khiljis, the Tughlaqs, and the Mughal emperors provided state patronage to the scholars. This was a golden period of the Unani system of medicine as it virtually spread all over the country finding immediate favor with the people at large. The Unani system suffered a temporary setback dur-

ing the colonial rule but was revived soon after independence, with the government recognizing it as one of the Indian systems of medicine and taking initiative for its development and propagation. During its over 1200 years' history in India, the Unani system of medicine made major advancements by the fusion of traditional knowledge of ancient civilizations like Egypt, Arabia, Iran, China, Syria, and India to get leadership at global level and successfully applied its principles to the Indian geo-human environment to emerge as one of the effective and commonly used systems of medicine. The Unani system of medicine also accommodated itself to Indian climate, temperament, culture, and medicinal resources successfully and catered to the health needs of all strata of the country's population (Anonymous 2016).

10.4 Unani (Greco-Arab) System of Medicine

The Unani system of medicine gave great importance to the Hippocratic "humoral theory" given in the fourth century BC, which emphasizes the unique character of living things/organic matter and their distinction from nonliving things/inorganic matter by explaining that the elements are metabolized and converted into humors, which are organic and possess the additional properties of living things. The theory describes four humors (*Akhlāṭ*, singular: *Khilt*) and elaborates that the most important of these principles is temperament (*Mizāj*) which classifies living human body into four (hot, cold, wet, and dry) qualitative types of states: (1) hot and dry, (2) hot and wet, (3) cold and wet, and (4) cold and dry. These are represented as earth, water, fire, and air. Temperament of human beings depends upon the dominant humor. Since each humor possesses a particular quality, it is possible to convert humoral temperament into qualitative temperament and then can easily be correlated with human states, drugs, and diets. The basic principles comprise of (a) the seven natural factors (*Umūr Ṭabī'iyya*), essential factors for the constitution of human body, (b) the basics of pathology (*Kulliyāt-i 'Ilm al-Amrāḍ*), (c) the principles of diagnosis (*Uṣūl-i Tashkhīṣ*), and (d) the principles of treatment (*Uṣūl-i 'Ilāj*). The Greek ideas were put forward by Arabian physicians as seven natural factors (*Umūr Ṭabī'iyya)*, which are responsible for the body constitution, its health, and diseased conditions (Anonymous 2016). The brief descriptions of these natural factors are as given below:

1. **Basic elements (*Arkān*)**
   Basic elements in Unani medicine are simple indivisible matters which provide the primary components for the human body. They can't be further resolved into simpler entities. The various substances (compounds) in nature depend for their existence on their chemical compositions (*Imtizāj*). Everything in the universe is composed of four basic elements in varying amount and proportion. Each element has two sets of basic qualities (*Kayfiyāt*): hot or cold and dry or wet (Fig. 10.1). The four elements are fire (*Nār*), air (*Hawā*), water (*Mā*'), and earth (*Arḍ*). Their basic qualities are fire (hot and dry), air (hot and wet), water (cold and wet), and earth (cold and dry).

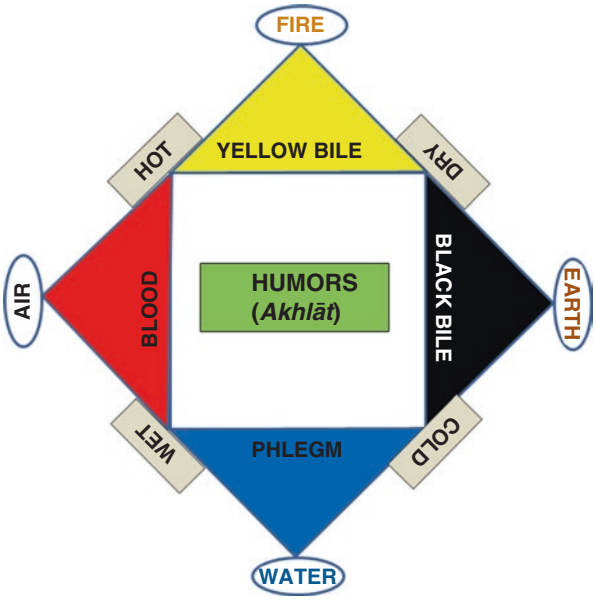

Fig. 10.1 Schematic diagram of four humors

Table 10.1 Humors (*Akhlāṭ*) and their basic qualities

Humors	Basic qualities
Blood (*Dam*)	Hot and wet
Phlegm (*Balgham*)	Cold and wet
Yellow bile (*Ṣafrā'*)	Hot and dry
Black bile (*Sawdā'*)	Cold and dry

2. **Humors (*Akhlāṭ*)**
   Humors (*Akhlāṭ*) are the wet and liquid parts of the body which are produced after normal metabolism. Their right proportion in terms of quality and quantity (homeostasis) is vital in maintaining health and any derangement results in disease. Their main function is to support biological processes related to nutrition, growth, repair, and production of energy for the preservation of individual's health. The four humors are characterized by the dominant basic qualities, and each humor has its own temperament (Fig. 10.1 and Table 10.1). The humors are formed within various organs, primarily in the liver, and run together in the blood vessels.
3. **Temperament (*Mizāj*)**
   Every individual has a unique *humoral* composition which represents his specific temperament. Entire human beings are classified accordingly as sanguine, phlegmatic, choleric, and melancholic, on the basis of the amount of dominant humor prevalent in the human body (Fig. 10.1 and Table 10.2). The temperament of man can be diagnosed by ten categories of bodily and mental parameters (*Ajnās 'Ashara*), which includes complexion, built, texture, hair, dreams, and

Table 10.2 Elements and basic qualities of humors on temperament

Elements	Basic qualities	Temperament
Air	Hot and wet	Sanguine (*Damawī*)
Water	Cold and wet	Phlegmatic (*Balghamī*)
Fire	Hot and dry	Choleric (*Ṣafrāwī*)
Earth	Cold and dry	Melancholic (*Sawdāwī*)

others. The pathological change of temperament, in whole man or in particular body parts and organs, can also be diagnosed by changes in these parameters.

4. **Organs (*A'ḍā'*)**
   The physical body is made up of tissues and organs (*A'ḍā'*). Organs are classified into two types: simple (*Basīṭ*) and compound (*Murakkab*). The simple organs are tissues like fat, bone, and so on, while compound organs are structures made up of poly-tissue such as the heart, brain, liver, and others.
5. **Pneumas (*Arwāḥ*)**
   The *Rūḥ* (p. *Arwāḥ*) is subtle, physical substance made from the subtle components of the humors. It possesses vitality and provides life to the organism. It also acts as the seat and carrier of the physiological powers or faculties (*Quwā*) which are responsible for producing the corresponding physiological processes including nutrition, growth, sensation, and movement. Humans possess three pneumas, each one generated in vital organs: vital pneuma (*Rūḥ Ḥaywānī*) in the heart, psychic pneuma (*Rūḥ Nafsānī*) in the brain, and natural pneuma (*Rūḥ Ṭabī'ī)* in the liver.
6. **Faculties (*Quwā*)**
   Faculties are the physiological power that performs the corresponding physiological functions of nutrition, growth, reproduction, propagation of species, respiration circulation, coordination, intelligence, sensation, and response. The primary faculties are Natural Faculty (*Quwwat Ṭabī'iyya*), Generative Faculty (*Quwwat Muwallida*), Vital Faculty (*Quwwat Ḥaywāniyya*), and Psychic Faculty (*Quwwat Nafsāniyya*) and their corresponding pneumas as Natural, Vital, and Psychic, respectively.
7. **Functions (*Af'āl*)**
   Af'āl refers to the functions and movement of all organs in the body due to the operation of faculties or physiological powers. In a healthy body, all the organs are in a proper shape or without any structural abnormality and perform their respective functions in an efficient manner. The humors themselves are assigned temperaments—blood is hot and moist, phlegm cold and moist, yellow bile hot and dry, and black bile cold and dry. There is a unique humoral constitution in every person representing his state of health. To maintain a healthy balance, there is a power of self-preservation or adjustment called *Quwwat-e-Mudabbira* (*medicatrix naturae*). A weakening of this power causes imbalance in the *humoral* composition and causes disease. What the Unani physician actually tries to do is to prescribe a medicine, which helps the body to regain this power and thereby restore the humoral balance. Correct diet and digestion is integral to this system.

10.4.1 Unani (Greco-Arab) System of Medicine and Cancer (Sartan)

The origin of the word cancer is credited to the Greek physician Hippocrates (460–370 BC). He has used the terms karkinos (carcinos), the Greek word (*καρκίνος*) for crab or crayfish, and carcinoma to connote ulcer- and non-ulcer-forming tumors. In Greek, these words used for a crab and most probably applied to the disease because the physical appearance of the cut surface of a solid malignant tumor, with the veins stretched on all sides (fingerlike spreading projections) as the crab has its feet. Celsus (ca. 25 BC–50 AD) translated karkinos into *cancer*, the Latin word for crab. Galen (130–200 AD), another Greek physician, for the first time used the term "*oncos*" (Greek word for swelling) to depict tumors. The crab analogy given by Hippocrates and Celsus is still in use to describe malignant tumors (ACS 2014). Hippocrates believed that a person is healthy, if all *humors* of the body are balanced, and if any *humor* is too much or little, then the disease will appear. An excess of black bile in body sites was considered to cause cancer. Hippocrates stated that cancer is a non-treatable disease. If treated, the patient may die; if not treated, patient may lead a longer life. It connotes that ablation and cauterization of cancer lesion may result in its infliction of vital organs (Tabri 2010). The Hippocratic theory of cancer was passed on and comprehends by influential Galen, who promulgated the concept in his medical education, which remained indisputable standard through the Middle Ages for over more than thousand years.

Arab-Muslim scholars in medicine, like Al Razi (Rhazes 860–930 AD), Al-Zahrawi (Abulcasis 936–1013 AD), and Ibn Sina (Avicenna 980–1037 AD), were well aware about the cancer. Al Razi explained most types of cancers known at his time and suggested several treatments for cancer on the basis of his knowledge on burnt black bile in the affected organs. He recommended the evacuation of black bile from the organ by emesis and purgation and using cold tempered foods and medications. Abulcasis was the first Andalusian scholar who conducted surgical removal of the cancerous breast and suggested that cancer can be managed surgically only in its initial stages, if complete removal is possible. Likewise, Avicenna also inferred that cancer is extremely difficult to treat caused by an excess of burnt black bile, which also produced excessive heat in the body. Avicenna believed that imbalance of any humor may lead to the inequilibrium of all the four humors. He differentiated benign tumor from a cancerous one by certain symptoms like pain, throbbing, and rapid growth and noted that cancerous tumors send out "crab like tracks" and predominantly occur in the hollow organs. Avicenna also stated that cancers often inflict muscles, tendons, and lymph nodes. Moreover, Abulcasis recognized that a cure was most likely if the cancer was detected at its earliest stage, and the ultimate goal should be to halt the cancerous growth (Said et al. 2011). Abulcasis for the first time clearly mentioned the cancer of kidney and was able to distinguish between acute kidney inflammation and cancer. All the Arab scholars including Rhazes, Abulcasis, and Avicenna realized that the possibilities of healing cancer are prognostically maximized if the cancer is diagnosed at an early stage

(Rhazes 925, Avi Senna 1037). According to Rhazes and Avicenna, cancer is a tumor that is complex to treat. They stated that a "cancerous tumor is destructive and progressively increases in size, spreading its roots which insinuate themselves in the tissue elements." Avicenna promulgated that if one of the body's humors goes out of balance, then all the four humors would also be disturbed. Excess of black bile generates excessive heat in the body which may cause all sorts of cancers. Avicenna further elaborated that cancers often involve muscles, tendons, and lymph nodes; cancerous lesions send out "*crab-like tracks*" and predominantly occur in the "*hollow*" organs. A benign tumor could be differentiated from a cancerous one on the basis of certain obvious symptoms such as throbbing pain and rapid growth. Diamandopoulos (1996) classified the tumors into three major types: (1) *onkoi* (lumps or masses), (2) *karkinomas* (non-ulcerating cancers), and (3) *karkinos* (malignant ulcers).

The first aim of the treatment strategy should be to arrest the growth of cancerous lesion. Avicenna advocated "When cancer starts, it may be possible to keep it as it is, so that it will not increase and keep it non-ulcerated. It may happen sometimes that the stating cancer may be cured. But when it is advanced, verily will not." The first description for treating the cancer was put forth by Avicenna in *Canon of Medicine*, wherein surgical removal of small and accessible tumor not closer to vital organs was described. It stated that the radical excision should be done to remove all diseased tissue, including amputation or the removal of veins extending in the direction of the tumor. Avicenna stated, "*can be arrested with anything, it can be so by vigorous excision…. including all the (blood) vessels supplying the tumor so that nothing of these will be left*." The other citation by Avicenna states "*It was told by one of the predecessors that a physician had excised a cancerous breast radically then cancer developed in the other breast. My opinion is that the second breast might have been on its way to cancerization (a dormant cancer) which fits this case and it is possible to be a spread of the material (cancerous from the first breast) and this is more evident (opinion)*." Avicenna also recommended for purifying the body from excess of black bile by advising a nutritious and balanced diet to the patient in order to retain purity and strengthening the immune system before surgery. He has also attempted the earliest pharmacological treatments for cancer with a herbal drug, *Cichorium intybus* (Hindiba), which later was identified by Ibn al-Baitar as having "anticancer" property that could also be used for the treatment of other types of tumors and neoplastic diseases (Rhazes 925; Avi Senna 1037; Ibn AlBitar 1874, Avi Senna 1993; Saad et al. 2005, 2008; Khan 2006; Said et al. 2011).

10.4.2 Etiopathogenesis of Cancer (Sartan)

Sartan is a melancholic inflammation due to increased combustion of black bile (*Sawdā*') that produces more intrinsic heat, thus becoming pathological. Avicenna noted that cancer may be caused by combustion of pure yellow bile, black bile mixed with that of yellow one, or pure black bile. It is characterized by pain,

burning, and pulsation and increases in size very rapidly due to excess morbid matter and swelling. Cancer gives off newer vessels, blackish or greenish in color, which resemble those of crab's walking legs. Neovascularization in cancerous lesions simulates to the legs of crab which are penetrated deep into the skin as well as subcutaneous tissues and turn hard. The vessels of the cancerous organ filled the cancerous matter, get dilated, and solidified (Tabri 1995). It is primary in its origin, not occurring secondary to any other disease. It is a sensitive lesion that commonly occurs in the hollow organs; hence, it is more prevalent in women and is equally common in nervous organs. Initially, it remains dormant and then appears clinically in the form of tumor and swelling followed by signs and symptoms (Ibn Sina 1992). Rhazes noted that cancer in its initial stage is treatable. Chronic cancer may be treated with surgical removal of tumor. The three most significant complications of surgical ablation are severe hemorrhage, severe pain, and recurrence of cancer (Razi 2002).

Averroes (also known as Ibn Rushd) stated that the disease occurs either due to disturbance in quantity (*Kamiyat*) and quality (*Kaifiyat*) or both in melancholic bile. This derangement in normal constituent of black bile at times may be traced to those substances which are stimulated by excess and prolonged intake of cold (*Barid*) and dry (*Yabis*) edibles and predominance of cold and dry (*Sard Khushk*) or hot and dry (*Har Khushk*) dis-temperament on alimentary organs (Ibn Rushd 1980). The leading cause of *Sartan* may be any abnormal change occurring in the black bile (*Sawdā'*). There are five types of abnormal changes that occur in *Sawdā'*: (1) excess production of normal black bile, (2) burning of normal black bile into abnormal, (3) formation of black bile due to combustion of blood, (4) formation of black bile due to combustion of phlegm, and (5) formation of black bile due to combustion of (*Safra*) bile (Quraeishi 2002; Samarqandi 2010). When abnormal black bile is excessively produced in the human body, spleen succumbs to its complete absorption; later on, the same is spread to the blood circulation from which the organs get their nutrients resulting into a lethal disease. Cancer results due to these abnormal humors which may be of two kinds: ulcerative cancer due to combustion of abnormal black bile and non-ulcerative cancer due to combustion of normal black bile (Ibn Rushd 1980). The ulcerative lesion has intense burning sensation as though sparkle is put on it with its effect onto the internal organs. The margins of ulcer are lusterless, thick, dark red, and externally averted (Qamari 2008). External factors such as occupation and environment or both may result in derangement of black bile. At times, the causes to the abnormality in black bile either qualitative or quantitative may be related to hereditary factors such as leprosy (Ibn Rushd 1980). Nerves, veins, and arteries are commonly inflicted with the spread of cancerous lesions. It may also inflict the esophagus and larynx (Qamari 2008). As the morbid matter of cancer lesion is solid and thick, the tumor neither dissolves nor moves from its site. Foul smelling pale water oozes from the ulcerative lesions as some parts of the lesion are combusted, whereas some are infected (Samarqandi 2010). It is initially circular in shape, firm in consistency and small in size with earthy tinge, and later on develops into a big abscess (Ibn Qiff Maseehi 1995).The size ranges from gram to that of watermelon (Qamari 2008). It is a firm and solid tumor with its

roots deeply seated into the organs. It is manifested by rough texture and distension with greenish vessels spread in its surroundings. The pain increases with the increase in its size. It starts with severe pain with no relief in pain from any drug. It is hot on touch. Some of its types may be without pain which is curable and has good prognosis (Baghdadi 2004).

Sartan is comparatively more common in women due to their softness of body which easily accepts the morbid thick matter; whereas the solid body as in case of men hardly accepts these matters, hence less prevalent in men (Qamari 2008). It is very common in wet organs such as women's breast, uterus, intestine, palate, face, and stomach; it may inflict any part and organ of the body, whereas cancer of intestine, genitals, and mouth is more common in men (Tabri 1997).

10.4.3 Management of Cancer (Sartan)

The pathological changes of temperament in particular body parts or organs amount to the emergence of a disease, which can be treated or cured by applying drugs and nondrug factors. In Unani system of medicine, there is a correlation in between the temperament and other nondrug factors (such as environmental, diet, mental state, and others), which directly or indirectly affect the temperament. The Unani system advocates to utilizing these nondrug factors very frequently and extensively. The principle modes of treatment in the Unani system of medicine include (1) regimenal therapy (*Ilāj bi'l-Tadbīr*), (2) dietotherapy (*Ilāj bi'l-Ghidhā*), (3) pharmacotherapy (*Ilāj bi'l-Dawā*), and (4) surgery *(Ilāj bi'l-Yad*).

The humoral theory of Unani system deciphered cancer as a result of diffusion of burnt black bile into the body organs which lead to "boiling" of the black bile in that cancerous site. The first step propounded in the management of cancer was to evacuate the black bile out of the body by the use of medications and regimental methods. According to Avicenna, medications for cancer have four purposes: (1) complete arrest of cancer lesion, (2) to arrest its progress, (3) to stop ulceration, and (4) healing of ulceration. Galen in his "methods of treatment" has hypothesized that cancer is a metabolic ailment associated with black bile which is difficult to be diagnosed at initial stages, and it should be treated systemically rather than locally. In order to treat cancer, Galen has proposed that removal of black bile from the body by administering a suitable purgative and then the production and buildup of black bile in the vessels should be prevented as far as possible. In case this method is not possible, then black bile should be evacuated from the body at regular time intervals (Said et al. 2011; Shamsi et al. 2016).

It is very important to know the stage of cancer before its treatment. The advanced stage of cancer is very difficult to treat. Avicenna and Galen clearly cautioned to treat the cancers which are developed inside the body and could not be observed by naked eyes. Initially, the evacuation of morbid substance (black bile) should be accomplished using steady purgation or venesection (*Fasd*) with highly nutritious,

cold temperament balanced diets given to produce normal blood in patients, and then it can be treated safely (Razi 2002).

The patients of cancer (*Sartan*) can be treated on the principle of (1) correction of temperament (*Taadil-e-Mizaj*) and (2) cleansing of morbid substance (*Tanqia-e-Mawad*). In cancer, there is imbalance in black bile (*Sawdā*') having cold and dry temperament, and therefore, by adopting the measures of *Ilaj-bil-zid*, the drugs and food having hot and wet temperament are given to antagonize the hotness of cancer. The diet in small quantity should be light (*Latif*), healthy (*Saaleh*), and easily digestible (*Jaiyyadul Kaimus*), having cold and wet temperament (Shamsi et al. 2016).

10.4.3.1 Correction of Temperament (*Taadil-e-Mizaj*)

Most of the ancient Unani physicians recommended the use of plants with cold temperaments in order to antagonize the cancer hotness. Rhazes and all the preceding Unani scholars had recommended cold-tempered plants in the category of third and fourth degrees such as *Brassica oleracea* (Karamkalla), *Bryonia syriaca*, *Lactuca sativa* (Kahu), *Lactuca serriola*, *Linum usitatissimum* (Alsi/Bazarul Kattan), *Malva sylvestris* (Khubbazi), and *Portulaca oleracea* (Khurfa). The recommended foods were also with cold temperament: milk, beans, *Hordeum vulgare*, *Portulaca oleracea* (Khurfa), *Cichorium intybus* (chicory), *Cucurbita maxima* (Kaddu), *Amaranthus polygamus* (Cholaee), *Chenopodium album* (Bathua), *Cucumis sativus* (Kheera), *Punica granatum* (anar), *Spinacia oleracea* (spinach), *Prunus amygdalus* (badaam), whey water (Ma-ul Jubn), barley water (Maus-Shaeer), Mufarreh, and exhilarant fruit juices (*Mashroobaat*) like apple juice (*Sharbat-e-saib*). *Arq-e-Neelofar* and *Arq-e-baid-Mushk* can effectively be used as a cardiac tonic (Samarqandi 2007a; Said et al. 2011; Shamsi et al. 2016).

10.4.3.2 Cleansing of Morbid Substance (*Tanqia-e-Mawad*)

Regimenal therapy (*Ilāj bi'l-Tadbīr*) is one of the most popular methods of treatment described by most of the ancient Unani physicians. Hippocrates, Galen, Rhazes, Avicenna, and Abulcasis described various regimens including *Munzij-o-Mus'hil* therapy for the management of diseases, either independently or in combination with other therapies. The regimen consists of two phases: (1) concoction (*Nuzj*) and (2) purgation (*Ishāl*).

Concoction is a method by which viscous *humors* (morbid materials) are modified into a form which could easily be expelled out of the body with the help of purgatives. Purgation (*Ishāl*) is the extraction of the morbid materials from the vessels and other deeper tissues. Concoction is essential in case of all chronic disorders as well as the diseases having a duration of more than 40 days soon after the signs of concoction (*Nuzj*) completion appear; the purgative (*Mus'hil*) drugs are added to the *Munzij* drugs. Purgative (Mus'hil) drugs bring out the purgation (*Ishāl*) of the morbid *humors* (*Phlegm*, *Sawdā*', and *Safra*) that have been evacuated by the action

of concoctive drugs (*Munzija*t). The *Munzij-o-Mus'hil* therapy can be used to evacuate the disease causing morbid materials out of the body (Kabiruddin 2006; Shamsi and Rehman 2012; Shamsi et al. 2016).

10.4.3.3 Concoctive Drugs (*Munzij-e-Sawdā'*)

Althaea officinalis (Khitmi), *Borago officinalis* (Gaozaban), *Cichorium intybus* (Tukhme Kasni), *Cordia dichotoma* (Sapistaan), *Foeniculum vulgare* (Badiyan), *Fumaria officinalis* (Barg-e-Shahatra), *Glycyrrhiza glabra* (Asl-ussoos), *Lavandula stoechas* (Ustukhuddus), *Rosa alba* (Gule Surkh), *Vitis vinifera* (Maweez Munaqqa), *Zizyphus vulgaris* (Unnab), and others.

10.4.3.4 Purgative Drugs (*Mus'hil-e-Sawdā'*)

Aloe barbadensis (Aelwa), *Cassia angustifolia* (Sana-e-Makki), *Cuscuta reflexa* (Afteemoon), *Emblica officinalis* (Amla), *Lavandula stoechas* (Ustukhuddus), *Polypodium vulgare* (Bisfayaj), *Terminalia bellerica* (Balela), *Terminalia chebula* (Halela Zard), and others.

10.5 Important Unani Formulations for *Munzij* and *Mus'hil* Therapy for Cancer

Different Unani formulations for *Munzij* and *Mus'hil* regimen are mentioned in classical text that can be used for the evacuation of morbid *Sawdā*' (Shamsi et al. 2016):

1. Unnab (*Zizyphus vulgaris*), 5 pieces; Gaozaban (*Borago officinalis*), 7 g; Shahatra (*Fumaria parviflora*), 7 g; Badranj boya (*Nepeta ruderalis*), 7 g; Badiyan (*Foeniculum vulgare*), 7 g; and Asl-us-Soos-muqashar (*Glycyrrhiza glabra*), 5 g. All the drugs are soaked in hot water overnight, and after serving add Gulqand, 20 g, or Turanjbeen (*Alhagi pseudalhagi*) 20 g (Azmi 2002).
2. Ayarjaat like Ayaraj-e-feyqra (*Aloe barbadensis*) or Ayaraj-e-Jalinoos or Ayaraj-e-Roofas to eliminate the morbid *Sawdā*' and for Taadil-e-Mizaj (Samarqandi 2007b).
3. Asl-us-Soos (*Glycyrrhiza glabra*); Gaozaban (*Borago officinalis*), 5 g; Gul-e-Gaozaban (flowers of *Borago officinalis*), 5 g; Badiyan (*Foeniculum vulgare)*; Beekh-e-Badyan (root of *Foeniculum vulgare*); and Tukhm and Bekh-e Kasni (seed and root of *Cichorium intybus*), 7 g each to be partially grounded. Gule Surkh (*Rosa alba*); Ustukhuddus (*Lavandula stoechas*), 7 g; Shahtara (*Fumaria officinalis*), 7 g; Tukhme Khatmi (*Althaea officinalis*), 7 g; Aloo Bukhara (*Prunus domestica*) 5 nos., medium size; and Maweez Munaqqa (*Vitis vinifera*) 9 nos., medium size. All the drugs to be soaked overnight in water followed by boiling

in the morning. Approximately 40 g Gulqand (sweet preserve of rose petals) to be added in filtrate. The preparation to be given as a drink (Kabiruddin 2006).

4. Kharbaq Siyah (*root of Picrorhiza kurroa*) 375 mg; Habbul Neel *(Ipomoea hederacea*), 256 mg; Shahtara (*Fumaria officinalis*) leaves and flowers, 375 mg; Habbul Ghar (*Laurus nobilis*), 375 mg; Hanzal (*Citrullus colocynthis*) fruit pulp, 512 mg; Afsanteen (*Artemisia absinthium*) whole plant, 1024 mg; Ghariqoon (*Agaricus alba*), mushroom, 1024 mg; Ayarij-e-Faiqra (Unani compound formulation), 1.75 g; Milhe Nafti (black salt), 630 mg; and Saqmonia (*Convolvulus scammonia*) resins, 630 mg; make powder of all drugs, and sieve it through silk cloth, and make pepper-sized tablet using cabbage water as a binder. Oral administration of 12 g tablet once in a week (Tabri 1995).

10.5.1 Dietotherapy (Ilāj bi'l-Ghidhā) in Unani System of Medicine

The Unani system of medicine lays rules on prevention of disease and promotion of health through a balanced lifestyle, which revolves around six essential factors (*Asbāb Sitta Ḍarūriyya*): air (*Hawā*), food and drinks (*Ma'kūl o Mashrūb*), bodily movement and repose (*Ḥarakat o Sukūn Badanī*), psychic movement and repose (*Ḥarakat o Sukūn Nafsānī*), sleep and wakefulness (*Nawm-o-Yaqẓa*), and evacuation and retention (*Istifrāgh-o-Iḥtibās*). Noncompliance with these principles leads to an errant lifestyle and ultimately leads to disease. The Unani system of medicine believes that a physician is not the healer but merely an assistant to physique (*Tabiyat*) of the body, which is the true healer; hence, the diet should be in accordance with it. Diet improves the health condition by preventing the complications. Since, *Sartan* is considered to be associated with black bile, hence, *Sawdā*' producing diet/foods may be harmful to cancer patients. The diet for cancer patient must be easily digestible (*Jaiyyadul Kaimoos)* and light (*Latif*) with cold and moist *temperament* including *Cucurbita maxima* (Kaddu), *Amaranthus polygamus* (Cholaee), *Chenopodium album* (Bathua), *Cucumis sativus* (Kheera), *Punica granatum* (Anar), whey water (*Ma-ul Jubn*), and barley water (*Maus-Shaeer*). Many items including wild animal beef, cow beef, red lentils, cabbage, some dry fruits, and concentrated liquor are possible contraindicated foods that should not be used in cancer patients (Tabri 1995; Alam et al. 2013; Anonymous 2016).

10.5.2 Pharmacotherapy ("Ilāj bi'l-Dawā") in Unani System of Medicine

The three primary sources of drugs (*Mawālīd Thalātha)* which are used for medication are plants, animals, and minerals. These drugs are either used singly or in compound formulations. Avicenna and other ancient scholars, like modern chemotherapy,

used materials derived from mineral, mainly to stop and prevent cancer development. Minerals such as blue vitriol, zinc, and iron were applied onto the cancer lesion or immediately after surgical removal (Said et al. 2011). Avicenna advised to avoid irritant medications; however, he clarified to use good medications as washed (pure) minerals such as tutty (copper sulfate) mixed with rose grease oils and oil of yellow gillyflower. Medications for the prevention of ulceration were described as "smears that prevent cancer progress provided they will not be irritant; all of them are useful, particularly if mixed with the mixture of lead stone and stone pounder of aromatics."

Unani drugs/formulations for ulcerated and non-ulcerated lesions are described in classical Unani text. Sprinkling of Baid Saada (Kohna) powder on cancer ulcers and washing of cancerous wounds with decoction of Chunar leaves, liniment of *Curcuma longa* (Haldi), *Boswellia serrata* (Kundur), and *Aloe barbadensis* (Sibr) in equal quantity to be applied on ulcer mixed with rose oil (Roghan-e-Gulab) (Razi 2002). Ointment of Zoofa and Lu'ab Bahi Dana mixed with Roghan-e-Banafsha (Tabri 1995). Roghan-e-Gul, Kishneez Sabz, and Mako Sabz can be used to block cancerous growth. Pus formation can be prevented by the use of A'b-e-Kahu and Roghan-e-Zaitoon (Samarqandi 2010). Local application of suppository made up of Afyun, Nakhuna, and Za'fran may be used in eye and nasal cancers (Baghdadi 2004). Paste of pomegranate is highly efficacious in cancer ulcer, while bartang leaves (*Lahi'ya-tut-tees*) can be used for checking bleeding (Jurjani 2010). In case of uterine cancer, "sitz bath" can be taken by using the decoction of Khatmi, Methi, Alsi, and Ikleel-ul-Mulk. The paste of Methi, Alsi, Qinnab Seeds, dry Banafsha, Habbul Ghar, Babuna, Guggul, Sandal Surkh, Khatmi, and Soya can also be used (Majoosi 2010).

10.5.2.1 Plant Origin Unani Drugs with Anticancer Properties

Plants are the major source of Unani drugs. The plant origin drugs are prescribed with an intention to keep the tumor in its dormant stage, to stop its further growth, to dissolve it, and lastly to surgically remove it (Aslam et al. 1981). In the advancement of modern analytical tools and techniques, some of the single Unani drugs have been evaluated for anticancer activities (Table 10.3), but negligible attention has been paid to explicate the safety and efficacy of Unani compound formulations to treat the cancer patients through clinical trials.

Table 10.3 Important Unani medicinal plants (single drugs) with anticancer activity

Botanical name (family)	Unani name	Anticancer activity	References
Abelmoschus moschatus (Malvaceae)	Habbul-Mushk	Hydroalcoholic seed and leaf extracts exhibited antiproliferative activity against colorectal adenocarcinoma and retinoblastoma human cancer cell lines	Gul et al. (2011)
Abrus precatorius (Fabaceae)	Ghunchi	High antitumor activity of agglutinin protein purified extract from the seeds. *In vitro* anticancer activity of aqueous extract of leaves on the murine mastocytoma cancer cell line (P815)	Panneerselvam et al. (2000) and Lébri et al. (2015)
Acorus calamus (Araceae)	Waj-e-Turki, Bach	Methanolic and ethanolic extracts and essential oil of rhizome showed anticarcinogenic and antiangiogenic effects on AGS cells, and their components might act against the proliferation of AGS cells	Rahamooz Haghighi et al. (2017)
Aegle marmelos (Rutaceae)	Belgiri (Bel), Safarjale-Hindi	Leaf extracts inhibited the growth of leukemic K562, T-lymphoid Jurkat, B-lymphoid Raji, and breast cancer cell lines MCF-7 and MDA-MB-231 and increase *ERa* gene expression in MDA-MB-231 due to antineoplastic effects. The ethanolic fruit extract has cytotoxic effect on SKBR3 cells *in vitro*. Extracts also showed ability to inhibit the *in vitro* proliferation of human tumor cell lines, including the leukemic K562	Lampronti et al. (2003), Moongkarndi et al. (2004), and Jagetia et al. (2005), Subramaniam et al. (2008), Baliga et al. (2012), and Baskar et al. (2012)
Alpinia galanga (Zingiberaceae)	Khulanjaan	Antiproliferative activity and induction of apoptosis in human breast carcinoma cell line (MCF-7) and nonmalignant (MRC-5). ACA (1′S-1′-acetoxychavicol acetate) obtained from rhizome exhibited anti-colorectal adenocarcinoma activity in SW480 cell lines	Samarghandian et al. (2014) and Baradwaj et al. (2017)

(continued)

Table 10.3 (continued)

Botanical name (family)	Unani name	Anticancer activity	References
Alstonia scholaris (Apocynaceae)	Kashim	Demonstrated the chemopreventive potential of bark extract in DMBA-induced skin tumorigenesis in Swiss albino mice. Alkaloid fraction studied *in vitro* in cultured human neoplastic cell lines (HeLa, HepG(2), HL60, KB, and MCF-7) and in Ehrlich ascites carcinoma-bearing mice. Increase in the antineoplastic activity was observed in HeLa cells	Jagetia and Baliga (2006) and Jahan et al. (2009)
Ammi majus (Apiaceae)	Atrilal	8-MOP inhibited cell growth in several cancer cell lines. The SK-N-AS and SW620 cells were the most sensitive to the compound. 8-MOP impaired the PI3K/AKT signaling pathway and, independently of photoactivation, reported to inhibit the growth of neuroblastoma and colon cancer cells by induction of apoptosis via intrinsic and extrinsic pathways	Bartnik et al. (2017)
Argemone mexicana (Papaveraceae)	Satyanasi	Alkaloids strongly inhibited the cell proliferation in human colon cancer cell (SW480) line. Aqueous and methanolic extracts inhibited a cytotoxic effect on A549, SiHa, and KB immortalized cell lines	More and Kharat (2016) and Singh et al. (2015)
Artemisia absinthium (Asteraceae)	Afsanteen (Qaisoom)	Crude extracts of the aerial parts inhibited cell proliferation and promote apoptosis in a human breast carcinoma estrogenic-unresponsive cell line (MDA-MB 231) and an estrogenic-responsive cell line (MCF-7). Methanol extracts of flowers exhibited considerable cytotoxic effect on breast cancer cell line (MCF-7). Decoction of the plant reported to be potent inducer of *in vitro* apoptosis of B chronic lymphocytic leukemia (CLL) cells	Shafi et al. (2012), Gordanian et al. (2014), and Mirkin et al. (2017)

(continued)

Table 10.3 (continued)

Botanical name (family)	Unani name	Anticancer activity	References
Artemisia nilagirica (Asteraceae)	Nagdon (Branjasif)	Exhibited considerable cytotoxic activity against breast cancer cell line (MCF-7). Essential oil (EO) induced apoptosis in human promyelocytic leukemia (HL-60) cells. Inhibition of proliferation and apoptosis in a human monocytic leukemia (THP-1) cell line via mitochondria-dependent and death receptor-dependent apoptotic pathways	Gordanian et al. (2014), Saleh et al. (2014), and Gul et al. (2016)
Asparagus racemosus (Liliaceae)	Satawar	The isolated shatavarin showed potent cytotoxicity (tumor volume, packed cell volume, viable tumor cell count, and increased nonviable cell count) in tumor-bearing mice. Inhibited growth of renal cell carcinoma cell (RCC UOK 146) lines	Mitra et al. (2012) and Verma et al. (2014)
Averrhoa carambola (Oxalidaceae)	Kamrakh	Effective against hepatocellular carcinoma (HCC) in Swiss albino mice. Showed the cytotoxic effects of DMDD (2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione) against human breast, lung, and bone cancer cells *in vitro*; DMDD suppressed the growth of breast carcinoma cells	Singh et al. (2014) and Gao et al. (2015)
Bacopa monnieri (Scrophulariaceae)	Jal-Brahmi	Aqueous extract showed biological activity of apoptosis in Ehrlich ascites tumor (EAT) cell lines. *Bacopa monnieri* water extract treatment of EAT cells produced apoptotic morphological characteristics, and *in vivo* DNA fragmentation was able to induce apoptosis in EAT cells via Bax-related caspase-3 activation and also exhibited the cytotoxic activity of ethanolic extract of dichloromethane (DCM) fraction on two different cell lines MCF-7 and MDA-MB 231) under *in vitro* conditions	Kalyani et al. (2013), Mallick et al. (2015), and John et al. (2017)

(continued)

Table 10.3 (continued)

Botanical name (family)	Unani name	Anticancer activity	References
Butea monosperma (Fabaceae)	Tesu (Dhak), Darakht-e-Palasha	The chemopreventive and antiangiogenic effects evaluated in a HBV-related X15-myc mouse model of hepatocellular carcinoma (HCC). Butanol fraction (Bmbu) from bark showed cytotoxic and apoptosis inducing activity in MCF-7 breast cancer cell lines. Butrin, a novel compound isolated from *Butea monosperma* flowers on suppressing the expression of SIRT1 and Aurora B kinase-mediated apoptosis in colorectal cancer cells (SW480 CRC)	Choedon et al. (2010), Kaur et al. (2017), and Subramaniyan et al. (2017)
Cannabis sativa (Cannabinaceae)	Qinnab (Bhang)	Cannabinoids inhibited angiogenesis and decrease metastasis in various tumor types in laboratory animals. It showed specific cytotoxicity against tumor cells while protecting healthy tissue from apoptosis. It also displayed potent anticancer activity against tumor xenografts, including tumors that express high resistance to standard chemotherapeutics	Caffarel et al. (2012), Velasco et al. (2016), and Bogdanović et al. (2017)
Centella asiatica (Apiaceae)	Brahmi Buti	Anticancer potential of asiatic acid on human ovarian cancer cells (SKOV3 and OVCAR-3), through inactivation of the PI3K/Akt/mTOR pathway. MicroRNA (miR)-1290 promoted asiatic acid-induced apoptosis in non-small cell lung carcinoma cells (A549). **A**n aqueous extract (AE) exhibited activity against mouse melanoma (B16F1), human breast cancer (MDA-MB-231), and rat glioma (C6) cell lines	Pittella et al. (2009), Kim et al. (2014), and Ren et al. (2016)
Cichorium intybus (Asteraceae)	Kasni	Exerted significant activity against amelanotic melanoma C32. Strong antiproliferative effect against HepG2 cell lines. Showed notable effects on the leukemia cell lines. Root extract exhibited tumor inhibitory activity against Ehrlich ascites carcinoma in mice	Hazra et al. (2002), Conforti et al. (2008), Yook et al. (2015), and Esmaeilbeig et al. (2015)

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Table 10.3 (continued)

Botanical name (family)	Unani name	Anticancer activity	References
Curcuma longa (Zingiberaceae)	Chob Zard (Haldi)	Extracts exhibited antiproliferative effects in cholangiocarcinoma (CCA) cells by inducing pro-apoptotic signals and modulating signal transduction molecules. Curcumin significantly induced apoptosis and cell cycle arrest in pancreatic cancer (PC) cell lines. Curcuma C20-dialdehyde exhibited anticancer effects against colon and cervical cancer cell	Chaithongyot et al. (2015), Zhou et al. (2016), Calaf and Roy (2017), and Leelawat and Leelawat (2016)
Cuscuta reflexa (Convolvulaceae)	Aftimun	Exhibited antiproliferation activities in HCT116 colorectal cell lines. Induced apoptosis in Hep3B cells through the upregulation of p53 and BAX and downregulation of Bcl-2 and survivin	Suresh et al. (2011) and Riaz et al. (2017)
Glycyrrhiza glabra (Fabaceae)	Asl-us-Soos	Licochalcone A (LCA) suppressed the oxidation of cancer cells and markedly inhibits the proliferation of cells. Aerial parts showed remarkable activity against human cancer cell lines (HeLa, MCF-7, MDA-MB-231, Caco-2, and PC3). Inhibited proliferation of the colon cancer cell line HT-29. Isoangustone-A (IAA), a novel flavonoid from licorice root, suppressed proliferation of human melanoma cell lines SK-MEL-28	Song et al. (2013), Nourazarian et al. (2015), Aiello et al. (2017), and Chen et al. (2017)
Justicia adhatoda (Acanthaceae)	Arusa	Isolated 2-acetyl-benzylamine screened for potent anticancer properties against leukemia cells: CEM, NB-4, MOLM-14, Jurkat, IM-9, K562, and HL-60. Significant cytotoxic properties reported against MOLM-14 and NB-4 cells. Vasicine acetate was obtained by acetylation of vasicine and showed prominent cytotoxic activity *in vitro* against A549 lung adenocarcinoma cancer cell line	Duraipandiyan et al. (2015) and Balachandran et al. (2017)

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Table 10.3 (continued)

Botanical name (family)	Unani name	Anticancer activity	References
Melia azedarach (Meliaceae)	Bakayin	Seed kernel extract exhibited highest cytotoxic activity and selectivity to cancer cell lines (HT-29, A549, MCF-7, and HepG-2 and MDBK). Hexane extract of the fruits exhibited cytotoxic activities against leukemia (HL60), lung (A549), stomach (AZ521), and breast (SK-BR-3) cancer cell lines	Jafari et al. (2013) and Pan et al. (2014)
Nigella sativa (Ranunculaceae)	Habbatus sauda (kalonji)	Thymoquinone (TQ), a major ingredient of black seed oil (*Nigella sativa*), exhibited antimetastatic capacity in prostate cancer DU145 and PC3 cells. TQ exhibited *in vitro* anticancer activity in animal models of cancer via numerous mechanisms of action likewise, antioxidant activity, interfering with DNA structure, affecting carcinogenic signaling molecules and immunomodulation activities. Moreover, the treatment of seed extract and seed oil significantly reduced the viability of human lung cancer cells (A549 cells)	Al-Sheddi et al. (2014), Asaduzzaman et al. (2017), Kou et al. (2017), and Mostofa et al. (2017)
Withania somnifera (Solanaceae)	Asgandh	The methanolic leaf extract showed a strong antiproliferative activity against MCF-7, HCT116, and HepH2 cell lines. Withaferin A (WFA) exhibited strong antiproliferative activity against osteosarcoma U2OS (a rare type of osteocancer), affecting human cell lines, via generation of ROS and disruption of mitochondrial membrane potential. Crude water extract of roots exhibited potent cytotoxic effect on human malignant melanoma A375 cells. Anticancer activity of withaferin A human and murine B cell lymphoma cell lines	Halder et al. (2015), McKenna et al. (2015), Alfaifi et al. (2016), and Li et al. (2017)

10.6 Current Status of Unani (Greco-Arab) System of Medicine in India

Nowadays, the pursuit for alternate to the conventional oncologic therapies has increased tremendously. The US National Cancer Institute (NCI) supports complementary and alternative medicine and has been instrumental in funding and collaborating with the institutions/group working on traditional Chinese medicine, Ayurveda, and other traditional therapies (Jia 2012). In India, the initiative on oncological research has been taken by Central Council for Research in Unani Medicine (CCRUM), New Delhi; so far EMR project "Evaluation of anti-proliferative activity of Unani drugs in cancer prevention- A mechanistic approach" has been completed, but its results are yet to come in public domain. Other projects that are in progress are Screening of Anticancer potential of Indian Medicinal Plants, Investigation of Anti-Cancer activities of some selected medicinal plants and their molecular targets on oral cancer cell lines, Analysis of efficacy of *in vitro* raised plant (*Catharanthus roseus*) extracts in protecting chemically induced carcinogenesis in model rat, and Screening and purification of plant extracts used in the Unani system of medicine against microbial flora of oral cavity: antimicrobial and carcinogenic activity (Anonymous 2017a).

Recently, the Ministry of AYUSH, Government of India, through CCRUM has started a program to integrate Unani Medicine with National Program for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS) in Lakhimpur district of Uttar Pradesh. The major objective of the program is to prevent the population from fatal diseases by early diagnosis and reducing the complications and drug dependency by the use of Unani medicine. In this program, patients are encouraged to make lifestyle changes and advised to take Unani medicines along with standard treatment for prevention of cancer and its complications (PIB 2016). Besides these measures, the Council is also making efforts to introduce Unani medicines as adjuvant therapy to improve the quality of life of patients with terminal cases of cancer in collaboration with Tata Memorial Cancer Hospital, Mumbai (Anonymous 2017b). Moreover, the Council has also started a collaborative study with Institute of Cytology and Preventive Oncology (ICPO), Noida (ICMR), to validate the efficacy of Unani pharmacopoeial drugs in the prevention of cancer of cervix (Anonymous 2017c).

At present, the research on cancer in Unani medicine is not much enthusiastic as the limited work has been done on the cancer. Thus, there is immense need to maximize the efforts to conduct the cancer trials with animal models on most common cancer types such as breast and colon cancers. There must be main concentration of Unani researchers on the evaluation and validation of Unani single as well as compound formulations in cancer on animal models as the work being done on cancer cell line is not much promising as the same is available across the scientific platforms in abundance. The focus should be on preclinical trial of Unani drugs on cancer animal models incorporating diagnostic and prognostic tools and carcinogenic biomarkers so that their efficacy can be elaborated more scientifically. Unani

medicine should be studied as it incorporates herbs as whole or its part either singly or more than two in compound formulations. Each herb (drug) possesses various active principles that work synergistically and produce therapeutic effects and thereby lessen the prospects of adverse effects of formulations. The research work is already underway on single anticancer herbs. However, the question arises; does the findings give us any edge over other available conventional therapies. In current scenario, our main focus should be on compound formulations, which consists of two or more single drugs. The utilization of compound drugs in alleviation of cancer inflictions is still unexplored to a large extent. Hence, the clinical trial must be done with Unani compound formulation with minimum ingredients, i.e., two to three, so that their efficacy can be tested and proved scientifically.

10.7 Conclusions and Future Prospects

The thrust areas of research in oncologic therapies may encompass palliative pain management through Unani analgesics as an adjuvant therapy (immune-modulators such as *Khamīra Marwārīd* and *Ḥabb-e Jawāhar Mohra*) in management of hepatotoxicity (*Arqe Kasni*, *Majoon Dabidul Ward*), and nephrotoxicity (*Habb-e-Banadiqul Bazoor* and *Jawarish Zarooni*) develop due to anticancer agents, and also could be an alternate to the conventional therapies (Fig. 10.2). Interaction potential of some herbs with chemotherapeutic agents may be more critical. To avoid such

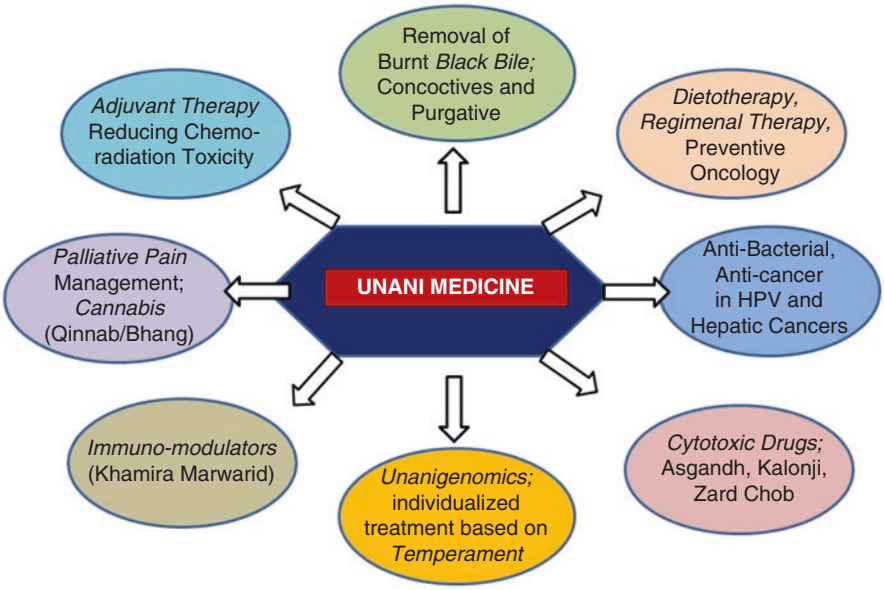

Fig. 10.2 Unani medicine and future strategies for research in cancer (*Sartan*)

circumstances, specific studies are warranted to optimize the dose requirement in cancer patients. The priority is to scientifically evaluate these valuable therapeutic options and generate evidence-based data for global acceptance of these age-old Unani medicines. But the path to these trends seems very difficult as the current status of Unani oncology is in its initial stage, but the same may be progressed on in a graded manner initiating with animal model trials on most prevalent cancer types in phases to the clinical trials. The future researches in Unani medicine may be undertaken to reduce the side effects of chemo- and radiotherapy in oncologic management as adjuvant to improve the quality of life of cancer patients. Moreover, Unani medicine is principally based on personalized medicine as the cure of every disease according to the humor involved, and patients are keenly monitored based on their specific condition. Thus, more clinical data are required to prove the effectiveness of the Unani system of medicine in the management of cancer.

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Chapter 11
Signaling Pathways of Anticancer Plants: Action and Reaction

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Abstract Insights into the alterations of the mammalian genome in neoplastic diseases and the mechanism of action of the therapeutic anticancer drugs are one of the extremely important, diverse, and challenging areas of study currently. By the virtue of lingering toxicity of the reputable chemical drugs, plant-derived anticancer substances, viz., vinblastine, vincristine, Taxol, topotecan, camptothecin, and podophyllotoxin derivatives, are highly safe and efficient in the treatment and management of this monstrous disease. Among the list of accessible targets of the therapeutic drugs, DNA replication and mitosis, hormonal regulation of cell growth, aberrant signaling pathways, cell surface receptors, and second messengers are noteworthy. Nowadays, newer therapeutic approaches are being followed, and an increased understanding into the mechanism of action of the therapeutic anticancer agents is evolving due to continuous and relentless efforts of the researchers. The aim of the present chapter is to highlight the application of medicinal plants and their secondary metabolites as anticancer substances and also focus on the signaling aspects of potential anticancer compounds to find out their mechanisms of action against cancer cells.

Keywords Cancer cell · DNA methylation · Epigenetic factors · Secondary metabolites · Therapeutic potential

11.1 Introduction

Cancer, a neoplastic disease, exhibits the hallmark properties of metastasis and invasion. Despite over 110 years of exhausting cancer research, the reason behind this grave disease is still a subject of debate between diverse theories hypothesizing

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either chromosomal or mutational alterations or epigenetic causes as reasons of cancer (Strauss 1992; Steinberg 2004). The cancer enigma is still mysterious as this debate has been monopolized by traditional theories of mutation, according to which the disease is a genetic one (Bishop 1987). The genetic reason of cancer development is the permanent mutations in the DNA of the cells resulting in the breakdown in the ability of the cell to control its own division. Normal cells repair most of the gene mutations while the cancer cells accumulate these mutations, and these become irreparable leading to abnormal proliferation of these cells (Krishnamurthi 2000). However, the reasons of cancer development are both genetic and epigenetic that result into the deregulated gene expression thereby leading to disruptive function (Jones and Baylin 2007). Moreover, epigenetic alterations unlike genetic ones are reversible hence the promising targets of the plant-derived drugs (Sharma et al. 2010; Best and Carey 2010).

Epigenetic mechanisms are indispensable for the maintenance of adult life. Deregulated gene expression is a consequence of disruption in the epigenetic processes which results in the development of grave diseases like cancer. Some of the important epigenetic components deregulated during the development of cancer include chromatin structure, DNA methylation, histone modifications, and noncoding RNAs. In view of the reversibility and reparability of epigenetic alterations, plant-derived epigenetic drugs or the drugs targeting epigenetic mechanisms have proven promising in cancer therapy (Mai and Altucci 2009; Sharma et al. 2010). Some of the most familiar epigenetic alterations that lead to the uncontrolled proliferation include methylation or deacetylation of histones and CpG (phosphorylated cytosine-guanine) islands methylation within the gene promoters (Esteller 2005; Jones and Baylin 2007; Best and Carey 2010). Epigenetic mechanisms pertaining to the alteration in the expression of certain important genes involved in cell proliferation and differentiation are often deregulated during the tumor development. Certain noncoding RNAs like miRNAs, siRNAs, etc. known for controlling the expression of plentiful cellular proteins by altering stability of the mRNA and/or translation constitute a vital part of the epigenome (Nelson and Weiss 2008; Guil and Esteller 2009). Evidences advocate that the epigenetic deregulation is only an introductory transforming event (Feinberg 2005). Epigenetic alterations commonly noticed in the preliminary-stage tumors include comprehensive DNA hypomethylation and hypermethylation of specific promoter sequences (Esteller 2007). This reveals that epigenetic changes comprise the early stages in the loss of cellular homeostasis and may pave way to the gene mutations and genomic instability. Deregulation of the epigenetic mechanisms lead to genetic instability which results in the inactivation of tumor suppressor genes leading to the activation of oncogenes (Ellis et al. 2009). Moreover, hypomethylation often takes place in the repetitive DNA sequences (heterochromatic DNA repeats and dispersed retrotransposons) (Ehrlich 2002).

11.2 Plant-Derived Anticancer Drugs

Even though a number of medicines are available to treat different types of cancers, no drug has been observed to be completely effective and harmless in anticancer therapy. The chief setback in the cancer chemotherapy is the lingering toxicity of the well-recognized chemical drugs. However, plant-derived products have proven safe and effective in the treatment as well as management of various types of cancers to an appreciable extent (Shukla and Mehta 2015). Herbal medicines are playing an irreplaceable role in the cancer prevention and treatment which implement their numerous medicinal effects and therapeutic benefits by restraining cancer, activating hormones and enzymes, promoting production of defensive enzymes including antioxidant enzymes, arousing DNA repair mechanism, and increasing immunity (Thakore et al. 2012). Vigorous investigations are in progress on a number of plant phytochemicals to gain insights into the antitumor mechanism of action against a variety of cancers, and a fair number of plants are under clinical trials for this purpose (Rao et al. 2008). A lot of natural products have been acknowledged as influential, effective, and intoxicating anticancer agents, and the anticancer properties of various phytochemicals or the plants as such are being identified. Plant-derived compounds have also proven promising in targeting these deregulated epigenetic processes as well as other genetic and cellular processes. The important anticancer drugs working through these different cellular and genetic as well as epigenetic mechanisms include polyphenols, terpenoids, alkaloids, and organosulfur compounds (Schneider-Stock et al. 2012). The aim of the present chapter is to highlight the application of medicinal plants and their secondary metabolites as anticancer substances and also focus on the signaling aspects of potential anticancer compounds to find out their mechanisms of action against cancer cells.

11.2.1 Alkaloids

Alkaloids are a remarkable assemblage of phytoprinciples, extracted from certain medicinal plants, having a diverse spectrum of effects ranging from analgesics and addictive to anticancer. These nitrogen-containing compounds do not follow into any proper classification, and no demarcated boundaries can distinguish them; however, most recent classifications rely on the carbon skeleton and the biosynthetic pathways (Meyers 2001). Owing to the substantial anticancer activity against a variety of cancers, these alkaloid structures are frequently mimicked and synthetic analogs in the form of chemical drugs are synthesized. Some of the important examples of anticancer alkaloids that are in wide clinical use throughout the world include vinblastine, vincristine, Taxol, camptothecin derivatives, topotecan, epipodophyllotoxins, etc. (Darwiche et al. 2007). The most commonly used anticancer mechanisms of the alkaloids involve cell cycle arrest at G2/M phase, microtubule

destabilization leading to apoptosis, microtubule polymerization prevention, and inhibition of topoisomerase I activity (Li et al. 2012).

11.2.2 Polyphenols

Polyphenols, acknowledged for their anticancer properties, are ubiquitous in vegetables, fruits, and beverages and safe and well tolerated by humans except for a few cases where toxic effects have been reported (Thomasset et al. 2007). Polyphenols possess strong antioxidant effect which is the key property accredited for the chemopreventive and therapeutic effects against cancer. Among the other mechanisms of anticancer effects induced by polyphenols include antiproliferation, anti-inflammation, induction of cell cycle arrest as well as apoptosis, and inhibition of angiogenesis and metastasis (Lambert and Elias 2010; Araujo et al. 2011). Polyphenols have been categorized into flavonoids, stilbenes, tannins, coumarins, and phenolic acids. However, the flavonoids constitute the major and most copious group (Quideau et al. 2011). The best studied polyphenols for the anticancer activity include curcumin, resveratrol, and genistein.

11.2.3 Organosulfur Compounds

Organosulfurs like phenols are plant-derived drugs with substantial anticancer effects. Characterized by their sulfur group, these compounds occur naturally in a lot of plants, mainly garlic, coffee, nuts, and some tropical fruits. Isothiocyanates constitute the major subgroup of organosulfur compounds differentiated by the presence of an isothiocyanate group (-N=C=S); the central carbon renders it exceedingly electrophilic (Fahey and Talalay 1999). Sulforaphane, an important organosulfur compound belonging to isothiocyanate group, is found in vegetables belonging to cruciferous group. Present in fair amounts in broccoli, cauliflower, cabbage, and hoary weed, it is derived from glucoraphanin (a glucosinolate) by the activity of myrosinase enzyme upon damage while chewing or cooking. Sulforaphane exerts its antitumor effect by different mechanisms like cell cycle arrest at G2/M phase which has been observed to be associated with distorted microtubule dynamics (Azarenko et al. 2008). Altered cdc2 kinase activity, increased expression of proteins like cyclin B1 and p21, and histone H1 phosphorylation are among the other cell cycle arrest mechanisms induced by sulforaphane (Parnaud et al. 2004; Azarenko et al. 2008).

11.2.4 Terpenoids

Terpenoids are a very large and diverse class of plant secondary metabolites comprising a miscellaneous treasure of phytochemicals in drug discovery predominantly in the field of cancer (Huang et al. 2012). Terpenoids, derived from isoprene units by mevalonic acid and methylerythritol phosphate (MEP) pathways, are divided into monoterpenoids, diterpenoids, triterpenoids, tetraterpenoids, and sesquiterpenoids depending upon the number of isoprene units. The anticancer potential of this class of compounds have been a focus of vigorous and passionate research only recently. Several sesquiterpene lactones have been recently put to test during the clinical trials to investigate their anticancer potential, and promising results have been obtained (Ghantous et al. 2010). These studies have revealed that terpenoids prove as potent anticancer drugs at several tumor formation stages by inhibiting several processes (cell proliferation, angiogenesis, metastasis, and cell death). These terpenes induce apoptosis in the tumor cells by targeting specific molecular mechanisms, like inhibiting certain important signaling pathways like NF-κB, JAK-STAT, etc., blocking the activity of Ca^{2+} ATPase pump as well as the activities of DNA topoisomerases of both I and II classes, and modulating various epigenetic mechanisms, etc. (Huang et al. 2012; Ghantous et al. 2010). Furthermore, terpenoids are also known to induce cell death of malignant tumor cells by targeting extrinsic and intrinsic apoptotic pathways (Yang and Dou 2010). Parthenolide, betulinic acid, and triptolide are some of the important examples of terpenes inducing apoptotic pathways.

11.3 Plant-Derived Drugs as Epigenetic Modulators

Epigenetic variations and alterations are the hallmark of cancer. Insights into the cancer epigenetics have revealed the deregulation of epigenetic machinery components like chromatin structure, histone modifications, DNA methylation, and noncoding RNAs. Plant-derived compounds have proven promising in targeting epigenetic processes and are presently at the front of drug discovery. Besides targeting the proteins and enzymes at the genetic and cellular levels, plant-derived anticancer drugs also function by bringing about the alterations in the epigenetic mechanisms. Some of the important plant-derived anticancer products that function as epigenetic modulators include polyphenols, alkaloids, organosulfur compounds, and terpenoids.

11.3.1 Polyphenols as Epigenetic Modulators

Polyphenols include a very important class of secondary metabolites with strong anticancer properties (Scalbert et al. 2005; Zhao et al. 2010a). Flavonoids, the most important and best characterized class of polyphenols, include the flavonols, flavones, flavanones, anthocyanidins, catechins, and isoflavonoids (Jamison 2003). A number of polyphenolics intercede their anticancer and antitumor activities through the modulation of the acetylation pattern of some of the vital genes as well as by the inhibition of hypermethylation of several essential tumor suppressor genes, a landmark in the development of cancer (Hauser and Jung 2008). Noteworthy among these are anacardic acid and its derivatives, curcumin and its derivatives, epigallocatechin-3-gallate (EGCG), genistein, and resveratrol. Anacardic acid derivatives are major HATs inhibitors used in a variety of cancers including kidney, cervical, breast, and prostate cancers as well as in myeloid and lymphoid leukemias. Curcumin (diferuloylmethane), a natural flavonoid derived from the *Curcuma longa* rhizome, alters the functions of HATs and HDACs thus restricting the uncontrolled cell growth epigenetically through modulation of histone acetylation (Fu and Kurzrock 2010). Promotion of the deacetylation activity or the inhibition of the transferase activity leads to the decrease in the acetylation of H3 and H4 histones in particular as well as other histone proteins (Prives and Manley 2001; Chen et al. 2007). EGCG, the principle bioactive polyphenol found in green tea, is known for its antitumor properties like antiproliferative, anti-angiogenic, and apoptotic properties (Shankar et al. 2007; Shankar et al. 2008). Its anticancer functions are endorsed to the epigenetic modulation through histone modification and methylation of DNA (Mittal et al. 2003). Genistein (4′,5,7-trihydroxyisoflavone) is the most copious isoflavone found in *Glycine max* which has been in use as a chemopreventive drug against several cancers (Barnes 1995). Genistein displays estrogen agonist as well as antagonist effects and is known to suppress oxidative stress and inhibit angiogenesis and certain protein tyrosine kinases (Li et al. 2009). Similar in function to EGCG, genistein is known to decrease methylation of promoter sequences of many tumor suppressor genes thus reactivating them. Resveratrol, a phytoalexin found abundantly in nuts and red grapes, is well recognized for its diverse health benefits in neuro- and cardioprotection and prevention of breast, lung, kidney, and skin cancers (Wang et al. 2008; Lin et al. 2010). Resveratrol also reduces HAT activity of many important promoter sequences and deacetylates NF-kB leading to reduced transcriptional activity in many cancer types (Bourguignon et al. 2009; Yeung et al. 2004).

It has been recently shown that plant extracts that are rich in polyphenols possess epigenetic activities in a number of types of cancers by bringing about the modulation of some important promoter sequences of decisive tumor genes through methylation (Fini et al. 2007). Moreover, modulation of TBP-interacting protein (TIP60), p300, and PCAF in these cancers is also brought about by the anacardic acid and its analogues (Balasubramanyam et al. 2003; Chandregowda et al. 2009). Different polyphenols target different genes during the epigenetic modulation. An extract of chlorogenic and caffeic acids, catechin, and epicatechin inhibits the expression of

two colon cancer genes (DNMT1 and DNMT3b) and reactivates the tumor suppressor genes by decreasing the methylation of important promoter sequences (*hMLH1*, *p14*, and *p16*) (Fini et al. 2007). Contrary to this, polyphenol extract (green tea rich) has been observed to decrease the methylation of CDX2 and p16 promoter sequences in the cells of primary gastric carcinoma as well as colon and gastric cancers (Yuasa et al. 2005, 2009). Green tea extract has further been found to be associated with the reduced promoter methylation of genes which are usually hyper-methylated in gastric cancers (Yuasa et al. 2009). On the other hand, polyphenol extracts (black tea) have been observed to inhibit HDAC1 expression, thereby modulating histone acetylation in the cells of hepatocarcinogenesis (Murugan et al. 2009).

11.3.2 Alkaloids as Epigenetic Modulators

Alkaloids, basic nitrogen-containing compounds, mostly comprise the well-recognized antitumor drugs, viz., the mitotic inhibitors, colchicines, vinblastine, and vincristine (Savel 1966). Procainamide is a non-nucleoside drug used against cardiac arrhythmias and shows potential anticancer (myeloid leukemia cells) activity through epigenetic modulation by affecting DNA methylation, specifically inhibiting the activity of DNMT1 in colon cancer cells (Lee et al. 2005). In addition, decreased methylation of RARβ, estrogen receptor (ER), and p16 promoter regions has also been endorsed to procainamide alkaloids which are proven effective against breast cancers (Segura-Pacheco et al. 2003). Mahanine, an alkaloid obtained from *Murraya koenigii* (curry leaf plant), not only reactivates the tumor suppressor gene (*RASSF1A*) which is epigenetically silenced during the cancer. In addition to the inhibition of the methylase activity, reactivation of *RASSFA1* has also been endorsed to the cell cycle arrest by specifically decreasing the expression of cyclin D1 in prostate cancer cells (Jagadeesh et al. 2007). Sanguinarine, a cell-permeable alkaloid purified from the roots of certain poppy and fumaria species like bloodroot or *Sanguinaria canadensis*, is a very effective cell growth inhibitor and inhibits the activity of NF-κB (Selvi et al. 2009; Chaturvedi et al. 1997). Unlike phenols and alkaloids like mahanine, sanguinarine has been observed to modulate histone expression other than DNA methylation. Further, it diffuses through cell membranes and has a strong affinity with the DNA. Its association with the core histones and chromatin and intercalation with the DNA consequently helps it in the alteration of the chromatin structure (Selvi et al. 2009).

11.3.3 Organosulfur Compounds as Epigenetic Modulators

Organosulfur compounds have been extensively applied in the avoidance of platelet aggregation as well as in cancer therapy (Moriarty et al. 2007). Sulforaphane (a derivative of glucoraphanin, extracted from the cruciferous vegetables like broccoli)

is a vital organosulfur compound with miscellaneous biological effects comprising apoptosis, cell cycle arrest, and induction of heme oxygenase and phase II detoxifying enzyme, viz., NAD(P)H:quinone reductase (NQO1). Upregulation of phase II metabolism by sulforaphane helps in removing the genotoxins of the body helping to prevent the cancer at the initial phase (Ho et al. 2009). The anticancer effects of sulforaphane also mediate through epigenetic mechanisms, and it is a well-recognized histone deacetylase (HDAC) inhibitor (Ho et al. 2009). Diminished activity of HDAC leads to the enhanced promoter-specific acetylation of H3 and H4 in the cancer cells of the colon, kidney, and prostate (Myzak et al. 2004, 2007). Sulforaphane has recently been observed to inhibit HDAC6 specifically leading to the acetylation and subsequent degradation of Hsp90, an androgenic receptor (AR). In the absence of this AR chaperone, its target gene (*E-twenty-six-related gene*, *ERG*) expression is inhibited (Gibbs et al. 2009). ERG, an important transcription factor, is observed to be overexpressed in prostate cancers (Tomlins et al. 2005), which leads to increased growth and invasiveness (Tomlins et al. 2008).

11.3.4 Terpenoids as Epigenetic Modulators

Terpenes, hydrocarbon derivatives of isoprene units, have proven promising against cancer and inflammation and are in clinical trials of cancer at present (Ghantous et al. 2010). Parthenolide, a sesquiterpene lactone, is commonly obtained from *Tanacetum parthenium* commonly called as European feverfew herb. It is presently one of the most promising drugs against cancer in clinical development that is known to target cancer stem cells selectively without any effect on the normal ones (Guzman and Jordan 2005). Lycopene acts as an epigenetic modulator and brings about the GSTP1 (tumor suppressor gene) promoter demethylation, thereby expressing it in the breast cancer. It also demethylates HIN-1 and RARβ2 tumor suppressors partially but does not induce their expression in the breast carcinomas as in GSTP1 (King-Batoon et al. 2008). Certain terpenoids mediate a range of anticancer activities concurrently by modulating an array of epigenetic mechanisms. Triptolide obtained from *Tripterygium wilfordii* and thymoquinone purified from *Nigella sativa* inhibit polycomb group of proteins through modulation of chromatin modifications. It also inhibits histone methyltransferases, by decreasing the level of transcripts of catalytic subunits of polycomb recessive proteins (Zhao et al. 2010a). Triptolide has been recently observed to decrease the expression of HDAC8 mRNA and protein in myeloma cells (Zhao et al. 2010b). On the contrary, it enhances the methylation of *MMP*-9 promoter leading to the silencing of its expression in sarcoma cells (Yang et al. 2009). Thymoquinone upregulates p73, a regulator of cell cycle checkpoint, and stimulates apoptosis of lymphoma cells through the caspase-dependent mechanism (Alhosin et al. 2010).

11.4 Treatment of Cancer Involving Cellular Mechanisms

An orchestra of events is involved in cellular division and DNA replication and a set of protein phosphorylation cascades and many checkpoints which oversee these two crucial events and eventually lead to the completion of the cell cycle. Deregulation of the cell cycle is one among the ten cancer transformation hallmarks. Targeting one of its vital modulators (cyclins, cyclin-dependent kinases, and tumor suppressor proteins) is a very critical step in cell cycle arrest which consequently induces apoptosis of the cancer cell (Giacinti and Giordano 2006; Foster et al. 2010). A plethora of reports claim the pharmacological potential of biologically active compounds derived from the plants, especially with mounting exploration of fresh, innovative, and vastly successful candidates in the anticancer therapy. Phytochemicals have been observed to be implicated with various inflammatory phenomena as well as oncogenic transformation in a number of mammalian cells and tissues including cell cycle control alteration, angiogenesis, metastases, and evasion of apoptosis (Surh 2003). These plant-derived anticancer drugs are synergistic in action with those of the other pharmaceutical chemopreventive drugs which facilitate them in overcoming the resistance development in the uncontrolled proliferative cells, thus enabling the utilization of very small amounts of the anticancer drugs with enlarged anticancer efficiency (Liu 2004). The action of a phytochemical may include complete blockade of the metabolic alteration of a pro-carcinogen or suppression of the malignant transformation of a just commenced preneoplastic cell. Modulation of the various cellular networks and signaling events implicated in proliferation, invasion, and metastases is another important aspect and worth mentioning (Aggarwal and Shishodia 2006). Insights into the fundamental pharmacokinetic mechanisms through which these plant-derived drugs evoke the anticancer response involve a number of molecular targets. This panel comprises protein kinases of different classes like protein kinases A and C (PKA, PKC), mitogen-activated protein kinase (MAPK), and tyrosine kinase (TYK2), caspases and Bax apoptotic proteins, anti-apoptotic proteins (TRAF1, bcl2, survivin), transcription factors (p53, Ap1, Nrf2, NF-κB), growth factors (EGF, FGF, TNF, PDGF), cell cycle proteins (CDK1, CDK2, cyclin D, p21, p27), and cell adhesion molecules (ICAM-1, VCAM).

11.4.1 Phenols as Cellular Mechanism Modulators

Phenolics like flavanones, isoflavones, and lignans check the binding of the ligands (estrogen) to the cancer cell receptors, thereby reducing their proliferation. Apigenin is the flavone found in parsley, celery, and chamomile. Certain flavanones like apigenin extracted from chamomile and celery-like plants induce cell death in adenocarcinoma cells of the lungs by targeting leptin/leptin receptor pathway. This apoptosis of human EGF receptor 2 (HER-2) is extrinsic and caspase dependent which leads to the overexpression of BT-474 in the cells of breast cancer. Signal

transducer and activator of transcription 3 (STAT3) signaling is inhibited in this overall process which leads to the caspase-dependent apoptosis (Seo et al. 2015). Curcumin, another important anticancer polyphenol obtained from the rhizomes of *Curcuma longa*, modulates a number of molecular components and halts the proliferation of the human glioblastoma cells. This modulation includes the upregulation of p21, p16, and p53; early growth response proteins (Egr-1); c-Jun N-terminal kinase (JNK); a member of ETS oncogenic family (ElK-1); extracellular signal-regulated kinase (Erk); caspase 3, 8, and 9 proteins; and Bcl-2-associated X protein (Bax) (Vallianou et al. 2015). It also downregulates the levels of cell division cycle protein 2 (cdc-2), NF-κB, cell division cycle protein 2 (cdc-2), mechanistic target of rapamycin (mTOR), p65, retinoblastoma protein (pRB), cyclin D1 proteins, B cell lymphoma protein (Bcl-xL), protein kinase B (Akt), cellular myelocytomatosis oncogenes (c-myc), etc. (Vallianou et al. 2015). Crocetin, a carotenoids obtained from *Crocus sativus* and *Gardenia jasminoides*, is very effective against cardiac hypertrophy. It has been observed to function through MEK-ERK1/2 pathway and GATA-binding protein 4 (Cai et al. 2009). Ellagic acid extracted from pomegranate restrains metastasis in many cancerous cells and stimulates apoptosis in cancer cells of the breast and prostate. Repression in the ornithine decarboxylase activity, enzyme signaling rapid proliferation of cells, and circumventing apoptosis by epigallocatechin gallate (EGCG) are an important cellular mechanism in a number of cancers. Obstruction of the epithelial mesenchymal transition is brought about by luteolin. Certain phenols (fisetin and hesperetin) alter an array of complex signaling networks like JAK-STAT, mTOR, MAPK, PI3K/Akt, NF-κB, and Wnt pathways leading to the cell cycle arrest in certain promyelocytic leukemias (Adan and Baran 2015). Genistein, an isoflavone obtained from soybean, inhibits the Akt and NF-κB signaling pathways (Li et al. 2012); gingerol inhibits the Erk1/2/JNK/AP-1 signaling and stimulates extrinsic caspase-dependent apoptosis of cancer cells (Radhakrishnan et al. 2014). Kaempferol acts via Erk1/2, Src, and Akt pathways and inhibits the growth as well as the migration of pancreatic cancer cells (Lee and Kim 2016). Cyanidin glycosides obtained from red berries execute anticancer and antioxidant functions through different mechanisms like suppression of the COX-2 expression in the cells of prostate carcinoma leading to their apoptosis and inhibition of matrix metallopeptidase-9 (MMP-9) expression in lung carcinoma cells (Singh et al. 2011).

11.4.2 Alkaloids as Cellular Mechanism Modulators

Alkaloids like vinca alkaloids and camptothecins have served as the well-reputed anticancer compounds (Darwiche et al. 2007). These compounds, mostly with ring structure containing basic nitrogen, provoke anticancer mechanisms mostly through arresting G2/M phase of the cell cycle and destabilize microtubule assembly leading to apoptosis, preventing polymerization of microtubules and topoisomerase I

inhibition (Darwiche et al. 2007).The mechanisms of action are different for different alkaloids. In order to understand the diversity in the mechanism of action of the alkaloids, a handful of them have been discussed as follows.

Vinca alkaloids (antimicrotubule agents), alkaloids obtained from periwinkle, have been observed to be sedative, hypotensive, and anticancerous. These (vincristine, vinblastine vinorelbine, vindesine) alter the mitotic spindle dynamics by irreversibly binding to the receptor sites on β-tubulin obstructing microtubules depolymerization. Microtubules are very dynamic polymers (α,β-tubulin heterodimers). Two binding sites for the vinca alkaloids per mole of tubulin dimer have been observed. Vincristine prevents the chromosome separation at the metaphase, and vinblastine is known to arrest the mitotic phase of cell cycle by inappropriate kinetochore and mitotic spindle formation leading to apoptosis (Jordan 2002). Vinca alkaloids have been in use for more than 40 years in the treatment of different cancers, lymphomas, leukemias, Kaposi's sarcoma (KS), and breast, lung, and testicular cancers (Balunas and Kinghorn 2005). Likewise, taxanes (paclitaxel) also act as microtubule disruptors leading to cell cycle arrest and apoptosis (Jordan and Wilson 2004; Khazir et al. 2014). Taxanes inhibit this microtubule disassembly by stabilizing this dynamicity and alter the conformation of these microtubules (Prota et al. 2013). Taxanes have also been found to decrease the binding of proteins associated with the microtubules. Although binding of microtubule-associated proteins (MAP) induces conformational rearrangements of the tubulin proteins that lead to an overall stabilization, binding of taxanes to the MAP-microtubule complex further stabilizes this complex (Xiao et al. 2012). Such disturbances prevent the normal mitotic spindle formation leading to cell cycle arrest avoiding cell proliferation (Ganguly et al. 2010; Priyadarshini and Aparajitha 2012).

Colchicine, one among the most important bioactive nitrogen-containing alkaloids, is isolated from Liliaceae members like *Colchicum autumnale* and *Gloriosa superba* and has been in use as antimitotic drugs, since long (Dias et al. 2012). The antitumor properties have been endorsed to its irreversible binding to tubulin similar to those of vinca alkaloids, leading to hindered microtubule formation and in turn inhibiting the progression of the cell cycle as well as inducing apoptosis. Some of the colchicinoid drugs act as vascular disrupting agents (VDA) causing swift disruption and shutdown of the tumor vasculature which leads to tumor starvation and disproportionate cell death (Atkinson et al. 2010). Berberine, an isoquinoline alkaloid with potent apoptotic properties in cancer cells, is however different in action in comparison with the abovementioned alkaloids and interferes at a number of tumor progression stages and metastasis. Molecular targets of this alkaloid are diverse. Some of the crucial and noteworthy targets include DNA topoisomerases, telomerase, p53, COX-2, NF-jB, metalloproteinases, etc. (Tillhon et al. 2012). Additionally, berberine-induced antitumor effects have been observed in several hematological cancers involving G1 or G2/M phases of cell cycle arrest, intrinsic as well as extrinsic caspase-dependent apoptosis, involvement of Bcl-2 family proteins, and ROS production (Sun et al. 2009).

11.4.3 Organosulfur Compounds as Cellular Mechanism Modulators

Organosulfur compounds are reputed to modulate the activity of a group of related antioxidant enzymes imperative in carcinogen detoxification, namely, glutathione S-transferases (GST) (Sparnins et al. 1986). NRF2 (nuclear factor erythroid 2-related factor 2), an important transcription factor, is the chief regulator of the expression of antioxidant molecules within the cell (Sporn and Liby 2012). NRF2 arouses anti-stress signaling to repress electrophilic or oxidative stress and halts carcinogenesis thus leading to a protective response (Lee and Surh 2005). The phase II carcinogen detoxification enzymes (glutathione transferases, NAD(P)H:quinone oxidoreductase I, UDP-glucuronosyltransferase, and heme oxygenase-1) which function via ARE-NRF2 pathway are induced by sulforaphane, thereby helping to eliminate a diverse collection of oxidative and electrophilic toxicants prior to their devastation to vital cellular macromolecules (Misiewicz et al. 2004). Sulforaphane, a type of isothiocyanates, arrests cell cycle in G2/M phase which has been observed to be associated to the distorted microtubule dynamics (Jackson and Singletary 2004; Azarenko et al. 2008). This disturbed microtubule polarization leads to aneuploidy which is followed by apoptosis (Azarenko et al. 2008). Altered cdc2 kinase activity, enhanced expression of cyclin B1 and p21, and histone H1 phosphorylation are the other mechanisms of cell cycle arrest induced by sulforaphane (Azarenko et al. 2008; Mi et al. 2008). Additionally, improper activation of cdc2 which leads to cell cycle halt at G2/M phase is believed to be the reason for the discernible mitotic catastrophe (Jackson and Singletary 2004). Confirmation of the cell death in MCF-7 cells by mitotic catastrophe after exposure to sulforaphane has been observed by abnormal mitosis and micro-nucleation (Jackson and Singletary 2004).

11.4.4 Terpenoids as Cellular Mechanism Modulators

Several stages of tumor progression (cell proliferation, cell death, angiogenesis, and metastasis) are inhibited by the application of terpenoids. These target various precise molecular mechanisms like blockade of the calcium ATPase pump of sarcoendoplasmic reticulum; inhibition of JAK-STAT, NF-κB, AP-1, MMPs, and DNA polymerases; and activation of p53 leading to the programmed cell death of the cancer cells. Specific and alternative mechanisms of cell death have been endorsed to these alkaloids noteworthy among which are triptolide, parthenolide, and betulinic acid.

Parthenolide, a sesquiterpene lactone originally extracted and purified from *Tanacetum parthenium*, has grabbed substantial attention in the discovery of cancer drugs (Ghantous et al. 2013). It induces intrinsic as well as extrinsic apoptosis in a vast array of tumor cells mainly by disrupting NF-κB; regulating MAPK, JNK, and Bcl-2 family members; activating p53 signaling; interfering with STAT3; and gen-

erating ROS (Kreuger et al. 2012). Triptolide, a diterpenoid isolated from *Tripterygium wilfordii* roots, is a potent modulator of the transcriptional machinery and has been observed to act together with RNA polymerase leading to the modulation of transcription of some of the key transcription factors (NF-κB, p53, HSF-1, and NF-AT) (McCallum et al. 2007). It was established very recently that triptolide inhibits overall transcription in cancer cell genes due to the proteasome-mediated degradation of RNA polymerase II large subunit (Wang et al. 2011). Furthermore, it induces apoptosis in a wide spectrum of tumors containing wild-type as well as mutant forms of p53 (Yang et al. 2003). Moreover, this diterpenoid has also been observed to inhibit the expression of p53 regulator (MDM2) and also the downregulation of the apoptosis inhibitor (XIAP) in a p53-independent manner. It also inhibits the heat shock protein (HSP70) which has been found to be upregulated in tumor formation (Yang et al. 2003; McCallum et al. 2007; Phillips et al. 2007).

Betulinic acid, a triterpenoid, induces cell death by affecting the intrinsic apoptotic pathway leading to the increased permeability of mitochondrial membrane resulting in the increased liberation of cytochrome c. The other routes of inducing apoptosis by this terpenoid include inhibition of NF-κB activity and the regulation of members of Bcl-2 family proteins. Additionally, betulinic acid is known to have anti-metastatic and anti-angiogenic activities (Gheorgheosu et al. 2014; Csuk 2014). Furthermore, betulinic acid treatment has been shown to induce apoptosis via both ROS-mediated mitochondrial pathway and the ER pathway in HeLa cells (Xu et al. 2014).

11.5 Clinical Trials of Plant-Derived Drugs

Though a good number of plant-derived anticancer drugs have been put to clinical trials and their synthetic analogues have been prepared after analyzing their efficacy, we are restricting our discussion to only a few important ones. Curcumin and its synthetic analogues have been found significantly effective in inhibiting growth factors that mediated corneal neovascularization and angiogenesis *in vivo* as well as *in vitro* (Arbiser et al. 1998). In addition to these in vitro antitumor effects, curcumin is known for its effectiveness against gastric and colon cancers in rodents (Ikezaki et al. 2001). The protective effect of curcumin is endorsed to its ability to restrain the proliferation of several tumors and modulation in the activity of certain angiogenesis-associated genes (Arbiser et al. 1998; Toi et al. 2001). Additionally, curcumin has been put to trials and found effective in inhibiting the growth of about 19 diverse strains of *H. pylori* (Mahady et al. 2002).

Vinca alkaloids (vinblastine, vincristine) extracted from periwinkle plant opened new doors of anticipation in the use of plant-derived components as anticancer drugs. The initial clinical trial was carried out to make the possible use of these alkaloids as anticancer agents (Cragg and Newman 2005). These two vinca alkaloids have been put to trial along with other chemotherapeutic medicines for fighting a variety of tumors and cancers including leukemias, advanced testicular cancer,

lymphomas, lung and breast cancers, and Kaposi's sarcoma (Cragg and Newman 2005). Similarly, paclitaxel extracted from *Taxus brevifolia* has revealed immense anticancer potential against advanced breast and ovarian cancer and lung cancer (Rowinsky et al. 1992). Camptothecin isolated from *Camptotheca acuminate* was also put to clinical trials; however, owing to a number of disputes on its severe bladder toxicity questioned its use as an anticancer drug (Potmeisel and Pinedo 1995). Its semisynthetic derivatives like irinotecan and topotecan have been exploited effectively in the treatment of ovarian and colorectal cancers as well as small-cell lung cancers (Creemers et al. 1996; Bertino 1997).

The chemoprotective effects of resveratrol have yet to be established in humans though these have been well standardized in animals. The shortcomings of resveratrol to establish its anticancer effect in humans are its meager availability and speedy metabolism limiting its occurrence in the bloodstream (Tome-Carneiro et al. 2013). Additionally, the actual metabolite(s) accountable for the resveratrol anticancer effects are still a mystery. In view of this, passionate research to enhance its bioavailability through encapsulating it alone or after combining it with standard chemotherapeutic drugs is being conducted (Mohan et al. 2014). In spite of these shortcomings, a good number of clinical trials are being conducted to analyze the effects of resveratrol on human health. Two inimitable alkaloids (schischkinnin and montamine) isolated from the seeds of *Centaurea schischkinii* and *Centaurea montana* when put to clinical trials showed remarkable anticancer potential in the cell lines of human colon cancer (Shoeb 2006). The exceptional structural features of these two alkaloids can be used as a template to a considerable level to develop drugs with improved anticancer activity.

11.6 Conclusions and Future Prospects

Presently the interpretation and understanding into the mechanism of cross talk between epigenetics and genetics are still illusive; however, pharmaco-epigenomics is continuously evolving and the approaches to target cancer cells using epigenetic mechanisms are becoming more comprehensive. Both types of alternative strategies (inhibition as well as activation) to counteract processes and enzymes (DNA methylation/demethylation; HATs/HDACs) are being followed. A perfect drug will alter histones like enhancing their degree of acetylation in the region of passive tumor suppressor genes of the chromatin to reactivate them. Such an ideal epi-drug will also lead to modification of the chromatin around the oncogenes to inhibit their activity. Equally, a drug of such kind can also encourage global DNA hypomethylation in tumor cells but will avoid such a change in normal cells. Further, such a drug will reduce promoter DNA methylation of tumor suppressor genes inactivated during the development of tumor to reactivate them by carrying out efficient promoter hypermethylation of the oncogenes leading to their reduced expression. Moreover, a number of medicinal plant products are being aimed at targeting several other genetic and cellular targets of destabilized and mutated cancer cells in

addition to the epigenetic targets. Since the use of phytoproducts as anticancer agents is very promising and is increasing swiftly, the appropriate scientific study with miscellaneous and biologically important chemical structures of phytochemicals as well as their thorough anticancer role along with the clinical aspects can prove to be fascinating subject of cancer research in the future.

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