Mohd Sayeed Akhtar
Mallappa Kumara Swamy *Editors*

Anticancer Plants: Mechanisms and Molecular Interactions

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This Book is dedicated to
First teacher and beloved parents
Without them it is quite impossible for us to reach up to this milestone.

When we speak up they have given sound to words,
When we analyze things they have given wisdom,
When we grew up they have given courage,
When we act they have given the good deeds.

Foreword

Cancer is regarded as a high-profile disease both in industrialised and less-industrialised countries. The report published by the WHO few years back shows that 7.6 million people have died from cancer-related diseases with the majority from low-income countries. UK Cancer Research also has reported that 14.1 million adults were diagnosed with cancer in 2012, and 8.2 million people passed away globally. In the Americas, this disease is the cause of 1 in 4 deaths. Recent estimates have revealed that the incidence of mortality and prevalence from major types of cancer on global basis lie around 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer. The projections are that by the end of 2030 there will be 26 million new cancer cases and 17 million cancer deaths. There are more than 200 different human cancer types, but they share similar characteristics such as insensitivity to signals which inhibit cell growth making their replication limitless. Cancer affects body systems, mostly starting due to gene changes that happen over a person's lifetime and rarely starting due to inherited faulty genes. In many cases these are cured, but some are not. Major cancer groups are brain tumours, carcinomas, leukaemias, lymphomas and sarcomas.

A constant battle is going on all around the world with a lot of development in cures and preventative therapies. As of today, the treatments include chemotherapy, radiotherapy and chemically derived drugs. These therapies in general put the patients under a lot of strain with further damage to their health. Latest focus is on using alternative treatments and therapies. The medicinal and aromatic plants have been used for thousands of years in folk medicines. The major compounds identified and extracted from plant taxa with anti-cancer properties include polyphenols, steroids, flavonoids, anthocyanins, flavones, flavonols, chalcones, taxols and several others. Many of the plant species are used currently to treat or prevent the development of cancer. The development of naturally derived compounds as anti-cancer agents is attracting much attention, in particular those derived from different plant taxa and their natural products. Anti-cancer drugs derived from plants are desired for cancer treatment as they are natural and readily available, and can be readily administered orally as part of patient's dietary intake. The plant extracts with a combination of anti-cancer compounds are able to show killing activity specific to

cancer cells. They show no effect on normal human lymphocytes and fibroblasts, and this makes plant extracts more desirable as anticancer therapeutic agents. However, a huge demand for medicinal plants is putting high pressure on the plant diversity, many of these plants are now cultivated for informal trade.

With a constant increase in demand, high-value medicinal plants are threatened by extinction if overexploitation continues. In Europe, China and India, some of these plant taxa are cultivated on a large scale to keep up with increasing demands. Attention is being drawn towards foods with medicinal properties like cruciferous vegetables and fruit berries. The use of herbal medicines offers a way to alleviate the crisis, and the main disadvantages in this connection are lack of international standardization. Raw by-products from industries also are now utilized to extract anticancer agents. The typical examples are grapes and 'grape seed extract'. Both are found as ingredients of food products due to their human health benefits. The grape stems also are a raw by-product of wine making with high organic load, which is acidic to the environment. But, its high polyphenolic content makes it advantageous for anti-cancer drug development. Grape stem extracts show antioxidant features, prevent DNA damage from reactive oxygen species and have anti-carcinogenic potential against many cancer cell lines.

This volume on *Anticancer Plants: Mechanisms and Molecular Interactions* by Dr. Mohd Sayeed Akhtar and Dr. Mallappa Kumara Swamy is a timely reviewed effort to fill this gap. It includes 14 chapters. Chapters 1 and 2 deal with the omics and miRNA and phytomolecules as anti-cancer therapeutics, respectively. Chapter 3 discusses the potential of herbal medicine in the colorectal carcinoma, while Chapter 4 describes the mechanisms of anti-cancer plants extracts against the tumour cells. Chapter 5 by the Malaysian researchers targets the protein phytocompounds in the drug development for breast cancer through computational approaches. Chapter 6 describes the unique anti-cancer compounds, andrographolide as an anticancer agent, while Chapter 7 focuses on the anti-cancer mechanisms of action of herbal medicines. Chapter 8 deals with the metabolomic study of anti-cancer phytochemicals, and Chapter 9 describes the applicability of CADD studies in the anticancer drug research. Chapter 10 explains the role of herbal mitocan in affecting the powerhouse of cancerous cells, and Chapter 11 focuses on phytoestrogens for the treatment of colon cancer. Chapter 12 focuses on genomics and post-genomics bioinformatics approaches of anti-cancer plants, and Chapter 13 describes the molecular innovations in anti-cancer compounds using the fruits of family, Rosaceae. Chapter 14 features the mechanism of action of anti-cancer herbal medicines. This book is written in the hope that it will prove to be highly beneficial to the academicians, researchers and students.

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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 time immemorial. Though several synthetic medicines are useful in treating cancer, they are still 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 acuminate*, *Andrographis paniculata*, *Crateva nurvala*, *Croton tonkinensis*, *Oplopanax horridus,* etc., are important sources 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, and 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 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, antitumorigenic properties, and suppression of carcinogen activities.

Anticancer Plants: Volume 4, Mechanisms and Molecular Interactions is timely reviewed in this direction. This volume contains 14 chapters from distinguished contributors across the world. This book deals with the elucidation of computational and molecular mechanisms involved in the disease diagnosis and cancer therapy, and also focuses on the bioinformatics challenges in designing and operating anticancer compounds or drugs. In conclusion, this book is a worthwhile source of

scientific verifications of plant compounds anticancer mechanisms against various cancers, and provides useful information for the students, teachers, and healthcare professionals involved in the drug discovery, clinical and therapeutic research.

We are highly grateful to all our contributors for readily accepting our invitation for not only sharing their knowledge and research, but also admirably integrating their expertise on scattered information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We greatly appreciate their commitment. We also thank Professor Munir Ozturk for his suggestions and writing the foreword for this volume. We also thank Springer Nature team and Dr. Mamta Kapila for their generous cooperation at every stage of the book production.

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About the Editors

Dr. Mohd Sayeed Akhtar is working as an Assistant Professor in Gandhi Faiz-e-Aam College, Shahjahanpur, Uttar Pradesh, India. He has received his Ph.D. degree from Aligarh Muslim University (AMU), India, in 2008. He has conducted his post-doctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and Chonbuk National University (CBNU), Republic of Korea (2011), respectively; and has also worked as Assistant Professor, Department of Biology, College of Natural Sciences, Jimma University, Jimma, Ethiopia (2011–2014), and Fellow Researcher UDQ9 at the Institute of Tropical Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia (2014–2015). Dr. Akhtar has 14 years of research and 8 years teaching experience in soil microbiology, applied microbiology, environmental microbiology, molecular biology, plant pathology, and plant nanobiotechnology. Dr. Akhtar has received several prestigious fellowships at national and international levels till date. His promising approach and dedication stands him in the row of foremost scientist in the field of plant-microbe interaction and plant nanobiotechnology. He is author and co-author of more than hundred articles in peer-reviewed journals, conference proceedings, and book chapters in the books published by Springer-Verlag, and also edited five books with international publishers. He is serving the scientific community as editorial board member and reviewer of several high-impact international journals. His current research is focused on the rhizospheric plant-microbe interactions and their molecular biotechnology, bioremediation, biomineralization, nanofertilizers, and nanobiotechnology.

Dr. Mallappa Kumara Swamy is presently a Postdoctoral Researcher at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. He has obtained his Ph.D. (Biotechnology) from Acharya Nagarjuna University, Guntur, India, in 2013. He has worked as Assistant Professor, Associate Professor and Head, Department of Biotechnology,

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Chapter 1
Omics: A Holistic Approach in Cancer Treatment

Madhumati Bora and Pratibha Parihar

Abstract Omic technologies advocate a holistic approach for the biomolecules aiming at the absolute detection in any biological sample. This high-dimensional biology when integrated with bioinformatics can target the high-throughput detection and sequencing of genes, RNA, proteins, metabolites, phytochemicals, and pharmacology or their combined use. Cancer is one of the most prevalent causes of death in many countries. According to the World Health Organization, 8.2 million people worldwide in 2012 died due to cancer, and by 2035 it is expected to increase to 24 million. Anticancer drugs used are highly expensive and develop resistance with their scrupulous side effects; thus there are unprecedented efforts to uncover new treatments. With the advent of chemotherapy, omic technologies established in recent years embrace a universal view of understanding the molecular system of biomolecules. Phytochemicals like camptothecin derivatives, vinblastine, vincristine, withanolides, withaferin A, topotecan, irinotecan, etoposide, and paclitaxel (taxol) are part of the armamentarium to treat cancer. Various approaches can be used in studying molecular markers to distinguish subtypes of disease and predict mutation that aid in cancer diagnosis and prognosis. The practice of high-end emerging omic technologies, including cDNA-AFLP, SAGE, cDNA microarray, oligonucleotide microarray, and micro-RNA, can be expanded from crop plants to medicinal plants, to expedite medicinal plant breeding and transform them into subsist industries of medicinal compounds. Therefore, the aim of this chapter is to focus on developing substitutes of these therapies to treat cancer with no pain and strain to patients.

Keywords Bioinformatics · Omics · Metabolome · Medicinal plants · Transcriptome

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1.1 Introduction

Cancer is an uncontrolled growth of cells having property of spreading to other body parts called metastasis. It is a multifaceted cellular disease caused by mutations (hereditary or somatic) or environmental factors (Cho 2010). Cancer is considered as a group of diseases, and a large number of factors can alter the normal growth of a cell, and hence, it is very difficult to predict a single treatment for it. There are many anticancer compounds used in the cancer therapy, but a major issue is the resistance of tumors to the current therapeutic agents. Therefore, efforts to discover new anticancer compounds with high sensitivity of cancer cells are extending. Several phytochemicals are also identified as anticancer compounds. Most of the cancers have angiogenesis potential; therefore investigations are being made to explore new compounds with antiangiogenic properties and higher sensitivity against cancer cells. Ups and downs in the attainment of biomedical research are mainly dependent on its success and failure in the cancer disease. The Human Genome Project paved the way to the scientific era of omics in understanding about the cancer disease in detail (Keusch 2006; Nicholson 2006; Finn 2007; Hamacher et al. 2008). "Omic" technologies not only involve the study of genomic, transcriptomic, proteomic, and metabolomic levels in a defined biological sample but their interaction with each other. This type of study involves the combination of modern tools and techniques (Horgan and Kenny 2011) to understand the cause and remedy of the disease. The Greek suffix "ome" or "omics" means gathering the information of DNA, RNA, protein, metabolites, metabolic processes, and physiological activities as a system. It also explains the vital networking between different units, for example, DNA (gene), RNA (transcript) and translated proteins, other biomolecules, and cells of an organism (Keusch 2006). For the systematic analysis of cancer genome, we require to gather data based on a sequence from a large number of tumors. Such information will help in studying new insight on how individual tumors develop and in designing new markers for the diagnosis with new curative approaches. Other emerging areas dealing with cancer are studying of genes and their transcripts with fragile site on chromosome leading to cancer (Geurts van Kessel 2010).

These omic technologies help in facilitating the rapid diagnosis and therapy of cancer. In-depth knowledge of cancer at the molecular level can be easily understood by omics which will help in its authentic diagnosis and therapy also. Holistic approach is usually applied in the milieu of therapeutic area. The idea behind this is to treat the patient as a whole with its mental, psychological, and social condition rather than his affected organ or system. Cancer-specific biomolecules are aimed in specific biological samples mainly for enabling the type of tumor and its diagnosis. Omic techniques have thus emerged as significant tools in the development of systems biology or high-dimensional biology (Mousumi et al. 2010).

With the omic technologies, different aspects of any system can be realized, understood, and analyzed better if studied as a single entity. Traditional studies are largely assumption based, whereas systems biology and omic experiments use

holistic approaches where no theories are known or approved but all data are acquired and analyzed to define or make a rule which can be verified further. A large number of medicinal plants known for their anticancer properties are used to treat cancer (Koehn and Carter 2005).These naturally occurring anticancer compounds are eco-friendly too than man-made compounds. Recent studies could explore the prospective of terrestrial plant extracts for the preparation of potential nanomaterial-based drugs for diseases including cancer (Sivaraj et al. 2014). The major groups of phytochemicals having anticancer activities are polyphenols, flavonoids, and brassinosteroids. These metabolites have shown anticancer properties through their antioxidant activity, inhibiting cancer cells, promoting cell apoptosis, and targeting the transformed cells and toxicity for cancer cells (Cao et al. 2013; Gupta et al. 2014; Kumar et al. 2014; Malíková et al. 2008). At present, cancer treatments include chemotherapy, radiotherapy, etc., but these therapies are putting strain on patients and their health. Therefore, the aim of this chapter is to focus on developing substitutes for these therapies to treat cancer with no pain and strain to patients.

1.2 Omic Technologies in Cancer

Understanding on the factors affecting human diseases like cancer and their effective treatments has taken an innovative move through the application of omic technologies. Various molecular properties like gene expression, alterations of the genome, epigenetics, and mutations, as well as their influence on the metastatic behavior and therapeutic responses have a most profound impact on the diagnosis and prognosis of cancer (Cho 2010). Omics thus has emerged as a magic in molecular biology research involving the study of genes, their transcript, gene product-protein, and its functional form metabolite. Thrill begins with the discovery of gene sequences and their product-proteins, and then it shifts dramatically to their vibrant roles and connections in a biological system. The wonder of "omic" technologies lies in its highly advanced technologies, which can simultaneously detect a large number of proteins/genes. The prime role of omic technologies is to analyze a particular biological sample for all its gene products (Mousumi et al. 2010). For an early detection of the cancer, "omic" technologies have paved a new path toward detecting the cancer through marker molecules and various indicator molecules linked with the growth and death and the metabolic status of the cell (Horgan and Kenny 2011). Through omics, it is possible to have a better interpretation on the complex biological processes which are associated with cancer and to design a more precise diagnostic tool to detect and treat cancers. A rapid progress in omic technologies can be witnessed in the drug discovery and their toxicity measurement (Gerhold et al. 2002; Kell 2006). In oncology pharmacogenomics, which is the joint adventure between genomics and pharmacology, is used to personalize and optimize the drug therapy (Evans and Relling 2004) so that the characteristic features of cancer drugs like general toxicity and their erratic efficiency can be circumvented (Watters and McLeod 2003). Genomics is the study of an organism's genome, the

complete DNA sequence of a nuclear DNA. The genome includes both coding and noncoding sequences. The functions of genome can be well explored by genomics. Transcriptomic means an absolute search of genes that are transcribed into RNA. Each cell is having a differential expression of genes in its developmental stages and under various physiological conditions. Therefore, molecular signatures based on the expression profiles can be defined and used to find normal cells or tissues into their correct category, while proteomics is the study of analyzing gene products (i.e., proteins) on a large scale. Proteomics have made a tremendous progress in the cancer research through (1) protein expression profiling of tumors, tumor fluids, and tumor cells; (2) protein microarrays; (3) mapping of cancer signaling pathways; (4) pharmacoproteomics; (5) biomarkers for diagnosis, staging, and monitoring of the disease and therapeutic response; and (6) the immune response to cancer (Kolch and Mischak 2005). In biomedical and drug development research, proteomics is becoming a major tool. Since proteins play a central role in the life of an organism, proteomics is instrumental in the discovery of biomarkers, such as markers that indicate a particular disease like cancer. Overall, omic technologies offer the simultaneous identification, analysis, and monitoring of the biological functions of genes, transcripts, proteins, and intermediary metabolites involved in a large number of key cellular pathways. These new markers assure component and safer pharmaceutical products for cancer screening and better choice of therapy (Mac Gregor 2004).

1.2.1 Genomics

Completion of the Human Genome Project in 2003 led to breakthrough advancement in the omic technology. Genomics has greatly advanced in the field of cancer research due to dropping in the price for DNA decoding and sequencing machines (Black et al. 2015). Genomic technologies are significantly involved in the detection of abnormalities and mutations in the chromosomes and associated genes which drive the growth and progression of different types of cancers. Further, such information enhanced our understanding of metabolism and genetics of cancer in developing and improving strategies to diagnose and treat the diseases which are personalized to patients' with a tumor. Such an approach is known as personalized cancer medicine (PCM) (Palma and Hanahan 2012). According to the National Cancer Institute, personalized medicine as a medication is associated with a person's genetic constituents to detect, treat, or prevent disease. Further such information can be used to develop drug for the cancer by (1) inhibiting enzymes and associated metabolic pathways that trigger the abnormal growth and development of cancer cells, (2) blocking the expression of a gene linked with cancer cells, and (3) obstructing molecular signaling pathways in cancerous cells. Garraway and Lander (2009) reviewed about international projects such as the Cancer Genome Project (CGP; http://www.sanger.ac.uk/science/groups/cancer-genome-project), the Cancer Genome Atlas Project (TCGA; http://cancergenome.nih.gov), and the

International Cancer Genome Consortium (ICGC; http://www.icgc.org) which provide powerful genomics platforms and tools for whole-genome expression analysis, identification of variation, and alteration in the genome specifically associated with cancer, chromosomal rearrangements, and aberrant methylation. Wieacker and Steinhard (2010) reviewed about the detection of chromosome aberrations and genetic disorder in the fetus of a pregnant woman by prenatal diagnosis and eschew amniocentesis.

Advancement in the next-generation sequencing (NGS) technology is proving stonework for an efficient and rapid detection of mutation/variants in the field of cancer genomics. Whole-genome sequencing (WGS) is the backbone for in-depth sequencing of cancer tissue with a high redundancy. Shyr and Liu (2013) discussed second- and third-generation technologies in the NGS platform. Recent NGS technologies include ion semiconductor method by Ion Torrent®, Oxford Nanopore Technologies® MinION, Qiagen GeneReader®, and 10x Genomics® technology. The latest launched Illumina NovaSeq® platform is a step ahead in the low-cost sequencing technology that can sequence a human genome in less than $1000 (Kamps et al. 2017). In spite of a reduced cost of sequencing, few drawbacks include short read length, complex sample preparation, lengthy and laborious process, and requirement of amplification and significant data storage and interpretation.

The genes associated with the tumor can be detected using targeted sequencing, whole-exome sequencing, and whole-genome sequencing which further assist in the identification of variation in gene expression, gene rearrangement, posttranscriptional modifications, single nucleotide polymorphism (SNP), inherited cancer syndrome, alternative spliceogenic variant transcripts, and small and long noncoding RNAs (Serrati et al. 2016). The major potential of whole-genome sequencing is the discovery of rearrangements of chromosomes and repetitive regions, transposition in the human genome, and somatic mutations in the noncoding regions. Targeted genome sequencing is the study of the specific subset of a protein-coding region of interest like the whole exome or the genes associated with the cancer. Exomes are the region in the genome encoded for the protein, which roughly constitute 1% in the whole genome (Ng et al. 2009). This strategy has reduced the cost per sample and enhanced the accuracy by targeting only the sequence region of interest. Whole-exome sequencing (WES) can be used for the detection of diseases with unknown causative genes (Bamshad et al. 2011; Singleton 2011). It confines to uneven capture efficiency across exons and sometime nonspecific hybridization. Beltran et al. (2015) reported the utility of WES in precision medicine and novel biomarkers associated with the tumor and statistically correlated the clinical response with the genetic and molecular data.

Kidder et al. (2011) reviewed ChIP (chromatin immunoprecipitation) sequencing as a powerful technique to interrogate gene regulation in many biological processes, specifically in a mutated state by combining ultrahigh-throughput parallel sequencing with ChIP. It allows *in vivo* mapping of interactions between DNA and protein at genomic level and evaluates the genome-wide DNA-binding site in response to the cancer state. It has higher accuracy. The limitation of current microarray ChIP design is that it requires knowledge of sequence of interest such as a

promoter, enhancer, or RNA-coding domain condition. Nevedomskaya et al. (2014) detected positive effect of aromatase inhibitors (AI) in curing estrogen receptor-positive breast cancers by ChIP sequencing.

Bisulfite sequencing is considered as a gold standard technology for identification of methylation in the DNA. An efficient approach to identify 5-methylcytosine with a single base-pair variation by bisulfite conversion of genomic DNA combined with NGS. It compares the level of expression of genes associated with regulatory activity between normal and tumor or within the tumor. It is widely used in personalized medicine. However, it fails to read the whole target region at a stretch, and further a high background noise limits its application (Li and Tollefsbol 2011). Legendre et al. (2015) reported 21 novel DNA hyper-methylation signatures in metastatic breast cancer patients, which is the indicator of high risk of recurrence. Upadhyay et al. (2015) reported NGS analysis of herb tulsi (*Ocimum tenuiflorum*) and genes involved in the production of apigenin, eugenol, luteolin, ursolic acid, and rosmarinic acid pathway showing anticancer properties. Genome sequence analysis of African medicinal plants showed that *Tulbaghia violacea* and *Cotyledon orbiculata* extracts have anticancer splicing activity on the BCLX and the AXL apoptosis genes. Grozav et al. (2015) *in vitro* tested and compared 16 hydrazinyl-thiazolo arene ruthenium complexes on tumor cell lines (HeLa, A2780, and A2780cisR) and on a normal cell line (HFL-1), modulated by p53 signaling pathway for their anticancerous activity. Further, gene expression profiling confirmed that association of hydrazinyl-thiazolo arene ruthenium complexes with cisplatin can induce apoptosis in ovarian cancer by preventing cisplatin resistance. Hao et al. (2017) stated the role of monoterpene thymoquinone and isoquinoline alkaloid berberine molecules in Ranunculaceae family with anticancerous properties. It arrests cell division, induces apoptosis of cancer cells, boosts the immune system, and reverts back the multidrug resistance in tumor cells. Further they specified omics platforms could depict differential expression of phytometabolites on the phenotypically heterogeneous tumor cells. Radiogenomics or imaging genomics is an emerging promising field in high-throughput comparison of imaging traits associated with gene expressions in human cancers. Radiogenomics can be useful in generating information linked with intratumor, intertumor and peritumor heterogeneity (Bai et al. 2016).

1.2.2 Transcriptomics

Transcriptomics or expression profiling is the study of level of expression of mRNAs in a given set of population under a specific condition. The characterization of gene expression can be done using microchip technology or transcriptome sequencing (RNA-seq). Fluorescence in situ hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR) regularly used in clinical practices are restricted to a single gene at a time. However, the foremost utility of microarray and transcriptome sequencing approaches is the proficiency in the identification of novel

genes as well as multiple gene rearrangements in a single experiment. Transcriptomics data of medicinal plants can be utilized to detect putative genes and metabolic pathways involved in different phytochemical production and associated transcription factors, response elements, and effector genes and their products (Cabral et al. 2011; Chen et al. 2011).

Gene expression profiling in the cancerous cell can be done by measurement and comparison of expression pattern of multiple genes under different environmental conditions as wild-type genes (control) versus tumor in order to measure their phenotypes, cancer stratification, and temporal evaluation (Maruyama et al. 2014). Moreover, it is also useful for analyzing large mammalian transcriptomes in the cancer drug development (e.g., drug sensitivity on cancerous cells) and clinical research that requires rapidly assessing specific genes in thousands of samples simultaneously (Kim et al. 2004). Lu et al. (2001) used the cDNA microarray method to estimate and compare gene expression profile from early to developmental phase of squamous cell carcinoma of the esophagus. Different stages like normal, dysplasia I (minor dysplasia), dysplasia II (temperate dysplasia), carcinoma in situ (CIS), and squamous cell carcinoma of the esophagus (SCC) were compared. The principal component analysis showed α-TNF (AA699697), keratin 6B (AA936779), and S100 calcium-binding protein A9 (AA864554) genes are involved in cancer development. OncoScan microarray is a recently developed strategy for the diagnosis of structural and somatic mutations, single-nucleotide polymorphisms (SNPs), copy number variations (CNV), and loss of heterozygosity (LOH) efficiently and successfully from formalin-fixed, paraffin-embedded (FFPE) tumor samples (Jung et al. 2017). Besides incredible utility, limitations of microarray technique include prerequisite knowledge of genome sequence and annotation, background noise during hybridization and scanning, and probe saturation interference with low-level and high-level detection.

Transcriptome sequencing (RNA-seq) technology enables genome-wide expression measurement of mRNA isoforms. Micro-RNAs (miRNAs) are a family of small noncoding RNA molecules around 21bp long and regulate a wide array of biological processes including carcinogenesis. These miRNAs have been found to be heavily deregulated in tumor cells (Rupaimoole et al. 2016). The miRNAs regulate the target gene expression specific to the cancer cells and suppress tumorigenesis by persuading apoptosis, arresting cell cycle, and suppressing metastasis or angiogenesis (Hong et al. 2015). Around 2500 human miRNAs have been detected which are associated with various posttranscriptional gene expression such as embryonic development, proliferation, differentiation and progression of cell, programmed cell death, autophagy, angiogenesis, and metabolism (Palmini et al. 2017). The earliest evidence indicating the role of miRNA in human cancer was given by Dr. Croce's group during his search for tumor suppressor genes at chromosome 13q14 region in B-cell chronic lymphocytic leukemia cells (Calin et al. 2002). Mao and Wang (2015) reviewed involvement of miRNAs in suppressing tumor or oncogenes depicting role of miRNAs as analytical, therapeutic, and prognostic markers of hepatocellular carcinoma (HCC). The complete genome-wide expression profile can be done by combining RNA-seq with high-resolution microarray quantitation.

Van Moerkercke et al. (2013) used RNA-seq method to developed metabolic pathway database, Catha Cyc, for *Catharanthus roseus* to detect key regulator(s) for metabolic pathway engineering. Further, Verma et al. (2014) sequenced transcriptome of *C. roseus* and annotated RNA-seq data and determined expression profile of genes associated with terpenoid indole alkaloids (TIA) biosynthesis pathway. They further concluded that TIA pathway genes were more active and upregulated in leaves and roots as compared to the flowers which are major source as anticancer. Gupta et al. (2015) generated leaf and root transcriptomes in *Withania somnifera* and detected genes encoding enzymes involved in intermediate steps of terpenoid backbone biosynthesis having anticancer properties.

Hong et al. (2015) reported 14 phytochemicals in Chinese medicinal plants that inhibit tumorigenesis and metastasis targeted by different miRNAs. Among all miRNAs, miR-2 was found to be extensively useful against various tumors. The miR-21 miRNA regulates PTEN and PDCD4 target genes with anticancer effect. Xie et al. (2016) reviewed different miRNAs in medicinal plant like miR159 which is found in both raw and cooked foods in broccoli and also detected in breast cancer tissues. Synthetic 2-O-methylated miR159 when targeted to 3′ UTR (untranslated region) of transcription factor 7 (TCF7) was found as a breast cancer cell proliferation suppressor. Yamazaki et al. (2013) conducted a deep transcriptome analysis in *Ophiorrhiza pumila* and identified *candidate* genes linked with the biosynthetic pathway for the monoterpenoid indole alkaloid, camptothecin. It is an anticancer alkaloid which inhibits DNA topoisomerase I activity. Winzer et al. (2012) analyzed transcriptomic data in the opium poppy (*Papaver somniferum*) capsule in the variety HM1 (high morphine 1) and revealed expression of ten genes encoding alkaloid. Mapping of F2 populations showed that these genes are tightly associated with morphine. He also found noscapine as an antitumor alkaloid that binds to tubulin, arrests metaphase, and induces cell death in active human cells.

1.2.3 Proteomics

Proteomics is the study of the entire proteins found in a biological fluid, an organelle, a cell, a tissue, an organ, a system, or the whole organism. Proteins play an essential role in various biological functions in living organisms. Therefore, detailed information on proteins will certainly benefit in the understanding of several diseases. Proteomics is a powerful technique to study mutations and its effect on physiological conditions, changes in response to external factors and adaptation (Lao et al. 2014). Thus, they are useful in the identification of mutated proteins as potential drug targets, mechanism of drug action, development of advanced methods for disease diagnosis, and remedies and potential treatment of the disease at an infant stage. Further, posttranslational modification may be useful in determining protein function and protein-protein interactions *in vitro* and *in vivo*. Protein profiles obtained from the cancer patients are being used to improve screening and early detection of cancer, identify novel targets for cancer therapy, reveal diagnostic

cancer biomarkers and protein signatures, and decipher the molecular mechanisms of cancer (Sallam 2015). The future goal of cancer proteomics is in personalized treatment. Koomen et al. (2008) elaborated the role of proteomics in diagnosis and prognosis of ovarian and lung cancers.

Proteomics also help in detecting the posttranslational modifications (PTMs) of protein such as phosphorylation, acetylation, lipidation, glycosylation, methionine oxidation, proteolysis, and ubiquitination and sequence variants in the medicinal plants (Mann and Jensen 2003; Zhang and Ge 2011). Khan et al. (2015) stated histones PTMs used as a potential marker for tumor detection and progression, for example, breast cancer, renal cell carcinoma, and pancreatic adenocarcinoma, reported loss of H3K4me2/me3, as a prognosticator of clinical outcomes. Yu et al. (2015) reported about PTMs like protein phosphorylation/dephosphorylation, ubiquitination, and sumoylation and their role in regulating abscisic acid-insensitive 5 (ABI5) which is a key regulatory molecule for abscisic acid (ABA) signaling pathway. In the medicinal plant, ABI5 is a basic leucine zipper (bZIP) transcription factor involved in controlling dormancy in seed, germination, time of flowering, and plant growth.

Protein expression profiles are progressively being used to determine, validate, and characterize biomarkers using mass spectrometry (MS)-based techniques such as matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) and electrospray ionization (ESI). In MALDI, the mass of the analyte is measured by time of the flight analyzer. MALDI, when combined with Fourier transform (FT-MS), increases sensitivity and accuracy. In cancer diagnosis, MALDI-TOF can be used to compare healthy (control) and cancerous tissues by biomarker discovery, tissue imaging, and quantifying biomarker levels which may prove to be clinically useful (Rodrigo et al. 2014).

An isotope-coded affinity tag (ICAT) is a high-throughput isotope labeling method that enables direct quantitative and qualitative comparisons of complex mixture of protein by mass spectrometer. The heavy isotope is added to mutated or diseased sample (e.g., cancer cells), whereas the lighter isotope is added to normal sample (healthy cells). Both the cells are pooled and subjected to proteolytic digestion using trypsin and/or Lys-C. The resulted peptides are evaluated in a mass spectrometer. Thus, a comprehensive outlook of protein present in cells or tissues in two different states can be assessed (Gygi et al. 1999). Dekker et al. (2005) developed the MALADI-TOF-MS-based method to study protein expression patterns in the cerebrospinal fluid. Samples from 106 breast cancer patients and 45 from the normal ones were taken and compared, and it was found that 164 peptides were differentially expressed. Petricoin et al. 2002 used surface-enhanced laser desorption and ionization (SELDI) approach in mass spectrometry to detect ovarian cancer. They developed a bioinformatics algorithm to distinguish neoplastic from non-neoplastic disease within the ovary in proteomic patterns in serum.

One-dimensional polyacrylamide gel electrophoresis and two-dimensional electrophoresis (2-DE) when coupled with tandem mass spectrometry-based isobaric tags are beneficial for the systematic quantitative and qualitative mapping of the whole proteome during disease conditions (Hussain and Huygens 2012). Several

compounds with anticancer properties (Al-Daghri et al. 2012) were identified, including gingerol, cedrene, zingerone, vanillin, and eugenol in the fenugreek extract using GC-MS analysis. Alsemari et al. (2014) further confirmed the usage of fenugreek as an autophagy inducer in human cells. Hew et al. (2013) examined protein profile of *Gynura procumbens* (Lour.) Merr. using LC/Q-TOF technique and found inhibition in the breast cancer cell line (MDA-MB-23) growth. They also detected proliferation markers, Ki67 protein and proliferating cell nuclear antigen (PCNA), invasion markers, and chemokine (C-C motif) ligand 2 (CCL2) in the protein fraction SN-F11/12-treated MDA-MB-231 cell lines which revealed the possible route of cytotoxic mechanism. Wang et al. (2015) reviewed that traditional Chinese medicines (TCM) are a good source of anticancer drugs. Proteomics analysis revealed secondary metabolites present in them like terpenoids, flavonoids, and glycosides act as tumor suppressor by specifically targeting the mitochondria in tumor cells. Yang et al. (2015) used stable isotope labeling by amino acid (SILAC) technique for comparative analysis of differential proteome expressions in bladder cancerous cells with the normal one. The different human cells were compared like HCV29 (normal bladder epithelia), KK47 (low-grade non-muscle-invasive bladder cancer, NMIBC), and YTS1 (metastatic bladder cancer) to study molecular mechanisms and cell signaling, labeled with three stable isotopes of arginine and lysine. Labeled proteins were evaluated by 2D ultrahigh-resolution liquid chromatography (linear trap quadrupole) LTQ Orbitrap MS. They found differentially regulatory proteins like COL6A2 and COL6A3 (collagen α3 (VI) chain) expressions reduced in urine in bladder cancer patients.

1.2.4 Metabolomics

Metabolomics are defined as "quantitative measurement of dynamic multi parametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Nicholson et al. 2002). Likewise, Oliver et al. (1998) defined them as the "study of the complete set of metabolites/low molecular intermediate, which are contexts dependent, varying according to the physiological, developmental or pathological state of cell, tissue and organ or organism." Metabolites can be defined as the end products of cellular metabolic processes, and their amount fluctuates in a biological system due to alterations in genetic or environmental factors (Simon-Manso et al. 2013). Their variations in structures make any analytical process more difficult (Fiehn 2002).

The total number of metabolites present in a particular cell, organ, or organism is called as metabolome, and the metabolic status of a living system is defined by metabolomics. Thus, omic technologies claim to give us metabolic profile (including carbohydrates, amino acids, lipids, organic acids, etc.) of any biological sample at a given time (Van Ravenzwaay et al. 2007). Metabolomics is the comprehensive

and unbiased systematic study of metabolites used in metabolic activities of an organism thus permitting the identification and quantification of each metabolite. In recent years, it has come out as an important objective of research for its ample uses in the field of drug discovery and drug development. Parallel with proteomic and functional genomic studies, metabolomic approaches are also driving with full force. Currently the study of metabolomics is targeted to understand different diseases common to human, to have a fast detection, and to develop novel and more reliable strategies for treatment. In addition, applications of metabolomic studies are also used in areas like toxicology and pharmacology, crop breeding, and plant biotechnology. In this chapter, we strongly support the benefits of metabolomics in cancer. In cancer chemotherapy various phytochemicals and their derivatives, for example, vincristine, vinblastine, taxol, camptothecins, etc., are well-established anticancer metabolites. The potential of these plant-derived natural products used in traditional Chinese medicine has been documented by the scientific community in the Western countries also. In agriculture, metabolomics is used to develop various herbicides and pesticides. The quality of food can be monitored by metabolomics, for example, in food processing and quality control or in plant breeding for improved crop varieties and in the development of novel foodstuffs. The information made available through metabolomics on various cancers would be a powerful move toward its diagnosis, prognosis, and therapy. With rapidly progressing technology, new methods will come up for better and faster analysis of a large number of human samples simultaneously. Metabolomics is aimed more on the identification of potential biomarkers for cancer (Oskouie and Taheri 2015) and other life-threatening diseases.

An important application of metabolomics is in the diagnosis of human diseases in particular cancer, because cancer cells are highly proliferative and even in presence of oxygen tend to generate energy through aerobic glycolysis also known as the Warburg effect (Kasture et al. 2012). Cancerous cells have a different need of metabolites as compared to noncancerous cells; thus they do not perform many important functions of a normal cell (Serkova and Glunde 2009). At present, different metabolites have been identified and proposed that would serve as markers for several tumor processes and other diseases. Despite the fact that various DNA markers and protein markers are used commonly in the diagnosis of cancer like AFP for liver cancer, BCR-ABL for chronic myeloid leukemia, BRCA1/BRCA2 for breast/ovarian cancer, BRAFV 600E for melanoma/colorectal cancer, CA-125 for ovarian cancer, CA19.9 for pancreatic cancer, CEA for colorectal cancer, EGFR (non-small-cell lung carcinoma), HER-2 (breast cancer), PSA (prostate-specific antigen) for prostate cancer, and S100 for melanoma, there are a lot of disparities in translating biomarker research into the clinical use. With the help of these validated biomarkers, we may satisfy some frequently raised queries of the patient after diagnosis of his cancer regarding its type, its optimal dose of drug, its recurrence, etc.

1.2.5 Phytochemomics

In spite of tremendous progress in modern medicine, death due to cancer is alarming in the whole world. The need of the hour is to have a safer and more effective chemotherapy for cancer patients. It would be better if we go with the node "prevention is better than cure" as cancer is largely a preventable disease and can be avoided to a greater extent by changing lifestyle. Foods or nutraceuticals constitute varied concentration of several bioactive phytochemicals. These phytochemicals have always been used for the treatment of cancer for they are safe, less toxic, and easily available. Studies based on different mass of population suggest that people consuming more vegetables and fruits are always at lower risk of getting cancer. Promising phytochemicals not only disrupt aberrant signaling pathways leading to cancer but also synergize with chemotherapy and radiotherapy (Pratheeshkumar et al. 2015). Thus, the cancer chemoprevention and therapeutic potential of naturally occurring plant products need more attention. Phytochemicals are a big group of chemicals which have fascinated many. Some protect against cancer when isolated, but others are not related with cancer at all. It is well established that many phytochemicals in their natural food forms are protective in cancer by their interaction with other phytochemicals and the cells in our bodies. Thus phytochemicals can be used to develop treatment methods for various diseases. Several medicinal plants are reported for their anticancer effects (Sharma et al. 2011; Teiten et al. 2013). These phytochemicals are able to decrease cell proliferation, induce apoptosis, retard metastasis, and inhibit angiogenesis (Hajzadeh et al. 2006; Tavakkol-Afshari et al. 2006; Mortazavian et al. 2012; Mortazavian and Ghorbani 2012; Sadeghnia et al. 2014; Shu et al. 2010). In addition some of these natural compounds are widely used in chemotherapy of cancerous patients. Various antitumor compounds like taxol from *Taxus*, vincristine and vinblastine from *Catharanthus roseus*, and podophyllotoxin from *Podophyllum* plants are having an important role in the treatment of cancer patients (Saklani and Kutty 2008). Through this chapter, we wish to recommend the use of phytochemicals or nutraceuticals in controlling and curing of cancer.

In the near future, the chances of getting cancer will be increasing in the aged people. Plant-derived various chemicals, which are nonnutritive, will find a promising position as modulators of main cellular signaling pathways having confirmed anticancer effects. Therefore with a better understanding of the molecular basis of phytochemicals on human health, new personalized drugs of specific phytochemicals for each clinical situation can be developed. Materials used for connoisseur food consumptions are considered to be the excellent sources of bioactive compounds with anticancer activities. These include curcumin, resveratrol, sulforaphane, isothiocyanates, silymarin, diallyl sulfide, lycopene, rosmarinic acid, apigenin, gingerol, etc. (Wang et al. 2012). Bioactive constituents from plants are a fascinating yet mysterious group of chemicals protecting against cancer and other diseases. It is clear that these phytochemicals, in their natural food forms, can protect us from cancer. Various medicinal plants like green tea, *Allium sativum*, *Aloe*

vera, *Punica granatum, Withania somnifera*, and plant metabolites like camptothecin, curcumin, resveratrol, and combretastatins are reported for their efficacy in treating or reducing some symptoms of cancer (Hosseini and Ghorbani 2015). Preventive measures of health point out the role of diets rich in fruits and vegetables against cancer on the basis of their bioactive metabolites with antitumor activity. This big group of compounds called "phytochemicals" impart flavor and color to edible plants and the beverages derived from them. The underlying mechanisms include the detoxification and enhanced excretion of carcinogens, the suppression of inflammatory processes such as cyclooxygenase-2 expression, inhibition of mitosis, and the induction of apoptosis at various stages in the progression and promotion of cancer (Geurts van Kessel 2010). Phytochemomics is an interdisciplinary concept of food, medicine, and cosmetic sciences intending to increase the data on bioactivity of phytochemicals. These achievements are based on modern analytical tool like mass spectrometric approaches. Phytochemomics together with other omics is essential for authorizing or rejecting nutrition and health claims made on foods. On the basis of the data collected by using omic approaches, a cause-effect relationship may be established between a food category, a food or one of its constituents, and the claimed effect.

1.2.6 Pharmacogenomics

Pharmacogenomics deals with the genetic testing that predicts the relationship between the variation in the genome and its effect on clinical drug response (Monte et al. 2012). Pharmacogenomics refers individual genetic constituent to select the drug type and dosage which suits it best. It evaluates the relationship between drug efficacy and toxicity in drug transporters, metabolizing enzymes, protein receptors, and drug targets (Crews et al. 2012). Variation in a sequence may affect its product which ultimately affects phenotypes. Genetic researchers use several types of studies to establish and explore gene-phenotype relationships. Dean (2015) reported irinotecan (Camptosar) as a type of chemotherapy commonly used for the treatment of colorectal cancer. UGT1A1 gene encoding for enzymes called UDP-glucuronosyltransferases is responsible for metabolizing irinotecan. The synthesis of UGT1A1 depends on the genetic makeup of the person. Lower production of UGT1A1 results in higher levels of irinotecan deposition in the body. In such a case, if high doses of drugs are given to the patient, it may cause severe and potentially life-threatening side effects. So, based on the pharmacogenomics of the patient above, a test is done for prescribing drug doses. Acute lymphoblastic leukemia in children can also be cured by pharmacogenomic testing. Thiopurine methyltransferase (TPMT) is responsible for metabolizing chemotherapy. To avoid severe side effects, children with lower TPMT levels receive lower doses (Cheok et al. 2009). Fluorouracil (5-FU) is also a type of chemotherapy. It is used to treat several types of cancer, including colorectal, breast, stomach, and pancreatic cancers.

Seo et al. (2016) concluded in his study that scopoletin which is a coumarin compound present in many *Artemisia* species might serve as a probable lead compound for drug development against cancer. It also showed positive response for tumor cells having ABC transporter expression. The nuclear factor (NF-κB) pathway activation may be considered as a resistance factor for this compound. Computational analysis using molecular docking software showed interaction of scopoletin with NF-κB and its regulator IκB. Efferth et al. (2007) reviewed on cancer therapy based on pharmacogenomics of traditional Japanese herbal medicine (Kampo). Kampo medicine may be used as synthetic or semisynthetic derivatives of natural products to develop novel drugs against cancer. In order to exploit its utility and minimalize its side effects, Kampo medicines can be used as chemotherapeutic agents and appropriately selected for an individual patient. Marsh and Hoskins (2010) reviewed irinotecan as an analog of camptothecin used as an anticancer drug and associated with toxicities which are potentially life-threatening. Variation in gene (UGT1A1) expression and related promoter (UGT1A1*28) polymorphism is strongly linked with the toxicity, although this association is dependent on dose. The mutation in other genes like transporter ABCC2, XRCC1, and TDP1 also plays an important role in both toxicity and response. Kadioglu and Efferth (2015) reported anticancerous property of *Salvia officinalis*. Molecular docking study showed that ursolic acid and pomolic acid are two constituents of *S. officinalis* that bind to key molecules of the NF-κB pathway (NF-κB, I-κB, NEMO). The classical drug-resistance mechanisms (oncogenes, ABC transporters, and tumor suppressors) involving cross resistance can play a promising role in both plant acids for cancer chemotherapy.

1.3 Bioinformatics Approaches in Detecting Cancer

Bioinformatics comprises of advanced computing, statistics, mathematics, and different technological platforms to physically store, manage, analyze, understand, and transfer the data. The present revolution in clinical science and medicine is the result of sequencing of the human genome along with model organisms which accelerated the growth of technologies associated with proteomics, transcriptomics, and functional genomics. Cancer bioinformatics is a tool or an approach to carry out an investigation in systems clinical medicine, disease-specific biomarkers with personalized medicine, altogether pugnacious cancer and thereby enhancing the quality of life of the patients affected with cancer. Bioinformatics tools are used in the detection and authentication of biomarkers, especially in early diagnosis of disease, to observe the advancement of the disease and the answer to treatment, and predictors to improve the quality of life of patient (Wu et al. 2012). Clinical bioinformatics, an emerging technique which includes clinical informatics, bioinformatics, medical informatics, information technology, mathematics, biostatistics, biochemistry, and omics science together (Wang and Liotta 2011), plays an important role in addressing clinically related challenges in early detection of any disease. Network

biology is an in silico approach which embraces intracellular interactions, protein annotations, and signaling pathway in the cellular system (Kreeger and Lauffenburger 2010). The recent advancements in network and system biology are being utilized in the discovery, validation, and optimization of cancer-related biomarkers (Guo and Wan 2014).

The National Cancer Informatics Program (NCIP), which is a part of NCI's Center for Biomedical Informatics and Information Technology (CBIIT), regulates all tumor bioinformatics research activities. NCIP is associated with genomics, clinical and translational studies, and data retrieving, sharing, investigation, and visualization. The TARGET program developed by NCIP generated data for genetic alterations in pediatric cancers. The NCI Genomic Data Commons (GDC) provides a single source for cancer research projects and the tools for mining and generating data. Recently developed, Cancer Genomics Cloud Pilots can be useful to explore innovative methods for accessing, sharing, and analyzing molecular data. The Cancer Biomedical Informatics Grid (caBIG™) initiated and funded by the US National Cancer intended to link cancer research centers and other premier institutions in a network, or grid, in order to store, retrieve, share, transfer, and evaluate biomedical data and develop novel approaches for identifying, treating, and preventing cancer. It embraced more than 800 people from over 80 organizations linked with more than 70 projects ranging from management of clinical trials to tools for analysis of gene expression data. The list of various cancer databases and their links is given in Table 1.1.

The list of softwares developed by caBIG™ are (1) caTissue core (collect and track the cancer samples), (2) caTissue CAE (annotate cancer samples with molecular and clinical data), and (3) caTIES (extract of structured data from free-text pathology reports). Precision medicine integrated with cancer bioinformatics provides the safe, efficient, and most effective therapeutic approach to apprehend the genetic characteristics of each patient and his/her tumor (Garraway et al. 2013). It integrates three types of molecular data including mRNA gene expression (GE), DNA copy number variation (CNV), and DNA exome sequencing (mutation). Modern personalized medicine considered an individual's genetic profile and disease history for the treatment, while traditional personalized medicine completely relied on a patient's family history, social circumstances, environment, and lifestyle. Besides genomic information, proteomic data also supports personalized medicine. By comparing protein profile of a healthy person with the tumor patient, all the genes associated with the cancer can be characterized. The Human Proteome Project (HPP) has developed a molecular map of protein-based foundation of the human biology at the cellular level and become a resource to initiate the development of diagnostic, predictive, therapeutic, and precautionary medical applications. Clinical DNA sequencing can be useful in detecting targeted multiple-gene panels for genetic or somatic mutations associated with the tumor. It can also be useful for the medical practitioners in the selection of therapies or medications against a particular tumor. Tumor sequencing can also identify germline mutations associated with a hereditary cancer syndrome. Overall, cancer bioinformatics is an emerging strategy and advanced approach in clinical research for improved diagnosis of tumor and has

Table 1.1 List of various cancer databases

Name	Database type and details	Website	References
ITTACA	Both microarray gene expression and clinical data of tumors	http://bioinfo.curie.fr/ittaca/	Elfilali et al. (2006)
HPTAA	Potential tumor-associated antigens	http://www.bioinfo.org.cn/hptaa/	Wang et al. (2006)
Ethy cancer	Database of human DNA methylation and cancer	http://methycancer.psych.ac.cn	He et al. (2008)
CT database	Cancer-testis antigen database	http://www.cta.lncc.br/	Almeida et al. (2009)
CCDB	Cervical cancer gene database	http://crdd.osdd.net/raghava/ccdb/faq.php	Agarwal et al. (2011)
OrCGDB	Oral cancer gene database	http://www.tumor-gene.org/Oral/oral.html	Gadewal and Zingde (2011)
DDPC	Dragon database of genes associated with prostate cancer	www.cbrc.kaust.edu.sa/ddpc/	Maqungo et al. (2011)
COLT-cancer	Functional genetic screening resource for essential genes in human cancer cell lines	http://colt.ccbr.utoronto.ca/cancer	Koh et al. (2012)
SomamiR	Somatic mutations altering micro-RNA-ce RNA interactions	http://compbio.uthsc.edu/SomamiR/	Bhattacharya et al. (2013)
Cancer DR	Cancer drug resistance database	http://crdd.osdd.net/raghava/cancerdr/	Kumar et al. (2013)
canSAR	Public integrative cancer-focused knowledgebase	https://cansar.icr.ac.uk	Bulusu et al. (2014)
Intogen	Integrative oncogenomics	https://www.intogen.org/search	Rubio-Perez et al. (2015)
G-DOC	The Georgetown database of cancer: a precision medicine platform	https://gdoc.georgetown.edu/gdoc/	Bhuvaneshwar et al. (2016)
Colorectal cancer atlas	Genomic and proteomic pertaining to colorectal cancer cell lines and tissues	http://www.colonatlas.org	Chisanga et al. (2016)
DriverDBv2	Database for human cancer driver gene annotation research	http://driverdb.tms.cmu.edu.tw/driverdbv2/	Chung et al. (2016)
TS gene	Tumor suppressor gene database	https://bioinfo.uth.edu/TSGene1.0/	Zhao et al. (2016)
COSMIC	Catalogue of somatic mutations in cancer	http://cancer.sanger.ac.uk/cosmic	Forbes et al. (2017)

become an aid for cancer research to solve the mysteries behind cancer progression and drive an impact on the expansion of effective targeted therapies.

1.4 Markers Used in Detecting the Anticancer Property of Medicinal Plants

A biomarker can be a biomolecule secreted by a cancerous cell or a specific response of the body or an indicator to reveal the presence. Biomarker can be defined as a cellular, biochemical, and/or molecular (including genetic and epigenetic) characteristic that can be measured and evaluated as a pointer of normal or abnormal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Mikeska and Craig 2014). Biomarkers may be proteins (e.g., hormone, receptor or an enzyme), phytochemicals, nucleic acids (e.g., noncoding RNA like miRNA), antibodies, small metabolites, sugar and oligopeptides, or entire tumor cell. A biomarker can also be a collection of alterations, such as gene expression, proteomic, and metabolomic signatures. Thus a cancer biomarker is a "biological molecule produced by cancer affected cells, used to measure and evaluate as an indicator of tumorous processes within the body" (Fuzery et al. 2013). Cancer biomarkers can be identified in the circulation (whole blood cell, serum, or plasma) or excretions or secretions (stool, saliva, urine, sputum, or nipple discharge) or derived from tissue, evaluated by either biopsy or special imaging (Kulasingam and Diamandis 2008). The approaches used in the detection of biomarkers linked with tumor-associated genes are gene microarrays, next-generation sequencing, and mass spectrometry.

Biomarkers are detected by comparing the relative levels of mRNA for thousands of genes associated with normal and cancerous tissues, which are differentially expressed in malignant tissues and classified as prostate, lymphoma, leukemia, lung, oral, liver, and breast tumors. Once a group of genes detected associated with a specific cancer, the challenge is to translate this information into a robust assay for further clinical analysis. Biomarkers in cancer research can be used in three primary ways: (1) diagnostic, detection and diagnosis of early stage of cancers; (2) prognostic, to forecast sternness of disease; and (3) predictive, to predict response of patient to the treatment. Recently, Wei et al. (2016) compared around 1500 differentially methylated regions in colorectal cancer with normal tissue. Differentially methylated genes (ADD2 and AKR1B1) were mapped as potential screening markers for colorectal cancer. Popp et al. (2016) reported that expression of genes p53 and p21 in bowel mucosa is linked with inflammatory-related tumorigenesis present in ulcerative colitis which slightly increases the risk of colorectal cancer in patients. Thus p53 and p21 can be the most valuable tissue biomarkers for ulcerative colitis. Cretoiu et al. (2016) reviewed the prospective of circulating miRNAs as biomarkers for the implantation period to preeclampsia and their association in pathological processes like recurrent abortion and ectopic pregnancy. Further microarrays and

Table 1.2 List of biomarkers available for different cancers

Biomarkers	Type	Cancer type	Up/down regulation	References
PSA	Protein	Prostate	Up	Stamey et al. (1987)
CA-125	Protein	Ovary	Up	Devine et al. (1992)
Her-2/neu	Protein	Breast	Up	Ross and Fletcher (1998)
EGFR	Protein	Lung	–	Paez et al. (2004)
Lysophosphatidic acid	Metabolic	Detection, diagnosis, and prognosis (ovary)	–	Sutphen et al. (2004)
miR-15b, 16, 107, 223, 342, let-7c	miRNA	Acute promyelocytic leukemia	Down	Careccia et al. (2009)
PAM50	miRNA	Breast cancer	–	Parker et al. (2009)
miR-21, miR-29a, miR-92, miR-93	miRNA	Ovarian	Up	Resnick et al. (2009)
miR-200a	miRNA	Oral cancer	Down	Zeng et al. (2009)
miR-195 let-7a, miR-155	miRNA	Breast cancer	Up	Heneghan et al. (2010)
miR-21	miRNA	Pancreatic	Down	Hwang et al. (2010)
SNORD33, SNORD66, SNORD76	snoRNA	Non-small-cell lung cancer	Up	Liao et al. (2010)
let-7f miR-20b, miR-30e-3p	miRNA	Lung	Down	Silva et al. (2010)
piR-651	piwiRNA	Gastric cancer	Down	Cui et al. (2011)
miR-10b	miRNA	Glioblastoma and brain metastasis	Up	Teplyuk et al. (2012)
HULC	lncRNA	Pancreatic cancer	Up	Peng et al. (2014)
PCA3 p	lnc RNA	Prostate cancer	Up	Pellegrini et al. (2015)
hTERT	mRNA	Prostate cancer	Up	Quinn et al. (2015)
miR-18a	miRNA	Liver cancer	Up	Sohn et al. (2015)
piR-651	piwiRNA	Lymphoma	Down	Cordeiro et al. (2016)

real-time quantitative reverse transcription polymerase chain reaction confirmed that miR-320b, miR-146b-5p, miR-221-3p, and miR-559 were upregulated, while miR-101-3p was downregulated and can be considered as biomarkers for recurrent abortion and ectopic pregnancy. A list of various cancer biomarkers and their regulation in the gene is given in Table 1.2. Advancement in high-throughput sequencing

technologies has enabled the identification of protein-coding RNAs (i.e., mRNAs) and different types of noncoding RNAs (e.g., small nucleolar RNA, small nuclear RNA, micro-RNA, etc.) in human at transcriptome level.

1.5 Conclusions and Future Prospects

Cancer is a disease of tissue growth regulation. A normal cell can be transformed into a cancer cell when the genes that regulate cell growth and differentiation are altered. It is noticeable that some of the most innovative and intensive research work is being focused around genomics and related fields such as proteomics to explore new ways to understand susceptibility to cancer and therefore new targets for treatment. "Omic" technologies have a wide range of applications like detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) in any biological sample. If our knowledge of molecular biology, engineering, and bioinformatics is combined together, then understanding of the human diseases will gain a better place in the success of a cancer research as well as the potential treatment responses. Omic technologies are moving at a very high speed toward a goal. However, it has a very strong strike on diagnosis, prognosis, and therapy of cancer. A more customized approach would be to combine the data of genomic, proteomic, metabolomic, and pharmacogenomic with bioinformatics to explore pathophysiology and to illustrate more accurately an individual's risk for disease, as well as response to interventions. The most challenging constraint is to ensure that massive data accumulated through various omics is translated into clinically useful information and made accessible to physicians and patients both.

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Chapter 2
Plant miRNAs and Phytomolecules As Anticancer Therapeutics

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Abstract In Indian health-care system, plants are used as a source of medicine to cure various ailments and also provide high quality of food and raw materials for human beings. In due course of time, gradually the expertise developed in selective uses of different plants and their secondary metabolites in treating certain disease conditions. Many such plant parts are now used as alternative medicines for treating diverse forms of diseases including cancer. Research is going on to identify active component/phytomolecules present in plant extracts to cure certain ailments and to be used as therapeutics. In this chapter, we are emphasizing on the role of different plants and their phytomolecules in the treatment of cancer with a detailed overview, and the specific plant parts are discussed in the later part of this chapter. As a second line of thought, authors believe that one of the major genetic components, i.e., plant microRNA, has been overlooked since years and may prove to play a major role as a therapeutic molecule. MicroRNAs are attributed to control gene expression at a very fine level both transcriptionally and posttranscriptionally. Studies have indicated that aberrant expression of several genes leads to cancer and damages normal cellular processes related to many human diseases. Plant miRNAs may play a major role in regulating such gene expression, thereby impacting the development of physiology and development of the human body. Interestingly, many reports are suggesting the possible cross-kingdom regulation of mammalian gene expression by plant-derived microRNAs. The possibility that food-derived miRNA can inhibit cancer growth in mammals is appealing as plant-derived microRNAs are reported to pass through the gastrointestinal tract and are found in human serum regulating the expression of endogenous mRNA. The present chapter highlights the plants and their derived phytomolecules having anticancer properties and also explored the potential of miRNA as a new therapeutic in the field of cancer biology.

Keywords Cross-kingdom · Phytomolecules · miRNA · RISC · UTR

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2.1 Introduction

Cancer is a major life-threatening disease and is the second leading cause of death worldwide. Cancer is an uncontrolled growth and proliferation of abnormal cells in organisms that can lead to death. Cancer affects any part of the body at any age group of peoples. More than 100 types of cancer are reported, including breast cancer, colon cancer, lung cancer, skin cancer, prostate cancer, and lymphoma. There are many factors that are responsible for cancer-like genetic and environmental factors (Pandey and Sharma 2006). Every year lots of people are diagnosed with cancer, and annually it kills about 3500 million populations around the world (Prakash et al. 2013). A number of treatments are available to cure the cancer including chemotherapy, radiotherapy, and chemically derived drugs. These types of therapies produce side effects and other health problems. Therefore, there is an urgent need to develop alternative treatments with least side effects. Plant molecules are gaining much interest for their use as therapeutic agents because of their least side effects. India has a rich repository of the wide variety of medicinal plants and is called the "botanical garden of the world" (Mahima et al. 2012). These medicinal plants having therapeutic properties cure a range of diseases and also provide high nutraceutical value to world population. It has been reported that about 70–80% of world population rely on natural medicines to combat their primary health-care needs due to their safer mode of action and least side effects (Akhtar et al. 2014a, b; Swamy et al. 2016). Plants and plant-derived molecule have medicinal properties, and they have been used to cure human diseases. In the current scenario, plant-derived natural products have the ability to control cancer progression, and in clinical trial natural drugs cover more than 50% of all tested modern drugs. World Health Organization reports that 80% of world population use natural products or plant-derived molecules for their primary health problems (Sivalokanathan et al. 2005). Many studies reported that about 60% of cancer patients depend on plant products to cure their disease. In stressful environmental conditions, different plant parts are producing a number of secondary metabolites to maintain plant homeostasis. These secondary metabolites are gaining much interest due to their high medicinal properties. It has been reported that secondary metabolites like alkaloids, terpenes, flavonoids, and polyphenols possess anticancer and antimutagenic properties. Along with medicinal properties, these plant-derived molecules/secondary metabolites are also able to influence miRNAs of organism (Fig. 2.1).

MicroRNAs (miRNAs) are small noncoding RNAs which are endogenous in nature and are known to play a major role in gene regulation and cell signaling. The miRNAs are a class of small (19–24 nucleotide), noncoding regulatory RNAs that function as regulatory molecule by base pairing with either 3′ untranslated region (UTR) or coding sequence (CDS) region of putative mRNA (Reinhart et al. 2002; Duursma et al. 2008; Zhang et al. 2012) resulting in gene silencing. MiRNAs not only control normal biological activities, but they also may regulate many pathological activities like evolution, pathogenesis, and progression of cancer (Goldie 2001; Li et al. 2010). Recently, miRNAs have taken a central stage in the field of

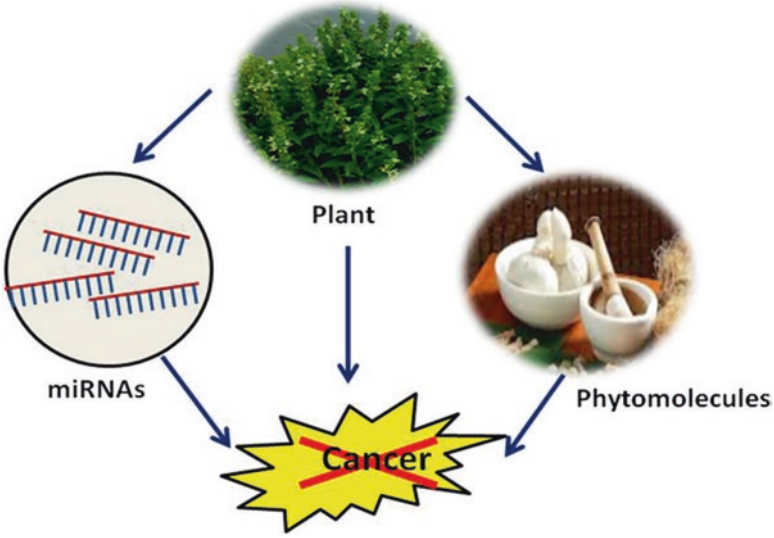

Fig. 2.1 Schematic diagram shows plant molecules/miRNAs inhibit cancer progression

plant molecular biology, developmental biology, and oncology as scientist and researchers are unraveling their role in respective fields. These miRNAs are relatively conserved among diverse species and are associated with various developmental events (Sunkar et al. 2012). In humans, it has been reported that more than 60% protein-coding genes contain a minimum of one conserved and several non-conserved binding sites for miRNA (Friedman et al. 2009). Due to conserve binding sites, miRNAs can regulate several biological processes in animals, viz., apoptosis, cellular metabolism, immune responses, cell signaling, etc. (Alvarez-Garcia and Miska 2005; Miska 2005; Zhang et al. 2007; Bushati and Cohen 2007). In plant cells, miRNAs also control flowering, nutrient homeostasis, and biotic and abiotic stress responses (Dugas and Bartel 2004; Kruszka et al. 2012). Interestingly, now there are growing evidences of cross-kingdom gene regulation by plant miRNAs. Zhang et al. (2012) have detected the presence of plant miRNA in mammalian serum and plasma when taken orally through food. Further they have also demonstrated the regulation of the target gene expression by these exogenous plant miRNAs in animal system. Several plant miRNAs have now been identified in various edible crops like papaya and tomato and certain members of Cucurbit family like *Lagenaria siceraria*, *Cucurbita moschata*, *Cucurbita pepo*, and *Citrullus lantus* (Sunkar et al. 2012; Aryal et al. 2012). Such miRNAs characterized from medicinal plants can be further investigated for their role in cross-kingdom gene regulation and as therapeutics in certain diseases. Owing to their participation in several biological phenomenon including human diseases, miRNAs became a new hope for the pharmaceutical industry. The present chapter highlights the plants and their derived phytomolecules having anticancer properties and also explored the potential of miRNA as a new therapeutic in the field of cancer biology.

2.2 Anticancer Activities of Plants and Their Derived Compounds

Cancer is one of the diseases that drastically diminish the quality of life and life expectancy. Though a lot of efforts and treatments have been worked to treat and prevent untimely death due to cancer, it is still the most dreadful disease causing maximum deaths worldwide. Chemotherapy now can be substituted by phytochemicals, thus preventing overexposure and side effects of chemicals on the human body. In Indian system of medicine, plants and its compounds have been used for the treatment of several chronic and acute diseases from ancient time. Especially in developing countries, herbal medicine provides a new path to discover plant-based new drugs to cure cancer progression with no side effects. Lots of work have been done on these medicinal plants to cure cancer progression (Coseri 2009). Based on these scientific reports, some plant products have been recognized as anticancer drugs (Kharb et al. 2012). Many compounds have been extracted and identified from plants and are well known for their anticancer activity, viz., brassinosteroids, polyphenols, and taxols. Use of phytochemicals is very much prevalent in many alternative medicinal practices as an effective treatment to control and manage cancer, besides it being easily available with proven results. The search for plant-based anticancer agent started with the discovery of vinca alkaloids (vinblastine and vincristine) in the 1950s (Cragg and Newman 2005). The vinca alkaloids are the first anticancer agent isolated from *Catharanthus roseus*. Taxanes, podophyllotoxin derivatives, camptothecin derivatives, and homoharringtonine are plant-derived drugs that are clinically proved as anticancer agents (Itokawa et al. 1993; Lee and Xiao 2005; Kingston 2005; Rahier et al. 2005). There are a number of medicinal plants, which are being used traditionally for the treatment of cancer (Aggarwal and Shishodia 2006; Sarangi and Padhi 2014). Few medicinal plants having anticancer activity and their parts used to derive the phytochemicals are enlisted in Table 2.1.

2.3 Therapeutic Potential of miRNAs

MiRNAs are short, noncoding RNAs that can regulate gene expression of multitude biological processes like cell proliferation, differentiation, and apoptosis. They are conserved from virus to human and can control several mRNAs within cellular pathways and networks. Due to their participation in several biological phenomenon including human ailments, miRNAs became a novel therapeutic molecule for pharmaceutical industry. The first miRNA lin-4 reported by Ambros and Ruvkun controls the timing of larval development of *Caenorhabditis elegans* development (Lee et al. 1993; Wightman et al. 1993). Nowadays, two decades after the first miRNA was introduced, many miRNA-based drugs are in clinical trials that are much closer to market exposure (Rooij and Kauppinen 2014; Schmidt 2014; Lam et al. 2015).

Table 2.1 Some important medicinal plants having anticancer activity

Botanical name	Common name	Family	Active components	Parts used
Acorus *Calamus*	Bach	Araceae	Asarone, eugenol, methyl eugenol, palmitic acid, and champhene	Rhizome
Allium sativum	Garlic	Amaryllidaceae	*Allicin*	Bulb
Andrographis paniculata	Kalmegh	Acanthaceae	Napthoquinones and their analogues	Whole plant
Bruguiera exaristata	Rib-fruited mangrove	Rhizophoraceae	Caesalpins (α, β, γ, δ, ε) and homoisoflavone	Whole plant
Butea monosperma	Palash	Fabaceae	Butein	Bark, flower
Cajanus cajan	Arhar	Fabaceae	Quercetin, xanthone, biflavonoid, neoflavonoid	Leaf, seed
Camellia sinensis	Green tea, black tea	Theaceae	Chrysophanol, rhein, isochrysophanol, and β-sitosterol	Leaf
Cayratia carnosa	Amalbel	Vitaceae	Sesquiterpene lactone and lignin	Whole plant
Catharanthus roseus	*Vinca*	Apocynaceae	Vincristine and Vinblastine	Whole plant
Calotropis gigantea	Madar	Asclepiadaceae	Calotropain FI and FII, Taraxerols	Whole plant
Cissus quadrangularis	Hadjod	Vitaceae	Flavonoid, flavone, limonoid, limonene, nobiletin, and tangeretin	Whole plant
Curcuma longa	Turmeric	Zingibaraceae	Curcumin	Rhizome
Daucus carota	Carrot	Apiaceae	Beta-carotene, lutein, and polyacetylenes	Root
Ginkgo biloba	Ginkgo	Ginkgoaceae	Ginkgetin, ginkgolides A and B	Whole plant
Jatropha curcas	Danti	Euphorbiaceae	Phenolics, flavonoids	Leaves, seed, oils
Morinda citrifolia	Indian mulberry	Rubiaceae	Flavonoids, iridoids, alkaloids	Fruit
Mimosa pudica	Sleepy plant	Fabaceae	Alkaloid mimosine	Whole plant
Ocimum sanctum	Tulsi	Lamiaceae	Eugenol, eugenol derivatives, linolenic acid, rosmarinic acid	Whole plant
Panax ginseng	Ginseng	Araliaceae	Ginsenosides	Root
Podophyllum peltatum	Mayapple	Podophyllaceae	Podophyllotoxins	Root
Terminalia arjuna	Arjuna	Combretaceae	Phenolic acids (gallic acid, ellagic acid)	Bark
Tinospora cordifolia	Giloy	Menispermaceae	Arabinogalactan, syringine, cordiol, cordioside	Stem, root, leaf

(continued)

Table 2.1 (continued)

Botanical name	Common name	Family	Active components	Parts used
Taxus brevifolia	Taxol	Taxaceae	Paclitaxel	Bark
Vitex trifolia	Nichinda	Lamiaceae	Lamiaceae, vanillic acid, p-hydroxybenzoic acid	Leaf, stem bark
Withania somnifera	Ashwagandha	Solanaceae	Withanolides, Withaferins	Stem, root, leaf
Zingiber officinale	Ginger	Zingibaraceae	Gingerenone A, zingerone, gingerol	Rhizome

2.3.1 miRNA Biogenesis

The biogenesis of plant miRNA starts with transcription of the miRNAs encoding genes by RNA polymerase II in the nucleus. Initially, these are synthesized as dsRNA with hairpin loop structure of several hundred nucleotides termed primary miRNA (pri-miRNA). Each pri-miRNA codes one to six pre-miRNA precursors with the help of several proteins, e.g., Dicer-like 1 enzyme (DCL1), Hyponastic leaves 1protein (HYL1), serrate (SE), Dawdle (DDL), and CBP20 and CBP80 (Lobbes et al. 2006; Kim et al. 2008; Dong et al. 2008; Yu et al. 2008; Liu et al. 2012; Saxena et al. 2014). These pri-miRNAs are further cleaved by RNAse III family enzyme and form miRNA/miRNA* duplex (22 nt length) and are called as mature miRNAs (Xie et al. 2010). Once these miRNAs come out from the nucleus into the cytoplasm, they look for their target mRNAs and only one of the mature miRNA sequences which is complementary to its target mRNA interacts and binds with it.

The miRNAs of plants are reported to bind both the UTR as well as the coding regions of the target mRNA with perfect complementarity or with few bases/short segments of complementarity. In the case of perfect complementarity when miRNA binds to either coding or UTR region, it results in cleavage of mRNA; on the other hand, most cases of short segments of miRNA binding with few bases in the UTR region of target mRNA are reported to result in attenuated translation. Thus the whole process results in no expression of the gene or gene silencing also termed as posttranscription gene silencing (PTGS) (Fig. 2.2).

2.3.2 Plant-Based miRNAs in Therapeutics Development

Humans consume fresh vegetables, fruits, cereals, herbs, etc. to nourish their body and supply it with loads of carbohydrates, proteins, fat, minerals, and several other nutrients and meet the daily requirement. Along with these diets, we also consume its genetic material, i.e., DNA and RNA, including some small regulatory noncoding RNAs, i.e., miRNA. It is emphasized that these different miRNAs from distinct food sources play a significant role in gene regulation in host physiology once taken

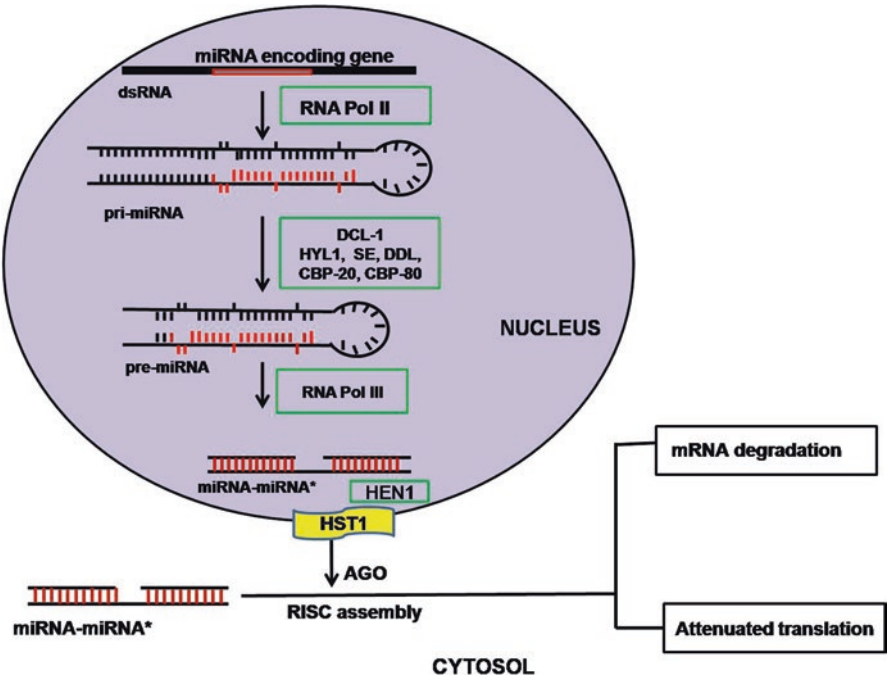

Fig. 2.2 miRNA biogenesis in plants

as a food. Such plant miRNAs have already been detected in animal sera when fed with food like rice orally (Zhang et al. 2012). The presence of *2′-O-methylation* on the 3′-terminal ribose is a unique feature found in plant miRNAs and distinguishes it with animal miRNA; hence plant miRNAs can be easily detected in animal serum when treated with periodate as it shows resistance for periodate oxidation.

In one of the early report, Xiang et al. used engineered *E. coli* expressing short hairpin (shRNA) against CTNNB1 (catenin-β1) and experimentally proved gene silencing in the intestinal epithelium and in human colon cancer xenograft mice. This study provided an example of trans-kingdom RNA interference in higher organisms (Xiang et al. 2006). Subsequently many studies demonstrated the presence of plant-specific miRNA in mammalian serum, plasma, secretions from the eyes, nasal tissue, etc. which can be further used as diagnostics and as a novel class of biomarkers identified for some diseases. For the very first time, Zhang et al. 2012 reported the presence of exogenous plant miRNA 168a in human serum, acquired orally through food intake. They emphasized in their study that epithelial cell lining intestine might absorb plant-derived miRNA from food, package them into microvesicles (MVs), and finally release these plant miRNAs into the circulatory system. Chinese population heavily consumes rice-dependent diet, and because of this, they contain miR168a in their serum. They conducted several *in vitro* and *in vivo* studies and found that human/mouse low density lipoprotein receptor-1 (LDRAP1) mRNA was a target to rice miR168a, which could bind and inhibit its

expression in the liver. However, the fact that plant miR168a decreases the LDLRAP1 protein concentration without affecting mRNA level suggests that rice miR168a acted like animal miRNA inside animal system and actually resulted in translational attenuation, unlike a plant miRNA which most likely binds the target mRNA with perfect complementarity resulting in complete degradation of mRNA itself. Generally LDLRAP1 is abundant in the liver which facilitates removal of LDL from the circulatory system; however, if expression of LDLRAP1 is inhibited due to it being a target of miR168a, LDL cholesterol in plasma may be elevated, thus increasing the risk of heart diseases and stroke. Another group with the same approach did comparative analysis between watermelon miRNAs and mixed fruit juice containing miRNAs. They found 16 miRNAs common in both. After oral administration in the healthy volunteer, their serum tested positive with consistent amplification for 10 watermelon miRNAs and 6 mixed fruit juice miRNAs (Liang et al. 2015).

Cross-kingdom gene regulation by plant miRNA is not limited to humans, but they also found it in animal sera that were fed plant diet. A study reported uptake of dietary miRNAs also called as "xenomiR" hypothesis (Witwer 2012) from commercially available plant-based, plant miRNA-rich substance (silk fruit and protein shake) when administered to pig-tailed macaques (Witwer et al. 2013). In another experiment Liang et al. 2014 showed presence of *Brassica oleracea* derived miRNA in mice. They extracted total RNAs in quantities of 10–50 μg and fed the mice by administering purified RNAs in its oral cavity using pipette tip, and in some experiments, they also added RNA solution in mice diet. Interestingly miR172, the most abundant plant miRNA in *B. oleracea*, survived through GI tract and was detected in the serum, stomach, intestine, and feces. Although the functions of these plant miRNAs in mammals are still under debate (Liang et al. 2014), the results are quite promising for cross-kingdom gene regulation hypothesis. Another interesting study revealed cross-kingdom miRNA transfer from mulberry plant to silkworm. *Bombyx mori*, also known as silkworm, is an insect that feeds only on mulberry leaves. When tested for the presence of mulberry-specific miRNA miR166b, the insect was found positive; miR166b was detected in its hemolymph and fat body. In subsequent experiments using synthetic miR166b, positive intake of it was found in insect hemolymph (Jia et al. 2015). Few more studies demonstrated the detection of maize-derived microRNAs in pigs where authors evaluated microRNA levels in cooked chow diets and showed plant miRNA is resistant to harsh cooking up to certain extent. Pigs were fed fresh maize, and then after 7 days maize miRNA was detected in porcine tissues and serum. This study has shown gene regulation of porcine mRNAs by maize miRNA in a cross-kingdom fashion (Luo et al. 2017).

In another study Chin et al. 2016 reported that western donor sera contained the plant miR159, and its presence inversely correlated with breast cancer incidence in patients. miR159 was detected in extracellular vesicle of human sera and found it to be resistant to sodium periodate oxidation which shows plant-originated miRNA because of the presence of *2'-O-methylation* on 3'-terminal ribose a unique feature of plant miRNAs. Further research was carried out on synthetic mimic of miR159 in breast cancer cells capable of inhibiting cell proliferation by targeting TCF-7 which encodes a Wnt signaling transcription factor, leading to decrease in myc protein.

Myc protein, a nuclear phosphoprotein, is known for multiple functions including its role in cell cycle progression, apoptosis, and cellular transformation, and any kind of mutation in Myc may lead to cancer-like condition.

Another very good example of cross-kingdom gene regulation is shown in the case of influenza virus. As we know virus infections always have been a threat to mankind, and millions of people carry these viruses themselves. A recent study by Zhou et al. 2014 reported plant microRNA miR2911, which directly represses influenza virus (IAV) by targeting PB2 and NS1 genes which play a significant role during influenza virus replication. miR2911 is found to be enriched in Chinese plant honeysuckle. Chinese have been drinking honeysuckle *(Lonicera japonica*) decoction to treat IAV infection, so this study reported miR2911 as the first active compound in honeysuckle decoction drink which actually inhibits virus replication and can be used as an augmented therapy against IAV infection.

If we talk about gene regulation by miRNA within the same species, e.g., in humans, miRNAs are found to transfer from individual (mother) to individual (newborn) by mammary gland milk production, which feeds the newborns and provides immunity as well. Mother's milk contains secretory antibody IgA, leukocytes, and some non-specific factors such as lysozyme, lactoferrin, and some oligosaccharides which have antimicrobial effects. A study by Kosaka et al. 2010 reported many miRNAs related to immunity were transferred to the infant via breast feeding during the first few months. Within the plant, it has been reported that miRNAs are found to regulate a number of genes involved in developmental processes like leaf, flower and embryo formation, flower onset, etc. (Saxena et al. 2014).

2.3.3 Plant Extracts/Phytomolecules Regulating miRNA

Fruits, vegetables, and medicinal plants are the important sources of phytomolecules. These phytomolecules exert their anticancer activity by targeting multiple signaling pathways including miRNAs in biological system. These plant-based natural molecules are gaining much attention to combat cancer progression in recent years. The plant extracts and phytomolecules, viz., curcumin, genistein, resveratrol, Epigallocatechin-3-Gallate (EGCG), Indole-3-carbinol (I3C), and 3,3′-diindolylmethane (DIM), could regulate miRNAs and eliminate cancer cell resistance to conventional treatment (Li et al. 2010). It has been reported that pomegranate juice used for the treatment of few cancers like prostate cancer (Wang et al. 2012a, b), breast cancer (Banerjee et al. 2012; Rocha et al. 2012; Martens-Talcott et al. 2013) by regulating miRNAs, which may play a role in prevention of cancer. Prostate cancer is the development of cancer in the prostate gland, a part of male reproductive system and is one of the nuisances. Nowadays, people are working to find out natural remedies to treat prostate cancer. So far no evidence of miRNAs have been described for the pomegranate, but the juice extracts of the pomegranate have been found to be effective by increasing concentration of tumor suppressor miRNAs and downregulating the level of several oncogenic miRNAs in case of prostate cancer.

In another report, the role of pomegranate juice has also been demonstrated, and it has been found that the juice extract administration leads to downregulation of miRNA-155 and miRNA-27a in breast cancer cells (Banerjee et al. 2012). Pomegranate juice has also been found to be very effective in completely stopping the cancer cell growth in breast cancer cell lines MCF-7 and MDA-MB-231(Wang et al. 2012a, b; Rocha et al. 2012). These reports are suggesting the role of pomegranate to regulate the human miRNAs, especially in the case of prostate cancer metastasis and breast cancer. Hence, pomegranate juice and its edible fruit parts may serve as a significant therapeutic in the treatment of cancer.

Curcumin is another natural agent derived from rhizomes of *Curcuma longa* having strong antioxidant, anti-inflammatory, and anticancer activities. It can inhibit cell proliferation and angiogenesis and also can induce cell cycle arrest and apoptosis on several cancers, viz., breast, cervical, colon, gastric, melanoma, prostrate, and pancreatic (Kingston 2005; Karunagaran et al. 2007; Gupta et al. 2010). Curcumin-treated human pancreatic cells showed upregulation of miR-22 and downregulation of miR-199a, and miR-22 targeted the genes SP1 and ESR1 (Bushati and Cohen 2007). These results revealed the anticancer properties of curcumin by influencing the miRNAs expression. Martens-Talcott and group showed the role of betulinic acid (BA) in inhibiting breast cancer growth. Betulinic acid, a terpenoid isolated from a tree bark, is found to decrease ER-negative breast cancer MDA-MB-231 cell growth. It is reported to downregulate expression level of several specificity proteins which are overexpressed in the tumor by inducing ZBTB10 expression (a putative sp-suppressor) and decreasing miR27a expression (Martens-Talcott et al. 2013). Likewise, few studies on plant-derived bioactive compounds, polyphenols, polyunsaturated fatty acids (PUFA), and short-chain fatty acids, reported modulation of host miRNA expression in colorectal cancer. Colorectal cancer is also known as bowel cancer or colon cancer and is one of the most commonly diagnosed cancers among males and females and cause of death worldwide (Siegel et al. 2016). The higher incidence of this cancer has once again been attributed to unhealthy modern lifestyles and changing food habits (Haggar and Boushey 2009).

Resveratrol is a natural molecule found in many plants, e.g., berries, grapes, peanuts, and plums. The anticancer activity of resveratrol is mediated by growth arrest of cancer cells and apoptosis. Resveratrol upregulated the expression of tumor suppressor miR-663 and downregulated many miRNAs that are generally found to be upregulated in human colon cancer cells such as miR-17, miR-21, miR-25, miR-26a, miR-92a-2, miR-103-1 and -103-2, and miR-181a2 (Tili et al. 2010). The target of miR-663 is transforming growth factor beta 1 (TGFβ1). Further study revealed that resveratrol is able to downregulate many miRNAs such as miR-17-92 and miR-106ab in prostate cancer cells (Dhar et al. 2011). These reports suggest that resveratrol plays a major role to arrest cancer progression in cells through regulation of miRNAs expression.

A phytomolecule, Epigallocatechin-3-Gallate (EGCG) is a major constituent of green tea with a potent antioxidant activity has shown anticancerous activity. It also shows protective effects against carcinogens in mouse model system. It has been reported that EGCG influence the expression of several miRNAs in HepG2 human

hepatic cancer cells. It also enhanced the expression of many miRNAs, out of which 13 miRNAs are shown over expression including miR-16 and 48 miRNAs are downregulated in human hepatic cancer cell. The miR-16 inhibits Bcl-2 protein by targeting it, and Tsang and Kwok also demonstrated that EGCG is able to reduce Bcl-2 that participates in HepG2 cell apoptosis (Tsang and Kwok 2010). In another study, it was reported that EGCG downregulate the expression of miR-98-5p in A549 lung cancer cells as a resulting enhanced effect of cisplatin. Due to this process, EGCG induced cell death and upregulate the expression of p53 gene (Zhou et al. 2014). These reports explored the potential of EGCG to inhibit the cancer growth by the regulation of miRNAs. Besides that, another report suggests that ursolic acid, a triterpene derived from medicinal plants such as *Oldenlandia diffusa* and *Radix actinidiae,* induced apoptosis in U252 glioblastoma cells through miR-21. Ursolic acid downregulates the expression of miR-21 resulting induced expression of PDCD4 (Wang et al. 2012a, b). Similarly Garcinol, a polyisoprenylated benzophenone, is another phytomolecule isolated from *Garcinia indica* extracts, reverse Epithelial-Mesenchymal Transition (EMT) in breast cancer cell lines (MDA-MB-231, BT-549) by upregulate the expression miR-200b, miR-200c, let-7a, let-7e, and let-7f (Ahmad et al. 2012). Quercetin, another natural phytomolecule, which is flavonoid in nature, is found in green tea, red wine, and apples, having loads of medicinal properties. It is well known that quercetin-rich food diet can modulate the expression of several miRNAs. These quercetin-mediated miRNAs have been reported to inhibit cell proliferation, induce apoptosis, upregulate tumor suppressor miRNAs, and decrease metastasis and invasion (miR-125a, miR-155, miR-183, miR-146a and let-7 family, etc.) (Lam et al. 2012). Del Follo-Martinez and group demonstrated that quercetin and resveratrol combination induced apoptosis in colorectal cancer cells through downregulation of oncogenic miR-27a (Del et al. 2013). There is a lot of evidence available in the form of research publication which claims that plant molecules inhibit cancer through regulation of miRNAs.

2.4 Conclusions and Future Prospects

Edible fruits, vegetables, and medicinal plants and their derived molecules have been prime sources of natural drugs for severe medical conditions like cancer in alternative practices, viz., Ayurveda and Unani medicines. These natural drugs maintain the human health, enhance body immunity, and are also able to cure various types of cancer. In these days, plants and its phytomolecules gain much attention in cancer therapy due to their safe mode of action and no side effect. A number of plant-based molecules have played a significant role in the development of cancer therapeutics, some of which are successfully undergoing for clinical trials. Vinblastine, vincristine, and *Vinca rosea* alkaloids are the most popular drugs used in cancer therapy. Out of 1000 medicinal plant species, some have been reported for their anticancer activity in biological system, so further research must be undertaken to reveal the anticancer activity of remaining plants. Taxol isolated from *Taxus brevifolia* has

figured high in the therapeutic segment of cancer. Along with chemopreventive nature, the plants and its phytomolecules, viz., curcumin, resveratrol, and EGCG, can influence the expression profiles of miRNA. Cancer is caused by defects in multiple genes, and phytomolecules show pleiotropic effects, implicating that phytomolecule-induced miRNA can target multiple gene/s or pathway/s at the same time. Due to the above characteristics, miRNAs are a new hope for the cancer therapy.

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Chapter 3
Potential of Herbal Medicines in Colorectal Carcinoma and Their Mechanism of Action

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Abstract Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the world. Although colon cancer is often treated successfully with the surgery, it requires an aggressive systemic therapy to completely cure. Several past studies have suggested the combined use of chemo- or radiotherapy with herbal medicines to enhance the efficacy and diminish the side effects caused by these therapies. In this regard, some herbal compounds such as vinca alkaloids, turmeric, astragalus, ginseng, and ginger have been well studied for their anti-colorectal cancer activities. The identification of active herbal compounds emphasizes on the development of an effective anticancer medicine, which remains as an essential step in the advanced cancer treatments. Many preclinical and clinical studies have proved that herbal medicines are safe, exhibit higher tumour suppressive activity, improve immune system, and increase sensitivity of chemo- and radiotherapeutics. The herbs are more promising as they prevent the invasion and proliferation of tumour by arresting cellular functions. Due to abundance, low cost, and safety in consumption, herbs remain with a tremendous potential to investigate as a combined formulation of chemotherapy to enable tumour growth suppression with less toxic side

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effect and to improve overall survival rate. The aim of the chapter is to focus on various plant and plant-derived compounds showing the promising anticancer activities against colorectal cancer and their potential mechanism of action.

Keywords Adjuvant therapy · Colorectal cancer · Herbal medicine · Mechanism of action

3.1 Introduction

Colorectal cancer (CRC), also known as colon or bowel cancer, develops from any part of the large intestine; begins as clumps of benign cells, called polyps; and may be formed in the last part of the digestive system (colon) or the final segment of the large intestine (rectum). Like other cancer forms, CRC results in an abnormal growth of cells, and it can metastasize (Xie and Itzkowitz 2008; Jemal et al. 2011). The symptoms of CRC vary depending on the tumour location, which may include blood in the stool, change in bowel movements, weight loss, and feeling tired all the time (Cunningham et al. 2010; Jemal et al. 2011). Major factors causing CRC are old age (above 50 years) and lifestyle, and only minor cases are due to genetic disorders. This may result in an altered bowel habits like constipation, diarrhoea, gastrointestinal bleeding, and unsatisfied excretion (Thompson 1989). Rarely, tumour may be very large to fill the entire lumen, like in the case of abdominal distension leading to a visible enlargement of the stomach or as in 'hydronephrosis', that is, distension of renal pelvis calyces resulting in kidney atrophy. CRC is more common in those people who are aged between 60 and 70 years, while cases before 50s are less common except where there is a family history. Persons, who smoke and consume high red meat and less fruits, vegetables, and poultry diets, will develop colon cancer. Alcohol consumption is also a significant risk factor, while regular physical exercise can reduce the chances of colorectal cancer (Chan and Giovannucci 2010). Screening is recommended for men and women over the age of 50; if earlier polyps persist before becoming cancerous, often surgery or chemotherapy is the treatment for CRC (Jasperson et al. 2010).

Growths confined to CRC can be diagnosed through a colonoscopy and/or sigmoidoscopy and, if detected at early stage, is often treatable (Moreno et al. 2016). According to the American Cancer Society report (2017), 90% of CRC cases discovered at this stage will survive a longer period of 5 years. But if tumour spreads into the lymph nodes, the prognosis becomes worse with 48% survival, and if it spreads further, only 7% survival is predictable. CRC is the third most common cancer in men (10% of the total) and the second in women (9.2% of the total) worldwide. It is the second most common cause of cancer death with 42% and 43% impact in both men and women. According to World Health Organization (WHO) report and World Cancer Report of 2014, about 8.2 million patients died from cancer in 2012. It has also been estimated that the number of annual cancer cases would have increased from 14 million to 22 million within the next two decades (Table 3.1).

Table 3.1 Global statistics of colorectal cancer

Incidence and mortality rate worldwide	References
148,810 new cases were diagnosed, accounting for 9% of cancer deaths in women and 8% in men	GLOBOCAN (2012)
1.4 million new cases and 694,000 deaths from the disease	WHO (2017)
26,300 male deaths and 24,530 female deaths	Siegel et al. (2013)
8.2 million patients died from cancer in 2012	WHO (2014)

By 2017, the estimated number of new cases of colon cancer and rectal cancer is 95,520 and 39,910, respectively, adding to a total of 135,430 new cases of CRC with 50,260 estimated deaths (American Cancer Society 2017).

TNM system is a staging tool used to diagnose and determine the stage of cancer for each person as tumour (T), growth of tumour into the wall of the colon or rectum; node (N), spreading of tumour to the lymph nodes; and metastasis (M), cancer metastasized to other parts of the body. Staging is a way of describing where the cancer is located, or whether it has spread and has affected other parts of the body. There are distinct stages described for several types of cancer. There are five stages in CRC, stage 0 (zero) and stages I to IV, which provide a common way of describing the cancer stages as provided by American Joint Committee on Cancer (AJCC) staging shown in tabular form (Table 3.2) (Edge and Compton 2010).

The most commonly used chemotherapy drugs for CRC include antimetabolites (e.g. methotrexate), monoclonal antibodies (e.g. bevacizumab), DNA-interactive agents (e.g. cisplatin, doxorubicin), antitubulin agents (e.g. taxanes), few hormones, and molecular drugs targeting CRC cells (Nussbaumer et al. 2011). However, clinical uses of these drugs are complemented with numerous side effects such as loss of hair, bone marrow suppression, drug resistance, few lesions in gastrointestinal tracts, neurologic dysfunction, and cardiac toxicity. Emphasizing the need for early detection of tumours and development of new and improved treatment regimens, and an increased understanding towards the disease, has reduced the mortality rate nearly by 5% in the last decades (Ramos et al. 2008). The survival of CRC patients depends largely on stage of disease at the time of diagnosis, and it also varies widely between stages (Lozano et al. 2012). The adjuvant (additional) therapy includes the use of herbal plants to reduce considerable risk in patients (Yin et al. 2013). Natural therapies involving medicinal plants and plant-derived products in cancer treatments may reduce the adverse side effects. Currently, many herbal products are being used to treat cancer, and plant products act as a major source of novel chemical structures for the drug discovery. Nowadays, more than 70% of anticancer drugs have their natural origin from plants. At present, a myriad number of plant products have shown their promising anticancer properties *in vitro*, but have yet to be evaluated acutely in humans (Dai and Mumper 2010; Yin et al. 2013; Ahmad et al. 2017). Currently, nanotechnology also aims to enhance the anticancer activities of herbal and herbal-derived drugs to control release of the compound known as nanomedicine, which aim to enhance plant-derived drugs activity (Greenwell and Rahman 2015). Further, studies are required to determine the efficacy of these plant products

Table 3.2 Various stages in CRC, according to American Joint Committee on Cancer (AJCC) (Edge and Compton 2010)

Stages	Stage grouping	Descriptions
0	Tis, N0, M0	The cancer is in its earliest stage. This stage is also known as carcinoma in situ or intramucosal carcinoma (Tis). It has not grown beyond the inner layer (mucosa) of the colon or rectum
I	T1 or T2, N0, M0	The cancer has grown through the muscularis mucosa into the submucosa (T1), and it may also have grown into the muscularis propria (T2). It has not spread to nearby lymph nodes (N0). It has not spread to distant sites (M0)
IIA	T3, N0, M0	The cancer has grown into the outermost layers of the colon or rectum but has not gone through them (T3). It has not reached nearby organs. It has not yet spread to nearby lymph nodes (N0) or to distant sites (M0)
IIB	T4a, N0, M0	The cancer has grown through the wall of the colon or rectum but has not grown into other nearby tissues or organs (T4a). It has not yet spread to nearby lymph nodes (N0) or to distant sites (M0)
IIC	T4b, N0, M0	The cancer has grown through the wall of the colon or rectum and is attached to or has grown into other nearby tissues or organs (T4b). It has not yet spread to nearby lymph nodes (N0) or to distant sites (M0)
IIIA	T1 or T2, N1, M0	The cancer has grown through the mucosa into the submucosa (T1), and it may also have grown into the muscularis propria (T2). It has spread to 1 to 3 nearby lymph nodes (N1a/N1b) or into areas of fat near the lymph nodes but not the nodes themselves (N1c). It has not spread to distant sites (M0)
	T1, N2a, M0	The cancer has grown through the mucosa into the submucosa (T1). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites (M0)
IIIB	T3 or T4a, N1, M0	The cancer has grown into the outermost layers of the colon or rectum (T3) or through the visceral peritoneum (T4a) but has not reached nearby organs. It has spread to 1 to 3 nearby lymph nodes (N1a or N1b) or into areas of fat near the lymph nodes but not the nodes themselves (N1c). It has not spread to distant sites (M0)
	T2 or T3, N2a, M0	The cancer has grown into the muscularis propria (T2) or into the outermost layers of the colon or rectum (T3). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites (M0)
	T1 or T2 N2b, M0	The cancer has grown through the mucosa into the submucosa (T1), and it may also have grown into the muscularis propria (T2). It has spread to 7 or more nearby lymph nodes (N2b). It has not spread to distant sites (M0)
IIIC	T4a, N2a, M0	The cancer has grown through the wall of the colon or rectum (including the visceral peritoneum) but has not reached nearby organs (T4a). It has spread to 4 to 6 nearby lymph nodes (N2a). It has not spread to distant sites (M0)
	T3 or T4a, N2b, M0	The cancer has grown into the outermost layers of the colon or rectum (T3) or through the visceral peritoneum (T4a) but has not reached nearby organs. It has spread to 7 or more nearby lymph nodes (N2b). It has not spread to distant sites (M0)
	T4b, N1 or N2, M0	The cancer has grown through the wall of the colon or rectum and is attached to or has grown into other nearby tissues or organs (T4b). It has spread to at least one nearby lymph node or into areas of fat near the lymph nodes (N1 or N2). It has not spread to distant sites (M0)

(continued)

Table 3.2 (continued)

Stages	Stage grouping	Descriptions
IVA	Any T, Any N, M1a	The cancer may or may not have grown through the wall of the colon or rectum (any T). It might or might not have spread to nearby lymph nodes (any N). It has spread to 1 distant organ (such as the liver or lung) or distant set of lymph nodes (M1a)
IVB	Any T, Any N, M1b	The cancer might or might not have grown through the wall of the colon or rectum. It might or might not have spread to nearby lymph nodes. It has spread to more than 1 distant organ (such as the liver or lung) or distant set of lymph nodes, or it has spread to distant parts of the peritoneum (the lining of the abdominal cavity) (M1b)

in treating CRC. The aim of the chapter is to focus on the various plant and plant-derived compounds showing the promising anticancer activities and their potential mechanism of action.

3.2 Colorectal Cancer (CRC)

3.2.1 Types and Causes

In CRC, the balance between the rate of cell growth and apoptosis is impaired progressively during disease development. It starts as a benign adenomatous polyp which changes into a propelled adenoma with high-rate dysplasia that advances to aggressive tumour (Simon 2016). Inherited genetic disorders, which can cause CRC, can be distinguished as familial adenomatous polyposis and hereditary non-polyposis colon cancer. However, these might represent only less than 5% of CRC cases. The continuous process of cell division and differentiation of intestinal epithelium can be subverted by genetic alteration that could switch the progenitor cells into tumour cells. Changes in the adenomatous polyposis coli (APC) gene have been linked to about 60% of colorectal neoplasia signifying that APC mutations may be a central event in the development of colorectal carcinogenesis (Abraha and Ketema 2016). Risk factors include older age; male gender; high intake of fat, alcohol, red meat, and processed meats; obesity; smoking; and lack of physical exercise (Johnson et al. 2013). Approximately 10% of cases are linked to insufficient physical activity. The risk for alcohol appears to increase at greater than one drink per day (Fedriko et al. 2011; Mustafa et al. 2016).

Another risk factor is the inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis. Drinking five glasses of water a day is linked to decrease the risk of CRC and adenomatous polyps (Lee et al. 2012a). Adenocarcinoma makes up 95% of all CRC cases which includes colorectal adenocarcinoma and gastrointestinal carcinoid tumours. In the gastrointestinal tract, adenocarcinoma develops in the cells of the lining inside the colon and/or the rectum. Among these

rarer types of CRC, gastrointestinal carcinoid tumours grow slowly to form in the neuroendocrine cell and make up to 1% of all CRC, whereas primary colorectal lymphomas develop in the lymphatic system and account for only 0.5% of CRC (Chung and Hunt 2006).

3.2.1.1 Inflammatory Bowel Disease

People with inflammatory bowel disease (ulcerative colitis and Crohn's disease) are at increased risk of colon cancer. The risk increases with how longer a person has the disease and the severity of inflammation. In people with considerable risk, prevention with aspirin and regular colonoscopies are recommended as precaution, while only 2% of people with inflammatory bowel disease turns out to have CRC yearly. In case with Crohn's disease, only 2% get CRC after 10 years, 8% after 20 years, and 18% after 30 years. In ulcerative colitis condition, approximately 16% develops either a cancer precursor or colon cancer in 30 years (M'Koma et al. 2011). Individuals with long-standing ulcerative colitis, an inflammatory disease of the large bowel, are three to four times more likely to develop CRC during their lifetime, compared with those without an inflammatory bowel disease (Rutter 2014; Rutter and Riddell 2014). These individuals also tend to have poorly differentiated carcinomas, which lead to a poor prognosis (Mikami et al. 2011). This link between inflammation and colon cancer is further supported by the association between the use of the anti-inflammatory compound aspirin and reduced colon cancer risk (Chan and Giovannucci 2010; M'Koma et al. 2011). Thus, bowel inflammation and other bowel diseases represent a potentially important target for intervention in colon cancer (Farraye et al. 2010; Rutter 2014; Rutter and Riddell 2014).

3.2.1.2 Genetics

Persons with family history in two or more first-degree relatives (such as a parent or sibling) have a two- to threefold greater risk of disease, and this group accounts for about 20% of all cases. A number of genetic syndromes are also associated with higher rates of CRC; the molecular bases of CRC include familial adenomatous polyposis (FAP), attenuated FAP (AFAP), and hereditary nonpolyposis colorectal cancer (HNPCC). The most common one is HNPCC or Lynch syndrome which is present in about 3% of people with CRC. Other syndromes that are strongly associated with CRC include Gardner syndrome and FAP (Lynch et al. 1993; Jo and Chung 2005). For people with these syndromes, cancer always occurs and makes up 1% of the cancer cases (Jo and Chung 2005). HNPCC consists of at least two syndromes: Lynch syndrome I, with hereditary predisposition for CRC having early (approximately 44 years) onset, with a proclivity (70%) for the proximal colon and an excess of synchronous and metachronous colonic cancers, and Lynch syndrome

II, featuring a similar colonic phenotype accompanied by a substantial risk for carcinoma of the endometrium. There are no known premonitory phenotypic signs or biomarkers of cancer susceptibility in the Lynch syndromes. The most frequent mutations in HNPCC are mutations in the MutS protein homolog 2 (*MSH2*) and MutL homolog 1 (*MLH1*), colon cancer, and nonpolyposis type 2 genes. The former is tumour suppressor gene and more specifically a caretaker gene, with those codes for a DNA mismatch repair (MMR) protein, while the latter one is a component of seven DNA MMR proteins that work coordinately in sequential steps to initiate repair of DNA mismatches in humans. Defects in MLH1 gene are also associated with microsatellite instability (MSI) and an elevated spontaneous mutation rate (mutator phenotype) (Boland and Goel 2010).

Epigenetic factors, such as abnormal DNA methylation of tumour suppressor promoters, play a role in the development of CRC. Although ~75% of colon cancer cases are sporadic, familiar predisposition also plays a vital role in percentage of occurrence of CRC. Germ line mutations in *APC* gene result in the syndrome, FAP, in which affected individuals develop hundreds to thousands of polyps as early as in their teens or early 20s. Individuals with FAP account for only ~1% of all known colon cancer cases, but these individuals have a 100% likelihood of developing cancer unless the colon is removed. The risks of CRC are variable and depend on specific germ line alterations. Fearon and Vogelstein proposed a model, whereby loss of function of *APC* initiates the formation of a benign lesion, followed by an activating mutation in *KRAS* (a proto-oncogene), allelic loss of the 18q locus, and mutation of *p53* gene, which all contribute to the progression to malignant disease. Most deaths due to colon cancer are associated with metastatic disease. A gene that appears to contribute to the potential for metastatic disease is metastasis associated in colon cancer 1 (*MACC1*). It is a transcriptional factor that influences the expression of hepatocyte growth factor. This gene is associated with the proliferation, invasion, and scattering of colon cancer cells in cell culture, tumour growth, and metastasis in mice. Thus, this gene implies to be a potential target for cancer intervention, and these mutations are highly associated with a 100% risk of developing cancer in a lifetime (Zarour et al. 2017).

3.2.2 Signalling Pathways

The risk for CRC is influenced by genetic predisposition, which is especially high for somatic mutations of the tumour suppressor genes, APC, causing familial adenomatosis coli and lifestyle factor. Beyond this, defects in signalling pathways like Wnt signalling pathway and other mutations should occur for the cell to become cancerous. The p53 protein, produced by the *TP53* gene, normally monitors cell division and kills cells if they have Wnt pathway defects. Eventually, a cell line acquires a mutation in the *TP53* gene and transforms the tissue from a benign

epithelial tumour into an invasive epithelial cell cancer. At times, it is not necessary for p53 to be mutated; BAX protein mutation may instead cause the disease (He et al. 2011a). Other proteins responsible for programmed cell deaths that are commonly deactivated in CRCs are transforming growth factor beta (TGF-β) and deleted in colorectal cancer (DCC) in segment of chromosomes. TGF-β has a deactivating mutation in at least half of CRCs. Sometimes TGF-β is not deactivated, but a downstream protein named SMAD is deactivated. To study about various hallmarks hereditary of CRC and its signalling network, naturally mutant or genetically modified animals, such as mutated APC in mice, are primarily used as inducible tumour models. Based on chemical carcinogens, many have been developed to mimic non-hereditary tumour development. Many distinctive models of genetic instability, subsequent clinical manifestations, and pathological behaviour have been characterized to know about different pathways (Esther et al. 2010). Recently, it has been established that many other systems and pathways are involved along with other pathways in the pathogenesis of CRC, which includes abnormal DNA methylation and inflammation, and discovered that microRNA (miRNA) can actively contribute to the carcinogenic process (Colussi et al. 2013; Eliane et al. 2013).

3.2.2.1 KRAS Signalling

The *KRAS* proto-oncogene, a 21-kDa guanosine 5-triphosphate (GTP)-binding protein, initiates the mitogen-activated protein kinase (MAPK) signalling pathway and downstream of epidermal growth factor receptor (EGFR) and another essential component of the EGFR signalling cascade. In 30–40% of CRC, *KRAS* mutations are observed, which shows to be inherently resistant to cetuximab and panitumumab treatment (De Roock et al. 2011). EGFR activates two main intracellular pathways: MAPK pathway and the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway. These pathways lead to the activation of various transcription factors that then impact cellular responses such as proliferation, migration, differentiation, and apoptosis (Rosty et al. 2013). High-level expressions of EGFR ligands epiregulin and amphiregulin are also linked to the benefit, resulting from EGFR MAb to therapy in terms of progression-free survival and overall survival in those with KRAS wild-type status (Yarom and Jonker 2011). KRAS is perhaps the only biomarker sufficiently developed for clinical utility; the predictive role of EGFR is limited, particularly for patients being considered for EGFR MAb therapy. To the downstream of KRAS in the MAPK signalling, *BRAF* gene encodes pathway for serine-threonine protein. Mutation in *BRAF* occurs as 5–22% of CRC, when separated by MIS status. *BRAF* mutations are as high as 40–52% of CRCs that arise to MIS pathway to form tumours. In a study with mice, mutations in *TGF-β1* gene were introduced into 129/Sv Rag2 mutant mouse, which accelerates adenocarcinomas with strong local invasion suggesting a role of genetic background in tumour

development. Colon-specific expression of activated mutant of KRAS protein results in development of single or multiple lesions. Oncogenic KRAS allele activated in colon epithelium induces expression of procarcinogenic protein kinase C-βII (PKCβII) and increases cell proliferation of epithelial cells, while in the distal colon the mutant form of KRAS has the opposite effects on PKCβII expression and cell proliferation. When mouse model was treated with the procarcinogen azoxymethane (AOM), it leads to formation of dysplastic microadenomas in the proximal but not in the distal colon, while in the intestine of mice, mutation in the *Muc2* gene causes adenomas and adenocarcinomas.

PIK3CA gene encodes phosphatidylinositol 3-kinase (PI3K), a key signal transducer in the PI3K-Akt pathway. Mutations in *PIK3CA* occur as 14–18% of colon cancers, and most mutations involve hotspots on exons 9 and 20. Interestingly, there is a strong association between *PIK3CA* exon 9 mutations and *KRAS* mutations, and Akt is a major downstream effector of PI3K (De Roock et al. 2011; Rosty et al. 2013). A study by Baba et al. (2011) reported the role of activated (phosphorylated) Akt expression in a large cohort of CRC. KRAS point mutations are generally observed as somatic mutations. Up to 90% of tumour activating mutations of the *RAS* gene are detected in codons 12 and 13, but less frequently also in codons 61 and 63. Regarding codon 12 and 13 mutations, only, 70% of mutations occur in codon 12 and 30% in codon 13 (Al-Shamsi et al. 2015; Módos et al. 2016; Lorentzen et al. 2016). However, KRAS mutation does not appear to account for all drug resistance. NRAS, BRAF, and PI3K activating mutations, as well as loss of PTEN6–9, may also render anti-EGFR-based therapy ineffective, although this is less well established than for KRAS mutations (Lorentzen et al. 2016). The recent success of the selective BRAF inhibitor PLX403222 in metastatic melanoma may herald a new class of targeted agents available for CRC (Grossmann and Samowitz 2011). Previous studies have demonstrated that Ras activation is sufficient to induce vascular endothelial growth factor (VEGF). These data suggest that VEGF may be an *in vivo* survival factor for tumour endothelium (Bruns et al. 2000). In the same study, alterations in PI3K were correlated with poor response, but this association was limited to a small subset of mutations. PI3K mutations have been reported in 15–18% of CRC and all mutations were detected in exon 9 and 20, which blocks binding of p110 catalytic subunit. KRAS testing is essential for determining patient eligibility for EGFR-targeted therapies in metastatic CRC (Li et al. 2016; Lorentzen et al. 2016).

3.2.2.2 Wnt Signalling Pathway

The Wingless-type MMTV integration site family member (Wnt) signalling pathway, the most frequent mutation, is the prominent cause for disease origin in the epithelial cell lining of the colon or rectum of the gastrointestinal tract that increases

signalling activity (Dihlmann and von Knebel 2005; Chiurillo 2015). One of the cadherin protein complex subunit β-catenins acts as an intracellular signal transducer of Wnt signalling pathway, and it is regulated and destroyed by the β-catenin destruction complex, APC protein. Therefore, genetic mutation of the APC gene is strongly linked to cancers and particularly in CRCs resulting from FAP (MacDonald et al. 2009; Fleming et al. 2012). The APC protein, a negative regulator, has been involved in controlling β-catenin concentrations, and it interacts with E-cadherin, involved in cell adhesion (Fleming et al. 2012; Gao et al. 2014; Morkel et al. 2015). Thus, mutations in the APC or β-catenin prevent phosphorylation and β-catenin activation and may result in CRC. Activating mutations of the Wnt signalling pathway are the only known genetic alterations present in early premalignant lesions in the intestine, such as aberrant crypt foci and small adenomas (Morkel et al. 2015; Novellasdemunt et al. 2015).

3.2.2.3 APC Gene

Adenomatous polyposis coli gene, the most common gene mutation in all CRC, produces mutation in APC protein. Around 80% APC mutation is of sporadic colorectal tumours which show diallelic inactivation of the APC gene (Fearnhead et al. 2001). A high percentage of remaining tumours show activating mutations in β-catenin or axin. APC protein prevents the accumulation of β-catenin protein. The normal function of this gene includes the negative regulation of signalling by Wnt cytokines. In the absence of APC function, the Wnt pathway is activated through β-catenin, leading to the transcription of tumour-promoting genes like *Myc* (Walz et al. 2014). Without APC, β-catenin accumulates to elevated levels and translocates (moves) into the nucleus, binds to DNA, and activates the transcription of proto-oncogenes. These genes are normally important for stem cell renewal and differentiation, but when inappropriately expressed at elevated levels, they can cause cancer. Mutation in APC inhibits β-catenin; some cancers show increased β-catenin because of mutations in β-catenin (CTNNB1) which blocks its own breakdown or have mutations in other genes with function like APC such as AXIN1, AXIN2, TCF7L2, or NKD1that results in increased β-catenin in cells (Markowitz and Bertagnolli 2009; MacDonald et al. 2009).

Mouse models of CRC were studied; APC mutant model provided a valuable biological system, to simulate human physiological conditions, suitable for testing therapeutics that can potentially benefit patients. In mutant mouse model of CRC, cyclooxygenase-2 (COX-2) expression was observed in an early event of carcinogenesis. These observations gave rationale to treat human patients suffering from familial form of the disease FAP with selective COX-2 inhibitor (Wang and DuBois 2010). In another experiment, combination of *Min* and *Mom1* mutations was found to increase the lifespan of FAP mouse (Mcilhatton et al. 2016). In zebrafish model, nonsense mutation of APC results in lethality under homozygous condition, while

less than 30% of heterozygous fish developed liver and intestinal tumours during 15 months of age onward. APC heterozygotes of zebrafish were treated with 7,12-dimethylbenz[a]anthracene to enhance tumourigenesis in intestinal, hepatic, and pancreatic tumours, with frequencies three- to fourfold higher than treated wild-types. The tumours displayed activated Wnt signalling, indicating conserved genetic pathway. Other study with two rat models is developed with the polyposis in the rat colon (Pirc) and Kyoto APC Delta (KAD) strains. Each carries APC gene mutations in the intestinal cancer; the heterozygous Pirc strain and the homozygous KAD strain reveal that these models closely mimic APC-dependent neoplasia of humans, and tumours form more frequently in the colon than in the small intestine. This occurs more frequently in males than in females (Irving et al. 2014; Robertis et al. 2011). Thus, it has been implicated in colorectal carcinogenesis, and its stability in the cell is regulated by APC (Schneikert and Behrens 2007; Kwong and Dove 2009).

3.2.2.4 Growth Factors

3.2.2.4.1 EGFR

EGFR has been shown to be overexpressed in colon cancer cell lines and is detectable by immunohistochemistry (IHC) in 70–80% of CRC tumours (Reyes et al. 2014; Mironea et al. 2016). Right-sided colon cancers, which are more often poorly differentiated, express EGFR more intensely than left-sided cancers. Overexpression of EGFR is associated with poor prognosis in most of the studies. EGFR represents an attractive target for anticancer therapies in a variety of malignant neoplasms, including CRC, non-small-cell lung cancer (NSCLC), head and neck carcinomas, and gliomas (Krasinskas 2011).

Recent studies on chemo-refractory colon cancers appear modest increase in copy number (three- to fivefold) present up to 50% of cases; however overexpression of the EGFR and its ligands, TGF-, has been correlated with poor prognosis. Ligand (TGF-) binds to EGFR, causing homo- or hetero- dimerization, enabling downstream signalling. Cancer cells under hypoxia secrete TGF-, thus ligand signals to EGFR in cell surface to stimulate downstream signalling cascade in involving RAS/MAPK and anti-apoptosis (phosphatidylinositol 3-kinase [PI3K]/Akt) and sequentially turn cell survival and cell proliferation. Overexpression of TGF- and EGFR, additionally by carcinomas, is correlated to poor prognosis and cancer metastasis, exhibiting resistance to chemotherapy (Sasaki et al. 2013).

Therapies in CRC treatment involves the EGFR which controls signalling pathways involved in cell differentiation, proliferation, and angiogenesis (Table 3.3). Similarly, overexpression of EGFR alone is correlated with poor differentiation and reduced survival of 1.5 years. EGF, VEGF, and their receptor expression were observed on tumour-associated endothelial cells and have been correlated to

Table 3.3 Components of the EGFR signalling pathway and its importance in CRC

Component (gene/ protein)	Functional protein	Causes/effects in CRC	Frequency of prevalence in CRC patients	Prognostic evidence
EGFR/EGFR	Tyrosine kinase receptor (transmembrane)	Abnormal protein expression, mutation, increased copy number of proteins	More than 90% (25–90%)	Unknown to controversial
KRas/KRas	Ligand-dependent signalling of GDP-/ GTP-binding proteins	Leads to activation of MAPK pathway	30–40%	Controversial
BRAF/B-Raf	Serine-threonine protein kinase (KRAS in downstream)	Mutation in protein	5–12%	MSS tumors-poor prognosis
PIK3CA/ PI3K	PI3K-Akt pathway inhibition	Mutation in exons 9 and 20	14–18%	KRas tumor-poor prognosis
PTEN/PTEN	PI3K inactivation by tyrosine phosphastase enzyme	Loss of heterozygous of protein expression due to mutation	13–19%	KRas tumor-poor prognosis

angiogenesis and tumour progression (López et al. 2012). Hence, inhibition of EGFR signalling pathways represents one of the good strategies for therapeutic intervention for CRC. The antigenic proteins, VEGFA and IL-8, were also strongly expressed in the microenvironment of tumours that produced TGF- (Grossmann and Samowitz 2011; Sasaki et al. 2013). In contrast, expression levels of VEGFA and IL-8 were considered unremarkable in TGF--deficient tumours, while imbalance of macrophages was observed in extracellular matrix (Sasaki et al. 2008, 2013). According to researchers, it is difficult to summarize significance of *EGFR* gene amplification/increased *EGFR* copy number, but studies report that *EGFR* gene is common in CRC. Thus, EGFR has been evolving its role as a prognostic and predictive biomarker in colon cancer (Krasinskas 2011; Joo et al. 2016).

3.2.2.4.2 VEGF

VEGF expression was observed in all surgical specimens, including normal mucosa, primary colon cancers, and metastatic tumours (>20 specimens). Many reports support the hypothesis that VEGF is an important angiogenic factor in all cancer and indicates that the vessel count and the expression of VEGF may be useful in predicting metastasis from CRC (Ellis et al. 2000). Studies with patients determine that it could serve as prognostic markers in node-negative CRC. Those results showed that patient with low VEGF expression had a significantly better survival than patients with high VEGF expression. Thus, VEGF appears to be the predominant angiogenic

factor in human colon cancer and is associated with metastases formation and poor prognosis (Ellis et al. 2000; Bendardaf et al. 2017).

Angiopoietins (Ang) are also expressed by human colon carcinoma. Using reverse transcriptase-polymerase chain reaction (RT-PCR), Ang-1 and Ang-2 expression was measured in normal colonic mucosa, colon cancer specimens, and colon cancer cell lines. Preliminary studies suggest that an imbalance of activity of Ang-2 over Ang-1 may play a role in colon cancer angiogenesis. Results showed a relatively equal frequency of expression of Ang-1 and Ang-2 in all tissues, whereas Ang-1 was not expressed in any of the cancer specimens, while Ang-2 was expressed in all of them. In *in vitro* study, Ang-1 serves as a survival factor for endothelial cells (EC), in conjunction with VEGF to help stabilize vascular networks (Goel et al. 2011; Pafumi et al. 2015). According to Yuan et al. (2009), Biel and Siemann (2016) examined the effect of Ang-1 and observed dose dependently inhibited apoptosis in human umbilical vein ECs (HUVECs), suggesting that Ang-1 acts in conjunction with VEGF and this response was indeed dependent on Tie-2 activation, which might have completely blocked the effects of Ang-1 (Dalton et al. 2016).

SU5416, tyrosine kinase inhibitor of VEGF, led to a decrease in tumour burden and improve survival in mice with liver metastasis. Increases in Src activity are also observed in the majority of colon tumour metastasis (Ellis et al. 2000; Dinarello 2011; Terracina et al. 2015). Studies suggest that the higher expression and specific activity of Src kinase in colon tumour cells can be augmented with the ability of hypoxia to induce VEGF. However, in HT29-AS15 cells, in which c-*Src* expression has been reduced fourfold, the ability of hypoxia to induce VEGF mRNA is severely impaired. These results suggest that in this colon tumour cell system, Src kinase regulates both inducible and constitutive pathways leading to VEGF production. Further confirmation of the ability of Src kinase to regulate inducible VEGF expression was derived from a study of Fleming et al. (1997) in which the ability of cell density was found to up-regulate VEGF expression. Our results suggest that constitutive Src activation may be a primary pathway leading to production of angiogenic factors in colon cancer. Other pathways resulting from genetic changes in colon cancer may also be responsible for the induction of angiogenic factors. These data suggest that the activation of anti-apoptotic pathways mediated by Akt and survivin in ECs may contribute to Ang-1 stabilization of vascular structures during angiogenesis. In another study, Tsai et al. (2015b) and Saif (2013) compared pre- and post-treatment VEGF expression by IHC in 57 patients with mCRC who underwent treatment with 5-fluorouracil (5-FU) and irinotecan (FOLFIRI regimen) combined with bevacizumab. Results indicated that decreased peri-therapeutic, low post-treatment, and VEGF expressions were significant predictors of response to therapy and 6-month progression-free survival (PFS) (Lee et al. 2015).

3.2.2.4.3 IGF

The insulin/insulin-like growth factor (IGF) system is a multifactorial signalling network that modulates energy metabolism, cell growth, and cancer. It consists of a family of six circulating IGF-binding proteins (IGFBPs) that may act as tumour suppressors by limiting IGF activity. According to Firth et al., IGFBPs may have IGF-independent effects on cancer growth, while Edward (2001) suggests multiple markers of hyperinsulinemia (e.g. low physical activity, high body mass index, central adiposity, and high IGF-1 levels) that are also correlated with higher risk of CRC. Followed with these results, the advent of sedentary lifestyle related with obesity and excess carbohydrates and saturated fatty acids increases CRC incidence. Moreover, it has been demonstrated that the increased blood levels of insulin in type 2 diabetes individuals, caused by insulin resistance, enhance the risk to develop colon cancer (Sridhar and Goodwin 2009).

According to Nahor et al. (2005), endogenous IGF-1R levels are reducing in a dose-dependent manner which is directly related to IGF-1R promoter, and hence mutated p63 and p73 are impaired of their ability to suppress IGF-1R in CRC. In case of hyperactivation of the IGF-Rs, HER2, and MET, CRC cells escape EGFR-dependent oncogene mechanism and increase IGF-1R signalling which is with less sensitivity to EGFR inhibition because of functional crosstalk between IGF-1R and the EGFR. Likewise, it is also associated with Akt activation and upregulation of anti-apoptotic protein Bcl-xl and the PI3K/Akt pathway, which was a study based on colon polyps from healthy subjects. In further experimenting with a human CRC cell line over expressing, the IGF-1R-HCT116/IGF-1R resulted in highly invasive tumour and produced distant metastasis in murine models (Vigneri et al. 2015)

People with type 2 diabetes and people with acromegaly, who have elevated levels of insulin and IGF-1, are at elevated risk of colon cancer in most studies (Lugo et al. 2012). Recently, studies that have directly assessed circulating concentrations of C-peptide, 2-h insulin, and IGF found that these predict risk of colon cancer and adenoma. Determinants like physical inactivity, high BMI, central adiposity, and markers like hypertriglyceridemia of insulin resistance and high IGF-1 levels are consistently related to higher risk of colon neoplasia (Vidal et al. 2012). High IGF and low IGFBP-3 are associated with increased risk of several common cancers, including those of the prostate, breast, colorectum, and lung. In addition to stimulating cell cycle progression, IGF-1 also inhibits apoptosis (Livingstone 2013). IGF-1 can stimulate the expression of Bcl proteins and suppress expression of Bax, which results in an increase in the relative amount of the Bcl/Bax heterodimer, thereby blocking initiation of the apoptotic pathway (Shamas-Din et al. 2013).

IGF-1R gene expression partly regulated by p53, but mutation, deletions, epigenetic silencing, or post-translational inactivation unleash IGF-1Rs oncogenic potential. Others like dysregulation of IGF-1 and enhanced activation of IGF-1R are also involved in exhibiting resistance over anticancer therapies like chemotherapy, hormonal agents, biological therapies, and radiation (Wang and Sun 2010). According to Denduluri et al. (2015), multidrug resistance-associated protein 2 (MRP-2) expression increases with IGF-1R signalling; this in turn reduces the intra-

cellular concentrations of multiple cytotoxic drugs. *In vitro* silencing of the IGF-1R suppresses MRP-2 in CRC cells and, thereby, increases the chemotherapeutic effect of intracellular drug concentration of 5-fluorouracil, mitomycin C, oxaliplatin, and vincristine. This effect is also mediated by the PI3K/Akt pathway, which causes nuclear translocation of nuclear factor-like 2 and reduces MRP-2 expression.

3.2.2.4.4 PIGF

Placental growth factor (PlGF) is an angiogenic protein belonging to the VEGF family which is upregulated mainly during the pathologic conditions. Accumulating reports have suggested that PlGF might be a useful prognostic marker of cancer progression. In a study with renal cell carcinoma patients, increased plasma PlGF levels correlate with tumour grade and survival and useful as a prognostic indicator of recurrence and survival in CRC (Van and Pot 2016). mRNA and protein level of PIGF in tumour tissues have also been found to be correlated with tumour stages in lung cancer and progression of disease and survival in case of CRC, with tumour stage and patient survival in gastric cancer, with recurrence, metastasis, and mortality in breast cancer and even with postoperative early recurrence in hepatocellular carcinoma (Kim et al. 2011).

3.2.2.4.5 TGF-Beta

TGF-beta signalling pathway is one of the most prevalent types of mutation during CRC progression. This pathway is involved in numerous processes in the development and homeostasis of adult tissues. This signalling is important in stem cell developments, and nearly all colon cancers have mutations that inactivate pathway components. TGF-beta ligands activate the signalling pathway by binding to TGF-beta receptor type II homodimers. Apoptosis is one of the anticancer effects by arresting cells in G1 stage during cell cycle. Ligand-bound receptor II recruits TGF-beta receptor I homodimers, which are subsequently transphoshorylated and thus activated by receptor type II. Phosphorylation of the intracellular mediator *SMAD* activated receptor I allows dimer formation with SMAD-4 and translocates to the nucleus, where the specific outcome of the signalling will depend on the cell type and the context of the cell itself. Mutations found in CRC affect mainly the TGF-beta receptor type II and the intracellular *SMADS*, SMAD-2, and SMAD-4, by abolishing the transcriptional effects mediated by TGF-beta.

3.2.2.5 Apoptosis

Apoptosis morphologically defined as a form of programmed cell death, a cellular process that is of tremendous current interest to clinicians who study and treat cancer. As a rule, it is thought that the equilibrium between the rates of cell growth and apoptosis sustains intestinal epithelial cell homeostasis, and this stability gets

disturbed during cancer expansion (Wong 2011). Abnormalities in apoptotic function contribute to both the pathogenesis of CRC and its resistance to chemotherapeutic drugs and radiotherapy, both of which act, at least in part, by killing cancer cells (Abraha and Ketema 2016). These epithelial cells have been shown to have a marked tendency to undergo apoptosis following DNA damage. The mechanism by which DNA damage induces apoptosis in the intestine has not been fully elucidated. MDB4 plays a significant role in detecting damage, and coupling this to apoptosis thereby suppresses neoplasia in APC Min/+ mice (Watson 2004; Abraha and Ketema 2016).

The molecular signals that create the stem cell niche at the base of the colonic crypt are currently being identified and have already been implicated in the regulation of apoptosis. Central to this niche is regulation of b-catenin/T-cell factor (Tcf) activity by the Wnt signalling pathway. In the absence of WNT signals, b-catenin is held in a complex with glycogen synthase kinase 3b (GSK3b), axin/conductin, and APC that is rapidly degraded (Stamos and Weis 2013). GSK3b functions to target b-catenin for destruction by ubiquitination. c-Myc, a target of the b-catenin/TCF signalling pathway, has two functional outputs: cell division and apoptosis. The capacity of c-Myc to induce cell division is potent but is not unleashed in normal cells unless apoptotic mechanisms are simultaneously inactivated. c-Myc sensitizes cells to many apoptotic stimuli, including DNA damage, which are sensed through the p53 pathway and mediated by the BH3-only proteins Puma and Noxa together with Bax (Stamos and Weis 2013). During each cell cycle, telomere shortening results in the risk of chromosomal instability (CIN) that increases with chromatin bridge breakage and the fusion of chromosomal ends. This phenomenon of telomere shortening and resultant CIN has been observed in patients with ulcerative colitis who subsequently develop CRC. Of course, normally such a serious genetic error would be expected to trigger apoptosis and thus eliminate the aberrant cell by protective mechanism, including p53 (Wong RS 2011).

The tumour suppressor gene p53 is mutated in 70% of CRCs. It is a transcription factor that binds to specific sequences in DNA and regulates expression of many pro-apoptotic genes (Rivlin et al. 2011). These include Bax and the BH3-only proteins Puma and Noxa. As discussed above, Bax activation inactivates Bcl-2 and Bcl-xL and triggers release of cytochrome c from mitochondria. p53 also increases expression of components of apoptosis effector mechanisms such as APAF-1 and caspase 6. Furthermore, p53 has essential elements of the extrinsic apoptosis pathway as transcriptional targets such as the death receptor Fas and DR5 as well as the BH3-only protein Bid that couples the extrinsic pathway to activation of the intrinsic pathway. It has been estimated that about half of all cancers express the anti-apoptotic proteins Bcl-2 or Bcl-xL. Retinoid, PPARc, and vitamin D receptor agonists all exhibit potential for reducing Bcl-2 or Bcl-XL expression in specific circumstances. Caution must be exercised as PPAR-agonists stimulate colonic neoplasia (Lin and Jian 2013; Alibek et al. 2014).

Ceramide is an important mediator of endothelial apoptosis following high-dose radiation damage, and it is antagonized by basic fibroblast growth factor. The ischaemic damage caused by endothelial apoptosis may be a key mechanism of action of radiotherapy, and apoptosis also plays a vital role as target for cancer treatment

(Lee et al. 2013; Abraha AM and Ketema EB 2016). The increased mitochondrial permeability and release of pro-apoptotic molecules such as cytochrome c into the cytoplasm to induce apoptosis in this pathway regardless of the pro-apoptotic molecules. This pathway is closely regulated by a group of proteins belonging to the Bcl-2 family, the anti-apoptotic proteins (e.g. Bcl-2, Bcl-XL, Bcl-W, Bfl-1, and Mcl-1). Although less thoroughly studied, p53 also trans-represses the important IAP gene survivin which may directly inhibit caspase activity. For example, p53 mutation renders HCT116 colon carcinoma cells more sensitive to Adriamycin and radiation but less sensitive to 5-fluorouracil. A further complication is that p53 appears to respond to RNA damage rather than DNA damage in response to 5-fluorouracil treatment (Pathak et al. 2015).

3.3 Current Treatments for CRC

CRC is the third most common cancer reported with 1200000 newly diagnosed cases each year and the second leading cause of cancer-related deaths with 600,000 deaths annually. Twenty percent of patients diagnosed for CRC with the symptoms unfortunately grow to have metastatic disease (American Cancer Society 2017). Furthermore, ~30% of patients who are diagnosed with early-stage CRC eventually develop metastatic disease. Nowadays overall survival in metastatic patients has improved approximately to 24 months, due to improved efficacy of standard chemotherapy-targeted agents. For more than 50 years, 5-fluorouracil has represented the backbone of all chemotherapy schedules, used both alone and combined (Braun and Seymour 2011; Burotto et al. 2012; Hocking and Price 2014). The addition of oxaliplatin and irinotecan to fluorouracil-based treatment has increased response rate and overall survival (Howells et al. 2010). Nevertheless, chemotherapy alone, reached 18–20 months survival plateau, was obtained by administering alternatively all active cytotoxic agents during treatment strategy (Goldberg et al. 2009; Giordano et al. 2014). Although the median overall survival of patients diagnosed with metastatic CRC has improved from 9 months to 30 months over the past decade, the 5-year OS remains at 5–15%. However, the poor treatment outcome from CRC metastasis patients, sounds for the development of new therapeutic options (Mousa et al. 2015) (Table 3.4).

According to Zeestraten et al. (2013), a five-step program can be used for the development of new biomarkers. Mainly six features are present in cancer cells that distinguish them from normal cells; one of the main characteristics is its ability to escape programmed cell death or apoptosis. Currently researchers are working to identify those key biomarkers in apoptotic pathways to determine cancer prognosis. There are about 26 potential prognostic biomarkers that are directly involved in apoptotic pathway and have been identified till now. In many cases, external death signals triggered by the extrinsic pathway in turn cause the formation of intracellular signalling complexes at the death receptors. This type of apoptosis is typically activated in immune responses. The second pathway, known as the intrinsic path-

Table 3.4 Current major chemotherapeutic drugs used in treatment of CRC

Drug name	Functions	Mechanisms of action	Type of agent	Side effects
Gefitinib	Autophosphorylation	Indirect-EGFR inhibitor	Small molecule inhibitor	Acceptable adverse drug reactions (ADRs)
Erlotinib	Autophosphorylation	Indirect-EGFR inhibitor	Small molecule inhibitor	Acceptable adverse drug reactions (ADRs)
Panitumumab	Tumor-specific antigens; induce apoptosis	Direct extracellular EGFR domain-inhibitor	Monoclonal antibody	Combined with radioactive particle and other anticancer drugs
Cetuximab	Tumor-specific antigens; induce apoptosis	Direct extracellular EGFR domain	Monoclonal antibody	Combined with radioactive particle and other anticancer drugs
Irinotecan	Type I topoisomerase inhibitors	Interferes DNA synthesis	Plant alkaloids (Camptothecin analogs)	Acceptable adverse drug reactions (ADRs)
Oxaliplatin	Cross-link subunits of DNA	Stop DNA synthesis	Alkylating agent (metal salts)	Severe neuropathies
5-Fluorouracil	Interfering with DNA components	Stop DNA synthesis	Antimetabolites (pyrimidine antagonist)	Neurological (CNS) damage
Mitomycin C	Induces ROS and DNA strand break/damage	Cell death	Antitumor antibiotics	Damage lung cells
Vincristine	Inhibits tubulin assembly in microtubules during cell cycle	Cell cycle toxic; inhibits cell division	Plant alkaloids (vinca alkaloids)	Neurotoxicity
Doxorubicin	Induces ROS and DNA strand break/damage	Cell death	Antitumor antibiotics	Cardiac toxicity
Sulindac	COX inhibitor	Indirectly induce apoptosis	Nonsteroidal anti-inflammatory drug (NSAID)	Damage the liver and pancreas; nonfatal myocardial infarction (MI)

way, is activated by many different stimuli, including growth factor deprivation and DNA damage, caused by factors such as UV or gamma irradiation or by chemotherapeutic agents (Ichim and Tait 2016).

3.4 Herbal Medicines in Anticancer Treatment of CRC

The effort for finding new anticancer agents with better efficacy and lesser side effects has gained researcher's interest for decades, and many traditional recommendations and experimental studies have reported anticancerous activities of numerous medicinal plants (Hosseini and Ghorbani 2015). Similarly, numerous studies have also indicated that many herbal medicines can be used along with chemo- or radiotherapy to diminish the side effects and complications in cancer treatment. In this chapter, many herbal medicines that are commonly used for treatment of cancer patients to reduce the toxicity induced by chemo- or radiotherapy (Yin et al. 2013) have been discussed. Alongside, many herbal plants also exhibit anti-proliferative, pro-apoptotic, anti-metastatic, and anti-angiogenic effects of several phytochemicals which have been shown in *in vitro* experiments or animal studies. But only a few medical plants have been tested on patients, and limited evidence exists for their clinical effectiveness (Melo et al. 2011). Mostly localized colon cancer is often successfully treated with surgery; advanced disease requires aggressive systemic therapy that has lower effectiveness. Approximately 30–75% of patients with colon cancer use complementary and alternative medicine (CAM), but there is limited formal evidence of survival efficacy (Hosseini and Ghorbani 2015).

Many herbal medicines possess antioxidant properties. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species (ROS); they are produced as by-products of biochemical reactions or as signalling molecules which include superoxide and hydrogen peroxides (Lü et al. 2010). When ROS-generating reactions are activated excessively, imbalance between antioxidants and ROS occurs, creating huge quantity of ROS which results in cellular damage. Most of human disease pathogenesis including cancer, aging, and atherosclerosis are directly linked with ROS. It is evident that their damage may be protected by herbal antioxidants which contribute to the total antioxidant defense system of the human body (Li et al. 2015). Previous studies suggest that phytochemicals present in herbal plants may stimulate immunocompetent cells and decrease side effects in patients treated with chemotherapy, but it may or may not affect the levels of antibodies in the blood. One of the essential steps is identifying these herbal sources, to develop better anticancer therapies (Lü et al. 2010; Birben et al. 2012; Nimse and Pal 2015).

Other than phytochemicals, some of minerals and fatty acids present in natural food also have anticancer properties, as chemopreventive agents in colon treatment. Selenium is associated with up to 50% decrease in the risk for colon cancer. It is an important dietary mineral found in broccoli extract, red wine, dietary fibre, pepper, soya, cloves, fenugreek, ginger, apple, and other vegetables. The brassica family of plants synthesizes yellow mustard oil, which also has potential anticancer properties. Mustard contains a complex mixture of long-chain polysaccharides, which may play a protective role in colon cancer formation, and essential oils such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and omega-3 fatty acids are also used to treat and prevent cancer and cardiac diseases. Particularly, the

consumption of fish and fish products reduces the risk of colon cancer progression due to its essential oil content. Vegetables and fruits, rich in fibre content also, may reduce the risk of colon cancer formation (Kuppusamy et al. 2014). Compared to patients treated by chemotherapy alone, patients treated with chemotherapy and herbal extracts showed less likely to experience nausea and vomiting or low white blood cell counts (Kuppusamy et al. 2014). This was proved with an experimental study consisting of 193 patients, up to a 10-year follow-up study by San Francisco Bay-Area centre for Chinese medicine (Pine Street Clinic, San Anselmo, CA). Study done with patients choosing short-term treatment lasts the duration of chemotherapy/radiotherapy than those continuing with long term. Herbal medicine along with vitamins combined with conventional therapy has better effects when compared with conventional therapy alone, which reduced the risk of death in stage I by 95%, stage II by 64%, stage III by 29%, and stage IV by 75%, suggesting that this experiment with prospective trials combining herbal medicine with conventional therapy are justified to be effective (Yin et al. 2013).

3.5 Herbal Plants Against Cancer Mechanism of Action

3.5.1 Crude Plant Extracts and Their Mechanism of Action

3.5.1.1 *Radix astragali*

Radix astragali, frequently used in Asian population as health food supplement, serves as a leading herb in many traditional medicine formulations. The extract of this plant contains total astragalus saponins (AST), the major active constituent found in this herb, and it acts as anticancer agents. AST also shows prominent effects against colon cancer growth. Study, done with HT-29 cell with nude mice tumour xenograft, showed that AST could downregulate circulating VEGF level in serum through inhibition of COX-2, under both normoxic and hypoxic conditions. Study conducted by Law et al. (2012) revealed that AST could significantly reduce tumour growth in nude mice by inhibiting cell proliferation and promoting apoptosis. Similar study with HCT 116 colon cancer cells showed that AST caused PTEN upregulation, reduction in Akt phosphorylation, and subsequent activation of mTOR and suppression of HIF-1α and VEGF under $CoCl_2$-mimicked hypoxia (Lopez-Sanchez et al. 2014). These effects were exaggerated by combined treatment of AST with the mTOR inhibitor rapamycin which could attenuate cobalt chloride-evoked COX-2 activation, while such can cause effect on COX-2 and its downstream target VEGF. In another study, with HCT116 xenografted athymic nude mice, protein level of p-Akt, p-mTOR, VEGF, VEGFR1, and VEGFR2 was downregulated, which effectively reduced COX-2 expression in tumour sections compared to the untreated control (Law et al. 2012; Ran et al. 2016).

Another extract of this plant, Huangqi, is antineoplastic and the most important herb to maintain normal blood levels by the myelosuppressive actions. This is a master herb used in wound healing and may activate telomerase, extending the lengths of the shortest telomeres which protect the terminal DNA at the ends of all chromosomes. This prime herb, which helps in maintaining spleen and lung function, raises WBC count and increases phagocytosis and NK cells; Huangqi is used as intervention with chemotherapy. In a study with decoction of Huangqi, there was a significant reduction of nausea and vomiting in chemotherapy patients and decreased rate of WBC count, increased T lymphocyte (CD3, CD4, and CD8) was observed, but there was no significant difference in immunoglobulins (Taixiang et al. 2005). The decoctions of Huangqi, Chinese herbal medical plant compounds, may stimulate immunocompetent cells and decrease side effects like nausea and vomiting or low white blood cell counts in chemotherapy. This evidence suggests that the decoctions also stimulated cells of the immune system, but did not affect the levels of antibodies in the blood (Cheng et al. 2017). Taken together, these findings suggest that AST exerts anti-carcinogenic activity in colon cancer cells through modulation of mTOR signalling and downregulation of COX-2, which in turn reduce VEGF level in tumour cells that could effectively suppress angiogenesis in both *in vivo* and *in vitro* (Law et al. 2012).

3.5.1.2 Ginseng

Ginseng herb includes genus *Panax* of the family Araliaceae, especially Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*), and notoginseng (*Panax notoginseng*) that are used in CRC therapeutics. The major pharmacologically active constituents of ginsengs are ginsenosides, which can be classified as protopanaxadiol and protopanaxatriol groups. In a randomized controlled study, it was found that taking 2000 mg per day of American ginseng (containing 3% of the active ginsenosides) significantly improved fatigue symptoms in cancer patients (Wang and Yuan 2008). Wang et al. (2016) studied the anticancer activities of red Asian ginseng, red American ginseng, and red notoginseng. According to him, the major anticancer mechanisms of red ginseng compounds include cell cycle arrest, induction of apoptosis/paraptosis, and inhibition of angiogenesis. The structure-function relationship analysis has revealed that sugar molecules in ginsenosides inversely impact the anti-proliferative potential of these compounds (Jin et al. 2016). In an assay of American ginseng extract with 5-Fu applied SW-480 cells, extract can heighten the arrest of SW-480 cells in the S phase and increase the cell distribution in G2/M phase compared with 5-FU applied alone. The trend of increasing cyclin A was exhibited, like the increase of S and G2/M phase cells in SW-480 cells. The enhancement of S and G2/M phase arrest, rather than cell apoptosis, might be the mechanism of synergistic effects of ginseng extract with 5-FU (Li et al. 2009a, b). While ERK1/2 reactivation delayed in EGF-stimulated SW-480 cells,

phosphorylated ERK1/2 translocated into the nucleus following its primary activation. It remained in the cytoplasm during late-phase activation leading to protein trafficking, blocked reactivation, and concurrently increased caspase-3 activities and thus improved the efficacy of cancer therapies that target ERK signalling (Joo et al. 2016). Panaxadiol enhanced the anticancer effects of 5-FU on human CRC cells through the regulation of cell cycle transition and the induction of apoptotic cells (Wang et al. 2014, 2015a).

Exposure of HT-29 human colon cancer cells to ginseng extracts resulted in time-dependent inhibition of histone deacetylase (HDAC) activity, results in accumulation of acetylated histones H3 and H4 within cellular chromatin, and enhances more histones to bind to the promoter sequences of the tumour suppressor gene runt-related transcription factor 3 (RUNX3), as well as p21, a downstream target of RUNX3. These alterations were consistent with cell cycle arrest at the G0/G1 phase and induction of apoptosis (Zheng et al. 2013) while they inhibited the phosphorylation levels of the extracellular signal-regulated protein kinases 1/2 (ERK1/2) and (H3) in HCT116 cells (Yang et al. 2016a). These experimental studies provide new insights into the mechanisms of ginseng to human CRC cells. Taken together, these results suggest that the induction of autophagy and apoptosis is mediated through ROS generation and JNK activation in human colon cancer cells (Yu et al. 2015).

The cytotoxic mechanism includes the involvement of ROS and the mitochondrial-involved apoptosis via the modulation of Bax and Bcl-2 expression, resulting in the disruption of the mitochondrial membrane potential. Cytochrome c release from the mitochondria, resulting in the activation of caspase-9 and caspase-3 (Du et al. 2012) and concomitant poly(ADP-ribose) polymerase (PARP) cleavage, which are the indicators of caspase-dependent apoptosis (Lee et al. 2010; Kang et al. 2013, Kim et al. 2013). Decrease in the levels of anti-apoptosis regulator Bcl-2 blocks ROS by inhibiting catalase activation of NF-κB signalling and enhanced ginsenoside like Rh2, Rg3, and Rh2-induced cell death, suggesting that the anticancer effect of Rh2 can be enhanced by antioxidants (Li et al. 2011; He et al. 2011a, b; Kim et al. 2014). American ginseng increased Rg3 and Rh2 content and anti-proliferative activity significantly in NF-kappa B-dependent manner (Luo et al. 2008; Tang et al. 2009; Fishbein et al. 2009).

Mitochondrial damage, increased ROS, and apoptosis in CRC cells via its antioxidants properties with several ginsenosides like Rh2, Rg3, and Rk1 exhibited anti-proliferative and anti-angiogenesis effects *in vivo* and *in vitro*. The mechanisms involved behind this action include NF-kB pathway inhibition of ginsenosides which in turn inhibits cell proliferation and induces apoptosis in cancer cells due to cell cycle arrest in G1 phase and G1/S phase checkpoints (Li et al. 2010; Park et al. 2011). This cell cycle arrest is involved with upregulation of tumour suppressor proteins P53 and P21 tumours and downregulation of cyclin and CDK including the CDK 2, cyclin E, and D1 in G1 phase and G1/S checkpoint (Park et al. 2011; Seo and Kim 2011; Vayghan et al. 2014), thus resulting in apoptosis. Increased apoptosis increases NO production via PI3-kinase/Akt pathway, suggesting an effect of inhibiting angiogenesis (Chen et al. 2014a, ; Han et al. 2016).

3.5.1.3 Mistletoe (Viscum album)

Mistletoe, commonly known as mistletoe, is a semiparasitic woody perennial that grows on several species of tree, including elm, apple, pine, and oak. Mistletoe leaves and young twigs are used by herbalists, and its preparations are made to treat circulatory and respiratory system problems. Animal study and cell line works suggest that mistletoe extract may boost immune system and kill cancer cells. In an experiment, the whole plant mistletoe extract was given to 61-year-old man with a pancreatic adenocarcinoma as once in a week for 5 weeks. After course of treatment, microscopic examination was conducted which revealed dense perivascular lymphocytic infiltrate and increased monocytes, and it protects the DNA in white blood cells (WBC) (Ma et al. 2008).

According to an interesting experiment in University of Adelaide, the researchers conducted an experiment with an extract of mistletoe to focus on whether mistletoe could complement chemotherapy or replace chemotherapy as a treatment for colon cancer. They found that one of the mistletoe extracts, from the *Fraxini* species (which grows on ash trees), was more potent against colon cancer cells in cell cultures compared with other three types of mistletoe extract. It also increased the potency of chemotherapy when used in conjunction. The mistletoe extract was also tested for treatment prolonging survival time of patients with carcinoma of the colon, rectum, or stomach; breast carcinoma with or without axillary or remote metastases; or small cell or non-small-cell bronchogenic carcinoma, and treatment achieved a clinically relevant prolongation of survival time of cancer patients and appears to stimulate self-regulation. Pooled analysis of clinical studies suggests that adjuvant treatment of cancer patients with the mistletoe extract is associated with a better survival. To contradict, another study suggested that mistletoe extract does not seem to be active in metastatic CRC resistant to 5FU/LCV in terms of objective tumour response.

In a retrospective study on 127 patients with CRC, the use of mistletoe extracts was used as possible prognostic indicators. From previous study mistletoe has been established as a potent anticancer agent who could strongly reduce human colon cancer HT 29 cell line growth *in vitro* through MTT bioassay. Hence, randomized controlled study was done on postoperative patients CRC stage; 40 patients in Dukes C and 24 patients in D received 5-FU chemotherapy, six cycles (either the Mayo or the de Gramont protocol). These 64 patients were randomly allocated into three groups as only chemotherapy, for 21 cases; chemo and mistletoe biotherapy for 29 cases and 14 patients underwent only surgery was kept as control group. As a result, they observed that patient treated with chemotherapy and biotherapy had median survival significantly superior to those of patients receiving only postoperative chemotherapy. This finding demonstrates benefit in terms of survival from both combined postoperative chemotherapy and mistletoe biotherapy, either as adjuvant or palliative (Bar-Sela and Haim 2004).

The molecular and cellular mechanism by which mistletoe extracts exerted the cytotoxic and immunomodulatory antitumour effect is largely unknown. Harmsma et al. (2006) studied mistletoe preparations induced tumour regression by cell cycle

inhibition and/or interference with apoptotic signalling pathways in cancer cells. Also, possible effect on angiogenesis, which is a prerequisite for tumour growth *in vivo*, is studied in endothelial cell cultures. Mistletoe caused early cell cycle inhibition followed by apoptosis in a dose-dependent manner. Apoptosis was induced by activating the mitochondrial but not the death receptor-dependent pathway. Mistletoe also seemed to induce apoptosis via the death receptor route, which may explain the higher sensitivity of cancer and endothelial cells to this preparation (Harmsma et al. 2006). Anticancer mechanisms of Korean mistletoe lectin-induced apoptotic human colon cancer cell line reported that cell death was activated due to caspases and inhibition of anti-apoptotic proteins, in part through the tumour necrosis factor receptor 1 signalling pathway. Treatment of human colon cancer cells with mistletoe activated caspase-2, caspase-3, caspase-8, and caspase-9 and decreased expression of anti-apoptotic molecules including receptor interacting protein, nuclear factor-kappaB, X-linked inhibitor of apoptosis protein, and Akt/protein kinase B (Khil et al. 2007; Friedel et al. 2009).

Bock et al. (2014) examined the fatigue levels during first-line chemo- or radiochemotherapy protocols, which were supported by a pharmaceutical mistletoe preparation. Out of 181 patients, 16 patients (8.8%) were diagnosed with CRF in the supportive care group, whereas 86 out of 143 (60.1%) in the chemo- or radiochemotherapy group without supportive mistletoe medication turned with CRF. Clinically, mistletoe medication is the first candidate to be included in a supportive care modus into chemo- or chemo-radiotherapy protocols for colorectal patients to improve CRF without discernible toxicities.

3.5.1.4 Green Tea

Green tea, native to China and India, has been consumed and hailed for its health benefits for centuries globally and one of the most popular affordable drinks in the world. Several health benefits have been explored based on its active metabolites, and antioxidant compounds of green tea play a role in protection against several types of chemically induced cancer in animal models and human on consumption (Babu et al. 2008; Chacko et al. 2010; Hu et al. 2016). Green tea contains a high amount of antioxidant polyphenols, phytochemicals such as heterocyclic amines, flavones, and saponins that can alter xenobiotic-metabolizing enzymes, which effectively controlled cancerous growth in both *in vitro* and *in vivo* models (Kuppusamy et al. 2014; Pampaloni et al. 2014). These metabolites induce the signal transduction pathway which leads to induction of apoptosis and cell cycle arrest. Some studies have asserted that the high consumption of black tea is also associated with reducing the risk of digestive track cancers (Fujiki et al. 2015a; Seif 2016). Regular green tea consumption as three times per week for 6 consecutive months is found to be associated with reduced CRC in non-smokers (Yang et al. 2011). Many biologically active compounds are present in green tea which includes the predominant polyphenols in green tea; (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC) have

antioxidant activity. These chemicals, especially EGCG and ECG, scavenge free radicals present substantially and protect cells from DNA damage caused by ROS (Fujiki et al. 2015a, b). Tea polyphenols have shown inhibitory effect to tumour cell proliferation, induce apoptosis in laboratory and animal studies, inhibit angiogenesis and tumour cell invasiveness, protect against damage caused by ultraviolet (UV) B radiation, and improve immune modulatory system function (Babu et al. 2008; Chacko et al. 2010; Yang et al. 2011; Seif 2016).

Reports conducted from many experimental studies support green tea as a chemopreventive agent for CRC, but no quantitative summary of the epidemiologic evidence on the role of green tea and CRC risk has ever been performed. So currently meta-analysis study was performed by Sun et al. (2006) including 25 papers conducted in 11 countries across three continents (North America, Asia, and Europe). From the odds ratios (ORs), highest versus non-tea/lowest tea consumption levels were calculated based on fixed and random effects models, and the meta-regression and stratified methods were used to examine heterogeneity across studies. The combined results from eight studies indicated a reduced risk of CRC with intake (OR = 0.82), effective among three-colon cancer case-control study with OR=0.74, from rectal cancer study OR=0.99, irrespective of study design. Cohort studies of colon cancer (summary OR = 0.99, 95% CI = 0.79–1.24) were compatible with the null hypothesis. Despite the convincing evidence from *in vitro* and non-human *in vivo* studies in support of green tea as potential chemopreventive agents against CRC, available epidemiologic data are compiled in Table 3.5, to conclude various protecting mechanisms of green tea against CRC in humans (Hao et al. 2017).

In conclusion, supplementation of green tea extract (GTE) regulates targeted biomarkers related to CRC specifically to genes associated with WNT signalling (β-catenin), inflammation (NF-κB) and methylation (DNMT1). Combinations with other chemotherapeutic agents provide additional effects compared with either agent alone (Hu et al. 2016). Furthermore, green teas have been shown to activate detoxification enzymes, such as glutathione S-transferase and quinone reductase, which helps to protect against tumour development (Jin et al. 2010). Other beneficial properties including its antioxidant activity also help to prevent cancer, yet its mechanism has not been established. Researchers believe that elevated level of polyphenols in green tea helps to kill cancerous cells and stop them from growing. However, the exact mechanism by which tea interacts with cancerous cells is unknown (Hu et al. 2015a, ; Shin et al. 2017).

3.5.1.5 *Ganoderma Lucidum*

Ganoderma lucidum, medicinal mushroom, is commonly used as Chinese herb and an important ingredient in traditional Chinese medicine herbal formulations. *Ganoderma lucidum* extract (GLE) is rich in antioxidant activity, while anti-proliferative effect of extracts was observed in SW480 cells, and it also possess anticancer effect and may also help to decrease chemotherapy-induced side effects. GLE-1 inhibited DNA synthesis in the cells and reduced the formation of free radicals

Table 3.5 Role of green tea extracts as anticancer agent

Green tea constituents	Model	Activity	Causes/signalling pathway involved	Gene involved	References
EGCG, polyphenolic constituent	HT-29	Inhibits growth, invasion, and metastasis (tumorigenesis)	–	Matrix metalloproteinase (MMP7, MMP2, MMP9)	Kim et al. (2005)
		Inhibits topoisomerase I	Induces DNA damage, cell cycle arrest, and causes apoptosis	Anti-proliferative agent	Berger et al. (2001) and Hajiaghaalipour et al. (2015)
		Inhibits receptor tyrosine kinases	Inhibits tyrosine receptors IGF/IGF-1R and VEGFR2	IGF, IGF-1R, VEGF, VEGR, EGFR, HER2, and HER3	Shimizu et al. (2011)
	SW837 cells	Inhibition of tyrosine kinases receptor	Decreased IGF-1R and IGF-1 protein and increased IGFBP3 protein	Increase in the expression of TGF-beta2	Shimizu et al. (2005a, b)
		Non-steroidal anti-inflammatory drugs (NSAIDs)	Regulatory role of AMP-activated kinase (AMPK) in COX-2 expression	Decreased COX-2 promoter activity via inhibition of nuclear factor kappaB (NF-kappaB) activation	Peng et al. (2006), Hwang et al. (2007), and Park et al. (2009)
		Reactive oxygen species (ROS)			
	Mice (nude mice) cells	Inhibits cell proliferation	APC/b-catenin	Decreased COX-2, c-MYC, and cyclin D1 protein	Patel et al. (2008) and Sukhthankar et al. (2008)
				Reduced bFGF expression	
		Inhibits APC	Adenomatous polyposis coli (APC)	Repressed expression of cyclin D1, c-myc, and degradation of β-catenin	Oh et al. (2014), Chen et al. (2017), and Kim et al. (2017b)
			Downregulates Wnt/β-catenin		
		Inhibition	Mitogen-activated protein kinase (MAPK) and Akt pathways	Kinase inhibitors, Akt, ERK1/2, or alternative p38MAPK activity	Cerezo-Guisado et al. (2015)
		miRNA		Suppressed notch1, Bmi1, Suz12, and Ezh2 and upregulated self-renewal suppressive-miRNAs, miR-34a, miR-145, and miR-200c	Toden et al. (2016)

	HCT-116 cells	Hypoxia condition	Inhibits HIF-1 alpha	Suppressed NF-kB, VEGF/VEGFR expression	Navarro-Perán et al. (2008), Shimizu et al. (2010), and Sukhthankar et al. (2010)
		Methylation-sensitive colon cancer cells	Apoptosis	E3 ubiquitin ligase, UHRF1	Moseley et al. (2013)
Green tea extract + protopanaxadiol (PPD)	HT29 cells	Enhances antioxidants of GT	Inhibits the activation of NF-κB signalling	Tumor controls	Wang et al. (2013)
EGCG and Poly E	HT29 cells and FHC cell line	Inhibits phosphorylation of protein	Kinase and Akt signalling	Decrease EGFR and HER2, NFk-B, Cyclin D1, HER2, and HER3	Shimizu et al. (2005a) and Xu et al. (2010)

(Xie et al. 2006). To confirm cancer-preventive effects of GLE, Oka et al. (2010) performed a no-treatment and GLE-treated controlled trial on patients with colorectal adenomas, which inhibited G2/M phase of cell proliferation through downregulation of cyclin A and B1 and upregulation of p21 and p27, revealing tumour shrinkage of CRC in nude mice (Hsu et al. 2008; Na et al. 2017)

The mechanistic effects of GLE were focused on the PI3K/Akt/mammalian target in IBC SUM-149 cells, which resulted in reduced expression of mTOR, and its downstream effectors at preliminary treatment time, with time eIF4G levels, are reduced which was coupled with increased levels of eIF1E and reduced protein synthesis (Suarez-Arroyo et al. 2013). Moreover, there was greater degree of reduced small intestinal damage in 5-FU plus GLE-treated rats than in 5-FU alone treated, and regulation of cell survival and growth was observed in IBC SUM-149 cells. This results in reduced expression of mTOR indicating that GLE could have potential to be used as agent against colonic precancerous lesions and to treat the common adverse effects of chemotherapy (Watanabe et al. 2013). Potent anti-proliferative and anti-colony formation activities were exhibited on HT29 and HCT116 CRC, by inducing cell cycle arrest in G1 phase through the regulation of cyclin D1 and P53 expression, while in HCT-116 cells by inducing cell apoptosis and activating unfolded protein response and caspase-9 regulated pathways. During stress condition, cancer cells undergo autophagy, a stress adaptation mechanism which is suppressed by GLE treatment; hence apoptosis of HT29 cells might be triggered by GLE (Thyagarajan et al. 2010; Dan et al. 2016). In another study, GLE reactivated mutant p53 in CRC HT29 and SW480 cells while applied alone or together with 5-fluorouracil (5-FU). This reactivation further induced cell growth inhibition and apoptosis (Jiang et al. 2017; Na et al. 2017).

HCT-119 cells were used to study GLE antitumour activity; according to Liang et al. (2014), increased level of caspase-8 activity was observed which is related to apoptosis. Fas and caspase-3 protein expression was upregulated after GLE treatment. This was the first experiment demonstrating GLE mechanism through elevated intracellular calcium release and the death receptor pathway (Liang et al. 2014). According to study conducted by Kim et al. (2015b), Khz (a fusion mycelium of G. lucidum and Polyporus umbellatus mycelia), cytotoxicity was measured using MTT assay and found that Khz suppressed cell division and induced apoptosis via mitochondrial disruption by changing membrane potential, increasing calcium concentration and ROS generation. Increased caspase-3, PARP, caspase-7, and caspase-9 levels, but reduced Bcl-2 protein levels, lead to reduced cell viability. The activation of caspases-3, caspase-8, and caspase-9 is involved with GLE-stimulated apoptosis. Additionally, treatment with GLE promotes the expression of Fas and caspase-3 proteins while reducing the expression of cleaved poly(ADP-ribose) polymerase. As a result antitumour activity of CRC cells was observed through inhibition of migration and induction of apoptosis (Qi et al. 2010; Liang et al. 2015).

3.5.1.6 *Phyllanthus Watsonii*

Phyllanthus watsonii Airy Shaw is an endemic plant found in Peninsular Malaysia, although anticancerous property has been reported earlier, but cytotoxicity effect was report very less. The lignan compound, phyllanthin, is the known principal constituent, while sterol glucoside was also being detected from various marine organisms and alga was reported to possess few cytotoxic activities. Extracts show strong cytotoxicity and high sensitivity towards human gynaecologic and colon cancer cells when compared to normal lung fibroblast cells. Cytotoxic and apoptotic potential of the endemic *P. watsonii* was investigated for the first time by bioassay-guided approach, which indicated that extracts have arrested cell cycle at different growth phases in SKOV-3, Ca Ski, and HT-29 cells (Ramasamy et al. 2012). Extracts on human breast cancer cell MCF-7-induced cell death were mainly due to apoptosis, which is characterized by morphological changes, nuclear DNA fragmentation, and caspase-3 activation. Following *P. watsonii* extract treatment, evident of apoptotic cell death was observed which was preceded by S phase cell cycle perturbation.

3.5.2 Isolated Plant Extracts: Phytoproducts and Its Action Mechanism

3.5.2.1 Curcumin

Curcumin is a naturally occurring powerful anti-inflammatory medicine and active ingredient present in the spice turmeric. It has been shown with anticancer properties in various animals and cell culture studies (Patel et al. 2010). Epidemiological data shows incidence of CRC is lower in countries with regular use of turmeric, which is present in spicy curry dishes. Bowel disorders in combination with phytochemicals was found to inhibit colon cancer cells from multiplying and spreading by inhibiting the cytochrome P-450 enzyme activity (Hamam 2014). The antitumour effect of curcumin has been attributed in part to the arrest of cancer cells in S, G2/M cell cycle phase, inhibits the growth of DNA mismatch repair, and induces apoptosis in CRC (Sa and Das 2008). Curcumin shows inhibitory effects upon Cox-2 and cyclin D1 which is mediated through NF-κB restricted tumour cell growth. It can also downregulate the expression of various other pro-inflammatory cytokines including TNF, IL-1, IL-2, IL-6, IL-8, IL-12, and chemokines, through inactivation of the transcription factor NF-κB (Mao et al. 2007). With its ability to inhibit the growth of neoplastic cells via various mechanisms, curcumin is king against colon cancer.

Curcumin is the most important secondary metabolites for its anti-carcinogenic properties. It affects protein expression in molecular level and controls cancers cells via COX-2, VEGF, IL-1, IL-6, IGF, and chemokines. It shows lipoxygenase activity against cyclooxygenase-2 (COX-2) expression in malignant cells, and it modulates the action of TNF-α and NF-κB factors. Preliminary observations on COX-2 expression in inflammatory bowel disease (IBD) is correlated to colorectal neoplasia; gen-

esis and progression were clinically reduced with antitumour effects of curcumin. It has the ability to reduce pro-caspase-3 levels, polymerase-1 cleavage, and chromatin condensation. In a time- and dosage-dependent manner study, curcumin caused wild-type p53 HCT-116 cells to self-destruct, while HT-29 cells show a manner inhibition of COX-2, but not COX-1 (Howells et al. 2010). Curcumin selectively destroys cancer cells by triggering the death pathway by increasing the level of protein called genes activated during DNA damage (GADD45a). Despite p53 upregulation and activation, curcumin-induced apoptosis in colon cancer cells occurs independent of p53 status and oxidative stress, independently via G2/M phase arrest (Watson et al. 2010; He et al. 2011b).

According to Rahmani et al. (2014) and Shanmugam et al. (2015), curcumin reduced the indirect association of cortactin, and it significantly decreases the pTyr421-CTTN in HCT116 cells and SW480 cells, but was ineffective in HT-29 cells. It physically interacted with PTPN1 and activates it to reduce cell motility in colon cancer via dephosphorylation of pTyr421-CTTN (Radhakrishnan et al. 2014). It also promotes caspase-3-mediated cleavage of β-catenin, decreases β-catenin/Tcf-Lef transactivation capacity for c-Myc and cyclin D1, and activates caspase-7 and caspase-9 which induce downregulation of NF-κB. Furthermore, it inhibits EGFR activation, Src activity, and activity of some nuclear receptors (Vallianou et al. 2015). It also acts as a potent immunomodulatory agent by activating both immune system via T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells. The molecular mechanism of action of curcumin is via (1) Wnt/β-catenin, sonic hedgehog, notch, and PI3K/Akt/mTOR signalling pathways, (2) microRNA, and (3) multiple level at epithelial-mesenchymal transition.

Thus, curcumin acts as chemosensitizer towards CRC stem cells, and hence they sensitized anticancer therapy. Combination therapy using other anticancer agents like chemotherapeutic agents or other herbal agents, along with curcumin, might be more effective, for example, agents such as silymarin along with curcumin treatment result in high amount of cell death, compared with single treatment (Thamil et al. 2015). In a clinical study conducted, patients showed clinical benefits after administration of curcumin doses of up to 2.2 g per day for up to 4 months reduced bowel polyps. The anticancer effects of curcumin and its derivatives have typically been attributed to inhibition of cell proliferation, cell cycle arrest, and/or induction of apoptosis. It expresses antitumour activity by altering the deregulated cell cycle via (a) cyclin-dependent, (b) p53-dependent, and (c) p53-independent pathways. This provides explanations for how curcumin reverses the multidrug resistance (MDR) of cancer cells (Tuorkey 2014). Curcumin is a potent cancer fighter; through several mechanisms, it can kill a wide range of tumour cell types including MDR cancer cells.

3.5.2.2 Resveratrol

Resveratrol (trans-3,5,4-trihydroxystilbene) is a phytoalexin, toxic compound that is synthesized by plants in response to stress and invasion of other pathogens (Patel et al. 2010). This compound has anti-inflammatory, anti-oxidant, and anticancer activities. This plant-derived ellagic acid has previously been identified as a potent

anticancer agent with molecular targets as NF-kB, cyclin D1APC, and TP53 found in CRC (Doonan et al. 2017), and COX-2 protein observed to be inhibited when cells are treated with resveratrol (RSVL) (Chen et al. 2012; Gong et al. 2017). The grape bioactive compound RSVL potentiates grape seed extract (GSE)-induced colon cancer cell apoptosis at physiologically relevant concentrations like sulindac, without any gastrointestinal toxicity. This RSVL-GSE treatment also reduced the number of crypts containing cells with nuclear β-catenin (an indicator of colon CSCs) via induction of apoptosis by elevated p53, Bax/Bcl-2 ratio, and cleaved PARP (Reddivari et al. 2016).

Experiments were carried on HCT-116 cells to evaluate anticancer potential of RSVL and its action mechanism involved. According to Karimi Dermani et al. (2017), RSVL controls tumour growth through upregulation of miR-200c by regulating apoptosis, invasion, and switching of EMT to MET phenotype in CRC. In other study, p53 and BAX gene expression were elevated after RSVL treatment, suggesting arrest of cell cycle during G2/M phase (Demoulin et al. 2015; Khaleel et al. 2016). Cancerous cells exhibit increased Shh signalling causing increased HCT116 cell viability and migration, inhibited cell apoptosis, and upregulated the expression of Ptch, Smo, and Gli-1. When these were exposed to RSVL, it promoted cell apoptosis and suppressed the protein Ptch, Smo, and Gli-1, which may be mediated by hedgehog/Gli-1 signalling pathways (Du et al. 2016). A synthetic analogue of resveratrol, labelled as HS-1793, can inhibit cell growth and induce apoptotic cell death in a concentration-dependent fashion via cleavage of poly(ADP-ribose) polymerase, alteration of Bax/Bcl-2 expression ratio, and caspase activation (Kim et al. 2017b). In HT-29 cells, the expression of Bcl-XL gene was significantly increased after exposure to RSVL, causing controlled growth possibly by arresting cell cycle in S phase (Schroeter et al. 2015; Khaleel et al. 2016). RSVL shows arrest of cell cycle in the G0/G1 phase and promotes cell apoptosis in colon cancer stem cell-related studies done by Yang et al. (2015). In *in vitro* study, TGF-β1-induced EMT promoted the invasion and metastasis of CRC, reduced the E-cadherin expression and elevated the vimentin expression, and activated the TGF-β1/SMAD signalling pathway (Ji et al. 2015).

CRC when exposed to RSVL significantly inhibits cyclooxygenase-2, indomethacin, and prostaglandin receptor expression (Feng et al. 2016), topoisomerase (TOP) II (Schroeter et al. 2015), inhibition of epithelial-mesenchymal transition (EMT) factors (increased E-cadherin), transcriptional activity of cAMP-responsive element (CRE) (Wang et al. 2015c; Scherzberg et al. 2015), downregulation of NF-κB activation (MMP-9, caspase-3) (Buhrmann et al. 2015), and WNT signalling (Holcombe et al. 2015). These findings enhance the usage of RSVL to develop strategies for diet-derived agents designed to achieve cancer chemoprevention (Del et al. 2013; Cai et al. 2015). Mechanistic study demonstrates RAH inhibits cell cycle arrest through downregulation of cyclins and induces apoptosis by activation of caspase-3 in cancer cells, highlighting the improved anticancer properties of resveratrol-based aspirin prodrugs (Bottone and Alston-Mills 2011; Zhu et al. 2015). The molecular mechanisms are studied for the CRC chemopreventive activity of NSAIDs (i.e. aspirin, sulindac, and ibuprofen), COX-2 inhibitors (i.e. celecoxib), natural products (i.e. curcumin, resveratrol, EGCG, genistein, and baicalein), and metfor-

min. Thus, this provides a new mechanistic link between resveratrol and tumour downregulation and its significant benefits. A deeper knowledge of this anti-inflammatory agent's mechanism will provide insight into potentially safer drug (Fajardo and Piazza 2015; Jeong et al. 2015). One of the new findings provided evidence that resveratrol could inhibit EMT in CRC through TGF-β1/SMAD signalling pathway mediated Snail/E-cadherin expression (Ji et al. 2015; Osman et al. 2015).

Sporadic and nonhereditary mutations are the major causes in CRC; the loss of APC function and activation of the β-catenin/LEF signalling pathway, activating mutations in KRAS, are major causes in sporadic CRC. Thus, resveratrol can prevent the formation and growth of CRC by downregulating KRAS expression in sporadic case (Saud et al. 2014). The exogenous expression of PTEN inhibits the PI3K/Akt signal and promotes the anti-proliferative effects in HCT116 cells, whereas knockdown of PTEN increases PI3K/Akt signalling but reduces the anti-proliferative function of RSVL that may be mediated by regulating separately the PTEN/PI3K/Akt and Wnt/β-catenin signalling (Vanamala et al. 2010; Liu et al. 2014; Aires et al. 2013). RSVL also cause reduction of immune cells and cancer cells jointly in a dose-dependent production of cytokines (IL-6, IL-1ra, and IL-10) (Bergman et al. 2013). Overall, the clinical evidence of dietary phenolics against CRC is still weak, and the amounts needed to exert some effects largely exceed common dietary doses (Tak et al. 2012; Núñez-Sánchez et al. 2015).

3.5.2.3 Betulin

Recent clinical studies have shown that betulinic acid (BA) was effective against a variety of tumours by either inducing apoptosis and/or slowing the cell division (Alakurtti et al. 2006). BA has significant inhibitory effect on VEGF expression in human CRC xenografts in *in vivo* model (Ren et al. 2010). The mechanism of action of BA was dependent on cell context as proteasome-dependent and proteasome-independent downregulation of Sp1, Sp3, and Sp4 in SW480 and RKO cells, respectively. In RKO cells, the mechanism of BA-induced repression of Sp1, Sp3, and Sp4 was due to ROS-mediated repression of microRNA-27a, and induction of the Sp repressor gene ZBTB10, suggesting the anticancer activity of BA against CRC (Chintharlapalli et al. 2011; Su et al. 2017).

BA could induce autophagy and proteasomal degradation pathway in HT-29 cells (Dutta et al. 2016). It is a novel class of selective PPAR gamma modulators with potential for clinical treatment of colon and pancreatic cancer (Chintharlapalli et al. 2007). It has been found to activate two human apoptotic pathways: the mitochondrial apoptotic pathway and NF-κB pathway. DNA damage activates NF-κB. NF-κB leads to inflammation and the synthesis of ROS, cytokines, and chemokines including TNF, lymphotoxins, IL-6 and IL-8, and growth and angiogenic factors. NF-κB can lead to malignant proliferation, prevention of apoptosis, and an increase in metastasis and angiogenesis (Mullauer et al. 2011). In study based on *in vitro* sensitivity of cell line on BA, prevalent cancer-type cell line panels derived from lung, colorectal, breast, prostate, and cervical cancer were performed

to show a highest mortality in women and men cell types. These results substantiate the possible application of BA as a chemotherapeutic and/or adjuvant agent for the most prevalent human cancer types (Kessler et al. 2007; Potze et al. 2016).

3.5.2.4 Silibinin

Silibinin, a polyphenolic flavonoid, is the major biologically active compound of milk thistle. Silibinin (SB) and its crude form, Silymarin (SM), are used clinically and as dietary supplements against liver toxicity. Studies have demonstrated the inhibitory effects of silibinin on multiple cancer cell lines including human CRC. The anti-angiogenic effects of SM/SB are associated with the upregulation of VEGFR-1 (Flt-1) gene expression, another viable candidate for combination therapy to treat CRC (Yang et al. 2005). The protein bid was cleaved in SW480 cells indicating crosstalk between extrinsic and intrinsic apoptotic pathway, overwhelmed by the activation of both the extrinsic and intrinsic apoptotic pathways (Kauntz et al. 2011).

First, SB rapidly induced oxidative stress in CRC, SW480 cells due to ROS generation with a concomitant dissipation of mitochondrial potential and cytochrome C release leading to mild apoptosis as a biological effect, suggesting that SB harbours a deadly 'double-edged sword' against CRC cells, thereby further advocating its clinical effectiveness against this malignancy (Raina et al. 2013a, b; Kumar et al. 2014). Taken together, these data indicated that silibinin inhibits LoVo cell invasion with the reduction of MMP-2 presentation by attenuating AP-1 binding activity, as novel anti-metastatic compound against CRC (Kaur et al. 2009; Lin et al. 2012; Wang et al. 2012). Oral feeding of silibinin on NF-κB pathway in SW480 (COX-2 negative) and LoVo (COX-2 positive) tumour xenografts in nude mice showed inhibitory efficacy on tumour growth and progression via NF-κB activation in both xenografts (Sangeetha and Nalini 2015). Together, these findings are highly significant in establishing for the first time that SB suppresses CRC growth and progression possibly through its anti-inflammatory activity by interfering with NF-κB activation and thus has potential against human CRC (Raina et al. 2013a).

In a comparative study between the effect of chrysin and SB on HT-29 cells, chrysin caused cell cycle arrest in G2/M phase, while SB activated caspase-3 triggers the cells directly to apoptosis. Moreover, SB diminished the NF-kB activation by increasing the sensitivity of cells to apoptosis, and it inhibited topoisomerase IB activity concluding that this is an important target involved in the anticancer vanadium effects. Thus, the result represented that silibinin has a stronger and deleterious action than chrysin on HT-29 cells (León et al. 2015). The combined treatment of Colo 205 cells with metformin and SB induced apoptosis in human CRC cells at a dose that does not affect human colonic epithelial cells (HCoEpiC). This finding reveals a potential therapeutic strategy of SB for the treatment of CRC (Tsai et al. 2015a). Silibinin also restores promoter activity from a vitamin D response element (VDRE); 1,25D had no significant effect on HT-29 and SW480-R cell proliferation and migration, while co-treatment with SB restored 1,25D responsiveness to decreased proliferation and migration (Raina and Agarwal 2013). These findings

demonstrate that this combination may present a novel approach to target CRC in conditions of chronic colonic inflammation (Bhatia and Falzon 2015). Thus, over the years, preclinical studies have shown that SB has strong preventive and therapeutic efficacy against various epithelial cancers, including CRC (Raina et al. 2016).

3.5.2.5 Tanshinone

Tanshinone (Tan) is one of the most abundant diterpenes isolated from *Salvia miltiorrhiza Bunge* (Danshen in Chinese). It has been shown to possess many pharmacological activities like antioxidant, protecting and/or preventing angina pectoris and myocardial infarction (Lee et al. 2012b). Report has also shown its proliferative inhibition and cytotoxic effects on cell lines derived from various human carcinomas and also a significant percentage of reduction in the mortality of patients suffering from alcoholic liver cirrhosis.

Tanshinone IIA (Tan IIA) lowers HIF-1α levels and inhibits secretion of VEGF and bFGF, but also efficiently suppresses the proliferation, tube formation, and metastasis in colon cancer (Shan et al. 2009). Interruption of the HIF-1α/β-catenin/TCF3/LEF1 signalling pathway which occurs in the hypoxic microenvironment is disturbed by Tan IIA. Finally, Tan IIA sodium sulfonate exhibits anti-angiogenesis activity in CRC patients by reducing levels of angiogenin, VEGF, and bFGF expression (Sui et al. 2017). Tanshinone IIA also significantly inhibited *in vivo* metastasis of colon carcinoma SW480 cells by reducing levels of urokinase plasminogen activator (uPA) and matrix metalloproteinases (MMP)-2 and MMP-9 and by increasing levels of tissue inhibitor matrix metalloproteinase protein (TIMP)-1 and TIMP-2 and inhibition of the NF-kappaB signal transduction pathway, and also it inhibits the proliferation in Colo 205 cells, through downregulation of ErbB-2 protein and upregulation of TNF-alpha and caspase-3 (Su et al. 2008a; Su and Lin 2008; Zhang et al. 2016). In a study, COX-2 promoter and COX-2 plasmids were transfected into HCT-116 cells, and the result showed that Tan IIA could inhibit tumour growth and suppress VEGF level *in vivo* and exerts inhibitory effect on COX-2 and VEGF in a dose-dependent manner (Zhou et al. 2012).

The expression of p53, p21, bax, and caspase-3 increased in tanshinone IA (Tan IA)-treated cells, and the cell cycle analysis showed G0/G1 arrest in Colo 205 cells, suggesting Tan IA work through both mitochondrial-mediated intrinsic cell death pathways and p21-mediated G0/G1cell cycle arrest (Su et al. 2008b). Tan IA was also known to induce apoptosis in CRC cell lines, but interestingly, Tan I did not exercise much inhibitory effect on normal colon epithelial cells or CRC cells with mutant p53, indicating relative selectivity towards CRC cells with full normal p53 (Lu et al. 2016). Tan IA induce apoptosis through inducing caspase-3/caspase-9; a crosstalk between cytochrome c and apoptosis-inducing factor (AIF) was also reported (Wang et al. 2015b). Multidrug-resistant colon cancer cells SW620 showed increased viability after autophagy inhibition, indicating that autophagy induced by the two tanshinones was pro-cell death (Hong et al. 2017). These two tanshinones induce cell death in a p53-independent pathway, which could be useful in inhibiting the growth of apoptosis-resistant cancer cells with p53 defects (Hu et al. 2015b).

Results from Liu et al. (2013) and Kim et al. (2015a) studies suggest that Tan I-mediated cyclin D1 downregulation may result from proteasomal degradation and through its ERK1/2-mediated phosphorylation of threonine-286 which provides new mechanistic link between Tan I, cyclin D1 downregulation, and cell growth in human CRC.

Tanshinone IIA also inhibits the production of inflammatory cytokines, tumour necrosis factor α (TNF-α), and interleukin 6 (IL-6), which generated by macrophage RAW264.7. microRNA-155 (miR-155) was upregulated in macrophages, and it could be a potential target for the prevention of inflammation-related cancer (Tu et al. 2012; Gavrilas et al. 2016). Tan-IIA plus 5-FU could be used as potential therapeutic agents for human CRC, as it causes a reduction in the xenograft tumour volumes and decreased P-glycoprotein (P-gp) and microtubule-associated protein light chain 3 (LC3)-II expression compared to 5-FU alone (Su 2012; Chen et al. 2014b). These result promising Tan IA and IIA as a leading compound for the development of antitumour agent or are developed as an adjuvant drug for colon cancer therapy (Liu et al. 2013).

3.5.2.6 Quercetin

Quercetin is a natural antioxidant derived from fruits and plant resources and is a bioactive compound with anti-inflammatory, antioxidant, and anticancer properties. This flavonoid is rich in onions, tea, and apples, and they effectively induce apoptosis and suppress the proliferation of cancer cells in both *in vitro* and *in vivo* CRC studies. The proliferation, apoptosis, and differentiation processes are shown to be dysregulated in HT-29 and HCT15 cells when investigated with quercetin during cancer (Del et al. 2013). Activation of caspase-3 leads to increased cytosolic cytochrome c, which in turn decreased pAkt, pGSK-3β level, and cyclin D1 development. Though nuclear translocation of NF-kB and overexpression of ROS and COX-2 were observed in HT-29 cells, quercetin-treated HCT15 cells did not expressed COX-2. In-silico analysis provides evidence that partial inhibition of COX-2 enzyme is due to quercetin binding to COX-2 subunit A, which has peroxidase activity and serves as source of ROS (Raja et al. 2017; Zizkova et al. 2017).

MTT assay was conducted to investigate the effects of quercetin on HT-29 CRC cells, which showed cell shrinkage, chromatin condensation, and nuclear collapse in a dose-dependent manner. Significant cell cycle arrest in the S phase and increased CSN6 protein degradation were also observed. Therefore, this affects the expression levels of Myc, p53, B-cell lymphoma 2 (Bcl-2), and Bcl-2-associated X protein suggesting quercetin-induced apoptosis (Atashpour et al. 2015; Yang et al. 2016b). The TEF (5,3′-dihydroxy-3,7,4′-triethoxyflavone), a newly synthesized quercetin derivative, has also been evaluated on HCT-116 cells to induce apoptosis. It is confirmed by the presence of fragmented nuclei, reduced mitochondrial membrane potential, and elevated cytoplasmic and mitochondrial ROS levels. Molecularly TEF treatment causes elevation of IRE1-α and activates calcium ions (Ca^{2+}) with concomitantly increased JNK levels. Thus, elevated Ca^{2+} ion translocates from ER to

mitochondria which leads to ROS release and oxidative stress. Additionally, JNK inhibition was shown to suppress TEF-induced apoptosis. Therefore, this study reveals the apoptotic role of TEF against HCT-116 cell line via IRE1-α and mito-JNK pathway and inhibition of the major survival signalling pathways like the PI3K/Akt/mTOR and an induction of the pro-apoptotic JNK/JUN pathways (Refolo et al. 2015; Khan et al. 2016).

3.6 Conclusions and Future Prospects

Colon cancer is the second leading cause of cancer death, and out of the 140,000 people diagnosed with colon cancer each year in the USA, about 40% dies (Siegel et al. 2013). Treatment of CRC includes combination of surgery, radiation therapy, chemotherapy, and targeted therapy. If cancer cells are confined within the wall of the colon, it may be curable with surgical removal, while, when it has metastasized, it is usually not curable (Qi et al. 2010). However, depending on cancer condition and stages, all the cancer can be removed with surgery, and it also depends on persons overall health. Regular screening is recommended starting from the age of 50 to 75, for preventing and decreasing deaths from CRC. Initially, first-line chemotherapeutic agent oxaliplatin and/or 5-fluorouracil (5-FU) is the first choice for colon cancer treatment, which acts as a DNA synthesis inhibitor used for the CRC treatment (Cheng et al. 2017). If a large polyp or tumour is found, aspirin and other non-steroidal anti-inflammatory drugs are used to decrease the risk. Their routine use is not recommended for this purpose, however, due to side effects (Esther et al. 2010).

The anticancer effect of phytochemicals and its beneficial effects on cancer-related symptoms (e.g. fatigue, pain, vomiting, and anorexia) or on quality of life have been reported for their antitumour actions (Hosseini and Ghorbani 2015). In this chapter, we have focused overall beneficial effects of phytochemicals and mechanism of action of several types of herbal extracts against CRC. Polyphenols are natural phytochemicals used to treat various viral and fungal diseases. They are derived from various sources such as plants, seaweeds, marine algae, and microorganisms. Polyphenols include different organic constituents such as flavones, flavanols, isoflavones, catechins, EGCG, and epicatechins. Numerous plants are reported to have polyphenols in their extracts.

Nutraceutical is a term derived from nutrition and pharmaceutical and is sometimes termed ‘functional foods’. Nutritional phytochemicals have a strong historical background and significant applications in modern medicine. These compounds are used in medicinal and commercial industries for cosmetics, food aids, and additives (McCulloch et al. 2011; Palaniselvam et al. 2014). Previous seminal work has been summarized above, which demonstrates the key molecular mechanism of tumorigenes is inhibition by medicinal plants (Yaeger 2013). We have also outlined various mechanisms of EGFR inhibition (highly expressed in case of CRC) (Grossmann and Samowitz 2011) that are induced by naturally occurring chemopreventive

agents such as ginseng, green tea, and curcumin and describing its mechanism of action (Hamam 2014; Fujiki et al. 2015a; Pabla et al. 2015). Major advances in understanding of cell cycle regulation mechanisms provided a better knowledge of the molecular interactions involved in human cancer. Further mechanistic investigation is required to find out switches that connect common effector pathways that regulate cell behaviour, phenotype alteration, and cell death or lineage commitment. Human intervention studies of phytochemical, whether alone or in combination, are indicated against intermediate biomarkers and morphological stages of gastrointestinal tumorigenesis, thus providing a useful component of dietary or pharmacological treatment that aimed at reduction of the incidence and mortality from cancer (Khan and Mukhtar 2010).

We have reviewed on many evidences using cell culture model system, preclinical studies, and clinical trials (patients with CRC or at risk, familial adenopolyposis or aberrant crypt foci) investigating the protective mechanisms of phytochemicals and crude extract like curcumin, resveratrol, isoflavones, and green tea extracts (epigallocatechin gallate) (Table 3.6). From the data reviewed in this chapter, it can be concluded that these compounds inhibit cell growth, by inducing cell cycle arrest and/or apoptosis; inhibit proliferation, angiogenesis, and/or metastasis; and exhibit anti-inflammatory and/or antioxidant effects. Graphical overview of signal transduction pathway explains the role of herbal medicines in CRC (Fig. 3.1). In turn, these effects involve multiple molecular and biochemical mechanisms of action, which have been explained partially, still not completely characterized.

Table 3.6 Herbal medical plant extracts in treatment of CRC

Herbal composite	Plant source	Possible mechanism of anticancer CRC	References
Astragalus saponins (AST)	*Radix Astragali* (Huangqi)	Modulation of mTOR signalling, downregulates COX-2, VEFG	Law et al. (2012), Lopez-Sa´nchez et al. (2014), and Ran et al. (2016)
Ginsengs	Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*) Notoginseng (*Panax notoginseng)*	Antioxidants, cell cycle arrest, apoptosis, and inhibits angiogenesis	Li et al. (2009a, b), Zheng et al. (2013), Wang et al. (2014, 2015a, b, c), and Jin et al. (2016)
Mistletoe extracts	*Viscum album* (mistleotoe)	Cytotoxic, cell cycle arrest, apoptosis, and immune modulator	Harmsma et al. (2006), Horneber et al. (2008), and Ma et al.(2008)
(-)-Epigallocatechin gallate (EGCG)	Green tea (polyphenolic constituent)	Antioxidants, tyrosine kinase receptor inhibitor; inhibits cell proliferation, immune modulator, APC inducer, WNT/β--catenin, MAPK/Akt pathways	Jin et al. (2010), Fujiki et al. (2015a, b), Hu et al. (2015a, b, 2016), and Shin et al. (2017)

(continued)

Table 3.6 (continued)

Herbal composite	Plant source	Possible mechanism of anticancer CRC	References
Ganoderma lucidum extract (GLE)	*Ganoderma lucidum*	Inhibits DNA synthesis, cell cycle arrest, apoptosis via mitochondrial disruption	Xie et al. (2006), Qi et al. (2010), Oka et al. (2010), Liang et al. (2015), and Na et al. (2017)
Phyllanthin	*Phyllanthus watsonii*	Cytotoxicity, apoptotic effect	Ramasamy et al. (2012)
Curcumin	Turmeric (phytochemical)	Cell cycle arrest (p53 dependent and independent, cyclin dependent), inhibition of cell proliferation, apoptosis	Mao et al. (2007), Howells et al. (2010), and Shanmugam et al. (2015)
Resveratrol	Red grapes (phytoalexin)	COX-2, APC, NF-kB, cyclin D1, p53, apoptosis, miR-200c	Chen et al. (2012), Demoulin et al. (2015), Schroeter et al. (2015), Khaleel et al. (2016), Reddivari et al. (2016), Gong et al. (2017), and Karimi Dermani et al. (2017)
Betulinic acid (BA)	Barks of the plants	VEGF, ROS, DNA damage (NF-kB, p53), apoptosis	Alakurtti et al. (2006), Marcin et al. (2009), Ren et al. (2010), Mullauer et al. (2011), and Gomathi et al. (2014)
Silibinin, silymarin (crude form)	Milk thistle (a polyphenolic flavonoid)	Cell cycle arrest, cell proliferation, NF-kB, MMP2, p53, and apoptosis	Yang et al. (2005), Kaur et al. (2009), Kauntz et al. (2011), Lin et al. (2012), Wang et al. (2012), and Raina et al. (2013a)
Tanshinone	*Salvia miltiorrhiza Bunge* (diterpenes)	Inhibits VEGF, miR-155, TNF-alpha	Su et al. (2008a, b), Lee et al. (2012a, b), Hu et al. (2015a, b), Zhang et al. (2016), and Sui et al. (2017)
Quercitin	Fruits and vegetables (antioxidants, flavonoids)	JNK, p53, NF-kB, COX-2, cell cycle arrest, apoptosis	Fridrich et al. (2008), Atashpour et al. (2015), Refolo et al. (2015), Khan et al. (2016), Yang et al. (2016a, b), Raja et al. (2017), and Zizkova et al. (2017)

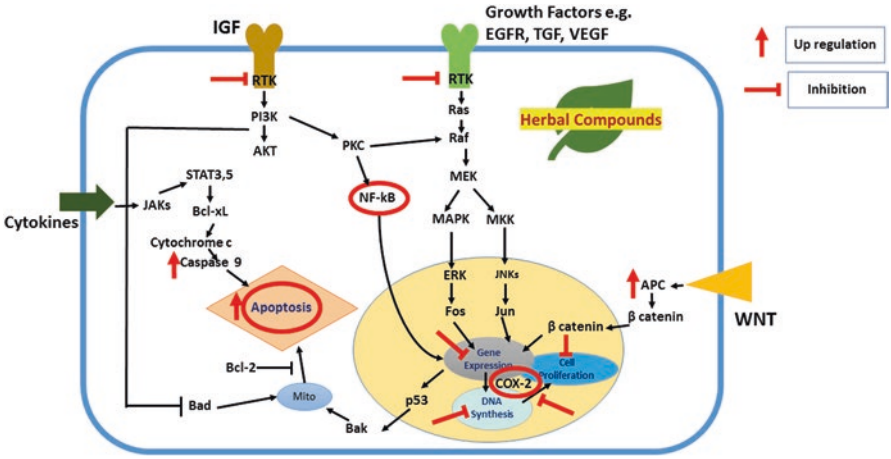

Fig. 3.1 Diagrammatic representation of signals transduction pathway. Black arrows and letters indicate regulators involved in signalling pathways and their components, while the herbal medicines targeting the pathway at different cellular level of the cascade are indicated in red colour as upregulators, inhibitors, and key regulators (circled)

Thus, caution is mandatory when attempting to extrapolate the observations obtained in CRC cell line studies to humans (Araújo et al. 2011). To date, chemopreventive properties of polyphenols are the most promising and potential future adjuvant in CRC management. Overall, the clinical evidence of mechanistic action of dietary phenolics against CRC is statically weak; still we have discussed here the possible reasons behind its mechanism of action as an antitumourigenic, anti-apoptotic, and anti-proliferative properties of compounds (Núñez-Sánchezet al. 2015). Moreover, if synergistic effects between herbal medicines and chemotherapy agents can be identified, reduction of chemotherapy dose in combination with herbs can further decrease dose-related drug toxicity and CRC. The identification of nontoxic anticancer herbal medicines remains as an essential step in advancing the treatment of cancer.

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Chapter 4
Elucidation of Mechanisms of Anticancer Plant Compounds Against the Tumor Cells

Radhakrishnan Narayanaswamy and Mallappa Kumara Swamy

Abstract Cancer is one of the noncommunicable diseases which is the second leading cause of deaths throughout the world. Chemotherapy is the major treatment approach, however, with a limited success rate accompanied by secondary adverse health effects. Moreover, in recent years, about 30–80% of cancer patients are developing resistance to chemotherapeutic drugs. Therefore, phytoconstituents have attained much attention among the researchers because of their effective multiple targeted cytotoxicity with a tolerable side effects and chemosensitizing potential. These are known to exhibit their anticancer activities in various ways of molecular mechanisms of action, such as arresting of cell cycle, inhibiting angiogenesis, inhibiting enzymes (cyclooxygenase, caspases, kinase matrix metalloproteinase (MMP), poly(ADP-ribose) polymerase 1 (PARP-1), etc.), inhibiting transcription factors, suppressing pro-inflammatory signaling pathways, inhibiting lipid signals, and inhibiting heat shock proteins. Though scientific evidences have suggested many plant compounds with chemopreventive potential, understanding the issues related to exposure time, bioavailability, toxic effects, and mechanisms of action will certainly help to identify the leads and utilize them against various cancer types. The present chapter deals with the anticancer effect of several compounds of plant origin and their mechanisms of action.

Keywords Anticancer plants · Apoptosis · Cell cycle · Inflammation · Transcription factors

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4.1 Introduction

Cancer is the second major noncommunicable disease and it caused about 8.2 million deaths in 2012 (Torre et al. 2015). Cancer is defined as a group of diseases in which normal tissue or organ is invaded by abnormal dividing cells. If left untreated, it spreads throughout the human body and even results in loss of human life (Eid et al. 2015). Carcinogenesis is the metabolic process, in which normal cells are transformed into cancer cells through three major stages such as initiation, promotion, and progression. The initiation (first) stage occurs in the normal cells due to exposure to carcinogenic (procarcinogens, epigenetic carcinogens, genotoxic carcinogens, etc.) or mutagenic (physical and chemical) agents. However, this first stage alone is not enough for tumor formation. In promotion (second) stage, tumor promoter agents help to convert the initiated cells into tumor cells. This second stage occurs very slowly, and it even takes several months to years depending upon changes in diet and lifestyle of the individual person. Progression (third/final) stage converts the tumor cells into high-degree malignant cells. At this progression stage, human diet has less impact on tumor progression (Reddy et al. 2003; Rajesh et al. 2015). The cancer incidence cases have been reported more from the developed countries compared to that of developing countries. Similarly, in the developing countries, breast, lung, and colorectal cancers are reported more among females (Jemal et al. 2011). Various risk factors have been reported in the cancer development which includes age, geographic area, and race (Millimouno et al. 2014). Chemotherapy is the major treatment approach; however, in recent years 30–80% of cancer patients are developing resistance to chemotherapeutic drugs. Therefore, plant-based substances (phytoconstituents) have attained much attention among the researchers. The present chapter deals with the anticancer effect of several compounds of plant origin and their mechanisms of action.

4.2 Mechanisms Involved in Cancer Chemoprevention and Treatment

Plant-based substances have been reported to induce cell cycle arrest and apoptosis by targeting multiple cellular signaling pathways such as (1) p53 pathway, (2) nuclear factor-kappaB transcription factor pathway, (3) nuclear factor-related factor 2 signaling pathway, (4) growth factors pathway, (5) signal transducers and activators of transcription (STAT) pathway, (6) Wnt/β-catenin pathway, (7) hedgehog (SHH) signaling pathway, (8) phosphatidylinositol 3 kinases (PI3K) pathway, (9) cyclooxygenase 2/prostaglandin E2 (COX 2/PGE2) pathway, (10) mitogen-activated protein kinase (MAPK) signaling pathway, (11) Cripto 1 protein signaling pathway, and (12) hypoxia signaling pathway. Thus, by targeting/modulating the abovementioned cellular signaling pathways, anticancer activity has been successful achieved by plant-based substances (Millimouno et al. 2014).

4.2.1 Cell Cycle Arrest

The cell cycle is the metabolic process by which cell progress and division consist of several biochemical and molecular signaling pathways. It exhibits four important stages or phases.

1. G1 (Gap 1) phase in which the cell grows and prepares to synthesize deoxyribonucleic acid (DNA)
2. S (synthesis) phase in which the cell synthesizes DNA (genetic material)
3. G2 (Gap 2) phase in which the cell prepares to divide
4. M (mitosis) phase in which cell division occurs and finally phase is termed as G0 (resting phase) in which cell leaves the cell cycle and quit dividing them

Cyclin-dependent kinases (CDKs) are group of enzymes which regulate the cell cycle transitions. These enzymes contain two subunits, namely, catalytic subunit (CDK) and regulatory subunit (cyclin). Each phase of the cell cycle has individual CDK cyclin enzymatic activity as shown below:

1. CDK2 cyclin E and A regulates G1 to S phase transition.
2. CDK1 cyclin A regulates late S to G2 phase transition.
3. CDK1 cyclin B regulates G2 to M phase transition.

In mammalian cells, about 10 CDKs and 20 cyclins have been reported; interestingly not all participate in cell cycle regulation. In normal cells cell cycle machinery controls the cell proliferation, which also includes repair mechanism with them. In the case of cancer cells, cell cycle machinery loses control and results in uncontrolled cell proliferation (Collins et al. 1997; Hwang and Clurman 2005).

4.2.2 Apoptosis

Apoptosis is the process of active cell death resulting in the breakdown of cellular structures, without causing any immune or inflammatory response to the host. It is also referred as "programmed cell death," which occurs in the several physiological and pathological conditions (Cummings et al. 1997; Iannolo et al. 2008). It is characterized by biochemical and morphological hallmarks such as cell shrinkage, chromatin condensation, cytoplasmic membrane blebbing, and nuclear DNA fragmentation (Fulda and Debatin 2006). It is essential for embryonic development, tissue homeostasis, immune function, and tumor suppression especially in multicellular organisms (Iannolo et al. 2008). It usually maintains balances between pro-apoptotic (BAD or BAK, BAK and BID) and anti-apoptotic (Bcl-2 and Bcl-Xl) signals. The accumulation of pro-apoptotic signals leads to apoptosis induction. Defects in apoptotic pathways have been observed in several diseases, including tumor and neurodegenerative disorders (Lowe and Lin 2000). Three important biochemical events occur during apoptosis; they are (1) activation of caspases activity,

(2) breakdown of DNA and protein, and (3) membrane modifications due to phagocytes (Wong 2011). The process of apoptosis is mainly divided into two pathways: (1) intrinsic pathway mediated by molecules released from mitochondrial membrane and (2) extrinsic pathway triggered by death receptor.

4.2.2.1 Intrinsic Pathway of Apoptosis

The intrinsic pathway is activated by physical or chemical stimuli such as cell detachments, cytokines, DNA damage, growth factor deprivation, hypoxia, and/or other stress signals. These stimuli modulate mitochondrial functions such as increases the mitochondrial membrane potential (MMP) and releases the cytochrome C into the cytoplasm. This cytosolic cytochrome C in turn interacts/binds with apoptotic protease-activating factor 1 (Apaf 1) and pro-caspase 9 (zymogen). Pro-caspase 9 activates caspase cascade such as caspases 3, 6, and 7, leading to DNA fragmentation and cell death. B-cell leukemia/lymphoma 2 (Bcl2) family proteins play an important role in regulating the intrinsic pathway by inducing or preventing the release of cytochrome C (Lowe and Lin 2000; Iannolo et al. 2008; Millimouno et al. 2014).

4.2.2.2 Extrinsic Pathway of Apoptosis

The extrinsic pathway is activated, when a death ligand binds to the extracellular domains of the death receptor and leads directly to caspase activation. For instance, Fas ligand (FasL) binds with its respective receptor Fas receptor (also called as Apo 1 or CD95), which forms death-inducing signaling complex (DISC) which contains the specific adaptor protein, namely, Fas-associated death domain protein (FADD) and caspase 8. Caspase 8 in turn activates caspase 3 and apoptosis in type 1 cells. The extrinsic pathway is quite similar to the intrinsic apoptotic pathway, which is also caspase-dependent; the one and only difference is that apoptotic signaling is initiated through membrane-bound death receptors (Wajant 2002; Iannolo et al. 2008).

4.2.2.3 Caspase-Independent or ROS-Mediated Apoptosis Pathway

Reactive oxygen species (ROS) are generated due to physiologic stress which is associated with the production of oxidative species through intracellular damage to DNA, lipids, proteins, and RNA. During cellular redox the excessive generation of ROS in turn induces oxidative stress, loss of cell function, and apoptosis. Granzyme A (enzyme belonging to serine proteases family) directly induces the ROS generation which in turn results in caspase-independent mitochondrial damage. Then ROS drives the endoplasmic reticulum (ER)-associated SET complex into the nucleus, where it activates apoptosis. ROS also mediates poly(ADP-ribose) polymerase 1

(PARP-1) activation, which is needed for apoptosis-inducing factor (AIF) release from mitochondria. Thus, AIF is the main pro-apoptosis factor involved in caspase-independent apoptosis pathway (Martinvalet et al. 2005; Lieberman 2010).

4.2.3 Necrosis

Necrosis is the process of passive cell death resulting in the breakdown of cellular structures, caused by specific physiological and pathological stimuli such as tumor necrosis factor (TNF), TNF-related apoptosis-inducing ligand (TRAIL), lipopolysaccharides (LPS), oxidative stress, and DNA damage (via PARP). Necrosis is characterized by biochemical and morphological hallmarks such as loss of membrane integrity, cell swelling, permanent loss of mitochondrial membrane potential, and DNA fragmentation (post-lytic/late stage). Specific physiological and pathological stimuli activate receptor-interacting protein (RIP1) kinase. This RIP1 kinase directly/indirectly transduces signal to mitochondria which leads to mitochondrial permeability and transition. This mitochondrial damage enhances enzymes such as protease (calpains, cathepsin) and phospholipase activities and finally results in plasma membrane destruction (sign of necrotic cell death). In contrast to apoptosis process, the mode of cell death is required for tumor regression, and thus necrosis plays important in anticancer therapy (Proskuryakov and Gabai 2010).

4.2.4 p53 Pathway

p53 (tumor suppressor gene) is stimulated by cellular stress like carcinogens, hypoxia, ionizing radiation, oxidative stress, and UV radiation. It regulates the apoptosis, genomic integrity, cell cycle, and DNA repair caused due to genotoxic stresses. p53 binds to regulatory DNA sequences as a tetramer and transactivates genes involved in apoptosis in response to DNA damage (ASPP1/2, BAX, Fas, NOXA, p53AIP1, PERP, PIDD, PUMA), cell cycle arrest (p21, cyclin G1, GADD45, 14-3-3, reprimo), angiogenesis (BA11, GD-AIF, maspin, TSP1), and genetic stability (DDB2, MSH2, p21, XPC). About 40 different isoforms of the p53 family members have been reported so far. Among these, p73 plays a significant role during neurogenesis, whereas p63 is essential in skin, limb, and craniofacial development. Interestingly, some p53 isoforms have oncogenic potential, while others have tumor suppressor activity (Millimouno et al. 2014; Pflaum et al. 2014).

4.2.5 NF-κB Pathway

Nuclear factor-kappaB (NF-κB transcription factor) is stimulated by cellular stress like carcinogens, cytokines, endotoxins, ionizing radiation, oxidative stress (free radicals), and UV radiation. It is involved in tumor initiation and progression. NF-κB is usually present in the cytoplasm via association with its IκB (endogenous inhibitor of NF-κB), which further phosphorylated by IκB kinase (IKK). IKKa activates metastasis in prostate cancer by inhibiting maspin (mammary serine protease inhibitor) and stimulates angiogenesis via activating interleukin 8 (IL8) and vascular endothelial growth factor (VEGF). The accumulation of the IκBα leads to activation of anti-apoptotic NF-κB, resulting in apoptosis. NF-κB pathway plays significant role in carcinogenesis by transactivating genes involved in angiogenesis, apoptosis, cell proliferation, metastasis, and tumor cell invasion (Millimouno et al. 2014).

4.2.6 Nuclear Factor-Related Factor 2 (Nrf2) Signaling Pathway

Nrf2 (belongs to the Cap 'N' Collar family) plays an important role in transcriptional activation of phase II detoxification enzymes (glutathione S-transferases) and plays as an important regulator of cell survival both in normal and cancer cells. It protects against chemical carcinogen by decreasing the ROS content and DNA damage in cells. Its defense role has been reported against various diseases such as acute pulmonary injury, aging, cancer, cardiovascular disease, diabetes, inflammation, photooxidative stress, and pulmonary fibrosis. Similarly, relationships between p21, p62, and Nrf2 have been reported. Nrf2 activators (from phytochemicals) have been shown to induce the Nrf2-mediated defense mechanism by enhancing phase II detoxification enzymes, antioxidants, and (ABC) transporters; this in turn protects the carcinogenic stimuli (Jaramillo and Zhang 2013; Millimouno et al. 2014).

4.2.7 Growth Factor Pathway

Growth factors (GFs) and growth factor receptors (GFR) play a vital role in physiological conditions such as growth and differentiation, wound healing, etc. Growth factors like colony-stimulating factor (CSF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and transforming growth factor (TGF) are few growth factors involved in carcinogenesis. Growth factor receptor activation leads to downstream signaling of PI3K-Akt and Ras-MAPK pathways and thus acts as target for numerous anticancer/antitumor agents. For instance, suramin (polysulfonated drug) inhibits the binding of growth factors like epidermal growth factor (EGF), fibroblast growth factor (FGF),

insulin-like growth factor (IGF1 and IGF2), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF-α) to their receptors and induces disassociation of bound growth factors from their respective receptors and thereby has exhibited anticancer activity against adrenal, prostate, and renal cancer (Rajkumar 2001; Millimouno et al. 2014).

4.2.8 Signal Transducers and Activators of Transcription (STAT) Pathway

STAT belongs to family members of interferon (IFN) signaling complex and plays a dual role as signal transduction and transcription activators. STATs are cytoplasmic proteins that are activated by tyrosine kinases resulting in phosphorylation and dimer formation and translocate into the nucleus to regulate the transcription of genes. Seven STAT family members have been reported so far; they are STATs 1, 2, 3, 4, 5a, 5b, and 6. STAT3 activation has been reported by both Janus kinase (JAK) and also by non-receptor tyrosine kinases (c-Src kinases). Thus, inhibition of JAK/STAT pathway has been recognized as novel chemopreventive and chemotherapeutic target (Millimouno et al. 2014; Xiong et al. 2014).

4.2.9 Wnt/β-Catenin Pathway

Wnt binds with frizzled family receptor (FZD) and leads to the cytoplasmic accumulation of β-catenin. Then β-catenin translocates into the nucleus, where it interacts with T-cell factor/lymphoid enhancer factor 1 (TCF/Lef 1) transcription complex and regulates the transcription of genes. Wnt/β-catenin pathway has been reported in several cancers, and Wnt inhibition has been recognized as new target for colorectal cancer treatment (Pai et al. 2017).

4.2.10 Hedgehog (SHH) Signaling Pathway

SHH (glycoprotein) binds and inactivates the patched 1 (PTCH1) receptor, which inhibits the protein smoothened (SMO) activity. This in turn leads to activation of glioma-associated (GLI) transcription factors. Then GLI translocates into the nucleus and regulates the transcription of genes. Abnormal hedgehog (SHH) signaling that has been reported in several cancers includes colorectal, glioma, pancreatic, and prostate carcinoma. GLI 1 small interfering RNA (siRNA) has been used to induce apoptosis in prostate cancer, and thus hedgehog (SHH) signaling has been recognized as new target for cancer treatment (Wang et al. 2012; Rimkus et al. 2016).

4.2.11 Phosphatidylinositol 3 Kinases (PI3K) Pathway

Activation of phosphatidylinositol 3 kinases (PI3K) leads to the production of phosphatidylinositols [P2 and P3 (Ptdlns 3, 4) and P3 (Ptdlns 5)], which are bound by Akt. Phosphatidylinositols are further activated by phosphoinositide-dependent protein kinase 1 (PDK1) and Akt. These activated phosphatidylinositols are translocated into the plasma membrane and regulate the cellular process. Phosphatidylinositol 3 kinases (PI3K) play a significant role in cellular transformation and cancer development, and thus inhibition of PI3K serves a new target for cancer therapy (Liu et al. 2009; Wang et al. 2012).

4.2.12 Cyclooxygenase 2/Prostaglandin E2 (COX 2/PGE2) Pathway

Inflammatory stimuli activate COX 2 activity, which in turn activates PGE2. This extracellular PGE2 binds with prostaglandin E2 (EP) receptors and initiates multiple intracellular signaling pathways. COX 2 is one of the pro-inflammatory mediators, found to be elevated at the early stage of tumorigenesis (Reader et al. 2011; Wang et al. 2012).

4.2.13 Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

The RAS (small G protein)/RAF (rapidly accelerated fibrosarcoma)/MEK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) signaling pathway involves complex network that regulates the proliferation, differentiation, cell survival, and apoptosis. The ligand (e.g., cytokine, growth factor, or hormone) binds with receptor tyrosine kinase (RTK). In this pathway, three protein kinases play a major role, namely, mitogen-activated protein kinase kinase kinase (MAPKKK or BRAF) that phosphorylates and activates mitogen-activated protein kinase kinase (MAPKK or MEK), which in turn activates mitogen-activated protein kinase (MAPK or ERK). This activated ERK translocates into the nucleus and regulates the gene expression. Numerous inhibitors of components of this pathway have been reported and recognized as good therapeutic approach for melanoma treatment (Kolch 2000; McCain 2013). Similarly, genistein (soy-derived isoflavone) has been reported to inhibit cervical cancer (HeLa and CaSki) cells via by inhibiting ERK1/2 activity and activating p38 MAPK pathway (Kim et al. 2009).

4.2.14 Cripto 1 and Its Allied Protein Signaling Pathway

Cripto 1 (CR1) mediates several cellular processes such as angiogenesis, cell growth, fetal development, inflammation, invasion, migration, tumor formation, and wound repair. CR 1 is a mitogenic protein, also known as teratocarcinoma-derived growth factor 1 (TDGF 1). CR 1 binds with growth factor receptors such as 78 kD glucose-regulated protein (GRP 78) or heat shock 70 kD protein 5 (HSPA5) and mediates several signal transduction pathway that includes nodal-dependent (Smad2/3) and nodal-independent (Src/p44/42/Akt) pathways. CR 1 functions as a chaperone protein by inducing cellular signaling via Wnt/β catenin and Notch/Cbf 1 signal transduction pathways. In cancer cells, activin is inhibited by the complex formed between CR1, activin, and activin receptor type II (ActRII). Alantolactone (*Inula helenium*) has been reported to exhibit antitumor activity via by inhibiting the activin signaling pathway (Wang et al. 2012; Klauzinska et al. 2015).

4.2.15 Hypoxia Signaling Pathway

Hypoxia plays a significant role in the number of pathological conditions including aging, cancer, diabetes, and ischemia/stroke. Hypoxia-inducible factor (HIF) is the main transcriptional factor in nutrient stress signaling and otherwise known as oxygen-sensitive transcription factor. HIF also plays a major role in autophagy, cell invasion, intracellular pH regulation, and metabolism. HIF also regulates the expression of two key angiogenic factors, namely, VEGF-A and angiopoietin 2 (Ang 2). HIF activity is predominately regulated through posttranslational modifications and stabilization of HIF 1α and 2α proteins. HIF-α protein activity is regulated by two key oxygen sensing/depending enzymes, namely, hypoxia-inducible factor prolyl hydroxylase 2 (HIF-PH2 or PHD) and factor inhibiting HIF (FIH or asparaginyl hydroxylase). Stabilization of HIF 1α activates transcription of genes that involved in angiogenesis, dedifferentiation, and invasion which are all important factors/pathways in carcinogenesis. Thus HIF pathway components serve as potential therapeutic targets against cancers (Pouysségur et al. 2006; Brocato et al. 2014; Elks et al. 2015). Apart from the important mechanism listed above, there are few other mechanisms not discussed. For example, plants/plant-based substances are known to have multitarget activity against multidrug resistance (MDR)-related proteins, thus providing solution to overcome drug resistance problem (Eid et al. 2015).

4.3 Plants Having Anticancer Activity

Whole/crude extracts from plants (more than 120,000 plant extracts belongs to 6000 genera) have been widely studied in the past few decades, and several plants found to have anticancer/antitumor activity. Some of these plants have been clinically proven as anticancer agents, while few others have been used as tools to elucidate the biochemical/molecular mechanisms involved in the growth and regulation of cancers/tumors. Plants are known to exhibit a wide range/spectrum of mechanisms of action such as blockers of cell cycle progression, antagonists of mitogenic signaling, anti-metastasis, upregulators of the immune system, and inhibitors of blood vessel formation (Mans et al. 2000). Among different plant families reported, the 11 predominant plant families such as Fabaceae, Asteraceae, Zingiberaceae, Euphorbiaceae, Apocynaceae, Meliaceae, Solanaceae, Rutaceae, Moraceae, Liliaceae, and Myrtaceae have been shown to exhibit anticancer activity (Table 4.1).

4.4 Phytoconstituents Having Anticancer Activity

Phytoconstituents are potent in the treating several cancers/tumors. Alkaloids, flavonoids, polyphenols, and terpenes are the few chemical classes that have been reported for anticancer/antitumor activity. According to Batra and Sharma (2013), flavonoids have modulated several cellular events in cancer cells such as apoptosis, cell differentiation, cell proliferation and vascularization, etc. Vinblastine, honokiol, magnolol, wedelolactone, oridonin, alantolactone, and costunolide are the few classical examples for phytoconstituents having anticancer activity (Millimouno et al. 2014). Apart from phytoconstituents, some marine-based substances also known to have anticancer activity include aragusterol A, ascididemin, bryostatin1, discodermolide, fascaplysin, indanone, jaspamide, lyngbyabellin A, melophlins A and B, salinosporamide A, and spisulosine (Mayer and Gustafson 2003). In the present chapter, Table 4.2 summarizes those 165 phytoconstituents shown to have anticancer activity by modulating several cellular signaling pathways.

4.5 Conclusions and Future Prospects

Despite much therapeutic advancements in the understanding of carcinogenesis processes, cancer still remains as a major health issue around the global. Plants have been recognized as rich source of anticancer drugs. At present more than 60% of commercially available anticancer agents are directly or indirectly obtained from

Table 4.1 List of medicinal plants with anticancer potential

Botanical name	Family name	References
Abrus precatorius	Fabaceae	Rahman and Khan (2013)
Achyranthes aspera	Amaranthaceae	Prakash et al. (2013)
Acorus calamus	Acoraceae	Pandey and Madhuri (2009)
Actinidia chinensis	Actinidiaceae	Chavan et al. (2013)
Aegles marmelos	Rutaceae	Costa-Lotufo et al. (2005)
Aegiceras corniculatum	Myrsinaceae	Rahman and Khan (2013)
Agapanthus africanus	Agapanthaceae	Kaur et al. (2011)
Agave americana	Agavaceae	Prakash et al. (2013)
Aglaia silvestris	Meliaceae	Prakash et al. (2013)
Agrimonia pilosa	Rosaceae	Prakash et al. (2013)
Agropyron repens	Poaceae	Prakash et al. (2013)
Ailanthus altissima	Simaroubaceae	Prakash et al. (2013)
Akebia quinata	Lardizabalaceae	Prakash et al. (2013)
Alangium salviifolium	Alangiaceae	Rahman and Khan (2013)
Albizia lebbeck	Fabaceae	Jaikumar and Jasmine (2016)
Albizia amara	Fabaceae	Jaikumar and Jasmine (2016)
Allium bakeri	Alliaceae	Pandey and Madhuri (2009)
Allium cepa	Alliaceae	Chavan et al. (2013)
Allium sativum	Alliaceae	Prakash et al. (2013)
Aloe spp.	Asphodelaceae	Chavan et al. (2013)
Alphitonia zizyphoides	Rhamnaceae	Pandey and Madhuri (2009)
Alpinia galanga	Zingiberaceae	Prakash et al. (2013)
Alstonia scholaris	Apocynaceae	Pandey and Madhuri (2009)
Amoora rohituka	Meliaceae	Rahman and Khan (2013)
Amorphophallus campanulatus	Araceae	Pandey and Madhuri (2009)
Andrographis paniculata	Acanthaceae	Prakash et al. (2013)
Ananas comosus	Bromeliaceae	Chavan et al. (2013)
Angelica sinensis	Apiaceae	Chavan et al. (2013)
Annona muricata	Annonaceae	Prakash et al. (2013)
Annona squamosa	Annonaceae	Jaikumar and Jasmine (2016)
Aphanamixis polystachya	Meliaceae	Chavan et al. (2013)
Apium graveolens	Apiaceae	Kaur et al. (2011)
Arctium lappa	Asteraceae	Chavan et al. (2013)
Aristolochia contorta	Aristolochiaceae	Prakash et al. (2013)
Artemisia diffusa	Asteraceae	Ko and Moon (2015)
Artemisia monosperma	Asteraceae	Solowey et al. (2014)
Artocarpus heterophyllus	Moraceae	Jaikumar and Jasmine (2016)
Aspalathus linearis	Fabaceae	Reddy et al. (2003)
Aster tataricus	Asteraceae	Prakash et al. (2013)
Astragalus membranaceus	Fabaceae	Prakash et al. (2013)
Avicennia alba	Avicenniaceae	Pandey and Madhuri (2009)
Azadirachta indica	Meliaceae	Kamkaen et al. (2006)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Bauhinia variegata	Fabaceae	Chavan et al. (2013)
Berberis aristata	Berberidaceae	Pandey and Madhuri (2009)
Betula utilis	Betulaceae	Chavan et al. (2013)
Bidens pilosa	Asteraceae	Prakash et al. (2013)
Bleckeria vitensis	Apocynaceae	Mans et al. (2000)
Blumea lacera	Asteraceae	Rahman and Khan (2013)
Boesenbergia pandurata	Zingiberaceae	Kamkaen et al. (2006)
Boesenbergia rotunda	Zingiberaceae	Rahman (2016)
Bolbostemma paniculatum	Cucurbitaceae	Prakash et al. (2013)
Broussonetia papyrifera	Moraceae	Jaikumar and Jasmine (2016)
Brucea antidysenterica	Simaraubaceae	Kaur et al. (2011)
Bruguiera exaristata	Rhizophoraceae	Pandey and Madhuri (2009)
Bruguiera gymnorrhiza	Rhizophoraceae	Rahman and Khan (2013)
Bruguiera parviflora	Rhizophoraceae	Pandey and Madhuri (2009)
Bryonia dioica	Cucurbitaceae	Prakash et al. (2013)
Bursera microphylla	Burseraceae	Kaur et al. (2011)
Caesalpinia bonduc	Caesalpiniaceae	Pandey and Madhuri (2009)
Cajanus cajan	Fabaceae	Pandey and Madhuri (2009)
Calophyllum inophyllum	Clusiaceae	Pandey and Madhuri (2009)
Calotropis procera	Asclepiadaceae	Rahman and Khan (2013)
Camellia sinensis	Theaceae	Prakash et al. (2013)
Camptotheca acuminata	Nyssaceae	Mans et al. (2000)
Canavalia ensiformis	Fabaceae	Prakash et al. (2013)
Cannabis sativa	Cannabinaceae	Prakash et al. (2013)
Catharanthus pusillus	Apocynaceae	Jaikumar and Jasmine (2016)
Catharanthus roseus	Apocynaceae	Mans et al. (2000)
Carissa opaca	Apocynaceae	Jaikumar and Jasmine (2016)
Cassia absus	Caesalpiniaceae	Pandey and Madhuri (2009)
Cassia garrettiana	Caesalpiniaceae	Jaikumar and Jasmine (2016)
Cayratia carnosa	Vitaceae	Pandey and Madhuri (2009)
Cedrus deodara	Pinaceae	Pandey and Madhuri (2009)
Ceiba pentandra	Bombacaceae	Pandey and Madhuri (2009)
Celtis africana	Ulmaceae	Pandey and Madhuri (2009)
Centaurea spp.	Asteraceae	Prakash et al. (2013)
Centella asiatica	Apiaceae	Prakash et al. (2013)
Cephalotaxus fortunei	Cephalotaxaceae	Basmadjian et al. (2014)
Cephalotaxus harringtonia	Cephalotaxaceae	Prakash et al. (2013)
Chelidonium majus	Papaveraceae	Prakash et al. (2013)
Chloranthus henryi	Chloranthaceae	Ko and Moon (2015)
Chimaphila umbellata	Ericaceae	Prakash et al. (2013)
Cichorium intybus	Asteraceae	Jaikumar and Jasmine (2016)
Cissus quadrangularis	Vitaceae	Pandey and Madhuri (2009)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Citrus limon	Rutaceae	Pandey and Madhuri (2009)
Clausena excavata	Rutaceae	Rahman (2016)
Cleistanthus collinus	Euphorbiaceae	Kaur et al. (2011)
Colchicum luteum	Liliaceae	Chavan et al. (2013)
Combretum caffrum	Combretaceae	Prakash et al. (2013)
Coscinium fenestratum	Menispermaceae	Kamkaen et al. (2006)
Coix lacryma-jobi	Poaceae	Prakash et al. (2013)
Crocus sativus	Iridaceae	Samarghandian et al. (2011)
Croton lechleri	Euphorbiaceae	Kaur et al. (2011)
Curcuma domestica	Zingiberaceae	Prakash et al. (2013)
Curcuma longa	Zingiberaceae	Gali-Muhtasib et al. (2015)
Curcuma wenyujin	Zingiberaceae	Ko and Moon (2015)
Curcuma zedoaria	Zingiberaceae	Reddy et al. (2003)
Curtisia dentata	Cornaceae	Pandey and Madhuri (2009)
Cuscuta reflexa	Convolvulaceae	Rahman and Khan (2013)
Cycas rumphii	Cycadaceae	Pandey and Madhuri (2009)
Cyclopia intermedia	Fabaceae	Reddy et al. (2003)
Cynodon dactylon	Poaceae	Jaikumar and Jasmine (2016)
Daphne mezereum	Thymelaeaceae	Prakash et al. (2013)
Datura metel	Solanaceae	Jaikumar and Jasmine (2016)
Decaspermum fruticosum	Myrtaceae	Pandey and Madhuri (2009)
Dendrobium moniliforme	Orchidaceae	Ko and Moon (2015)
Dendrophthoe falcata	Loranthaceae	Rahman and Khan (2013)
Dioscorea bulbifera	Dioscoreaceae	Rahman and Khan (2013)
Diphylleia grayi	Berberidaceae	Kaur et al. (2011)
Dracunculus vulgaris	Araceae	Jaikumar and Jasmine (2016)
Dryopteris crassirhizoma	Polypodiaceae	Prakash et al. (2013)
Duranta serratifolia	Verbenaceae	Jaikumar and Jasmine (2016)
Dysoxylum binectariferum	Meliaceae	Mans et al. (2000)
Dysoxylum caulostachyum	Meliaceae	Jaikumar and Jasmine (2016)
Echinacea angustifolia	Asteraceae	Chavan et al. (2013)
Echinops setifer	Asteraceae	Prakash et al. (2013)
Elaeis guineensis	Arecaceae	Jaikumar and Jasmine (2016)
Elephantopus scaber	Asteraceae	Jaikumar and Jasmine (2016)
Embelia ribes	Myrsinaceae	Rahman and Khan (2013)
Erythronium americanum	Liliaceae	Prakash et al. (2013)
Erythroxylum pervillei	Erythroxylaceae	Prakash et al. (2013)
Eucalyptus grandis	Myrtaceae	Reddy et al. (2003)
Eucomis autumnalis	Hyacinthaceae	Pandey and Madhuri (2009)
Eugenia aquea	Myrtaceae	Jaikumar and Jasmine (2016)
Eugenia caryophyllata	Myrtaceae	Pandey and Madhuri (2009)
Euonymus alatus	Celastraceae	Prakash et al. (2013)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Eupatorium cannabinum	Asteraceae	Prakash et al. (2013)
Euphorbia ingens	Euphorbiaceae	Pandey and Madhuri (2009)
Euphorbia peplus	Euphorbiaceae	Basmadjian et al. (2014)
Euphorbia semiperfoliata	Euphorbiaceae	Kaur et al. (2011)
Equisetum hyemale	Equisetaceae	Pandey and Madhuri (2009)
Fagopyrum esculentum	Polygonaceae	Chavan et al. (2013)
Fallopia japonica	Polygonaceae	Gali-Muhtasib et al. (2015)
Ficus benghalensis	Moraceae	Rahman and Khan (2013)
Ficus religiosa	Moraceae	Rahman and Khan (2013)
Fragaria vesca	Rosaceae	Prakash et al. (2013)
Fritillaria thunbergii	Liliaceae	Prakash et al. (2013)
Galium aparine	Rubiaceae	Prakash et al. (2013)
Garcinia celebica	Clusiaceace	Jaikumar and Jasmine (2016)
Gardenia jasminoides	Rubiaceae	Ko and Moon (2015)
Genista tinctoria	Fabaceae	Gali-Muhtasib et al. (2015)
Geranium robertianum	Geraniaceae	Pandey and Madhuri (2009)
Ginkgo biloba	Ginkgoaceae	Chavan et al. (2013)
Glycyrrhiza glabra	Fabaceae	Pandey and Madhuri (2009)
Glycine max	Fabaceae	Suthar et al. (2001)
Gossypium hirsutum	Malvaceae	Prakash et al. (2013)
Gunnera perpensa	Gunneraceae	Kaur et al. (2011)
Gymnosporia rothiana	Celastraceae	Reddy et al. (2003)
Gynura pseudochina	Asteraceae	Pandey and Madhuri (2009)
Harringtonia cephalotaxus	Cephalotaxaceae	Mans et al. (2000)
Hibiscus cannabinus	Malvaceae	Rahman (2016)
Hibiscus tiliaceus	Malvaceae	Rahman and Khan (2013)
Hydrastis canadensis	Ranunculaceae	Prakash et al. (2013)
Hypericum perforatum	Hypericaceae	Prakash et al. (2013)
Hypoxis colchicifolia	Hypoxidaceae	Kaur et al. (2011)
Hypoxis hemerocallidea	Hypoxidaceae	Pandey and Madhuri (2009)
Indigofera tinctoria	Fabaceae	Kaur et al. (2011)
Inula graveolens	Asteraceae	Jaikumar and Jasmine (2016)
Ipomoea batatas	Convolvulaceae	Mans et al. (2000)
Jatropha gossypiifolia	Euphorbiaceae	Rahman and Khan (2013)
Juncus effuses	Juncaceae	Prakash et al. (2013)
Justicia procumbens	Acanthaceae	Kaur et al. (2011)
Kaempferia parviflora	Zingiberaceae	Rahman and Khan (2013)
Lantana camara	Verbenaceae	Rahman and Khan (2013)
Larrea tridentate	Zygophyllaceae	Prakash et al. (2013)
Lavandula dentata	Lamiaceae	Jaikumar and Jasmine (2016)
Leea indica	Leeaceae	Rahman and Khan (2013)
Lepisorus contortus	Polypodiaceae	Yang et al. (2011)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Limonia acidissima	Rutaceae	Jaikumar and Jasmine (2016)
Linum album	Linaceae	Kaur et al. (2011)
Linum usitatissimum	Linaceae	Chavan et al. (2013)
Lithospermum erythrorhizon	Boraginaceae	Demain and Vaishnav (2011)
Lonicera japonica	Caprifoliaceae	Prakash et al. (2013)
Luisia tenuifolia	Orchidaceae	Pandey and Madhuri (2009)
Maclura pomifera	Moraceae	Greenwell and Rahman (2015)
Macrosolen parasiticus	Loranthaceaea	Jaikumar and Jasmine (2016)
Mallotus philippensis	Euphorbiaceae	Pandey and Madhuri (2009)
Malus domestica	Rosaceae	Chavan et al. (2013)
Mangifera indica	Anacardiaceae	Prakash et al. (2013)
Manilkara zapota	Sapotaceae	Jaikumar and Jasmine (2016)
Marrubium vulgare	Lamiaceae	Jaikumar and Jasmine (2016)
Martynia annua	Martyniaceae	Pandey and Madhuri (2009)
Medicago sativa	Fabaceae	Prakash et al. (2013)
Melastoma malabathricum	Melastomataceae	Jaikumar and Jasmine (2016)
Mentha arvensis	Lamiaceae	Pandey and Madhuri (2009)
Mollugo pentaphylla	Molluginaceae	Rahman and Khan (2013)
Morinda citrifolia	Rubiaceae	Chavan et al. (2013)
Moringa oleifera	Moringaceae	Costa-Lotufo et al. (2005)
Moringa peregrina	Moringaceae	El-Alfy et al. (2011)
Murraya koenigii	Rutaceae	Rahman and Khan (2013)
Mussaenda raiateenisis	Rubiaceae	Pandey and Madhuri (2009)
Nelumbo nucifera	Nelumbonaceae	Rahman and Khan (2013)
Nervilia fordii	Orchidaceae	Prakash et al. (2013)
Nigella sativa	Ranunculaceae	Chavan et al. (2013)
Nyctanthes arbortristis	Oleaceae	Rahman and Khan (2013)
Ochrosia elliptica	Apocynaceae	Chavan et al. (2013)
Ocimum sanctum	Lamiaceae	Chavan et al. (2013)
Oldenlandia diffusa	Rubiaceae	Chavan et al. (2013)
Olea europaea	Oleaceae	Prakash et al. (2013)
Origanum dayi	Lamiaceae	Solowey et al. (2014)
Ornithogalum spp.	Asparagaceae	Reddy et al. (2003)
Oroxylum indicum	Bignoniaceae	Costa-Lotufo et al. (2005)
Oxalis corniculata	Oxalidaceae	Rahman and Khan (2013)
Panax ginseng	Araliaceae	Chavan et al. (2013)
Panax quinquefolium	Araliaceae	Prakash et al. (2013)
Pandanus odoratissimus	Pandanaceae	Pandey and Madhuri (2009)
Paris polyphylla	Melanthiaceae	Kaur et al. (2011)
Pastinaca sativa	Apiaceae	Pandey and Madhuri (2009)
Penstemon deustus	Scrophulariaceae	Kaur et al. (2011)
Periploca aphylla	Asciepiadaceae	Pandey and Madhuri (2009)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Phaleria macrocarpa	Thymelaeaceae	Prakash et al. (2013)
Phellodendron amurense	Rutaceae	Ko and Moon (2015)
Phyllanthus emblica	Euphorbiaceae	Jaikumar and Jasmine (2016)
Physalis angulata	Solanaceae	Pandey and Madhuri (2009)
Physalis minima	Solanaceae	Rahman and Khan (2013)
Picrorhiza kurroa	Scrophulariaceae	Chavan et al. (2013)
Piper cubeba	Piperaceae	Jaikumar and Jasmine (2016)
Piper longum	Piperaceae	Pandey and Madhuri (2009)
Piper nigrum	Piperaceae	Jaikumar and Jasmine (2016)
Pittosporum viridiflorum	Pittosporaceae	Pandey and Madhuri (2009)
Plumbago zeylanica	Plumbaginaceae	Chavan et al. (2013)
Podophyllum emodi	Berberidaceae	Mans et al. (2000)
Podophyllum peltatum	Berberidaceae	Mans et al. (2000)
Polygala senega	Polygalaceae	Pandey and Madhuri (2009)
Polygonatum multiflorum	Liliaceae	Prakash et al. (2013)
Polygonum cuspidatum	Polygonaceae	Kaur et al. (2011)
Pongamia pinnata	Fabaceae	Pandey and Madhuri (2009)
Potentilla chinensis	Rolsaaceae	Prakash et al. (2013)
Premna obtusifolia	Verbenaceae	Pandey and Madhuri (2009)
Primula auriculata	Primulaceae	Jaikumar and Jasmine (2016)
Prosopis cineraria	Fabaceae	Jaikumar and Jasmine (2016)
Prunella vulgaris	Lamiaceae	Chavan et al. (2013)
Prunus spp.	Rosaceae	Pandey and Madhuri (2009)
Psoralea corylifolia	Fabaceae	Chavan et al. (2013)
Psychotria insularum	Rubiaceae	Pandey and Madhuri (2009)
Psychotria valentonic	Rubiaceae	Jaikumar and Jasmine (2016)
Pteris multifida	Pteridaceace	Jaikumar and Jasmine (2016)
Pterocarpus santalinus	Fabaceae	Rahman and Khan (2013)
Pterospermum acerifolium	Sterculiaceae	Pandey and Madhuri (2009)
Punica granatum	Punicaceae	Chavan et al. (2013)
Pygeum africanum	Boraginaceae	Prakash et al. (2013)
Pyrus malus	Rosaceae	Prakash et al. (2013)
Raphanus sativus	Brassicaceae	Prakash et al. (2013)
Rhaphidophora pertusa	Araceae	Pandey and Madhuri (2009)
Rhus chinensis	Anacardiaceae	Prakash et al. (2013)
Rubia cordifolia	Rubiaceae	Prakash et al. (2013)
Rubus idaeus	Rosaceae	Prakash et al. (2013)
Salix tetrasperma	Salicaceae	Rahman and Khan (2013)
Salvadora persica	Salvadoraceae	Jaikumar and Jasmine (2016)
Saururus chinensis	Saururaceae	Ko and Moon (2015)
Saussurea lappa	Asteraceae	Chavan et al. (2013)
Scilla natalensis	Hyacinthaceae	Prakash et al. (2013)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Scrophularia variegata	Scrophulariaceae	Jaikumar and Jasmine (2016)
Scrophularia nodosa	Scrophulariaceae	Prakash et al. (2013)
Scutellaria spp.	Lamiaceae	Prakash et al. (2013)
Sesamum indicum	Pedaliaceae	Pandey and Madhuri (2009)
Sesbania grandiflora	Fabaceae	Jaikumar and Jasmine (2016)
Silybum marianum	Asteraceae	Prakash et al. (2013)
Smilax china	Smilacaceae	Prakash et al. (2013)
Smilax chinensis	Liliaceae	Prakash et al. (2013)
Solanum anguivi	Solanaceae	Jaikumar and Jasmine (2016)
Solanum nigrum	Solanaceae	Rahman and Khan (2013)
Solanum torvum	Solanaceae	Jaikumar and Jasmine (2016)
Sonchus oleraceus	Asteraceae	Pandey and Madhuri (2009)
Strychnos nuxvomica	Loganiaceae	Prakash et al. (2013)
Sutherlandia frutescens	Fabaceae	Pandey and Madhuri (2009)
Syzygium cumini	Myrtaceae	Jaikumar and Jasmine (2016)
Tabebuia spp.	Bignoniaceae	Prakash et al. (2013)
Taraxacum officinale	Asteraceae	Prakash et al. (2013)
Taxodium distichum	Cupressaceae	Pandey and Madhuri (2009)
Taxus brevifolia	Taxaceae	Mans et al. (2000)
Tecoma stans	Bignoniaceae	Jaikumar and Jasmine (2016)
Tephrosia purpurea	Fabaceae	Jaikumar and Jasmine (2016)
Terminalia chebula	Combretaceae	Prakash et al. (2013)
Tetragonia tetragonioides	Aizoaceae	Pandey and Madhuri (2009)
Tetrastigma serrulatum	Vitaceae	Pandey and Madhuri (2009)
Thespesia populnea	Malvaceae	Pandey and Madhuri (2009)
Thuja occidentalis	Cupressaceae	Prakash et al. (2013)
Thymus vulgaris	Lamiaceae	Prakash et al. (2013)
Tinospora cordifolia	Menispermaceae	Chavan et al. (2013)
Tinospora crispa	Menispermaceae	Rahman and Khan (2013)
Toona ciliata	Meliaceae	Jaikumar and Jasmine (2016)
Tragia involucrata	Euphorbiaceae	Rahman and Khan (2013)
Trapa natans	Trapaceae	Pandey and Madhuri (2009)
Trichosanthes kirilowii	Cucurbitaceae	Pandey and Madhuri (2009)
Trifolium pratense	Fabaceae	Prakash et al. (2013)
Trigonella foenum-gracecum	Fabaceae	Jaikumar and Jasmine (2016)
Typhonium flagelliforme	Araceae	Rahman (2016)
Urtica membranacea	Urticaceae	Solowey et al. (2014)
Uncaria tomentosa	Rubiaceae	Reddy et al. (2003)
Vernonia amygdalina	Asteraceae	Prakash et al. (2013)
Vernonia cinerea	Asteraceae	Pandey and Madhuri (2009)
Vetiveriazizanioides	Poaceae	Jaikumar and Jasmine (2016)
Viscum album	Santalaceae (Viscaceae)	Chavan et al. (2013)

(continued)

Table 4.1 (continued)

Botanical name	Family name	References
Vitex negundo	Verbenaceae	Rahman and Khan (2013)
Vitex rotundifolia	Verbenaceae	Prakash et al. (2013)
Vitex trifolia	Verbenaceae	Jaikumar and Jasmine (2016)
Wikstroemia viridi	Thymelaeaceae	Kaur et al. (2011)
Withania somnifera	Solanaceae	Prakash et al. (2013)
Wrightia tinctoria	Apocynaceae	Jaikumar and Jasmine (2016)
Zingiber cassumunar	Zingiberaceae	Rahman (2016)
Zingiber officinale	Zingiberaceae	Prakash et al. (2013)
Zingiber zerumbet	Zingiberaceae	Rahman (2016)
Zizyphus spp.	Rhamnaceae	Prakash et al. (2013)
Miscellaneous		
Chlorella pyrenoidosa	Oocystaceae	Chavan et al. (2013)
Coriolus versicolor	Polyporaceae	Reddy et al. (2003)
Ecteinascidia turbinata	Perophoridae	Basmadjian et al. (2014)
Gyrophora esculenta	Umbilicariaceae	Chavan et al. (2013)
Halichondria okadai	Halichondriidae	Basmadjian et al. (2014)
Lentinus edodes	Marasmiaceae	Chavan et al. (2013)
Mylabris phalerata	Meloidae	Chavan et al. (2013)
Undaria pinnatifida	Alariaceae	Reddy et al. (2003)

natural sources including plants. New technologies in isolating bioactive compounds and screening for anticancer activities (high throughput screening) have been developed and studied for natural products. Further, scientific evidences have revealed that anticancer phyto-drugs prevent and destroy cancerous cells through the involvement of various kinds of molecular mechanisms of action. Simultaneously, new challenges are emerging due to safety concern, increased cases of drug resistance, and cost of tumor diagnosis. Apart from these, rapid growing obesity rate and increasing addict to smoking has been recognized as two more challenges/risk factors leading to high cancer incidence. The gold standards for assessing the safety and efficacy of drugs must be followed strictly and uniformly across the globe. In this regard, more number of *in vivo* studies should be encouraged to access the potential of lead plant molecules in future. Similarly, the placebo-controlled clinical trials must be carried out universally to have statistical significance value. In conclusion, natural product research is fascinating approach for discovering novel bioactive compounds with unique chemical structure and unique mode of action against different cancer types.

Table 4.2 List of phytochemicals with anticancer potential

Compound name	Mechanism of actions	References
Abrin A and B	Cytotoxicity, growth inhibition	Rahman and Khan (2013)
Ailanthone	Apoptosis induction and cell cycle arrest	Zhuo et al. (2015)
Alantolactone	Apoptosis induction and cell cycle arrest	Millimouno et al. (2014)
Allicin	Cell cycle arrest (S to G2/M phase)	Reddy et al. (2003)
Aloesin	Apoptosis induction, cell cycle arrest, anti-invasion and anti-migration activity	Zhang et al. (2017)
Amarbelin	Cytotoxicity, p53 and Bax upregulation, Bcl-2 and survivin downregulation	Rahman and Khan (2013)
9-Aminocampothecin	Inhibition of topoisomerase I	Mans et al. (2000)
Amooranin	Cytotoxicity, cell cycle, arrest (G2/M phase), caspase-activated apoptosis, growth inhibition through cyclin-dependent kinases (CDK2 and CDK1)	Rahman and Khan (2013)
Amygdalin	Apoptosis induction	Song and Xu (2014)
β-Amyrin	Cytotoxicity	Rahman and Khan (2013)
Anacardic acid	Cytotoxicity	Kubo et al. (1993)
Anthocyanidin	Induction of apoptosis, inhibition H3 and H4 acetylation, and inhibition Rb protein phosphorylation	Rahman and Khan (2013)
Apigenin	Cytotoxicity	Rahman and Khan (2013)
Arbutin	Apoptosis induction	Nawarak et al. (2009)
Arctigenin	Antiproliferative effect	Hirano et al. (1994)
Asiaticoside	Apoptosis induction	Huang et al. (2004)
Asimilobine	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Astaxanthin	Antiproliferation, apoptosis induction, and anti-invasion activity	Zhang and Wang (2015)
Astragalin	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Baicalein	Antiproliferative effect	Hirano et al. (1994)
Berberine	Apoptosis induction, complexes with DNA	Reddy et al. (2003)
Bergenin	Cytotoxicity, p53 and Bax upregulation, Bcl-2 and survivin downregulation	Rahman and Khan (2013)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Betulinic acid	Apoptosis induction by affecting the mitochondrial membrane permeability complex, enhancing the release of cytochrome C, regulating Bcl-2 family members, inhibiting NF-κB activity and anti-metastasis	Wang et al. (2012)
Biochanin A	Antiproliferation, apoptosis induction, anti-invasion and anti-migration activity	Bhardwaj et al. (2014)
Bruceantin	c-Myc (oncoprotein) downregulation	Cragg and Newman (2005)
Caffeic acid	Apoptosis induction via by the mitochondrial apoptotic pathway and caspase-3 activation	Chang et al. (2010)
27-*O-trans* caffeoylcylicodiscic acid	Cytotoxicity, growth inhibition	Rahman and Khan (2013)
Camaraside	Cell proliferation inhibition, caspase-3-dependent apoptosis, Bcl-2 downregulation and Bax upregulation	Rahman and Khan (2013)
Capsaicin	Apoptosis induction and cell cycle arrest	Ko and Moon (2015)
Cardols	Cytotoxicity	Kubo et al. (1993)
Carnosic acid	Forms DNA adducts	Reddy et al. (2003)
Catechin	Cytotoxicity	Rahman and Khan (2013)
Caryatin	Apoptosis induction	Rahman and Khan (2013)
β-Caryophyllene	Cytotoxicity	Rahman and Khan (2013)
Casticin	Antiproliferation effect	Millimouno et al. (2014)
Chalcinasterol	Cytotoxicity	Rahman and Khan (2013)
Chebulinic acid	Cytotoxicity	Rahman and Khan (2013)
Cholestane glycoside	Apoptosis induction	Reddy et al. (2003)
Chrysin	Antiproliferation, apoptosis induction, caspase activation and inactivation of Akt signaling pathway	Khoo et al. (2010)
Cleistanthin A	Inhibition of DNA synthesis and cell division	Pradheepkumar and Shanmugam (1999)
Colchicine	Hinders microtubule formation, inhibits cell cycle progression, and induces apoptosis	Wang et al. (2012)
Costunolide	Apoptosis induction and cell cycle arrest	Millimouno et al. (2014)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Crocetin	Inhibition of growth, induction of apoptosis, and hindering growth factor signaling pathways	Wang et al. (2012)
β-Cryptoxanthin	Stimulates expression of RB and p73 gene (tumor suppressor gene)	Reddy et al. (2003)
Coumarin	Cytotoxicity, p53 and Bax upregulation, Bcl-2 and survivin downregulation	Rahman and Khan (2013)
Cubebin	Apoptosis induction	Rajalekshmi et al. (2016)
Curcumin	Apoptosis induction and regulation of multiple cell signaling pathways including cell proliferation pathway (cyclin D1, c-myc), cell survival pathway (Bcl-2, Bcl-x, cFLIP, XIAP, c-IAP1), caspase activation pathway (caspase-8, 3, 9), tumor suppressor pathway (p53, p21), death receptor pathway (DR4, DR5), mitochondrial pathways, and protein kinase pathway (JNK, *Akt*, and AMPK)	Wang et al. (2012)
Daidzein	Antiproliferative effect	Hirano et al. (1994)
Dayscyphin C	Cytotoxicity	Khanna and Kannabiran (2009)
Delphinidin	Antiproliferation (cell cycle arrest, apoptosis) effect	Bin Hafeez et al. (2008)
Denbinobin	Apoptosis induction	Ko and Moon (2015)
Diosbulbin B	Apoptosis induction	Rahman and Khan (2013)
Dulcitol	Cytotoxicity, p53 and Bax upregulation, Bcl-2 and survivin downregulation	Rahman and Khan (2013)
β-Elemene	Cell cycle arrest (S to G2/M phase)	Reddy et al. (2003)
Ellagic acid	Apoptosis induction and inhibition of NF-κB activity	Edderkaoui et al. (2008)
Elliptinium	Inhibition of topoisomerase II	Mans et al. (2000)
Embelin	Apoptosis induction, cell cycle arrest, downregulation of Bcl-2, Bcl-xL, survivin, IAP-1, IAP-2, cyclin D1, and caspase-3 activation	Rahman and Khan (2013)
Epigallocatechin-3-gallate	Apoptosis induction, cell cycle arrest	Reddy et al. (2003)
Epipodophyllotoxin	Pro-apoptotic effects, cell cycle interference	Greenwell and Rahman (2015)
Etoposide	Inhibition of topoisomerase II	Mans et al. (2000)
Euglobal-G1	Apoptosis induction	Reddy et al. (2003)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Evodiamine	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Millimouno et al. (2014)
Falodone	Antiproliferative effect, cytotoxicity	Rahman and Khan (2013)
Fangchinoline	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Ko and Moon (2015)
E-*β*-farnesene	Cytotoxicity	Rahman and Khan (2013)
Ferulic acid	Modulates mitogenic signaling and induction of cell cycle (G1) arrest and apoptosis	Reddy et al. (2003)
Fisetin	Cell cycle arrest (G1 phase) and disrupted Wnt/*β*-catenin signaling	Wang et al. (2012)
Flavopiridol	Inhibition of cyclin-dependent kinases	Mans et al. (2000)
Formononetin	Apoptosis induction and inhibition of *Akt* signaling pathway	Jin et al. (2014)
Friedelin	Cytotoxicity	Rahman and Khan (2013)
Furanodiene	Cell cycle transition, anti-invasion, anti-metastasis	Ko and Moon (2015)
Gallic acid	Cytotoxicity	Rahman and Khan (2013)
Galangin	Apoptosis induction and anti-invasion	Cao et al. (2016)
Genipin	Apoptosis induction, inhibiting invasion/metastasis	Ko and Moon (2015)
Gentisic acid	Induction of apoptosis, inhibition H3 and H4 acetylation, and inhibition Rb protein phosphorylation	Rahman and Khan (2013)
Genistein	Regulation of multiple signaling pathways (PTK, *Akt*, NF-κB, MMP, and Bax/Bcl-2)	Lee et al. (2012)
Glycyrrhizic acid	Antiproliferation, apoptosis induction, and Fas and FasL upregulation	Haghshenas et al. (2014)
Gingerol	Apoptosis induction	Wang et al. (2012)
Ginsenoside	Apoptosis, cell cycle transition	Ko and Moon (2015)
Gymnemagenol	Cytotoxicity	Khanna and Kannabiran (2009)
Halofuginone	Antiproliferative effect	Juárez(2014)
Homoharringtonine	Inhibition of DNA polymerase α	Mans et al. (2000)
Honokiol	Apoptosis induction	Juárez (2014)
α-Humulene	Cytotoxicity	Rahman and Khan (2013)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Hypericin	Photocytotoxicity, induction of apoptosis and necrosis	Agostinis et al. 2002.
Indirubin	Inhibition of cyclin-dependent kinases (CDKs)	Cragg and Newman (2005)
4-Ipomeanol	Cytochrome P-450-mediated conversion into DNA-binding metabolites	Mans et al. (2000)
Isoalantolactone	Antiproliferative effect	Millimouno et al. (2014)
Isoliensinine	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), and inhibition of growth	Rahman and Khan (2013)
Isoliquiritigenin	Antiproliferation (cell cycle arrest, apoptosis induction) effect, anti-invasion and anti-metastasis activity	Peng et al. (2015)
Jaceosidin	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Millimouno et al. (2014)
Jatrophone	Antiproliferative effect and cytotoxicity	Rahman and Khan (2013)
Kaempferol	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth, and apoptosis induction	Rahman and Khan (2013)
Ent-kaurane	Cytotoxicity	Rahman and Khan (2013)
β-Lapachone	Inhibition of cell division cycle 25 (CDC25) phosphatases	Cragg and Newman (2005)
Lantadene A	Antiproliferative effect, caspase-3-dependent apoptosis, Bcl-2 downregulation, and Bax upregulation	Rahman and Khan (2013)
Leucocyanidin	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Liensinine	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Limonin	Antiproliferative effect	Hirano et al. (1994)
Liriodendrin	Inhibition of growth	Ran et al. (2013)
Liquiritigenin	Antiproliferative effect	Hirano et al. (1994)
Lupeol	Cytotoxicity	Rahman and Khan (2013)
Lunasin	Induction of apoptosis, inhibition H3 and H4 acetylation, and inhibition Rb protein phosphorylation	Rahman and Khan (2013)
Luteolin	Induction of apoptosis, inhibition H3 and H4 acetylation, and inhibition Rb protein phosphorylation	Rahman and Khan (2013)
Lycopene	Antiproliferation (cell cycle arrest, apoptosis induction) effect, anti-invasion, anti-metastasis	Ko and Moon (2015)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Machicendiol	Antiproliferative effect	Hirano et al. (1994)
Machilin A	Antiproliferative effect	Hirano et al. (1994)
Magnolol	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Millimouno et al. (2014)
Mahanine	Apoptosis induction	Rahman and Khan (2013)
Matairesinol	Antiproliferative effect	Hirano et al. (1994)
Methylcardols	Cytotoxicity	Kubo et al. (1993)
Mezerein	Induction of protein kinase C (PKC) activity	Saraiva et al. 2001.
Mollupentin	Cytotoxicity	Rahman and Khan (2013)
Morin	Anti-invasion and anti-metastasis effect	Ko and Moon (2015)
Myriceric acid B	Cytotoxicity, growth inhibition	Rahman and Khan (2013)
Myricetin	Apoptosis induction	Rahman and Khan (2013)
Myricetin-3-*O*-galactopyranoside	Apoptosis induction	Rahman and Khan (2013)
Naringenin	Antiproliferative effect	Hirano et al. (1994)
Neferine	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Nexrutine	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Ko and Moon (2015)
Nitidine	Inhibition of topoisomerase I	Cragg and Newman (2005)
Nobiletin	Antiproliferative effect	Hirano et al. (1994)
Noscapine	Cell cycle arrest (G2/M phase) and induction of variety of cell death mechanisms including autophagy and mitotic catastrophe	Wang et al. (2012)
Nuciferine	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Nyctanthic acid	Cytotoxicity	Rahman and Khan (2013)
Oleanolic acid	Cytotoxicity	Rahman and Khan (2013)
Olomucine	Inhibition of cyclin-dependent kinases (CDKs)	Cragg and Newman (2005)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Oridonin	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Millimouno et al. (2014)
Panduratin A	Cell cycle arrest and apoptosis induction	Kirana et al. (2007)
Parthenolide	Antiproliferation (cell cycle arrest, apoptosis induction) and cytotoxicity effect	Millimouno et al. (2014)
Phytic acid (inositol hexaphosphate)	Cytotoxicity	Norhaizan et al. (2011)
Phloroglucinol	Apoptosis induction and caspase 3 and 8 activation	Kang et al. (2014)
Pinoresinol	Cytotoxicity and antiproliferative effect	López-Biedma et al. (2016)
Pseudolaric acid B	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Millimouno et al. (2014)
Psoralen	Anti-metastasis	Ko and Moon (2015)
Pterostilbene	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Ko and Moon (2015)
Quercetin	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Quercetin-3-*O*-galactopyranoside	Apoptosis induction	Rahman and Khan (2013)
Quercitol	Apoptosis induction, cell cycle arrest, downregulation of Bcl-2, Bcl-xL, survivin, IAP- 1, IAP-2, cyclin D1, and caspase-3 activation	Rahman and Khan (2013)
Remrefidine	Cytotoxicity, p21-dependent cell cycle arrest (G1 phase), inhibition of growth	Rahman and Khan (2013)
Resveratrol	Cell cycle arrest, and regulation of multiple cell signaling pathways	Wang et al. (2012)
Rohitukine	Cytotoxicity, cell cycle arrest (G2/M phase), caspase-activated apoptosis, growth inhibition through cyclin-dependent kinases (CDK2 and CDK1)	Rahman and Khan (2013)
Rosmarinic acid	Apoptosis induction, inhibition of growth and anti-metastasis activity	Hossan et al. (2014)
Sauchinone	Apoptosis induction	Ko and Moon (2015)
Sesamin (asarinin)	Antiproliferative effect	Hirano et al. (1994)
Silvestrol	Apoptosis induction	Kim et al. (2007)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Silymarin	Cell cycle arrest, antiproliferation, induction of cyclin-dependent kinase inhibitors (p15, p21, and p27), downregulation of anti-apoptotic gene products (Bcl-2 and Bcl-xL), inhibition of cell survival kinases (AKT, PKC, and MAPK), and inhibition of inflammatory transcription factors (NF-kappaB), anti-invasion, anti-angiogenesis, and anti-metastasis	Agarwal et al. (2006)
β-Sitosterol	Cytotoxicity	Rahman and Khan (2013)
Stigmasterol	Cytotoxicity, p53 and Bax upregulation, Bcl-2 and survivin downregulation	Rahman and Khan (2013)
Sulforaphane	Cell cycle arrest associated with altered microtubule dynamic, cdc2 kinase activity, increased protein expression of cyclin B1, p21, and histone H1 phosphorylation	Wang et al. (2012)
Tehranolide	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Ko and Moon (2015)
Thapsigargin	Apoptosis induction	Cragg and Newman (2005)
Thymoquinol dimethyl ether	Cytotoxicity	Rahman and Khan (2013)
Tinoscorside A	Antiproliferative effect	Rahman and Khan (2013)
Triptolide	Antiproliferative effect and apoptosis induction	Wang et al. (2012)
Umbelliferone	Apoptosis induction, cell cycle arrest, and DNA fragmentation	Yu et al. (2015)
Ursolic acid	Apoptosis induction and anti-metastasis effect	Ko and Moon (2015)
Verbascoside	Apoptosis induction and activation of HIPK2-p53 signaling pathway	Zhou et al. (2014)
Vinblastine	Inhibition of tubulin polymerization	Mans et al. (2000)
Vincristine	Inhibition of tubulin polymerization	Mans et al. (2000)
Wedelolactone	Antiproliferation (cell cycle arrest, apoptosis induction) effect	Millimouno et al. (2014)
Wogonin	Antiproliferative effect	Hirano et al. (1994)
Xanthorrhizol	Antiproliferation (cell cycle arrest, apoptosis induction), caspase activation, regulation of MAPK pathway, inhibition of Akt/NF-κB pathway	Oon et al. (2015)
Yakuchinone A	Apoptosis induction through the Bcl-2-mediated signaling pathway	Lin et al. (2013)

(continued)

Table 4.2 (continued)

Compound name	Mechanism of actions	References
Zeaxanthin	Cytotoxicity, apoptosis induction, decreased the expression of anti-apoptotic proteins (Bcl-2 and Bcl-xL) and increased the expression of pro-apoptotic proteins (Bak and Bax) and caspase activation (caspases 3 and 9)	Bi et al. (2013)
Zingerone	Inhibition of c-Jun N-terminal kinases (JNKs) signaling pathway	Bae et al. (2016)
Aplidine	Inhibition of growth, apoptosis induction, cytotoxicity, and inhibition of VEGF secretion	Broggini et al. (2003)
Aragusterol A	Cell cycle arrest	Mayer and Gustafson (2003)
Ascididemin	Apoptosis induction	Mayer and Gustafson (2003)
Bryostatin1	Antiproliferation	Mayer and Gustafson (2003)
Curacin A	Inhibition of tubulin polymerization	Wipf et al. 2004.
Discodermolide	Apoptosis induction	Mayer and Gustafson (2003)
Dolastatin10 and 15	Apoptosis induction, inhibition of microtubule assembly, anti-mitosis, induction of Bcl-2 phosphorylation	Amador et al. (2003)
Ecteinascidin 743 (ET743)	Regulation of transcription-coupled NER pathway	Takebayashi et al. (2001)
Fascaplysin	Inhibition of cyclin-dependent kinase 4 (CDK4)	Mayer and Gustafson (2003)
Indanone	Inhibition of VEGF expression	Mayer and Gustafson (2003)
Jaspamide	Induction of polyploidization	Mayer and Gustafson (2003)
Lyngbyabellin A	Disruption of cellular microfilaments formation	Mayer and Gustafson (2003)
Melophlins A and B	Reversal of transformed phenotype to normal	Mayer and Gustafson (2003)
Salinosporamide A	Proteasome inhibition	Macherla et al. (2005)
Spisulosine	Disassembly of actin stress fibers	Mayer and Gustafson (2003)
Theopederin A–E	Cytotoxicity	Fusetani et al. (1992)

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Chapter 5
Computational Approach Towards Exploring Interaction of Target Protein-Phytocompounds in Drug Development for Breast Cancer

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Abstract Breast cancer is one of the leading cause of cancer related deaths in women, worldwide. In Malaysia, it is the most common cancer, where 1 in 19 Malaysian women are diagnosed with breast cancer. The incidence rate is increasing because of the lack of specific symptoms in the early stage of disease leading to delay in diagnosis. Unfortunately, recently used treatments such as chemotherapeutic, surgery and radiation therapies have not been fully effective against breast cancer. Therefore, there is a need of developing new approaches for the treatment of breast cancer. In this regards, plant derived compounds have been identified as a class of promising anticancer agents in the quest for novel pharmaceutical compounds to built the new target protein, their simulation and interaction with phytocompounds. The molecular docking method has been explored for its binding affinity towards the site of interactions. The best binding affinity is chosen based on the lowest binding energy, total intermolecular energy and highest number of hydrogen bonds to target the phytocompounds. Thus, the aim of this chapter is to focus on the potentiality of the phytocompound based drug discovery for the treatment of breast cancer.

Keywords Breast cancer · Target protein · Phytopcompound · Computational tools

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5.1 Introduction

At present, breast cancer is a major global health problem and the leading cause of deaths among women of all ethnic backgrounds. According to an estimate, 1.6 million new cases are diagnosed worldwide every year (WHO 2014). Based on World Health Organization (WHO 2014), 560,000 women's died because of breast cancer in 2015. In Malaysia, breast cancer is the most common cancer where 1 in 19 Malaysian women will develop breast cancer at some point in their lifetime. Every year, about 5000 Malaysian women are diagnosed with breast cancer. Most cases occur during age between 30 and 60 years. Furthermore, nearly half of those affected are below 50 years old. This is due to the poor prognosis such as lack of specific symptoms in the early stage of disease leading to late in detection, lack of adequate diagnosis and treatment facilities, disease aggressive nature, and high rate of local spread and metastasis at diagnosis time. Currently, the offered cancer treatments such as chemotherapeutic agents, surgery and radiation therapies have not been fully effective against the high incidences of breast cancer. Moreover, these modern treatments cause negative side effects such as anemia, edema, nerve problems, kidney failure, heart attack and other types of health problems (Hawkins and Hermiston 2001). Therefore, the development of a novel therapeutic approach to treat breast cancer still remains as one of the most challenging areas in cancer research.

The computational biology has become a potential tool for the discovery and design of drugs to breast cancer. Currently, this method is growing rapidly because it is less expensive, labour required and time consuming compared to experimental approaches. This when combined with phytocompounds enables a researcher to explore the available choices efficiently. Plant compounds also have been identified as a class of promising anticancer agents in the quest for novel pharmaceutical compounds as they can produce secondary metabolites with new chemical structure. Thus, the aim of this chapter is to focus on the potentiality of the phytocompound based drug discovery for the treatment of breast cancer.

5.2 Etiology of Breast Cancer

Researchers are not sure about the causes of breast cancer because it is very hard to say why one person develops the disease while another does not (Medical News Today 2015). However, there are some factors that can be attributed to the risks of developing breast cancer. It occurs due to the interaction between a defective gene and the environment which means the external agents such as physical (ultraviolet and ionizing radiation), chemical (asbestos, components of tobacco smoke, alcohol, aflatoxin (a food contaminant) and arsenic (a drinking water contaminant)) and biological (infections from certain viruses like hepatitis B (HBV), hepatitis C (HCV) virus and human papilloma virus (HPV), bacteria or parasites) carcinogens (WHO 2014). Besides that, unhealthy diet (low intake of vegetables and fruits), obesity and overweight and physical inactivity may also cause cancer.

5.3 Genetic Alteration of Breast Cancer

Breast cancer is a genetic disease, which means that it is caused by alterations in DNA. These mutations can be inherited in germ line or they can be acquired after birth. At the time of the initial detection of clinical cancer, several genetic changes already accumulated in the tumour cells. Mutation of TP53, BRCA1 (breast cancer susceptibility gene 1), BRCA2 (breast cancer susceptibility gene 2) and PTEN (phosphatase and tensin homolog) genes play a vital role in tumourigenesis (Dunning et al. 1999; Cavalier et al. 2006). These genetic alterations are candidate targets in the development of novel therapeutic approaches (Sunamura et al. 2002).

5.4 Role of Medicinal Plants in Breast Cancer

Phytocompounds are a class of promising anticancer agents. They have been explored intensively to treat different types of cancer. Vinca alkaloids were discovered and developed in the late 1950's. Recently, 60% of used anti-cancer agents are derived from natural sources. Moreover, numerous ethnobotanical studies were reported that herbal medicine was better than synthetic drug treatment for cancer. It is relatively simple, no side effects and effective therapeutic effect. Ethanopharmacology helps to find the novel biologically active compounds from plant sources. The combination of computational biology with medicinal plants has become an advantage for discovery and design drugs to breast cancer. The uses of medicinal plants in breast cancer are summarized in tabular form (Table 5.1).

5.5 Computer Aided Drug Design

Computational biology is a vital and promising branch of bioinformatics where a computer system is useful to analyse the biological data. Currently, this method is growing rapidly because it is less expensive and less time consuming than experimental approaches. This application of technology aids to identify novel therapeutic targets of protein phytocompounds in the drug development for breast cancer treatment.

5.5.1 Structure Based Drug Design

Structural biology provides information of structure. This information combined with the knowledge of biological target helps in development of new therapeutic targets with a rational drug design which is called as structure-based drug design

Table 5.1 Medicinal plants used in the breast cancer therapy

Plant's name	Bioactive compounds	Mechanisms	References
Allium sativum	Quercetin, cyanidin, allistatin I and allistatin II, allicin, alliin oligosaccharides, arginine, selenium	Mitotic arrest of breast cancer cells due to the alteration of the microtubule network, possibly as a consequence of the high reactivity of sulfur atoms against the thiol groups of different cellular macromolecules controlling crucial regulatory functions of cells	Liu et al. (1992)
Camptotheca acuminata	Camptothecin	Inhibits the activity of topoisomerase enzymes I	Mantle et al. (2000)
Lycopersicum esculentum	Lycopene	Inhibits the PI3K/AKT signalling pathways as well as inducing apoptosis in the cancerous cells	Sharoni et al. (2000)
Amoora rohituka	Triterpenic acid, amooranin	Induce apoptosis in breast cancer through up-regulation of caspase 3 activity	Rabi et al. (2003)
Podophyllum peltatum	Etoposide	Inhibits the activity of topoisomerase enzymes II	Cragg and Newman (2005)
Taxus baccata	Paclitaxel, docetaxel	Effects on cytoskeletal proteins which play a vital role in mitosis	
Vitis vinifera	Proanthocyanidins	Inhibits migration of human breast cancer cell by inactivating the inflammatory transcription factor NF-κB	Le Corre et al. (2005)
Garcinia mangostana	Garcinone E	Induces significant cell cycle arrest at G_0/G_1-phase which is indicative of its anti-proliferative properties	Jung et al. (2006)
Citrus sinensis	Polymethoxyflavones	Blocks the metastasis cascade pathway; inhibits mobility of breast cancer cells; increase the apoptosis process and angiogenesis	Sergeev et al. (2006)
Achillea santolina	Caffeic, p-coumaric acid, achillinin A guaianolide, flavonol, centaureidin, santoflavon, saponin	Suppress the proliferation of breast cancer cells by causing the down-regulation of BCL2 expression, and up-regulation of caspase 3 and BAX expression	Abu-Dahab and Afifi (2007)
Nicotiana tabacum	Narcotine, piperidine, N-methylpyrroline, pyrrolidine	Control the growth of breast cancer through reduces oxidative stress (ROS)	Alkhalaf (2007)

(continued)

Table 5.1 (continued)

Plant's name	Bioactive compounds	Mechanisms	References
Crocus sativus	Crocetin	Anti-proliferative	Chryssanthi et al. (2007)
Andrographis paniculata	Andrographolide	Inhibits breast cancer growth at G_0/G_1 phase through induction of cell-cycle inhibitory protein and decreased cyclin-dependent kinase expression activity	Sukardiman et al. (2007)
Zingiber officinale	Curcuminoids	Inhibits breast cancer cell secretion of osteolytic factors	Lee et al. (2008)
Punica granatum	Anthocyanins, catechins, kaempferol, quercetin, apigenin, luteolin, conjugated fatty acids, hydrolyzable tannins	Inhibition of intratumor cell proliferation, induction of apoptosis, and it altered the expression of BAX, BCL2, BAD, caspase-3, caspase-7, caspase-9, poly (ADP ribose) polymerase and cytochrome c	Gazala et al. (2009)
Curcuma longa	Curcumin	Inhibits mitogen-activated protein (MAP) kinase activity, interferes negatively with Janus kinase/signal transducers and activators of transcription(JAK/STAT) signalling pathways, and inhibits the expression of several transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-Kβ) and STAT	Jurenka (2009)
Oryza sativa	Anthocyanin	Suppress the growth of breast cancer cell through PI3K/Akt pathway and inhibition of NF-κB levels	Hui et al. (2010)
Soymida febrifuga	Methyl angolensate, Tetranortriterpenoid	Activates MAP kinase pathway to induce apoptosis	Kishore et al. (2010)
Phaleria macrocarpa	Phalerin, gallic acid, icaricide C, magniferin, mahkoside A, dodecanoic acid, palmitic acid, des-acetylflavicordin-A, flavicordin-A, flavicordin-D, flavicordin-A glucoside, ethyl stearate,lignans, alkaloids and saponins	Induces apoptosis in breast cells through intrinsic mitochondrial related pathway with the participation of pro and anti-apoptotic proteins, caspases, G_0/G_1 and G_2/M-phases cell cycle arrest by p53-mediated mechanism	Tandrasasmita et al. (2010)

(continued)

Table 5.1 (continued)

Plant's name	Bioactive compounds	Mechanisms	References
Mangifera indica	Catechins, antocyanines, flavones coumarins, lignans, phenolic acids, flavonoids, quinones, stilbenes, tannins, curcuminoids	Inhibits growth of breast cancer cells through reduced oxidative stress (ROS)	García-Rivera et al. (2011)
Raphanus sativus	Raphanin, vitamin C	Inhibits cell proliferation through the ErbB-Akt pathway	Kim et al. (2011)
Azadirachta indica	Azadirachtin, nimbolide	Induces apoptosis through caspases 3,8 and 9 activities	Othman et al. (2011)
Costus pictus	Alkaloids, steroids, terpenoids, glycosides, tannins, saponins, phenols, flavonoids	Decreases the growth rate and cell survival of breast cancer cells	Sathuvan et al. (2012)
Dillenia suffruticosa	Saponins, triterpenes, sterols, polyphenolic compounds	Induces apoptosis and the G_2/M cell cycle arrest	Armania et al. (2013)
Tinospora cordifolia	Rutin, quercetin	Increases intracellular ROS levels which causes cellular damages to breast cancer cells, altered pro and anti-apoptotic genes expression, decreases the ability of colony formation and induced programmed cell death in breast cancer cells	Maliyakkal et al. (2013)
Annona muricata	Acetogenins	Inhibits complex I (NADH-ubiquinone oxidoreductase), which prevents the formation of ATP, and in turn, prevents the nutrition of breast cancer cells causing their death	Moghadamtousi et al. (2015)
Moringa oleifera	D-allose	Inhibits the growth of breast cancer cells at G_1 through induction of specific thioredoxin interacting protein (TXNIP) and subsequent stabilization of p27kip1 protein without damaging the normal cells	Charoensin (2014)
Sarracenia alata	Ar-turmerone, β-caryophyllene, (E)-phytol, 6,10,14-trimethyl-2-pentadecanone	Cytotoxic activities, apoptotic induction and proliferation inhibition	Devi et al. (2016)
Pseudocedrela kotschyi	Saponins	Anti-proliferative	Elufioye et al. (2017)
Annona hypoglauca	Isoquinoline, Acetogenins	Disrupts cell cycle progression	Rinaldi et al. (2017)

(SBDD). This technique becomes possible and effective. Recently, this applied routinely in pharmaceutical and medicinal research. In past century, biologically active compounds and chemicals were screened and optimized them to produce suitable pharmaceutics by trial and error method because the structural information and target of biological compounds were unknown (Macarron 2006). Now, SBDD aids to pinpoint the target research and permits the drug discovery and design that have high affinity and selectivity against specific targets. Furthermore, with a complete understanding of the three dimensional (3-D) coordinates of the macromolecules, SBDD gives an opportunity to target allosteric sites, therefore expanding the possible pathway to modulate the targets functions.

5.5.1.1 Homology Modelling

Homology modelling is widely used because of the generation of high accuracy level of 3-D model of the protein of interest (Sanchez et al. 2000; Al-Lazikani et al. 2001). It predicts the 3-D model of target sequence based on its alignment to one or more template sequences. It involves several steps such as alignment of target with template, model building, refinement of model and model evaluation. Firstly, the target sequence is retrieved from National Centre Biotechnology Information (NCBI) in FASTA format. Then, Protein Basic Local Alignment Search Tool (BLASTP) against the RCSB Protein Databank is performed to identify a suitable template with existing 3-D model of template protein sequence. The target sequence is aligned with the template sequence through pair wise alignment. MODELLER or Swiss model program is used to predict the 3-D protein model based on a given sequence alignment and selected template. Then, the protein model is refined using molecular dynamics (MD) simulation tools such as AMBER, CHARMM and GROMACS. Finally, the stereo-chemical quality of generated protein model is evaluated using the protein model validation tools.

5.5.1.2 Molecular Dynamics Simulation of Target Protein

Molecular dynamics simulation describes the study of complex, dynamic processes that happen in biological systems such as protein stability, conformational changes, protein folding, molecular recognition including protein, nucleic acid and transport of ion in biological systems. In this study, MD simulation will be carried out for drug design. Target proteins will simulate using the Gromacs package 4.6.3 (van Der Spoel et al. 2005) adopting the GROMOS 53a6 force field parameter to explore and compare the protein internal dynamics before docked with phytocompounds. They will be simulated to determine the stability of structure at a particular temperature. The essential steps are involved in the MD simulation as follows: setup a cubic box of simple point charge (SPC) molecules of water for simulation; solvate the box, neutralize the protein system by adding sodium or chloride ions; setup energy minimization using steepest descent steps, setup the position-restrained molecular

dynamics (equilibration) at constant volume, temperature, particles number and pressure, setup the production stage and finally processing and analysing trajectories using GROMACS utilities. Root mean square (RMSD), root mean square fluctuations (RMSF), radius of gyration, potential energy and secondary structure analysis is also carried out and the validation of generated protein models is performed by GROMOS (van Gunsteren et al. 1996) and ANOLEA (Atomic Non-Local Environment Assessment) programs.

5.5.1.3 Identification of Active Sites on Target Protein

The active sites of target proteins are identified using Q-site Finder (Laurie and Jackson 2005). Q-Site Finder is a method for prediction of ligand binding sites. The area (cubic Å) and volume (cubic Å) of predicted active sites for target proteins is determined using Q-Site Finder.

5.5.1.4 Docking

Autodock is an automated procedure for predicting the interaction of ligands with biomacromolecular targets. Docking and ranking of a large number of compounds is helpful in identification of new inhibitors for drug development in breast cancer treatment. In this study, target protein is docked with phytocompound using Autodock Version 4.2 program (Sanner 1999). Firstly, target protein is downloaded to the working directory followed by the removal of water molecules and hydrogens, Kollamap and the Gasteiger charges is added to the target protein, respectively. Phytocompound is prepared as a ligand. Hydrogens, Kollamap and Gasteiger charges are added to the target protein and unwanted molecules such as water and small ion is removed. During docking, all torsions is allowed to rotate. The target protein and phytocompound is converted from the PDB format to the PDBQT format. Target protein is static, while phytocompound as flexible. Geometric features of molecules are used to find the surface of the molecules. Grid points is used to find the surface area using AutoGrid. One hundred Lamarckian Genetic Algorithm (LGA) runs with default parameter settings are performed. Docking analysis is carried out to predict the binding affinities based on the native Autodock scoring function. The stability of target protein and phytocompound complex is determined by their binding affinities. According to the highest dock score of best conformation, the lowest binding energy is calculated. The interactions of complex target protein phytocompound conformations including hydrogen bonds and bond length is analyzed.

5.5.1.5 Molecular Dynamics Simulation of Target Protein Phytocompound Complex

Molecular dynamics simulation of target protein phytocompound complex is carried out to determine their stability throughout the simulation period. It is performed in the same way as described in molecular dynamics simulation of target protein with an additional step applied before the system neutralization. The additional step is the generation of the ligand topology file using the PRODRG server. This step is carried out to add the heteroatom. This is because GROMACS has limitations to parameterize the heterogroup in the PDB file. RMSD, RMSF, hydrogen bonds, secondary structure, salt bridge and surface accessible area (SASA) between the target protein and phytocompound in the docked complex during the molecular dynamics simulation is analyzed using Gromacs analysis tools.

5.6 Conclusions and Future Prospects

The use of phytocompounds of medicinal plants can be a potential treatment for breast cancer. Identification of novel bioactive compounds helps in developing new and effective drugs. In this regards, the pharmacoinformatics is one of the approaches to understand the drug receptor interactions. The future researches should be more focused on the use of computational biology in designing of new, effective and more potent structure-based drug for the treatment of breast cancer with a better understanding of drug and receptor interactions.

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Chapter 6
Anticancer Potential of Andrographolide, a Diterpenoid Lactone from *Andrographis paniculata*: A Nature's Treasure for Chemoprevention and Therapeutics

Imran Khan, Mohd Aslam Yusuf, Irfan Ahmad Ansari, and Mohd Sayeed Akhtar

Abstract Cancer is one of the major causes of mortality in human population worldwide. The conventional drugs are known to be accompanied with severe side effects. Thus, the recent research is focused to find out the new chemotherapeutic agents of natural origin (plants derived) against cancer, which have the least side effects. In this regard, andrographolide, a major bioactive compound of a traditional medicinal plant, *Andrographis paniculata* has drawn much attention. It has shown a strong anticancer potential in several *in vitro* and *in vivo* studies against different cancers because of its ability to inhibit cell cycle progression in cancer cells. Moreover, it has also shown antimetastatic and antiangiogenic properties in different cancer cells through various underlying molecular mechanism of action. Recently, the roles of andrographolide in cancer progression via cellular developmental pathways have gained attention. Thus, the aim of this chapter is to summarize the anticancer potential of andrographolide and provide an insight in identifying new molecular targets for developing new cancer treatment strategies.

Keywords Andrographolide · Anticancer plants · Bioactive compound · Herbal medicine · Signaling pathways

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6.1 Introduction

Plants with medicinal properties have been extensively used in various human ailments from the time immemorial (Chaudhary et al. 2010). More than 80,000 plant species have now been identified around the world exhibiting medicinal properties (Joy et al. 1998). Throughout the world, especially in developing countries, medicinal plants are widely used in primary health care due to affordability, availability, and acceptability (Hajiaghaee and Akhondzadeh 2012). In the 1950s, the discovery of vinca alkaloids, vinblastine and vincristine, and podophyllotoxin boosted the search for potent anticancer agents from plant source (Da Rocha et al. 2001; Mans et al. 2005; Warber et al. 2006). Thereafter, the quest to find anticancer agents shifted toward the candidate therapeutic compounds from plant origin. Diverse research around the globe led to the identification of several classes of anticancer agents like etoposide and teniposide, derivatives of epipodophyllotoxin, paclitaxel, or Taxol which are currently being used in a large number of cancer treatments (Butler and Newman 2008). In addition to these compounds, there are several other potent compounds with strong anticancer potential that are currently under clinical trials including 4-ipomeanol, colchicines, combretastatins, genistein, lapachol, and curcumin (Da Rocha et al. 2001). The point of fascination lies in the fact that all the abovementioned compounds hold a strong ethnobotanical background, thus strengthening the fact that traditional knowledge can be further explored to treasure trove many other potent anticancer agents.

In this regard, *Andrographis paniculata* (Burm f.) Wall. ex Nees (family: Acanthaceae) is recognized as an important medicinal plant and commonly used as a traditional herbal medicine in India, Sri Lanka, China, Thailand, Malaysia, Indonesia, etc. (Chao et al. 2009; Chao and Lin 2010; Akbar 2011; Kabir et al. 2014). Due to the extremely bitter taste in all parts of plant body, it is also known as "king of bitters" and also by several other names like kalmegh, andrographis, Indian Echinacea, etc. (Singh et al. 2012). Extracts of different parts of this plant are widely used in various ailments like diabetes, fever, malaria, snake bite, and dysentery (Jarukamjorn and Nemoto 2008). Due to ethnobotanical background, *A. paniculata* is one of the most important medicinal plants used in Unani and Ayurvedic systems of medicines (Akbar 2011). *A. paniculata* has been reported to exhibit a wide array of pharmacological effects like antimicrobial, antihepatitis, anti-HIV, antimalarial, antidiarrheal, antihyperglycemic, antioxidant, anti-inflammatory, immunostimulatory, anticancer, cardioprotective, and hepatoprotective (Singh et al. 2001; Gabrielian et al. 2002; Wiart et al. 2005; Sheeja et al. 2006; Hossain et al. 2014). The traditional and pharmacological importance of the *A. paniculata* has been well documented previously by several research groups and also recently in an extensive review by Jarukamjorn and Nemoto (Jarukamjorn and Nemoto 2008; Varma et al. 2009). Furthermore, phytoconstituents of this plant have been reported for various pharmacological properties, and the major bioactive component of *A. paniculata* is andrographolide, a diterpenoid lactone (Sharma and Sharma 2013). Moreover, there has been an increase in the number of reports demonstrating the anticancer potential

of andrographolide around the world (Rajagopal et al. 2003; Jada et al. 2006). Thus, the aim of this chapter is to summarize the anticancer potential of andrographolide and provide an insight in identifying new molecular targets for developing new cancer treatment strategies.

6.2 Phytoconstituents of *Andrographis paniculata*

A. paniculata has been reported to have several active constituents like polyphenols, flavonoids, and diterpene lactones (Rao et al. 2004; Li et al. 2007a, b). However, andrographolide, a diterpenoid lactone, is the major constituent making 0.5–6%, 0.8–1.2%, and 4% in dried leaf, stem, and whole plant extract, respectively (Burgos et al. 1997; Cheung et al. 2001; Pholphana et al. 2004). Andrographolide is present abundantly in the leaves and has been extracted as crystalline solid (Matsuda et al. 1994; Chao et al. 2009; Chao et al. 2010a, b). The chemical structure of the andrographolide has been elucidated and reported to contain α-alkylidene γ-butyrolactone moiety and three hydroxyl groups at C-3, C-14, and C-19. Chemically, it is designated as (3-(2-(decahydro-6-hydroxy-5-(hydroxymethyl)-5,8-dimethyl-2methylene-1-naphthalenyl ethylidene) dihydro-4-4-hydroxy-2(3H)-furanone (Fig. 6.1) (Varma et al. 2009). Apart from andrographolide, the other diterpenoids present in *A. paniculata* are neoandrographolide, deoxyandrographolide, isoandrographolide, and 14-deoxy-11,12-didehydroandrographolide (Fig. 6.1). The other components from ethyl acetate-soluble fraction of ethanol or methanol extract containing flavones include 5-hydroxy-7,8-dimethoxyflavone, 5-hydroxy-7,8,2′5’-tetramethoxyflavone, 5-hydroxy-7,8,2, 3′-tetra methoxyflavone, 5-hydroxy-7,8,2′-trimethoxy flavone, 7-O-methylwogonin, and 2′-methyl ether (Reddy et al. 2003; Chao et al. 2010a, b; Radhika et al. 2010).

The andrographolide has a strong ethnobotanical background which explained its early reports of extraordinary multiple pharmacological properties (Yu et al. 2003; Maiti et al. 2006; Suo et al. 2007). It has been documented for immunomodulatory, anti-inflammatory, and hepatoprotective agent and implemented in treatment of diarrhea, cold, fever, and other infectious diseases (Iruretagoyena et al. 2005; Reddy et al. 2005; Trivedi et al. 2007). In recent years the various reports show its anti-HIV and cardioprotective properties (Rajagopal et al. 2003; Jada et al. 2007; Woo et al. 2008; Zhao et al. 2008). Early studies by past researchers have established the anticancer potential of andrographolide (Rajagopal et al. 2003; Jada et al. 2006). Since then, studies emerged with reports of anticancer activity of andrographolide involving different mechanisms like inhibition of cell cycle progression, reduced cell invasion, or apoptosis induction through targeting different target genes in different cancer cell lines (Lin et al. 2014; Li et al. 2015). Therefore, research groups around the globe have identified andrographolide as a potent anticancer agent and are in the quest to determine and understand its targeting mechanism for the development of anticancer drug from natural origin (Woo et al. 2008).

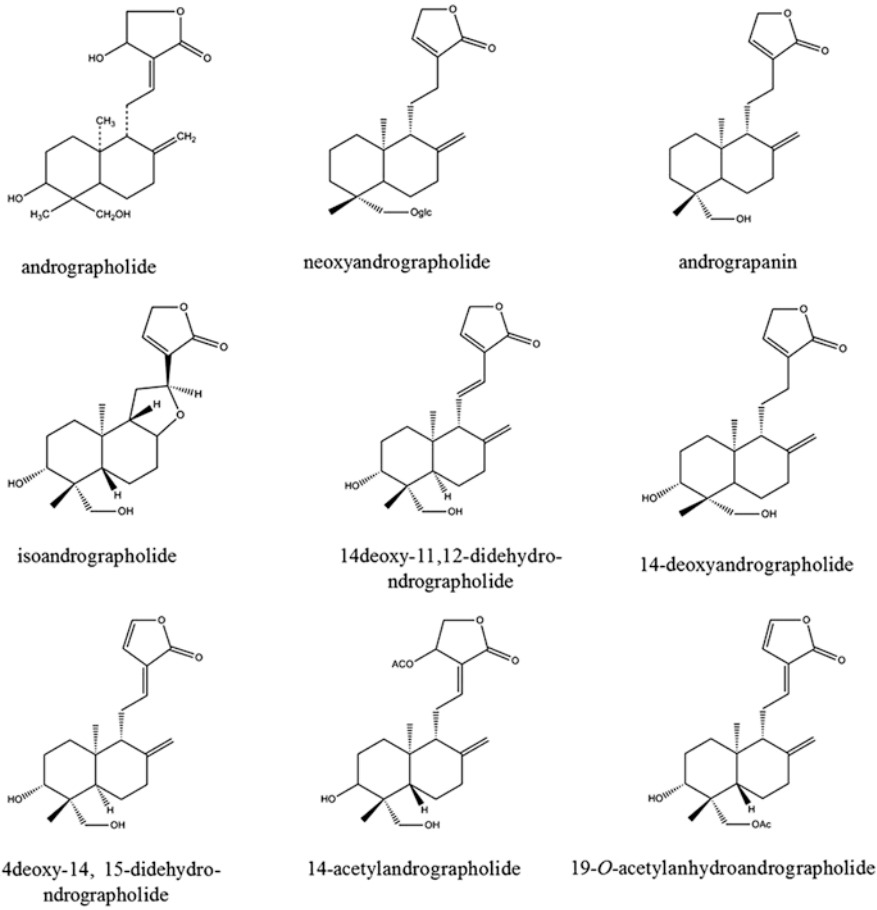

Fig. 6.1 Structures of major terpenoid compounds isolated from *Andrographis paniculata*

6.3 Andrographolide as a Potential Anticancer Compound

Carcinogenesis is a very complex process involving multiple cellular pathways. Thus, anticancer agents selected and employed in the modern medicine have the ability to inhibit the cancer cell proliferation via inducing apoptosis and inhibiting cell cycle progression and also immunomodulatory activities. It is also understood that anticancer compounds that can inhibit multiple procancer processes are more likely to inhibit a wider range of cancers and that are of greater importance (Boik 2001). Siripong et al. (1992) reported the cytotoxic effects of andrographolide on P388 (lymphocytic leukemia) and KB (human epidermoid leukemia) cancer cells (Siripong et al. 1992). This, perhaps, initiated the quest around the globe to investigate the antiproliferative potential of andrographolide against various cancers. Till date, andrographolide has been shown to exhibit anticancer activity against many

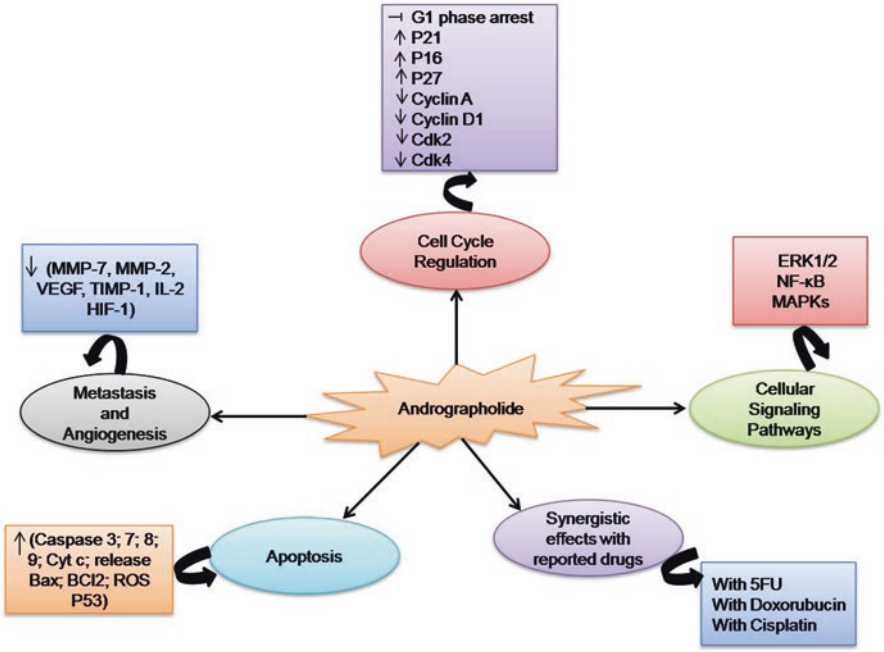

Fig. 6.2 Schematic representation depicting the anticancer properties of andrographolide ┤, inhibition; ↑, upregulation; ↓, downregulation

cancer cell lines via various mechanisms like disruption of the cell cycle progression, induction of apoptosis, and inhibition of metastasis and angiogenesis (Fig. 6.2) (Jada et al. 2006; Lin et al. 2014; Li et al. 2015).

6.3.1 Antiproliferative and Cytotoxic Activities Against Cancer Cells

One of the first significant demonstrations of cytotoxic potential of andrographolide was by Siripong et al. (1992) which showed that methanolic extract of *A. paniculata* significantly inhibited the growth of human epidermoid leukemia KB and lymphocytic leukemia P388 cell lines. Similarly, proliferation of colon cancer HT-29 cells was significantly inhibited by dichloromethane fraction of methanol extract of *A. paniculata* (Kumar et al. 2004). The major bioactive compound of *A. paniculata*, andrographolide, isolated from dichloromethane fraction, was also shown to inhibit the growth of different cancer cell lines (Kumar et al. 2004). Furthermore, andrographolide and iso-andrographolide, isolated from 85% ethanol extract of *A. paniculata*, exhibited higher antiproliferative activities in human leukemia HL-60 cells than other 16 *ent*-labdane diterpenoids (Chen et al. 2008). In another study, ethanol

extracts of *A. paniculata* showed effective cytotoxic activity against various human cancer cells like HepG2 (hepatoma), PC-3 (prostate), Jurkat (lymphocytic), and Colon 205 (colonic) cancer cells (Geethangili et al. 2008). Further, in an interesting research, andrographolide, at concentrations of 50 mg/kg body weight, efficiently prevented the tumor formation in the 12-dimethylbenz(a)anthracene (DMBA)-treated hamsters (Manoharan et al. 2012).

Interestingly, a semisynthetic analog of andrographolide, DRF3188, showed anticancer activities at a lower dosage than andrographolide (Satyanarayana et al. 2004). Synthesis and structure-activity relationships of andrographolide analogs revealed that intact α-alkylidene γ-butyrolactone moiety of andrographolide, the D12(13) double bond, the C-14 hydroxyl or its ester moiety, and the D8(17) double bond or epoxy moiety were responsible for the cytotoxic activities exhibited by andrographolide and its analogs (Nanduri et al. 2004). Moreover, in another similar study, the semisynthesized andrographolide derivative 3,19-isopropylideneandrogr apholide was found to be selective toward leukemia and colon cancer cells, whereas 14-acetylandrographolide was selective toward leukemia, ovarian, and renal cancer cells (Jada et al. 2007). The benzylidene derivatives of andrographolide showed more potent anticancer activities than andrographolide (Jada et al. 2007).

6.3.2 Induction of Programmed Cell Death in Cancer Cells

Apoptosis or programmed cell death is an active form of cell suicide involving concerted action of several cellular signaling pathways (Green and Reed 1998; Green and Kroemer 2004). Cells can undergo programmed cell death via intrinsic or mitochondrial-mediated pathway or death receptor-mediated or extrinsic pathway. Moreover, deregulated apoptosis is one of the mechanisms responsible for the uncontrolled cell proliferation of cancer cells, making it an important factor in developing new cancer chemoprevention approaches (Iannolo et al. 2008). Andrographolide has been reported to induce cell death in few cancer cell types through activation of extrinsic apoptotic pathway via activation of caspase-8 and caspase-3 (Kim et al. 2005). In some cancer cell types, the caspase-8 activation is sufficient to activate the effector caspase-3/7; whereas in majority of cancer cell types, it requires mitochondrial signal to activate the effector caspases (Zhou et al. 2006). For example, andrographolide has been shown to initiate a caspase-8-dependent Bid cleavage, followed by conformational change in Bax and its mitochondrial translocation, cytochrome c release from mitochondria, and finally the activation of caspase-9 and caspase-3 (Zhou et al. 2006). This study strongly suggested the key role of pro-apoptotic Bcl-2 family members, Bid and Bax, in andrographolide-induced cell death. According to Zhou et al. (2006) andrographolide treated-human cancer cells (breast, cervical, and hepatoma) showed elevated levels of caspase-3/7 up to eightfold compared to control. In another study, andrographolide has been shown to cause morphological changes and apoptosis in prostate cancer PC3 cell line via activation of caspases-8 and 3 (Kim and Milner

2005). In human leukemia HL-60 cells, andrographolide treatment induced the mitochondrial cytochrome c release followed by upregulation of Bax and down-regulated Bcl-2 protein expressions (Cheung et al. 2005). Recently a study attempted to elucidate the mechanism of andrographolide-induced apoptosis in rat VSMCs. Study demonstrated reactive oxygen species (ROS) formation and p53 activation accompanied with caspase-3 and Bax expression (Chen et al. 2013).

Additionally, andrographolide resulted in a dose- and time-dependent cell death in the Burkitt p53-mutated Ramos cell line, the mantle cell lymphoma (MCL) line Granta, the follicular lymphoma (FL) cell line HF-1, and the diffuse large B-cell lymphoma (DLBCL) cell line SUDHL4 (Yang et al. 2010). Furthermore, andrographolide significantly increased reactive oxygen species (ROS) production in all cell lines, and the apoptosis was accompanied by poly(ADP-ribose) polymerase cleavage and activation of caspase-3, caspase-8, and caspase-9 (Yang et al. 2010). Andrographolide also induced apoptosis in TD-47 human breast cancer cell line in a time- and concentration-dependent manner by increasing the expression of p53, Bax, and caspase-3 and decreasing the expression of Bcl-2 (Harjotaruno et al. 2007). Andrographolide was reported to induce apoptosis in hepatoma Hep3B cells in a previous study (Ji et al. 2007). Further in-depth study by the same group showed that the intracellular redox system plays important roles in regulating the cytotoxicity of andrographolide on hepatoma Hep3B cells (Ji et al. 2009). Interestingly, andrographolide initially increased intracellular GSH levels which then decreased later, while inhibition of cellular GSH synthesis by L-buthionine-(S,R)-sulfoximine (BSO) augmented andrographolide-induced cytotoxicity and apoptosis in Hep3B cells. Further results showed that andrographolide increased the activity of the GSH-related antioxidant enzyme glutathione peroxidase (GPx) and the production of intracellular reactive oxygen species (ROS) (Ji et al. 2009).

An important member of extrinsic apoptosis pathway, tumor necrosis factor-α (TNF-α)-related apoptosis-inducing ligand (TRAIL), has been shown to be upregulated in various human cancer cell lines upon andrographolide treatment (Zhou et al. 2008). Any drug or compound which enhances TRAIL expression in resistant cancer cell line could resensitize resistant cancer cells to TRAIL-induced apoptosis (Jin et al. 2004). In this regard, andrographolide could be a promising anticancer agent as it has been shown to enhance TRAIL expression in resistant cancer cells via upregulation of death receptor-4 (DR-4) (Zhou et al. 2008). Further studies in this direction might help to develop andrographolide as a sensitizer for TRAIL-induced apoptosis in various types of cancers.

6.4 Modulatory Effects on Cell Cycle Regulation

Recent discoveries have shown that cancer cells have upregulated cellular proliferation (MacLachian et al. 1995; Spataro 1998). Normal eukaryotic cells have strictly regulated cell proliferation through cell cycle (Pardee 1989). Cell cycle progression is a periodic process involving activation and inactivation of unique protein kinase

complexes consisting of cyclins (regulatory) and Cdk (catalytic) subunits. Active complexes of cyclin D1 and Cdk4, cyclin E and Cdk2, and cyclin A and Cdk2 have been explained to phosphorylate retinoblastoma (Rb) in G0 to G1 and G1 to S transition phase of cell cycle (Weinberg 1995). The cyclin/Cdk complexes regulating the G1 phase can be inactivated by binding of cyclin-dependent kinase inhibitors (CKIs) (Hunter 1993; Peters and Herskowitz 1994). Also, there are several other proteins reported to be associated with the inhibition of the G1 phase cell cycle arrest (Hall et al. 1995). Since cancer cells are different from normal mortal cells in having the uncontrolled cell division, this makes cell cycle and its regulatory proteins a potent target for anticancer compound.

Many studies have demonstrated that andrographolide effectively induces cell cycle arrest in several types of cancer cells at the G0/G1 stage (Cheung et al. 2005; Shi et al. 2008). Andrographolide induced dose- and time-dependent antiproliferative effects accompanied with G1-S phase cell cycle arrest in human colorectal cancer Lovo cells (Shi et al. 2008). Various other studies have demonstrated that andrographolide effectively induces cell cycle arrest in cancer cells at G0/G1 stage (Geethangili et al. 2008). A study, involving human acute myeloid leukemia HL-60 cells, reported a 27% increase in G0/G1 phase of cell cycle following andrographolide treatment (Cheung et al. 2005). A semisynthetic analog of andrographolide, DRF3188, also exhibited anticancer activities against MCF-7 breast cancer cells at a lower dosage than andrographolide through blocking cell cycle at the G0-G1 phase (Satyanarayana et al. 2004). Andrographolide also inhibited cell cycle progression by tempering the expression of cell cycle regulatory proteins. The G0/G1 phase cell cycle arrest was mainly due to the upregulation of cell cycle inhibitory proteins p16, p21, and p27 and marked decrease in the expression of cyclin A, cyclin D1, Cdk2, and Cdk4. Decreased expression of these cyclins and Cdk proteins prevents the phosphorylation of Rb and subsequently resulting the dissociation of Rb/E2F complex (Shi et al. 2008; Rajagopal et al. 2003; Satyanarayana et al. 2004). Interestingly, andrographolide induced cell cycle arrest and apoptosis of pancreatic cancer cells in a dose- and time-dependent manner by inhibiting STAT3 and Akt activation, upregulating the expression of $p21^{WAF1}$ and Bax, and downregulating the expression of cyclin D1, cyclin E, survivin, X-IAP, and Bcl-2 (Bao et al. 2013). Andrographolide has also been shown to exhibit growth inhibition and cytotoxicity against the androgen-independent (DU145 and PC3) and androgen-dependent (LNCaP) prostate cancer cell lines by inducing G_2/M cell cycle arrest which further led to apoptotic cell death (Wong et al. 2011). Mechanistically, andrographolide was found to downregulate CDK1 without affecting the levels of CDK4 and cyclin D1. Moreover, induction of apoptosis was associated with an increase in activation and expression of caspase-8 which induced cleavage of Bid into tBid. In addition, activation and enhancement of executioner caspase-9 and Bax proteins without affecting Bcl-2 protein levels were also observed (Wong et al. 2011).

Andrographolide arrested the cell cycle at G2/M in several hepatocellular cancer cell lines (Cheung et al. 2012). In HepG2 cells, it blocks G2 cells from entering mitosis and prevents mitosis from completion. Andrographolide also induced DNA damages, as indicated by the expression of phospho-H2AX in all cell lines. Heme

oxygenase 1 and heat shock protein 70 were among the proteins induced by andrographolide, which indicate the possible role of oxidative stress in the anticancer mechanism of this drug (Cheung et al. 2012). In another study, the cytotoxic effect of andrographolide on HepG2 cells was primarily attributed to the induction of cell cycle arrest at G2/M phase via the alteration of cellular redox status and caspase-independent cell death (Li et al. 2007a, b). Similarly, at nontoxic concentration, andrographolide inhibited the proliferation of human glioblastoma U251 and U87 cells through induction of G2/M arrest, which was accompanied by downregulating Cdk1 and Cdc25C proteins (Li et al. 2012). Additionally, andrographolide decreased the activity of PI3K/Akt signaling, as demonstrated by downregulation of the expression of phos-PI3K, phos-Akt, phos-mTOR, and phos-p70s6k in U251 and U87 cells (Li et al. 2012). In a recent study, andrographolide was shown to significantly inhibit HPV16 E6 oncogene expression in cervical cancer SiHa and CaSki cells in a dose-dependent manner. Interestingly, p53 protein was restored by andrographolide treatment in both the cell lines which was correlated to apoptosis and cell cycle arrest at the G2/M phase (Ekalaksananan et al. 2015).

6.4.1 Targeting Tumor Metastasis and Angiogenesis

Tumor metastasis is a complex process involving various key regulatory proteins, such as matrix metalloproteinases (MMPs). Tumor metastasis involves strictly regulated series of steps including vessel formation, cancer cell detachment, invasion, and proliferation (Fidler 2005). The basement membrane and stromal extracellular matrix (ECM) degradation are two crucial steps in tumor invasion and metastasis. Degradation of ECM is carried out by MMPs, family of human zinc-dependent endopeptidases (Parks and Shapiro 2001). Matrilysin also designated as MMP-7 is a low-molecular-mass (28KDa) protein lacking C-terminal domain. Activated MMP-7 has a broad proteolytic activity against a variety of ECM substrates, including laminin, proteoglycans, collagen, elastin, casein, and fibronectin. It is well documented to be produced by different malignant tumor cells including head and neck, prostate, lung, gastric, colorectal, and hepatocellular carcinomas (Zucker and Vacirca 2004; Adachi et al. 1999).

Tumor cells could express high amount of sialyl Lewis surface antigens which interact with adhesion molecules E- and P-selectins on endothelial cells. Cancer cell adhesion to endothelial cells followed by tumor extravasation results in metastasis. Andrographolide has been shown to inhibit the adhesion of cancer cells to the activated endothelium by blocking E-selectin expression (Sheeja et al. 2007). Andrographolide also inhibited angiogenesis for tumor metastasis via downregulation of matrix metalloproteinase-7 (MMP-7) expression by inhibiting PI3K/Akt signaling pathway (Shi et al. 2009; Lee et al. 2010). Andrographolide has shown promising results against the tumor invasion and migration in colon cancer (Shi et al. 2008; Chao et al. 2010a, b). The results of a study on Lovo colorectal cancer cells demonstrated dose- and time-dependent inhibition of tumor migration and

invasion, accompanied by decreased expression of MMP-7 protein (Shi et al. 2008). Similarly, in another study on CT26 colon cancer cells, andrographolide demonstrated inhibition of MMP2 activity. Andrographolide has also been shown to downregulate the expression of MMP2 in human colon cancer HT-29 cells which further supported its anti-migratory potential (Chao et al. 2010a, b). Andrographolide dose dependently inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced matrix metalloproteinase-9 (MMP-9) protein expression, enzyme activity, migration, and invasion in MCF-7 breast cancer cells. Furthermore, TPA-induced extracellular signal-regulated kinase (ERK) 1/2 and Akt phosphorylation and the DNA binding activity of activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB) were attenuated by pretreatment with andrographolide (Chao et al. 2013). Another study on non-small-cell H3255 lung cancer cells demonstrated the inhibitory potential of andrographolide in a dose- and time-dependent manner. Moreover, anti-migratory and anti-invasion potential was also found to be linked with downregulation of MMP-9 expression (Luo et al. 2013). Andrographolide inhibited dose dependently the migration and invasion of non-small-cell lung cancer (NSCLC) A549 cells under non-cytotoxic concentrations. Molecular data showed that the effect of andrographolide in A549 cells might be mediated via sustained inactivation of phosphatidylinositol 3-kinase (PI3K)/Akt signal involved in the upregulation of matrix metalloproteinases (MMPs). Andrographolide exerted an inhibitory effect on the activity and the mRNA and protein levels of MMP-7 but not MMP-2 or MMP-9. The andrographolide-inhibited MMP-7 expression or activity appeared to occur via activator protein-1 (AP-1) (Lee et al. 2010). In another study, andrographolide upregulated HLJ1, which is a novel tumor suppressor and is a potential druggable target for non-small-cell lung cancer (NSCLC), via jun B activation, which modulates AP-2α binding at the MMP-2 promoter and represses the expression of MMP-2 (Lai et al. 2013). This study also showed that andrographolide could affect several genes that are dominantly involved in the cell cycle, apoptosis, and adhesion-related biological signaling, including mitogen-activated protein kinase, focal adhesion, and tight junction pathways, indicating the diverse effects of andrographolide on invasion and proliferation (Lai et al. 2013).

Cancerous cells are well known to induce angiogenesis as a survival mechanism for continuous nutrient supply to the proliferating cancer cells. Andrographolide as an anti-angiogenic prospect has shown promising results in downregulating various angiogenic factors like vascular endothelial growth factor (VEGF) and nitric oxide and also upregulating tissue inhibitor of metalloproteinase (TIMP-1) and anti-angiogenic factors like IL-2 (Sheeja et al. 2007). Andrographolide had been reported to demonstrate antiproliferative activity against non-small-cell lung cancer A549 cell invasion and migration via downregulation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. This study also added to the andrographolide induced decrease in the expression of VEGF expression, thus, presenting andrographolide as a potential antiangiogenic agent against NSCLC in the future (Lin et al. 2011). In another report andrographolide also demonstrated VEGF inhibition accompanied by cell cycle arrest in transgenic mice models (Tung et al. 2013). Further, extending its anticancer effect via inhibiting the angiogenic properties of

cancer cells, andrographolide showed promising results against breast cancer cells. Andrographolide inhibited the proliferative and colony-forming ability of T47D and MDA-MB-231 breast cancer cell lines through decreasing the expression of HIF-1α protein followed by decreasing the expression of HIF-1α target and VEGF gene and protein (Li et al. 2015). Moreover, andrographolide has also been shown to suppress breast tumor growth in orthotopic NOD/SCID mice model (Kumar et al. 2012). The antitumor activity of andrographolide was correlated with downregulation of PI3 kinase/Akt activation and inhibition of pro-angiogenic molecules such as OPN and VEGF expressions (Kumar et al. 2012). Andrographolide inhibited tumor growth in nude mice bearing xenograft Hep3B cancer cells, concomitant with a reduction in tumor vessel counts (Shen et al. 2014). Andrographolide inhibited vascular endothelial growth factor A (VEGFA)-induced angiogenic responses *in vitro* and neo-angiogenesis *in vivo* (Shen et al. 2014). ANGL also inhibited VEGFA-induced phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR2) and its downstream targets such as the mitogen-activated protein kinases (MAPKs) (Shen et al. 2014). Andrographolide significantly inhibited melanoma tumor growth and metastasis by inducing cell cycle arrest and apoptosis. In addition, andrographolide significantly inhibited the TLR4/NF-κB signaling pathway (Zhang et al. 2014a, b). Andrographolide prevented human breast cancer-induced bone metastasis by suppressing RANKL-mediated and human breast cancer cell-induced osteoclast differentiation. Furthermore, andrographolide prevented osteoclast function by inhibiting RANKL-induced NF-κB and ERK signaling pathway at lower dose, as well as inducing apoptosis at higher dose (Zhai et al. 2014).

6.4.2 Targeting Other Cellular Signaling Pathways

Apart from inducing apoptosis, inhibiting cell cycle progression, and suppressing metastasis and angiogenesis, andrographolide has been shown to modulate a number of other cellular signaling pathways in cancer cells. For example, andrographolide showed anticancer effects on the nasopharyngeal carcinoma via downregulation of NF-κB target genes. The decreased expression of NF-κB target genes was linked to the apoptosis induction and cell cycle arrest caused by andrographolide treatment (Peng et al. 2015). It potently suppressed 7,12-dimethyl-1,2-benzanthracene (DMBA)-induced squamous cell carcinogenesis in the cheek buccal pouch of hamsters. Andrographolide reduced phosphorylation of p65 (Ser536) and IκBα (Ser32/36) to inhibit aberrant NF-κB activation, suppressed c-Myc and cyclin D1 expression and attenuated neoplastic cell proliferation, promoted cancerous cell apoptosis, and mitigated tumor-induced angiogenesis (Wang et al. 2011). Consistently, andrographolide retarded growth, decreased proliferation, and promoted apoptosis of Tb cells, a human tongue squamous cell carcinoma cell line, in time- and dose-dependent manners, with concomitant reduction of the expression of NF-κB targeted genes. Andrographolide also inhibited NF-κB activation in HL-60 cells and stimulated endothelial cells via decreasing the E-selectin gene expression

(Lowe 1994; Xia et al. 2004). Furthermore, another study group also reported andrographolide induced a decrease in E-selectin expression in gastric cancer cell lines, therefore resulting in the reduced cell surface adherence (Jiang et al. 2007). Andrographolide has also been shown to significantly inhibit tumor growth at both the early and the advanced stages of insulinoma through targeting the TLR4/ NF-κB signaling pathway (Zhang et al. 2014a, b).

Andrographolide has shown inhibitory effect on the Hep3B hepatoma cells via activation of mitogen protein kinases (MAPKs) along with p38 kinase, extracellular signal-related kinases (ERK1/2), and c-jun N-terminal kinase (Shimodaira et al. 1997). Andrographolide also inhibited human hepatoma cell growth by activating c-jun N-terminal kinase human hepatoma cell growth through activating c-jun N-terminal kinase (Manikam and Stanslas 2009). Further, a study, involving *in vitro* and *in vivo* investigation on primary astrocyte cells, demonstrated ERK activation by andrographolide treatment and suggested andrographolide as a potent therapeutic candidate for treating gliomas (Yang et al. 2014). A recent study has shown that andrographolide, in human leukemia HL-60 cells, could reduce cholesterol levels, accompanied with downregulation of Ras translocation to the membrane and downstream phosphorylation of Akt and ERK. The study further established that the apoptotic effect of andrographolide in HL-60 cells was due to interactions with the Ras/Akt/NF-κB/GLO1 and Ras/Raf/ERK/NF-κB/GLO1 pathways (Chen et al. 2015). Andrographolide has been shown to inhibit proliferation of non-small-cell lung cancer cell line H3255 in a concentration-dependent manner by inhibiting protein kinase C activity and reducing the levels of VEGF and TGF-β1 (Luo et al. 2014).

Aberrant activation of Src oncogene has been found to be associated with cancer initiation and progression, making Src as promising molecular target. In a study on an oncogene v-Src-transformed epithelial cell line, it was found that andrographolide notably downregulated the v-Src protein expression (Liang et al. 2008). Furthermore, the study also demonstrated that attenuation of ERK1/2 signaling pathway is crucial for andrographolide-induced inhibition of v-Src transformation (Liang et al. 2008). Further detailed study by the same group showed that andrographolide-induced inhibition of Src oncogenic activity was mediated by Hsp90 (Liu et al. 2014). The concentration- and time-dependent induction of Hsp90 cleavage that accompanied the reduction in Src was validated in RK3E cells transformed with either v-Src or a human truncated c-Src variant and treated with andrographolide (Liu et al. 2014). Notably, Hsp90 cleavage, decreased levels of Bcr-Abl (another known Hsp90 client protein), and the induction of apoptosis were also observed in human K562 leukemia cells treated with andrographolide (Liu et al. 2014).

In an interesting study, andrographolide inhibited cell viability and induces apoptotic cell death in both androgen-stimulated and castration-resistant human prostate cancer cells without causing significant toxicity to normal immortalized prostate epithelial cells (Chun et al. 2010). Moreover, treatment of andrographolide to mice bearing castration-resistant DU145 human prostate tumors that express constitutive IL-6 autocrine loop significantly suppresses tumor growth (Chun et al. 2010). Furthermore, andrographolide suppressed both IL-6 autocrine loop- and

paracrine loop-induced cell signaling including Stat3 and Erk phosphorylation (Chun et al. 2010). Taken together, these results demonstrated that andrographolide could be developed as a therapeutic agent to treat both androgen-stimulated and castration-resistant prostate cancers possibly by suppressing IL-6 expression and IL-6–induced signaling (Chun et al. 2010). Andrographolide has also been shown to significantly enhance autophagic markers in various cancer cell lines, including GFP-LC3 puncta and LC3-II level. Interestingly, andrographolide treatment also led to marked increase of p62 protein level, and addition of chloroquine (CQ) failed to further enhance either LC3-II or p62 level, indicating that andrographolide is likely to suppress autophagic flux at the maturation and degradation stage. Furthermore, andrographolide also inhibited autophagosome maturation not by affecting the lysosomal function but by impairing autophagosome-lysosome fusion. These observations collectively suggested that andrographolide could be a promising anticancer agent via its potent inhibitory effect on autophagy by disrupting autophagosome-lysosome fusion (Zhou et al. 2012).

6.4.3 Targeting Regulatory Developmental Pathways

Developmental pathways such as hedgehog (Hh), notch, wingless-related integration site (Wnt), and Bone morphogenic protein (BMP) are well documented and characterized in the developmental stages of embryo for establishing body pattern segmentation, cell position, cell fate, and polarity decision (Bertrand et al. 2012; Geissler and Zach 2012). Thus, it is not surprising that these developmental pathways are deregulated in various oncogenic processes (Geissler and Zach 2012). Several research groups around the globe have reported the crucial role of these developmental pathways in cancer development, progression, and chemoresistance, therefore, making these signaling pathways a potential target for development of chemoprevention strategies (Watt et al. 2008; Mazumdar et al. 2011; Sethi and Kang 2011; Zhong et al. 2012; Kamdje et al. 2017; Koury et al. 2017). Till date, there is no report of andrographolide describing its modulatory role on these developmental pathways. Our recent findings clearly indicated the potentiality of andrographolide in modulation of signaling pathways in colon cancer cell lines (data not published).

6.4.4 As an Adjunct in Synergistic Chemotherapeutic Strategies

Combinatorial chemotherapeutic strategies, in which more than one drug is used to target the cancer cells, have been proven to be more effective (Fitzgerald et al. 2006). Anticancer agents with similar or different modes of action may prove to work synergistically to increase the therapeutic efficacy, decrease drug resistance,

and reduce host toxicity (Yeh and Kishony 2007). On the other hand, some incompatible combinations may also cause antagonistic efficacy (Yeh and Kishony 2007). Therefore, the increased efficiency has brought a surge in cancer research to employ combination therapy as a treatment option for several solid tumors (Jackman et al. 2004). There are several good combinations like BEP (bleomycin, etoposide, cisplatin) or ABV (doxorubicin, bleomycin, vinblastine) which have proven effective via achieving positive biological interaction and also reduced toxicity (Fitzgerald et al. 2006). Natural compounds which have been reported to induce cytotoxic effect on various cancer cells present themselves as a possible option for the combinatorial chemotherapeutic strategies. Andrographolide has been shown to increase apoptosis in multidrug-resistant cancer cells, when used in combination with other chemotherapeutic agents like 5-fluorouracil (5-FU), cisplatin, and Adriamycin (Han et al. 2005). In a study, andrographolide was tested individually as well as in combination with 5-FU, and the results demonstrated that andrographolide, in combination with 5-FU, induced synergistic cytotoxic effect on human colorectal carcinoma SMMC-7721 cells (Yang et al. 2009). Surprisingly, the results showed significant increase in the caspase-8 and p53 expression, cytochrome c release, and decreased mitochondrial membrane potential, along with activation of caspase-9 and caspase-3 as compared to andrographolide alone (Yang et al. 2009). Similarly, another study showed that andrographolide enhanced the chemosensitivity of tumor cells to doxorubicin via inhibiting JAK-STAT3 pathway in human colon cancer HCT-116 and cervical cancer HeLa cell lines (Zhou et al. 2010). Recently, andrographolide was investigated for its synergistic effects on human colon cancer Lovo cells in coadministration with cisplatin (Lin et al. 2014). The results of this study further supported andrographolide as a potential candidate for synergistic chemotherapeutic approach against colon cancer and demonstrated synergistic apoptosis induction via altering Bax and Bcl-2 ratio, cytochrome c release from mitochondria, and activation of caspases (Lin et al. 2014). Additionally, andrographolide, in combination with gemcitabine, has also been shown to induce stronger cell cycle arrest and more prominent apoptosis in comparison to andrographolide or gemcitabine alone (Bao et al. 2013). The mechanistic study demonstrated that this synergistic effect was also dependent on the inhibition of STAT3 and Akt activations which subsequently regulate the pathways involved in the apoptosis and cell cycle arrest. Furthermore, both andrographolide alone and the combination treatments exhibited efficacious antitumor activity *in vivo* (Bao et al. 2013). In another recent study, andrographolide was found to be three times more active in the ovarian A2780 cisplatin-resistant cancer cell line as compared to that in ovarian A2780 cancer cell line, whereas cisplatin was less active in the A2780 cisplatin-resistant cell line than in the parent A2780 cell line (Yunos et al. 2013). A synergism between cisplatin and andrographolide was observed when administered in combination, and the percentage of apoptotic cell death was found to be greater for the combination of andrographolide and cisplatin as compared to single-drug treatments (Yunos et al. 2013). Therefore, results of these studies suggested a potential therapeutic strategy of combining andrographolide with standard chemotherapeutic agents to treat cancer.

6.5 Conclusions and Future Prospects

In the quest to identify the alternative chemotherapeutic agents of plant origin, andrographolide, a diterpenoid lactone from *Andrographis paniculata*, has emerged as a potent candidate. Undergoing research around the world has documented andrographolide for anticancer activity against several human cancers. Studies have shown that andrographolide induces cell cycle arrest and apoptosis and also inhibits the angiogenic and metastatic ability in cancer cells. The ability to act synergistically when used with conventional drugs further supports the chemotherapeutic activity of andrographolide for cancer therapies. Furthermore, andrographolide has also been documented to target specific molecular targets in cancer cell apoptosis, cell cycle arrest, metastasis, and angiogenesis. Several studies point toward the ability of andrographolide to affect cancer cells via targeting the specific key molecules of several cellular signaling pathways. Moreover, andrographolide may have modulatory effect on the developmental signaling pathways involved in the cancer carcinogenesis. Therefore, andrographolide stands as a potential candidate that can be further studied extensively and can be a viable candidate for developing novel chemotherapeutic strategies.

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Chapter 7
Anticancer Activity of Herbal Medicine: Mechanism of Action

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Abstract Cancer is an alarming disease and quite lethal in nature in developed and developing nations. Many new therapeutic agents and therapies are available in the market but have some severe side effects on human beings' organs. These therapeutic agents are quite costly and not easily available in some of the developing nations. Various scientific reports have shown that chemoprevention through naturally derived herbal and dietary phytochemicals is an innovative therapeutic tool against different cancer types. These herbal phytochemicals have shown their potential anticancer activity in both *in vitro* and *in vivo* studies. Further, many of them have been successfully proved for their chemopreventive property by inducing apoptosis equivalent to certain other chemical drugs without causing any side effects. The combinational role of herbal and dietary phytochemicals has proved to be very effective against cancer prevention. The present chapter summarised the effectiveness of herbal and dietary phytochemicals for chemoprevention and also highlighted their combinational role on various kinds of cancer.

Keywords Anticancer · Therapeutic agents · Herbal phytochemicals · Chemoprevention

7.1 Introduction

Cancer is one the most alarming diseases which is mainly distinguished by irregular and uncontrollable proliferating activities of the cells. According to a report, millions of people are dying from cancers (Jemal et al. 2011). It is mainly considered

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as the most alarming and more demising disease as compared to other diseases such as acquired immune deficiency syndrome (HIV/AIDS), malaria and tuberculosis combined (Vorobiof and Abratt 2007). The hyperproliferative nature involved in this mainly includes transformation, apoptosis dysregulation and many more. However, many cancer chemotherapies have only a limited success rate, i.e. approximately 2.2% in the developed nations (Mileshkin et al. 2005). As per the available literature, there is at least 3000 plants species having anticancer property possessed within them (Cragg and Newman 2009). Anticancer potential of plants is mainly shown by their secondary metabolites and their semisynthetic derivatives in cancer therapy (D'Incalci et al. 2005; Pan et al. 2010). It is found that phytochemicals isolated from natural source are used in the treatment of many human diseases including cancer and parasitic infection, and likewise some of the commonly used drugs for chemotherapy purpose derived from the natural sources include Taxol (*Taxus baccata*), camptothecin (*Camptotheca acuminata*), etoposide (*Podophyllum peltatum*) and docetaxel (*T. brevifolia*) (Demain and Vaishnav 2011).

Some herbal and dietary phytochemicals have shown their potent anticancer property against various types of cancers. Such types of phytochemicals have become more popular in both developed and developing nations due to many advantages such as the ease of availability, lower cost and having no adverse severe side effects (Bhanot et al. 2011). Dietary supplements are one of the most important constituents for the human health. These dietary supplements derived from many types of food supplements such as cereals, vegetables, spices, beans, etc. Many dietary phytochemicals present in the diet including cannabinol (*Cannabis sativa*), carnosol (*Rosmarinus officinalis*), genistein (*Puerarialobata radix*) and emodin (*Aloe arborescens*) act as anticancer agents (Bhanot et al. 2011). Likewise, vegetables, fruits, spices and beans contain various classes of important phytochemicals possessing anticancer property. Some of such phytocompounds including alkaloids, saponins, flavonoids, isoflavonoids and many phenolic compounds possess anticancer property. Edible items like fruits, vegetables, spices and cereals have attracted a major portion of the society, because of its cancer-supressing capabilities (Bhanot et al. 2011). Studies have shown that a regular utilisation of phytochemicals via diet can retard the chance of various kinds of cancers (Sporn and Suh 2002; Surh 2003; Russo et al. 2005). A wide group of dietary constituents act as inhibitory agents similar to anticancer chemical drugs, since they kill only cancerous cells with a low side effect on other cells. Phytoconstituents enhance the potential of curing in combination with a classical chemotherapeutic drug. The present chapter summarizes the effectiveness of herbal and dietary phytochemicals in the chemoprevention and also, highlights their combinational role against various kinds of cancer.

7.2 Herbal Phytochemicals

7.2.1 Curcumin

Curcumin is mainly derived from the rhizome part of *Curcuma longa* which is known to have a polyphenolic nature (bis-α,β-unsaturated β-diketone, commonly called diferuloylmethane) and belongs to the Zingiberaceae family. The anticancer activities of curcumin are mentioned in various reports providing evidence that it can cause cell death in cancer cell lines and retard tumour growth in animal models. Curcumin is actively reported in the suppression of many cancers like breast, head and neck, colon, oral and prostate cancers (Hanif et al. 1997; Elattar and Virji 2000; Mukhopadhyay et al. 2001; Aggarwal et al. 2004; LoTempio et al. 2005; Siwak et al. 2005; Lin et al. 2007; Wang et al. 2008).

The antitumour activities of curcumin are correlated to its complex chemical nature, i.e. benzene ring with unsaturated side chain of methoxy groups and β-diketone moiety in the centre, and thus allow it to interfere with many cellular signalling pathways of multiplication through directly interacting or modulating gene expression cascades (Araujo and Leon 2001). The anticarcinogenic activity of the plant is well reported in case of pancreatic cancer cell line in which the molecule suppresses the proliferation and cell death induction via NF-κB and IκB kinase downregulation (Mazzanti et al. 2009). Another scientific report has claimed that curcumin in combination with gemcitabine was found to supress pancreatic cancer growth in mice effectively (Kunnumakkara et al. 2007). The anticancer property of curcumin is also reported in several cell lines of colon cancer. In human colon cancer cell lines, curcumin showed its potential activity via inhibition of neurotensin-mediated activator protein-1, epidermal growth factor receptor (EGFR) and PGE-2 (prostaglandin E2), Ca^{2+} mobilisation, NF-κB stimulation and downregulation of MMP-2/9, COX-1/2 and IL-8 gene expression (Anand et al. 2008). A study conducted over male F344 rats demonstrated that curcumin has significantly reduced azoxymethane-induced colon tumourigenesis (Rao et al. 1995). *In vivo* study conducted over weanling male F344 rats demonstrated that curcumin has a chemopreventive effect during the progressive stage of colon cancer (Kawamori et al. 1999).

The effect of curcumin was tested over Caco-2 cancer cell line and it showed inhibition of proliferation as compared to other phytochemicals such as quercetin and resveratrol. It also showed apoptosis induction in Caco-2 cell line by an enhancement in ratio of Bax/Bcl-2, stimulation in caspase 3/7 and DNA fragmentation on nucleus (Sakuma et al. 2014). After curcumin treatment HCT-116 cell line showed p53 and p21-independent G2/M cell cycle arrest and apoptosis (Aruna et al. 2002). Curcumin had shown that it has potential to induce apoptosis in HCT-116 by

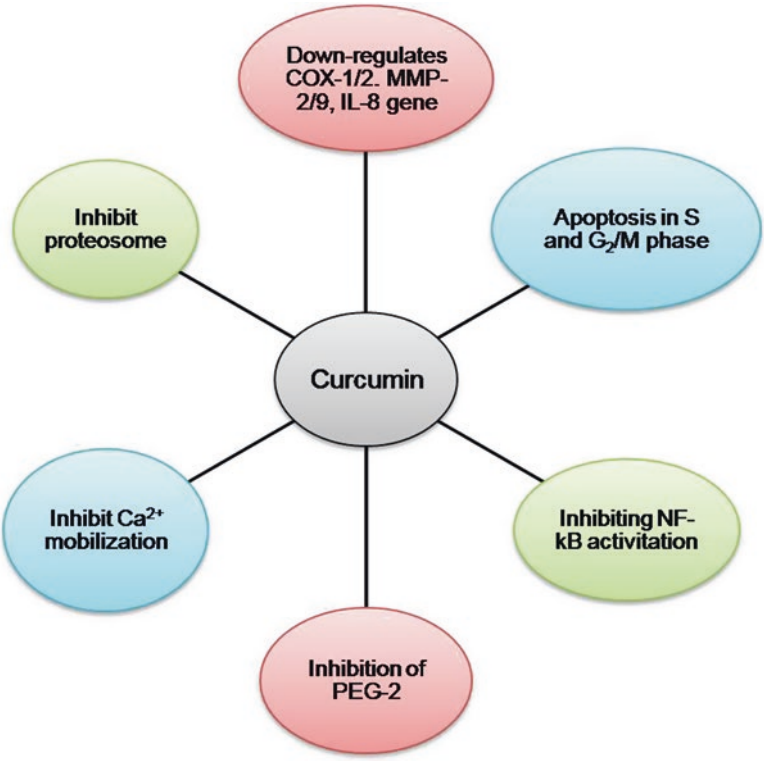

Fig. 7.1 Anticancer potential of curcumin by various mechanisms of action

activating c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) (Collett and Campbell 2004). Likewise, Milacic et al. (2008) reported that in SW480 and HCT-116 colon cancer cell lines, curcumin has the potential to supress proteasome and induce apoptosis. In another study, deficient COX-2 SW480 and expressed COX-2 HT29 cells treated with different concentration (0–50 μM) of curcumin for 72 h also revealed that apoptosis induction by curcumin in these colorectal cancer cell lines is directly related to the suppression of PEG-2 formation through COX-2 inhibition (Lev-Ari et al. 2006). Different kinds of molecular mechanisms of anticancer action mediated by curcumin are depicted in Fig. 7.1.

7.2.2 Genistein

Genistein is a phytoestrogen in nature which is mainly classified and included under isoflavone group. It was first isolated from *Genista tinctoria* (also known as dyer's broom) in 1899. Genistein is chemically known as 5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one (Fig. 7.2). It is helpful in supressing and treating

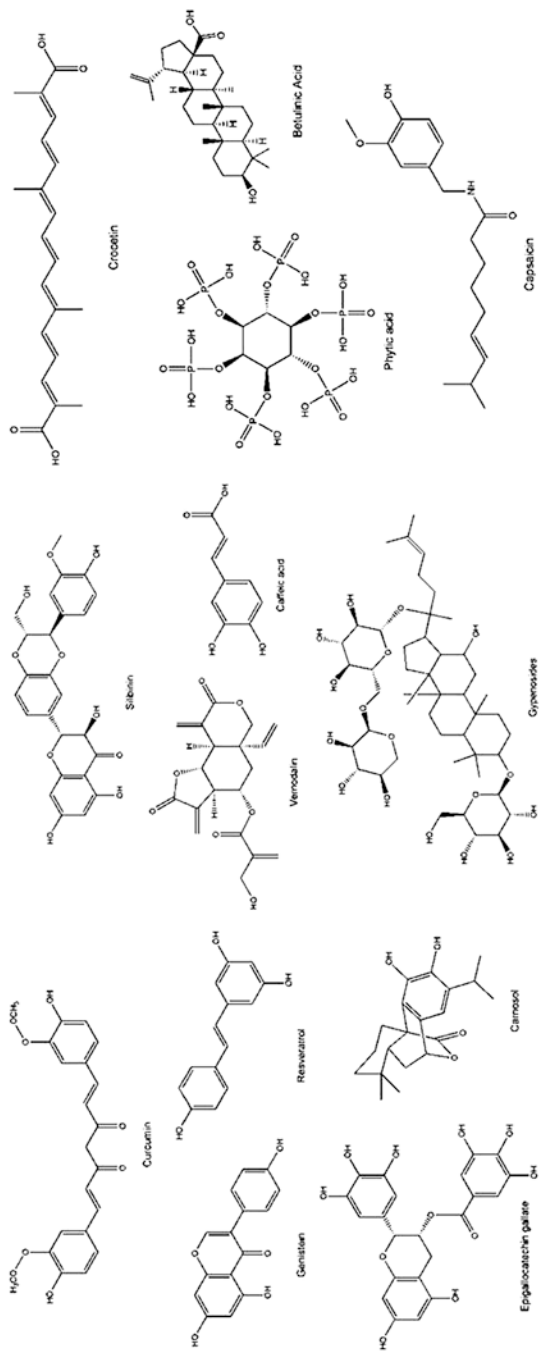

Fig. 7.2 Major herbal and dietary phytochemicals

cancers including prostate and breast cancers (Lampe et al. 2007). Various study findings have revealed that it is effective against both hormone-independent and hormone-dependent carcinomas (Lampe et al. 2007). The anticancer property of genistein in colorectal cancer cells (HT-29) was found to be through inhibiting the proliferation of cells and inducing apoptosis process. It was evidenced that an apoptotic death of HT-29 cells is due to G2/M phase arrest. Moreover, they also noticed an increased level of the expression of Bax and $p21^{WAFI}$; however, Bcl-2 level was found slightly decreased (Yu et al. 2004). An experiment was carried out with an aim to investigate the low-dose effect of genistein on prostate cancer cells (IA8-ARCaP and LNCaP/HIF-1α) and epithelial mesenchymal transition and its invasion (Zhang et al. 2008a, b). They found that it downregulated IA8-ARCaP and LNCaP/HIF-1α cells in a dose-dependent manner (with increasing concentrations from 15 to 75 μM/l). However, the concentration of genistein below 15 μM/l was found not effective in controlling the rate of cell proliferation (Zhang et al. 2008a, b). Phillip et al. (2012) evaluated the combined action of genicitin and vorinostat against prostate cancer cells and found that genicitin at 20 μM concentration causes CpG methylation effect, histone H3K9 acetylation and enhancement of the expression of histone acetyltransferase 1 (HAT1). Simultaneously, they also pointed out that genistein indulges in the histone deacetylase inhibitor, vorinostat, for inducing cell death in prostate cancer cells. LNCaP and PC-3 cells incubated with genicitin resulted in the suppression of clonogenic potential. The LNCaP and PC-3 cells incubated with estradiol at a low concentration influenced their survival. Later, it was concluded that genicitin and estradiol stimulate hyper-radiosensitivity (HRS) in LNCaP, PC-3 cells and abolish HRS during hormonal incubation (Hermann et al. (2008). A study was conducted to understand the mode of action of genistein on the inhibition of proliferation of breast cancer cell line, MCF-7. The results revealed that genistein at 50 and 100 μM arrested G2/M phase of cell cycle in MCF-7 cells and reduced the proliferation rate. Furthermore, they found that genistein stimulates heat shock protein 105 (HSP) mRNA and reduced mRNA expression of serum response factor (SRF), oestrogen receptor (ER), a disabled homolog 2 (DOC 2) and recombination activation gene 1 (RAG-1) (Chen et al. 2003). The treatment of genicitin and hydroxycamptothecin (HCPT) to bladder cancer cells and bladder epithelial cells (BDEC) showed an apoptotic activity under both *in vitro* and *in vivo* study models. The synergistic activity of genistein and HCPT significantly inhibited the growth and proliferation of bladder cells by promoting the cell cycle arrest at G2/M phase and apoptosis (Wang et al. 2013).

7.2.3 Resveratrol

Resveratrol, an antioxidant having polyphenol in nature, is a well-studied phytochemical with an abundant potential in health regards due to its inherent properties including antioxidant, anticancer and the like. Studies on resveratrol have elucidated that it has the potential of inhibiting cancer progression (Lucie 2000; Wang

et al. 2002; Nguyen et al. 2009; Cui et al. 2010; Lucie 2000). Resveratrol (3,5,4′-trihydroxy-trans-stilbene) (Fig. 7.2) is synthesised by plants in its own way in response to any injury or when plant is facing threat of external attack by microbes including bacteria and fungi (Lucie 2000). The main source of resveratrol includes grapes, blueberries, raspberries and mulberries. Other alternative sources include peanuts, cranberries, bilberries, turmeric and hops. Red wine, a commercial product of black grapes and cranberries, also contains a high amount of resveratrol (Wang et al. 2002). In human neuroblastoma cell lines such as SH-SY5Y, NGP and SK-N-AS, resveratrol has proved its anticancer activity in a time-dependent manner after treating cells with a concentration of 50–200 mM/l for 10 days (van Ginkel et al. 2007). There was a decrease in the survivability of cancer cells up to 85–90% after 5 days of treatment with IC_{50} values ranging between 70 and 120 mM/l in different cells. Its mechanism of action was based on induction of mitochondrial potential loss, enhanced proapoptotic caspases and induction of cell death. In human colorectal cancer cell lines such as DLD1 and HT29, resveratrol induced self-dependent anticancer potential by inducing apoptosis in a dose-dependent manner (Trincheri et al. 2007). Human colon cancer cells (HT-29), resistant to chemodrugs such as etoposide, were inhibited by resveratrol through various molecular mechanisms of action like inducing apoptosis, modulating signalling pathway of AMP kinase and production of ROS (Hwang et al. 2007). Likewise, Patel et al. (2010) conducted an *in vivo* study to confirm the chemopreventive potential of resveratrol on colorectal cancer. The major outcome of their experiment showed that it prevented the proliferation of tumour cells and controlled colon cancer. Later, Nguyen et al. (2009) carried out an experiment to determine the mode of action of resveratrol in colorectal cancer cells and found that it exhibits a chemopreventive property by inhibiting Wnt pathway target genes such as myc, cyclinD1, axinII, etc. Azoxymethane/dextran sodium sulphate-induced colon cancer in mouse was significantly inhibited by the treatment of resveratrol which showed its chemopreventive potential by decreasing markers of inflammation and supressing the inhibition of neutrophils (Cui et al. 2010). In another study, HT-29 and SW-620 cells treated with a combination of resveratrol and 5FU (an anticancerous drug) significantly increased the intracellular levels of ROS and lipid peroxides and, also, supressed the proteins of oncogenic potential such as Akt and STAT3 (Santandreu et al. 2011). Cytotoxic, apoptotic and antiangiogenic effects of resveratrol on HCT-116 and CaCo-2 human colorectal cancer cell lines include decreasing their glycolytic enzymes, stimulating citrate synthase and downregulating VEGF and leptin (Fouad et al. 2013). *In vitro* experiment on HT-29 and SW480 cell lines showed the antiproliferative activity of resveratrol (Vanamala et al. 2010). They reported that it increased the apoptosis rate and supressed signalling pathways of IGF-1R/Akt/Wnt and activated p53. Resveratrol inhibits bcl-2 and increases bax expression and cell cycle blockage at S phase in human colon cancer cells MCF-7 and MDA-MB-231 (Su et al. 2007; Chen et al. 2009). Resveratrol showed its anticancer property in only concentration-dependent manner by reducing the proliferative activity in oestrogen-positive and oestrogen-negative human breast cancer cells (Su et al. 2007). In MCF-7 and MDA-MB-231 cells, resveratrol showed a dose-dependent

manner of cytotoxicity by inducing COX-2 nuclear accumulation and facilitating pro-apoptotic activity of p53 (Tang et al. 2006a, b). Zhu et al. (2012) performed an experiment on adult women having risk of breast carcinoma. They reported that resveratrol has the potential to reduce the methylation of tumour suppressor gene RASSF-1α and prostaglandin. Resveratrol prevents epigenetic silencing and affects MCF-7 and BRCA-1 cell growth and progression (Papoutsis et al. 2010). Likewise, cancer preventive effect of resveratrol was also evidenced in MCF-7, S2-013 and CD18 cell lines (Golkar et al. 2007; Galicia et al. 2013). In human multiple myeloma cells, such as U266 and RPMI 8226, 50 μM of resveratrol inhibited cell proliferation rate by decreasing the proliferative and anti-apoptotic factors (Bhardwaj et al. 2007). Resveratrol was found to have anticancer potential in dose- and time-dependent manner in human multiple myeloma cell lines such as RPMI 8226, U266 and KM3 by suppressing cell proliferation, arresting cell cycle at G1 and S phase, inhibiting expression of NF-kB and inducing apoptosis with IC_{50} values of 131–187 mM after 24 h (Sun et al. 2006). In case of human T-cell acute lymphoblastic leukaemia cell lines such as MOLT-4, resveratrol induced apoptosis by increasing pro-apoptotic factors, Bax, p53 and p21waf, and modulated the p53 and PI3K-/Akt-mediated apoptosis pathway (Cecchinato et al. 2007). A study on peripheral blood and BM mononuclear cells of chronic lymphocytic leukaemia (CLL) patients showed that a combination of resveratrol and purine analogs such as fludarabine and cladribine exhibited apoptosis through DNA damage. They further revealed the reduction of Bcl-2/Bax ratio and noticed an increased activity of p53 and p21 genes (Podhorecka et al. 2011). The proliferation rate of 232B4 CLL cells was found to decrease with the arresting of cells at G0–G1 phase and promoting apoptotic activities via caspase 3 functions (Gokbulut et al. 2013). Resveratrol induces cytotoxicity in HL60 cell lines by inducing LASS genes and supressing SK-1 and GCS genes (Cakir et al. 2011). Likewise, an *in vitro* experiment conducted over human K562 chronic myeloid leukaemia cells to evaluate the cytotoxic potential of resveratrol reported a decrease in SK-1 genes and GCS genes and an increase of LASS genes (Kartal et al. 2011). Similarly, B16F10 and B16BL6 melanoma cells were also inhibited by the treatment with resveratrol (Bhattacharya et al. 2011). Resveratrol and black tea were tested in male/BALB/c mice to study their apoptotic effect individually and in combination. The study revealed that resveratrol downregulated cell proliferation and induced apoptosis of skin tumour by reducing the expression of MAPK proteins and increasing the activity of p53phospho/p3. They further concluded that their combination is more potent for chemopreventive purpose than any biological agents alone (George et al. 2011). The cytotoxic potential of resveratrol was also evident from *in vivo* and *in vitro* studies using mice and head and neck squamous cell carcinoma (SCC) cells FaDu, Cal27 and Det562. The viability of FaDu and cal27 cells was found to decrease, but Det562 cells were not affected. The cytotoxic effect was correlated to DNA damaging and apoptotic effect (Tyagi et al. 2011). Likewise, the growth and proliferation of human prostate cancer cell lines (PC- 3M-MM2 cells, DU145 and LNCaP) were inhibited through the expression of miRNA-21 with the treatment of resveratrol (Sheth et al. 2012). In the same way, both androgen-dependent prostate cancer (LNCaP) cells and androgen-independent

(C4-2) cell lines were also inhibited by resveratrol (Wang et al. 2010). Resveratrol inhibited human nasopharyngeal carcinoma (NPC) cell lines (TW076, CG-1 and TW04) by an increased apoptotic activity mediated by caspases 8 and 9, loss of mitochondrial potential and stimulation of the level of Bcl-2 family proteins (Huang et al. 2011). Later in another study, both poorly differentiated human NPC cell line (CNE-2Z) and well-differentiated human NPC cell line (CNE-1) proliferation rates were reduced by resveratrol treatment. It acted by inducing apoptosis by a modulation of cell growth controlling molecular signalling pathways (Zhang et al. 2013).

7.2.4 Epigallocatechin Gallate (EGCG)

Epigallocatechin gallate (EGCG) (Fig. 7.2), commonly recognised as epigallocatechin-3-gallate, is a derivative of catechin and an ester of epigallocatechin and gallic acid. EGCG is mostly found in white tea and green tea. The polyphenol EGCG possesses a powerful antioxidant activity compared to other polyphenols and helps in chemoprevention of cancers (Paschka et al. 1998; Nakazato et al. 2005; Lambert and Elias 2010). The antioxidant potential of green tea is mainly due to the presence of polyphenols (EGCG) and flavonoids (catechins) (Bettuzzi et al. 2007). It has been reported that EGCG and other tea catechins significantly inhibit the tumour progression and release TNF alpha from the treated cells. This may be due to free radical scavenging activity of flavonoids. The anticancer property of EGCG is reported in several kinds of cancers. Previous reports have shown that EGCG when treated along with ginseng exhibits a synergistic effect and enhances anticancer activity in colon cancer cells indicating more effective nature of green tea in cancer prevention along with other anticancer drugs (Du et al. 2013; Fujiki and Suganuma 2012; Bettuzzi et al. 2007). *In vitro* studies performed by several groups have proved that EGCG inhibits the expansion of oral squamous carcinoma cells and growth of oral epithelial cells (Ho et al. 2007; Yamamoto et al. 2007; Kato et al. 2008; Hong et al. 2009). Tea polyphenols and EGCG have the property to retard cellular propagation by inducing apoptosis in cancer cells of the ovary, head and neck and lungs (Huh et al. 2004; Pan et al. 2010), prostate (Bettuzzi et al. 2006; Johnson et al. 2010) and breast (Siddiqui et al. 2008), mouse embryonic fibroblast cell tumour (Hsieh and Wu 2008), human osteogenic sarcoma (Yagiz et al. 2007), human epidermoid carcinoma (Ji et al. 2006), laryngeal squamous carcinoma (Bhatia et al. 2001), and nasopharyngeal carcinoma (Luo et al. 2001).

7.2.5 Carnosol

Carnosol is a polyphenolic diterpene (Fig. 7.2) found in herbs such as rosemary (*Rosmarinus officinalis*) (Lo et al. 2002) and mountain desert sage (*Salvia pachyphylla*) (Johnson 2011; Guerrero et al. 2006) and is being commercially used in

food processing. Carnosol also has the potential to reduce risks of cancer. Carnosol was first extracted from mountain desert sage in 1942 (Johnson 2011). The anticancer property of carnosol is being well reported against prostate (Johnson et al. 2008), skin (Mengoni et al. 2011), breast (Hussein et al. 2007), blood (Zunino and Storms 2009) and colorectal cancer (Cheng et al. 2011). Cancer cells treated with the crude extracts of rosemary plant parts have shown an effective antitumour, antioxidant and antimutagenic activities. The anticancer potential of carnosol is mainly attributed by triggering various cellular pathways of cell cycle and apoptosis.

7.2.6 Silibinin

Some flavonoids are also quite well reported for inhibiting different cancers. Amongst several flavonoids reported so far, silibinin (Fig. 7.2) inhibits cancer cells effectively. This compound is an active constituent of silymarin isolated from milk thistle plant (*Silybum marianum*) and leaves of artichoke (*Cynara scolymus*) plants. Silibinin is also well known for its strong antioxidant activities (Singh and Agarwal 2009). Many researchers have documented cancer chemopreventive efficacy of silibinin using both *in vitro* and *in vivo* experiments (Bhatia et al. 2001; Singh and Agarwal 2009; Rouholamini et al. 2015). Silibinin effectively retards the growth of tumour initiation and invasion in various clinical models and cell lines including colon, lung, prostate and skin cancers. It shows its anticancer property by inducing the arrest of G1 phase of cell cycle through inhibiting the kinase activity of cyclin proteins involved in the cell cycle (CDK4, CDK6 and CDK2) and correspondingly elevated expression of Cip1/p21 and Kip1/p27 proteins. It also shows the chemoprevention of cancer by declining the expression of cyclin B1 and Cdc2 (Singh and Agarwal 2006). In breast cancer cells, silibinin also promoted apoptosis by modulating p21, p53, Bak and Bcl-XL pathways (Rouholamini et al. 2015).

7.2.7 Vernodalin

Throughout the centuries, medicinal plants are quite well practised for control and inhibition of diseases. In the present time, many anticancer drugs have been originated from the natural sources. The main anticancer compounds include Taxol derived from *T. baccata* and camptothecin derived from *C. acuminata* (Liu and Wang 2004). Apart from these well-known plant compounds, some of the plants are recently documented for their anticancer potential, and the traditional plant, *Centratherum anthelminticum* (L.), is one amongst them. Lambertini et al. (2004) revealed that its isolate showed antiproliferative effect on breast cancer. Similarly, the seed chloroform fraction showed the anticancer potential by suppressing TNF alpha of breast cancer cells. The potential component of *C. anthelminticum*

responsible for supressing the breast cancer was found to be vernodalin (Fig. 7.2). It caused apoptosis in MCF-7 cells through the fragmentation of DNA, shrinkage in cell size and deformation of cytoskeletal structure (Looi et al. 2013).

7.2.8 Gypenosides

Gynostemma pentaphyllum (Thunb) Makino (Jiaogulan) is a much admired folk medicinal plant used for treating hypertension, hepatitis and cancer in Taiwan and China. Gypenosides (Gyp) are the major chemical component of *G. pentaphyllum* solvent extracts (Fig. 7.2). Gyp are well known for their therapeutical activities against hepatitis, cancer, hyperlipoproteinemia and cardiovascular diseases. The anticancer effect of gypenosides on colorectal cancer cells, H460 and A549, has been evidenced by the research study conducted by Tsui et al. (2014). The antiproliferative activity of the extracted flavonoids showed its inhibitory effect on H460 and A549 with a varying IC_{50} value of 50.2 μg/ml and 19.8 μg/ml, respectively. On other hand, the isolated flavonoids arrested the cell cycle progression at S and G2/M stages. It also showed the altered expression of cellular proteins (cyclin A and B, p53 and p21) but failed to show such expressions in H460. Though Gyp effectively exhibit a diverse range of pharmacological effects including anticancer activity against many cancer types, it is very effective in inhibiting the invasion and migration of oral cancer cells (Lu et al. 2010a). Gyp showed overwhelming anti-metastatic activity and considerably reduced the invasion of oral cancer (SAS) cells. Gyp significantly inhibited several proteins such as NF-kB, COX-2, SOS, FAK and plasminogen activator in oral cancer cells. This may be the reason behind the impeded invasion and migration of carcinoma cells. On the other hand, Gyp decreased the level of mRNA of MMP-2, MMP-9 and MMP-7 but failed to express FAK and RhoA mRNAs in SAS cells (Lu et al. 2010a). Chen et al. (2006) investigated that Gyp is cytotoxic to human colon cancer (colo205) cells at an IC_{50} value of 113.5 μg/ml. In addition, it is also reported that Gyp treatment gradually diminished the expression of the anti-apoptotic proteins (Bcl-2 and Bcl-x) and upregulated expression level of the pro-apoptotic protein, Bax. Gyp-treated cancer cells also have increased the level of p53, stimulated cytochrome-c release and, subsequently, activated caspase-3 before commitment to apoptosis.

7.2.9 Crocetin

Saffron is a spice and accumulated in the stigma of saffron flower. It is a major food colourant and has significant antitumour activity in different experimental models (Bakshi et al. 2010; Gutheil et al. 2012). The active potential of crocetin (Fig. 7.2), anticancer agent, is reported for hepatocellular carcinoma, lung carcinoma,

pancreatic cancer cell line, skin carcinoma and several cell lines such as colon, breast and gastric cancer cell lines. The anticancer effect of crocetin and safranal is reported over the two breast cancer cells, MDA-MB-231 and MCF-7, and found that their antiproliferative potential on breast cancer cells is in a concentration-dependent inhibitory manner. Crocetin induced pro-apoptotic effects in MCF-7 cells through increased expression of Bax protein (Chryssanthi et al. 2007; Chryssanthi et al. 2011). Crocetin has been found effective in combination of certain poly(lactide-co-glycolide) (PLGA) and doxorubicin (DOX) nanoparticles (Langroodi et al. 2016). An experiment carried out for assessing effect of crocetin in combination with PLGA nanoparticles and doxorubicin on MCF-7 cell line found that it inhibits cell growth. Further, they reported that nanoparticles inhibited the growth more effectively than DOX or crocetin. Thus, PLGA-DOX-crocetin combination can be effectively used for chemopreventive purpose in breast cancer cells. Also, crocetin is relatively well known for decreasing the viability of HeLa and MCF cell lines in cultured condition (Tavakkol-Afshari et al. 2008). Escribano et al. (1996) showed that some other chemical derivatives from saffron such as crocin, picrocrocin and safranal inhibited the growth of HeLa cells and encouraged apoptosis. Furthermore, others have revealed that it interacts with tRNA at molecular level suggesting its cancer chemopreventive property. Colorectal cancer is one of the most serious problems of aged persons in the western hemisphere. Mainly some of the most popular ways for treatments involve mostly chemotherapy, surgery and radiation therapy but with severe side effects. To avoid such serious side effects of these therapies, persons opt for chemoprevention through some naturally derived phytochemicals. Crocetin and some other *Crocus sativus*-derived phytochemicals have shown their potential against colorectal cancer. The chemopreventive potential of crocetin on colorectal cancer is reported by Aung et al. (2007) on three different cell lines, viz. HCT-116, HT-29 and SW-480, and concluded the inhibition of proliferation of cancer cells without any side effects on normal cells. The experimental study of crocetin on MIA-PaCa-2 cell line indicated a significant alteration of cell proliferation rate and cell cycle proteins such as Cdc-2, Cdc-25 and cyclin B1. It was also observed that crocetin and its carotenoid derivatives have significantly induced apoptotic proteins (Bax and Bcl) resulting in higher cell death in cancer cells (Dhar et al. 2009). For *in vivo* efficacy of crocetin against pancreatic tumour cells, a palpable tumour was grown in athymic nude mice, and crocetin dosages of different concentration were given orally. They found a decrease in proliferation rate and concluded that crocetin is an effective agent for inhibition of pancreatic cancer (Bakshi et al. 2010). The cytotoxic activity of crocetin on lung carcinoma cells (A549) has revealed that it has an anticancer potential over lung carcinoma cells. They further revealed that aqueous extract of saffron has a good anticancer property and can be administered efficiently through caspase-dependent pathway activation (Samarghandian and Shabestari 2013). A summary of herbal phytochemicals for cancer chemoprevention is listed in Table 7.1.

Table 7.1 Summary of some herbal phytochemicals for cancer chemoprevention

Name of the compound	Plant	Effective against	Cell lines	Mode of actions	References
Curcumin ($C_{21}H_{20}O_6$)	*Curcuma longa*	Colorectal cancer	HCT-116	Induction of apoptosis and arrest of G2/M phase, induction of apoptosis by activating c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK)	Aruna et al. (2002)
		Pancreatic cancer	RLT-PSC cells, PSCs	Inhibition of proteasome and induction of apoptosis	Collbett and Campbell et al. (2004)
			Pancreatic cancer (PC)	Increase in proliferation by TGF-α	Tahara et al. (2013)
			Primary PSC s – outgrowth method, PC cell lines	Connective tissue growth factor (CTGF), secretion leading to enhancement of PC invasiveness	Eguchi et al. (2013)
			Immortalised human PSC line hPSC21-S/T and PC cell line	Inhibition of cell growth by olmesartan and type-1 collagen production in PSCs	Masamune et al. (2013)
Genistein ($C_{15}H_{10}O_5$)	*Genista tinctoria*	Colon cancer	HT-29	Induction of apoptosis and inhibition of antiproliferation	Chen et al. (2003)
		Breast cancer	MCF-7	Cell cycle arrest in G2/M phase and increasing the expression of Bax and p21WAFI but also decreasing Bcl-2 level slightly	Yu et al. (2004)
		Prostate cancer	DU145, PC3 and LNCaP	Arresting the growth of G2/M phase and decreasing the proliferation in S phase	Zhang et al. (2008a, b)
				Affecting histone H3K9 acetylation and increased expression of histone acetyltransferase1	Phillip et al. (2012)

(continued)

Table 7.1 (continued)

Name of the compound	Plant	Effective against	Cell lines	Mode of actions	References
Resveratrol ($C_{14}H_{12}O_3$)	*Vitis vinifera*	Colorectal cancer	DLD1 and HT29	Apoptosis induction in dose- and time-dependent manner	Trincheri et al. (2007)
		Breast cancer	MCF-7 and MDA-MB-231	Inducing antiproliferative activity in both oestrogen-positive and oestrogen-negative human breast adenocarcinoma	Tang et al. (2006a, b) and Su et al. (2007)
		Pancreatic cancer cell	S2–013 and CD18	Nuclear accumulation of COX-2 in MCF-7 cells and by facilitating p53-dependent pro-apoptotic activity	Golkar et al. (2007) and Bhardwaj et al. (2007)
			U266 and RPMI 8226	Inhibition of cell proliferation in both cancer cells at 100 mM in both time- and dose-dependent manner	Sun et al. (2006)
		Human multiple myeloma	RPMI 8226, U266 and KM3	Decreasing proliferative and anti-apoptotic factors by inhibiting proliferation	Cecchinato et al. (2007)
		T-cell acute lymphob-lastic leukaemia	MOLT-4	Suppressing cell proliferation, arresting cell cycle at G1 and S phase, inhibiting expression of NF-kB and inducing apoptosis	Kartal et al. (2011)
		Chronic myeloid leukaemia vcells	K562	Inducing apoptosis by increasing pro-apoptotic factors, Bax, p53 and p21waf, and modulating the p53 and PI3K-/Akt-mediated apoptosis pathway; decrease in SK-1 genes and GCS genes	
Epigalloca-techin-3-gallate (EGCG) ($C_{22}H_{18}O_{11}$)	*Camelia sinensis*	Lung carcinoma	A549	Suppression in cell growth, increased endostatin expression and suppressed vascular endothelial growth factor (VEGF) expression	Sakamoto et al. (2013)

		Prostate cancer	LNCaP, PC-3 and DU145	Apoptotic cell death observed through nuclear morphology and DNA fragmentation	Paschka et al. (1998)
		Colorectal cancer	HT29 and HCT-8	Affected the proliferation and apoptosis of HCT-8 and HT29	Zhang et al. (2011)
Carnosol ($C_{20}H_{26}O_4$)	*Rosmarinus officinalis*	Colon cancer	HCT-116 and SW480	Increased apoptosis and decreased viability in colon cancer cell lines occur	Yan et al. (2015)
Silibinin ($C_{25}H_{22}O_{10}$)	*Silybum marianum*	Breast cancer	MCF-7	Inhibit cell growth and significant increase in BRCA1, ATM, Bak and Bcl-XL gene expression at the mRNA	Pirouzpanah et al. (2015)
			MCF-7	Antiproliferation of MCF-7 cells, inducing apoptosis by caspase-3 activation in breast cancer cell	Rouholamini et al. (2015)
Vernodalin ($C_{19}H_{20}O_7$)	*Vernonia amygdalina*	Breast cancer	MCF-7 & MDA-MB-231	Cell size shrinkage, deformed cytoskeletal structure and DNA fragmentation, inhibited cell growth, induction of cell cycle arrest and apoptosis	Looi et al. (2013)
Gypenosides ($C_{48}H_{82}O_{18}$)	*Gynostemma pentaphyllum*	Lung carcinoma	H460 and A549	Inhibiting proliferation and arrested cell cycle in S and G2/M phase	Chen et al. (2006)
		Oral cancer	SAS	Decreasing several proteins including nuclear factor-kappa B (NF-kB), cyclooxygenase-2 (COX-2), extracellular signal-regulated kinase (ERK1/2), matrix metalloproteinase-9, matrix metalloproteinase-2 (MMP-9,MMP-2), Son of Sevenless (SOS) homolog, Ras, urokinase-type plasminogen activator (uPA), focal adhesion kinase (FAK) and RAC-α serine/threonine-protein kinase (Akt) in time-dependent manner	Lu et al. (2010a, b)

(continued)

Table 7.1 (continued)

Name of the compound	Plant	Effective against	Cell lines	Mode of actions	References
		Colon cancer	Colo 205	Decreased the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xl but increased the expression of the pro-apoptotic protein Bax	Tsui et al. (2014)
Crocetin ($C_{20}H_{24}O_4$)	*Crocus sativus*	Breast cancer	MCF-7 and MDA-MB-231	Supressed proliferation, increased expression of Bax protein and induced pro-apoptosis	Chryssanthi et al. (2007, 2011)
		Cervical cancer	HeLa cells	Suppression of growth and induction of apoptosis	Tavakkol-Afshari et al. (2008)
		Pancreatic cancer	MIA-PaCa-2	Induction of antiproliferative activity in pancreatic cancer	Dhar et al. (2009)

7.3 Dietary Phytochemicals

7.3.1 Phytic Acid

The various workers carried out their study on anticancer property of phytic acid (Fig. 7.2) on various kinds of cancers such as colon, lung, prostrate, blood, liver, mammary glands, etc. and noted its potential for potent anticancer property. Anticancer property of IP6 (phytic acid) was first noted in a tracheal cell culture transformation assay (Arnold et al. 1993). Many studies have also reported that phytic acid is quite effective in several haematopoietic lineages, including K-562 (Shamsuddin et al. 1992; Deliliers et al. 2002), leukemic haematopoietic cells and normal cells (Deliliers et al. 2002), which are unable to divide in both time- and dose-dependent ways. The phytic acid is also quite effective in cancer cell lines such as colon cancer cell line HT-29, breast adenocarcinoma cell line MCF-7 and common prostate cancer cell line PC-3 (Shamsuddin and Yang 1995; Singh et al. 2003; Husna et al. 2010; Al-Fatlawi et al. 2014). Shamsuddin et al. (1992) showed the anticancer activity of phytic acid in blood cancer cell lines such as K-562 and noted a decreased differentiation of carcinoma cells. The phytic acid mechanisms behind its anticancer property vary in different kinds of cancers. For instance, Coradini et al. (2000) had shown that the anticancer activity of phytic acid in colon cancer cell line HT-29 is predominantly through butyric acid production and lower gut pH and bile acid metabolism. The antiproliferative effect of phytic acid in hepatocellular carcinoma (HEPG2) cells is due to apoptosis induction (Norazalina et al. 2011).

7.3.2 Caffeic Acid

The caffeic acid is a stimulant isolated from *Coffea arabica* leaves. It is a natural compound having a polyphenolic ring and is also isolated from propolis (Fig. 7.2). It has potent antioxidant, antiproliferative, antitumour, anti-inflammatory and antineoplastic properties (Beauregard et al. 2015). In human cervical cancer cells, caffeic acid induced apoptosis via the mitochondrial pathways (Chang et al. 2010). CAPE (caffeic acid phenethyl ester) is known to specifically inhibit NF-kB and also supress the lipoxygenase pathway of arachidonic acid metabolism. CAPE is reported to be an anticancer agent towards various kinds of cancers including lung, colon, adenocarcinoma and prostate cancers. The possible mechanism of anticancer property is attributed towards cessation of the nucleotide turnover salvage pathway, oxidative stress, inhibition of 5-lipoxygenase by a complete uncompetitive mechanism, induction of apoptosis and angiogenesis activity in all cell types. It is reported

that CAPE effectively supresses the TGF-β-enhanced cell motility and phosphorylation of Akt in A549 cells; this study arises the hope that these may be used as a chemoreceptive agent on metastatic therapy (Shigeoka et al. 2004). Potent inhibitory potential of CAPE on NF-kB and 5α-reductase can be exploited for chemopreventive purpose of prostate cancer. The suppression of NF-kB is properly outlined in the prostate cancer cell line PC-3. CAPE regulates the paclitaxel and TNF alpha-arbitrated NF-kB activation. The activation of NF-kB is also correlated with the reduction in the cellular levels of the inhibitors of apoptosis proteins (XIAP, cIAP-1 and cIAP-2) in a dose-dependent manner (Mceleny et al. 2004). The antitumor activity of CAPE is quite well revealed in colon cancer and adenocarcinoma (CT26) cells by inducing the cytotoxicity in a dose-dependent manner (Liao et al. 2003). CAPE analogs are also reported to cause apoptosis in breast cancer cells by inducing cell growth arrest (Beauregard et al. 2015).

7.3.3 Capsaicin

Amongst the naturally occurring phytochemicals, capsaicin (Fig. 7.2) is the most abundant ingredient of hot chilli pepper family belonging to family Solanaceae and genus *Capsicum* which is the most widely used vegetable around the world. Several reports have been published on its beneficial effects of various physiological and pharmacological effects (Govindarajan and Sathyanarayana 1991; Szallasi and Blumberg 1999). These published reports also had shown that capsaicin has potential for antiproliferative effects against several cancer cell lines including leukaemia (Zhang et al. 2003; Ito et al. 2004; Tsou et al. 2006), multiple myeloma (Bhutani et al. 2007), cutaneous cell carcinoma (Hail and Lotan 2002), hepatocarcinoma (Jung et al. 2001; Huang et al. 2009), glioma (Lee et al. 2000; Amantini et al. 2007), tongue cancer (Ip et al. 2012a), oesophageal carcinoma (Wu et al. 2006), nasopharyngeal carcinoma (Ip et al. 2012b), gastric cancer (Kim et al. 2004), pancreatic cancer (Zhang et al. 2008a, b; Pramanik et al. 2011), breast cancer (Chou et al. 2009), colon carcinoma (Lu et al. 2010b), non-small cell lung cancer (Brown et al. 2010) and prostate cancer (Mori et al. 2006; Sánchez et al. 2007). The potential of anticancer activity of capsaicin is mainly attributed by the induction of apoptosis, arrest of cell cycle, retardation of growth signal transduction pathways and regulation of transcription factor expression.

The effects of capsaicin are well reported over this oral cancer (KB cell line). The effect of capsaicin over KB cell line was found to induce apoptosis in a dose-dependent manner and also significantly reduce cell proliferation/viability. The effect of capsaicin is also reported on cell cycle as it arrests in the G2/M phase. Moreover, the effect of capsaicin is also found to increase membrane permeabilisation, disrupt mitochondrial membrane potential as well as modulate the cell cycle and activate caspases 9 and 3 and poly-(ADP-ribose) polymerase in KB cells (Lin et al. 2013). The cytotoxic potential of capsaicin is also quite well impacted on T-cell leukaemia. The *in vitro* effect of capsaicin is studied on three ALT cell lines.

It was noticed that capsaicin treatment to these cell lines inhibited the growth in both time- and dose-dependent manner compared with the untreated cells. The effect of capsaicin was mainly attributed due to the induction of cell cycle arrest and induction of apoptosis. Capsaicin has shown decrease in nuclear factor (NF)-kappa B/p65 DNA attachment activity and also Bcl-2 level (Zhang et al. 2003). The capsaicin has also induced its anticancer potential on HepG2 cell line. The *in vitro* study of capsaicin on human hepatocellular carcinoma cells has shown decrease in cell viability and also that capsaicin downregulates the release of LPO, LDH and NO production in a dose-dependent manner (Amruthraj et al. 2014). The anticancer property of capsaicin is quite well tested over human colon cancer cell line HCT-116. Capsaicin has profound antiproliferative activity on colon cancer cell. It is reported that it induces anticancer property through regulating cell cycle G0/G1 phase arrest and inducing apoptosis which directly increases p21 level, Bax and cleaved PARP (Jin et al. 2014).

7.3.4 Betulinic Acid

Betulinic acid (3β, hydroxy-lup-20(29)-en-28-oic acid) (Fig. 7.2) is a natural product with a potent antitumour activity. The anticancer property of betulinic acid is principally due to the induction of controlled cell death (apoptosis) in cancer cells via activation of mitochondrial pathways. The betulinic acid has shown its potent anticancer property on various kinds of cancer cells lines including colon, blood and breast cancer cells. In the case of colon cancer cell it has an apparent effect on 4 different cancer lines such as U937, HepG2, HT29, MCF-7 and non-cancerous human peripheral blood mononuclear cells. They pointed out that betulinic acid derivative is a potential promoter of apoptosis and most potent inhibitor of cell growth and proliferation. They further found that anticancer property of betulinic acid is mainly due to increased ROS production and caspase activation and DNA degradation (Dutta et al. 2015). A summary of dietary phytochemicals for anticancer property is listed in Table 7.2.

7.4 Combined Role of Herbal and Dietary Phytochemicals for Anticancer Property

7.4.1 Curcumin Combination with 5-FU, Doxorubicin and Cisplatin

Sivanantham et al. (2015) carried out a combinational approach study of using curcumin and antineoplastic agents such as 5-FU, doxorubicin and cisplatin to improve the anticancer property over NT8e cancer cells. The combined effect of curcumin and these chemotherapeutic agents showed ceased inhibition and an increase in

Table 7.2 Dietary phytochemicals for anticancer property

Name of the compound	Source	Chemical formula	Types of cancer	Study on	Mode of action	References
Phytic acid	Rice bran, wheat bran	$C_6H_{18}O_{24}P_6$	Hepatocellular carcinoma	HEPG2	Cell cycle arrest at G2/M phase, induced apoptosis	Norazalina et al. (2011)
			Breast cancer	MCF-7	Inhibit proliferation and apoptosis in MCF-7 cells by modulating the expression of apoptosis regulatory genes	Al-Fatlawi et al. (2014)
			Colorectal cancer	HT-29	Inhibited growth, induced apoptosis	Husna et al. (2010)
Caffeic acid	Coffee beans	$C_9H_8O_4$	Breast cancer	MCF7 and MDA-MB-231	Induced cell death by inhibiting NFκB and by inducing pro-apoptotic pathways	Beauregard et al. (2015)
			Cervical cancer	HeLa	Significant reduction in the proliferation of HeLa cell in time-dependent manner	Chang et al. (2010)
Capsaicin	Red chili	$C_{18}H_{27}NO_3$	Colon cancer	Colo205	Induced cytotoxic and increased reactive oxygen species (ROS) and decreased the level of mitochondrial membrane potential	Lu et al. (2010a, b)
			Human KB cell	KB cell	Reduced cell proliferation/viability and induced cell death in a dose-dependent manner compared. Cell cycle arrest at G2/M phase. Capsaicin induced disruption of the mitochondrial membrane potential as well as activation of caspase-9 and caspase-3 and poly-(ADP-ribose) polymerase in KB cells	Lin et al. (2013)
Betulinic acid	*Dillenia indica*, *Orthosiphon stamineus*	$C_{30}H_{48}O_3$	Leukaemia	K562	Induced apoptosis and inhibitory activity on cell	Liu and Wang (2004)
			Breast cancer	MCF-7	Observed decrease in angiogenesis, proliferation and invasion	Damle et al. (2013)
			Colon cancer	HT-29	An increase in ROS production, caspase activation, apoptotic activities and DNA degradation	Dutta et al. (2015)

apoptosis rate of NT8e cancer cells by reducing inhibition of Bcl-2 and increasing the activity of apoptotic Bax, caspase-3 and poly-ADP ribose polymerase (PARP) in NT8e cells. The combinational approach of curcumin and anticancer drugs such as doxorubicin, 5-FU and cisplatin further showed antitumourigenic activity by arresting cell cycle at G1/S phase and an elevated level of p21. Further, they revealed that this combination of curcumin and other antineoplastic agents downregulated the signalling molecules such as EGFR-ERK1/2 which has direct impact over the cell proliferation. This experiment further concluded that combination of curcumin and other anticancer agents can be more effective in cancer chemoprevention purpose.

7.4.2 Combination of Curcumin and Quercetin

The combinational approach of curcumin and quercetin was studied by Zhang et al. (2015) over gastric cancer cell line MGC-803. They reported a synergistic inhibitory effect of curcumin and quercetin on MGC-803 cell growth by reducing the mitochondrial membrane potential, release of cytochrome c and declined phosphorylation of AKT and ERK. They concluded that the combination of curcumin and quercetin can be used effectively for designing potential anticancer drugs against gastric cancer cells.

7.4.3 Combination of Garcinol and Curcumin

Parasramka and Gupta (2012) carried out an *in vitro* study to evaluate the effect of garcinol along with curcumin against the pancreatic cancer cell lines Panc-1 and BxPC-3. They observed that the combined use of these compounds over cells significantly reduced the cell viability in a dose-dependent manner and increased apoptosis in treated cells compared to control cells and suggested their potential effect against pancreatic cancer cells. Also, this combined treatment methods will be suggested for further clinical trial studies in pancreatic cancer chemoprevention.

7.4.4 Combination of Genistein and Vitamin C

The combined effect of genistein and ascorbic acid was studied over radiation-induced pancreatic cancer cells by Diaka et al. (2015). The study was done in triple combination of genicitin, ascorbic acid and radiation (Rad-Gn-VitC). The result found that it has effectively induced apoptosis in pancreatic cells and inhibition of ROS. This triple combination (Rad-Gn-VitC) can be effectively applied for clinical trial.

7.4.5 Combination of Resveratrol and Quercetin

The combined effect of resveratrol and quercetin was studied for anticancer potential against colorectal cancer cell line HT-29 (Follo-Martinez et al. 2013). This combinational approach of resveratrol with quercetin in a 1:1 ratio showed decrease in ROS by 2.25-fold with an increase in antioxidant activity up to threefold higher in HT-29. This combinational approach not only induced twofold higher caspase-3 cleavage but also PARP cleavage with a significant decrease in Sp1, Sp3 and Sp4 mRNA through decrease in protein expression. A summary of the combined effect of herbal and dietary phytochemicals on cancer prevention is listed in Table 7.3.

Table 7.3 Combined effect of herbal and dietary phytochemicals on cancer prevention

Name of the compound	Combination	Types of cancer	Cell lines	Mode of actions	References
Curcumin	Curcumin with 5-FU, doxorubicin and cisplatin	Head and neck	NT8e cells	Inhibition of Bcl-2 and increasing Bax, caspase-3 and poly-ADP ribose polymerase (PARP) in NT8e cells. Arresting cell cycle at G1/S phase and an increased level of p21, downregulated the signalling molecules such as EGFR-ERK1/2	Sivanantham et al. (2015)
	Curcumin with quercetin	Gastric cancer cell line	MGC-803	Inhibition of cell proliferation rate with decrease in mitochondrial membrane potential, release of cytochrome c and decreased phosphorylation of AKT and ERK	Zhang et al. (2015)
	Curcumin with Garcinol	Pancreatic cancer cell line	BxPC-3 and Panc-1	Decrease in cell viability and an enhancement of apoptosis in treated cell in dose-dependent manner	Parasramka and Gupta (2012)
Genistein	Genistein +ascorbic acid+radiation (Rad-Gn-VitC)	Pancreatic cancer cell line	LNCaP prostate cancer cell lines	Induction of apoptosis in pancreatic cells and inhibition of ROS	Diaka et al. (2015)
Resveratrol	Resveratrol with quercetin	Colon cancer	HT-29	Significant decrease in ROS level with an increment in antioxidant potential, caspase-3 cleavage and PARP cleavage, decrease in protein expression	Follo-Martinez et al. (2013)

7.5 Conclusions and Future Prospects

The use of herbal and dietary phytochemicals as chemopreventive agents against various cancers exhibited antiproliferative activity through diverse mechanisms of action including suppression of PEG-2, COX-2 inhibition, activation of JNKs and MAPKs, cell cycle arrest, activation of oncogenes, reduction of the mitochondrial membrane potential, increase of ROS, etc. These herbal and dietary phytochemicals derived from various sources showed their anticancer activity either alone or in combination and significantly enhanced the chemopreventive activity against cancer cells, but these studies are very much limited. Thus, the future researches should be more focused on the combined evaluation of phytoconstituents for a better cure against different kinds of cancers.

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Chapter 8
Metabolomic Study of Chemo-preventive Phytochemicals and Their Therapeutic Prospects

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Abstract The survival rates of cancer patients are decreasing over the years which are possibly due to selection of poor conventional anti-cancer drugs. At present complementary and alternate medicines (CAM) are high on demand as they show few or rather zero side-effects. Metabolomic analysis is considered to be a complex as well as efficient bridge between CAM and plants and its therapeutic possibilities as optimized and subscribed medicines. Metabolomics, considered to be the smallest domain comprises approximately 5000 metabolites, is chemically and physically more complex as compared to genomics (30,000 genes) and proteomics (100,000 or more proteins) associated with anti-cancer properties as it involves diverse groups of biological molecules. Stress on the cellular activity is inflicted directly by changes in the metabolome of an organism which underlines the importance of metabolomics in disease diagnosis and drug discovery. The present chapter focuses on the recent progresses in metabolomics which have transformed it to become a robust systems biology tool in studying both chemical and biochemical events that contribute to the cancer prevention activities of plant preparations or their bioactive components. Variations in the metabolome of cancer cell lines on treatment with plant extracts are also discussed. The current status of metabolic engineering efforts is highlighted for *in vitro* production of different chemo-preventive compounds viz. paclitaxel, geraniol, methyl cinnamate, Δ^9-tetrahydrocannabinol, etc. in their respective medicinal plants. The aim of present chapter is to explore the feasibility of metabolomic analysis of potent anticancer

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plants in order to search for the lead chemo-preventive compounds and also, to integrate the traditional cancer therapy with metabolomic profiling for the cure of cancer.

Keywords Cancer cell lines · CAM · Metabolomics · Phytochemicals · Screening

8.1 Introduction

Cancer is the leading cause of death worldwide, accounting for 8.8 million deaths in 2015 (Forouzanfar et al. 2016). According to WHO, the global burden of Cancer is predicted to increase by 50% to 15 million by 2020. In the present, scenario the search for novel chemo-preventive bioactive compounds have gathered momentum considering the dose limiting toxicity of conventional cancer treatment approaches. Complementary and alternative medicines (CAM) derived from plants are used in cancer treatment as these show reduced adverse side effects (Ravishankar and Shukla 2007). Treatment of cancer with Ayurveda, the traditional Indian medicine (TIM) dates back to seventh century BC, where early stages of cancer was treated using herbal medicines by Dhanwanthri and Atreya (Ravishankar and Shukla 2007; Poornima and Efferth 2016). In Ayurveda, cancer is described as an inflammatory and non-inflammatory swelling called as *Granthi* (minor neoplasm) or *Arbuda* (major neoplasm) (Patwardhan et al. 2005).

The approach or methodology used by the pharmaceutical companies for production of plant-derived drugs for the past decades was too expensive or time-consuming or both at the same time. Consequently, the former approaches have been regarded as the major bottleneck in exploiting the therapeutic potential of plants (Kim et al. 2010). Metabolomics have proved to be a promising tool as a new strategy for detection of bio-active compounds and such new strategies was the need of the hour to get natural products research out of its deadlock (Rochfort 2005; Verpoorte et al. 2005; Merzenich et al. 2007; Trenerry and Rochfort 2010). It is an 'omics' science that gives an overview of the metabolites present in a biological system. Its exposure under different conditions provides a better understanding about the biochemical reaction, disease development, selection of bio-markers, and patho-physiological interactions in the biological system (Sumner et al. 2003; Kim et al. 2010; Tomita and Kami 2012; Zhang et al. 2015). The downstream expression of the genome, transcriptome, proteome is represented by the metabolites thereby closely reflecting the phenotype of an organism at a specific time. Moreover, assessing the metabolite variation in cells on treatment with plant extracts or plant-derived compounds not only help us to monitor response of the cells but also to detect the activities of the bio-active compounds (Kim et al. 2010). The aim of present chapter is to explore the feasibility of metabolomic analysis of potent anticancer plants in order to search for lead chemo-preventive compounds and also to integrate the traditional cancer therapy with metabolomic profiling for the cure of cancer.

8.2 Metabolomics in Cancer Research and Diagnosis

Metabolomic approaches have brought metabolomics into the forefront of cancer research. The existing diagnostic modalities are not only expensive but also less sensitive in tumour detection (Soga et al. 2011). Conventional methodologies for detecting hepato-cellular carcinoma are by measuring serum-level of α-fetoprotein along with ultrasound imaging (Nicholson and Lindon 2008). However, other liver diseases may also result in high blood levels of α-fetoprotein resembling that of hepato-cellular carcinoma and thereby making α-fetoprotein an insensitive biomarker (Sumner et al. 2003; Robert and Morvan 2013). High-throughput metabolomics enables us to screen an array of biomarkers by comparing a diseased individual with healthy individuals (Fig. 8.1). Emerging metabolomics approaches have led to identification of biomarkers and associated candidate gene which is to be regulated for a particular type of cancer.

8.2.1 Metabolomics of Medicinal Plants

As vast number of primary and secondary metabolites having the potential therapeutic importance are synthesized by medicinal plant. The medicinal plant-based drugs and the use of metabolomics study is of paramount importance in the cancer therapy. Several secondary metabolites (about 2,00,000) from plants have been explored (Sumner et al. 2003). For example, ~5000 secondary metabolites have been derived from *Arabidopsis thaliana* and approximately 1500 and 2500

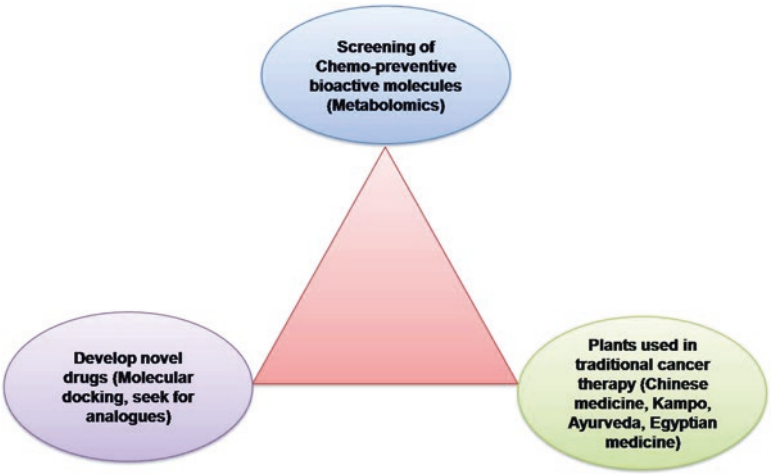

Fig. 8.1 Integrating traditional knowledge and high throughput metabolic approaches for discovering novel anticancer drugs

from microorganisms and animals, respectively. Different genomics-based 'phyto-chemical arrays (genome, transcriptome, proteome and metabolome)' have been established for measurement and analysis of several aspects including metabolite profiling in plants. Potential anticancer activity have been reported to be present in plant secondary metabolites like paclitaxel (taxol), camptothecin (irinotecan, topotecan) and podophyllotoxins (etoposide, teniposide) etc. (Schattka et al. 2011). Hence, medicinal plants or natural products are being considered as alternative application. It includes metabolite fingerprinting, which can be applied in different aspects like qualitative and quantitative analysis of target compound, identification of a set of compounds, quantification of all metabolites and rapid analysis of metabolites. This study has given rise to special emphasis on phyto-medicine research. It can be very useful in shifting the paradigm in drug discovery and development from natural resources. An overview of strategies pertaining to the enhancement of bioavailabity of the chemo-preventive bioactive molecule(s) from their respective plants is shown in Fig. 8.2 (Wang et al. 2012) (Table 8.1).

8.2.2 *Metabolomes of Cancerous Cell Lines Vary on Treatment with Natural Products*

Metabolomics have been applied to study the phytochemical induced chemo-preventive influences in carcinoma cells. Dose-dependent metabolic changes in breast cancer cells were revealed by NMR-based metabolomics on treatment with

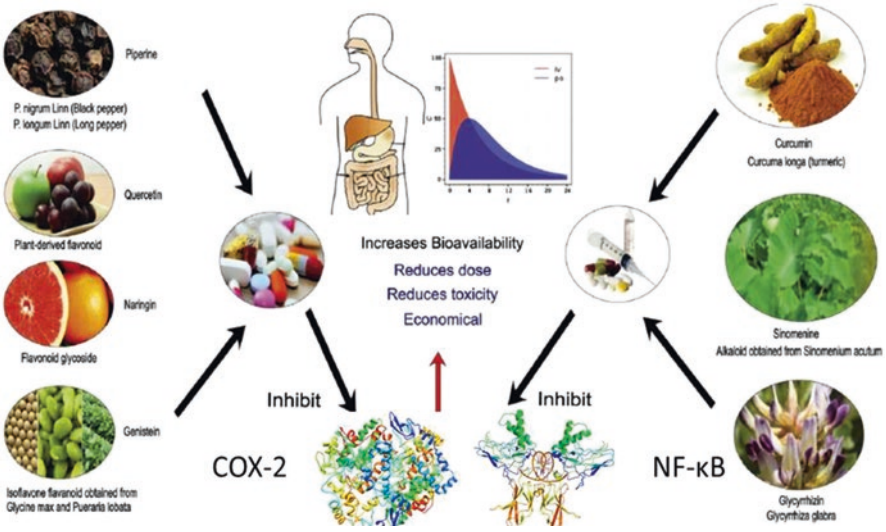

Fig. 8.2 Enhancing bioavailability of potent chemo-preventive bioactive compounds (Modified from Wang et al. 2012)

Table 8.1 Some major secondary metabolites derived from plants with potent anticancer properties

Major constituent	Molecular target	Plant source	References
Boswellic acid	↓leukocyte elastase, ↓5-LOX	*Boswellia serrata*	Safayhi et al. (1992, 1995) and Ammon et al. (1993)
Curcumin	↓NF-κB, ↓AP-1, ↓Egr-1, ↓COX-2, ↓LOX, ↓iNOS, ↓MMP-9, ↓uPA,↓ TNF	*Curcuma longa*	Bharti et al. (2003) and Villas-Boas et al. (2005)
Flavopiridol	↓CDK1, ↓CDK 2, ↓CDK 4, ↓CDK 7, ↑TNF, ↑doxo-rubicin, ↑etoposide	*Dysoxylumbi nectariferum*	Losiewicz et al. (1994) and De Azevedo et al. (1996)
Guggulsterone	↓iNOS, ↓IAP, ↓Bfl-1/A1, ↓bcl-2, ↓cFLIP, ↓cyclin D1, ↓c-myc, ↓MMP-9, ↓COX-2, ↓VEGF	*Commiphora mukul*	Aggrawal (2004) and Shishodia and Aggrawal (2004)
Resveratrol	↑p21, ↑p53, ↑Bax, ↓cyclin D1, ↓cyclin E, ↓bcl-2, ↓bcl-XL, ↓cIAPs, ↓NF-κB, ↓AP-1, ↓Egr-1, ↓IκB α-kinase, ↓JNK, ↓MAPK, ↓Akt, ↓PKC, ↓PKD, ↓COX-2, ↓5-LOX, ↓VEGF, ↓IL-1, ↓IL-6, ↓IL-8	*Vitis vinifera*	Manna et al. (2000), Banerjee et al. (2002), Estrov et al. (2003) and Agrarwal et al. (2004)
Zerumbone	↓COX-2, ↓IL-1β	*Zingiber zerumbet*	Kitayama et al. (1999) and Muakami et al. (2003)

↓ = Down regulation; ↑ = Upregulation

curcumin which is an active chemo-preventive curcuminoid present in the rhizome of *Curcuma longa* (Xie et al. 2015). Robert and Morvan (2013) reported increased glutathione level in breast cancer cells at low dose of curcumin and decreased glutathione level at high dose, suggesting that glutathione biosynthesis was up-regulated at low dose while the consumption of glutathione elevated at high dose. In addition, the effects of curcumin treatment on lipid metabolism, including accumulation of polyunsaturated fatty acids and decrease of glyerophospholipids, were also observed (Xie et al. 2015). In another investigation, the chemo-preventive effect of American ginseng on progression of colorectal carcinogenesis in genetically modified mouse was reported (Noorolahi et al. 2016). Gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and liquid chromatography time-of-flight mass spectrometry (LC-TOFMS) analysis of the serum shows significant alteration in the metabolites *viz.* amino acids, carbohydrates, fatty acids and organic acids which were attenuated by American ginseng and simultaneous histo-pathological improvement along with reduced tumor initiation, progression and gut inflammation (Noorolahi et al. 2016). Moreover, intestinal tissues of ginseng treated mice shows reduction of anti-inflammatory cytokines. Besides, ginseng extract independently exhibits chemo-preventive effects by anti-oxidant and anti-inflammatory mechanisms as it induces alteration of metabolites involved in inflammation and oxidation *viz.* tryptophan, arachidonic acid, glutamate, docosahexanoate and fructose (Noorolahi et al. 2016).

Metabolomic studies of *Aloe vera* extract on cancerous lymphoma cells showed altered levels of amino acids when compared with the untreated cells (Yagi et al. 2003). Glycoproteins present in the *A. vera* extract was described to be anti-ulceric and anti-tumor and observed to increase the rate of proliferation of normal human dermal cells (Singh et al. 2000; Yagi et al. 2003). A substance named *Aloin*, an anthroquine andthe main ingredient of *A. vera* has been shown to possess anticancer potential activities, as it blocks signal transducers and is an activator of transcription III activation by inhibiting effect on detoxification and are associated with carcinogen metabolism. The microsomal and cytosolic proteins were increased in the *A. vera* treated mice indicating the possibility of its involvement in the initiation of protein synthesis (Wang and Chen 2013).

8.2.3 Metabolomics Approaches for Investigation of Chemo-preventive Phytochemicals

8.2.3.1 Selection and Preparation of Sample

Based on the aims of metabolomics investigation, plants (extracts), bio fluids of animal models (treated *in vitro* with chemo-preventive plant extracts), tissue and cell extracts can be chosen for identifying bioactive phytochemicals *in vitro* and examining the impact of phytochemicals on metabolism on animal system (Fig. 8.3). In general, a power analysis should be conducted to ensure that a sufficient number of samples are included and the data can be statistically validated. In order to minimise or avoid the formation of new chemical species or degradation of metabolites during and after sample collection, the integrity of chemical composition in acquired samples, experimental techniques, storage conditions viz. (snap freezing in liquid nitrogen, quenching in preservation solution or freeze clamping) should be optimized (Villas-Boas et al. 2005; Wang and Chen 2013).

Preparation of the sample for the analytical platform is the key for success in metabolomic analysis, especially for MS-based approach. Metabolomic analysis of phytochemicals mediated chemoprevention demands ideal sample preparation strategies so as to efficiently extract small-molecule chemo-preventive phytochemicals from plants, human materials, and animals and also remove incompatible matrices such as salts and macromolecules. The chemical and physical properties of sample determine the extraction and preparation techniques to be applied. Liquid-liquid extractions, super-critical fluid extraction, solid-phase extraction, micro-wave assisted extraction, protein precipitation and dialysis are the widely applied (Dettmer et al. 2007). The obstacles for detecting phytochemicals in MS-based analysis is not only the concentration of bio-active compounds in samples but also their non-optimal performance in MS systems such as insufficient ionization in MS and poor retention in Liquid chromatography (Zhou et al. 2012). Chemical derivatization is an effective approach in this regard as it enhances the chromatographic and spectroscopic performance of these phyto-chemicals (Halket et al. 2005). This approach is

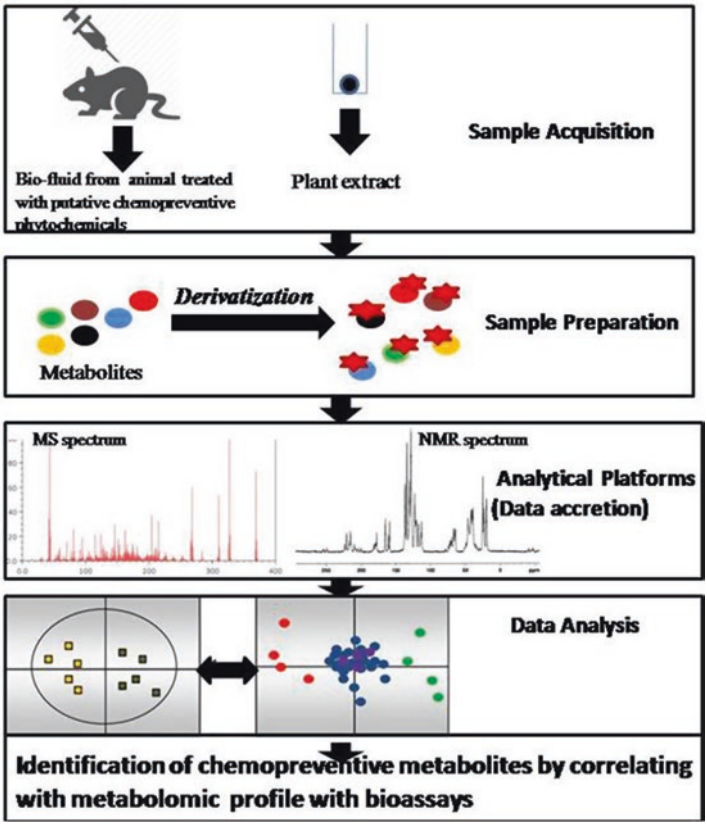

Fig. 8.3 Workflow of metabolomic approach for identification of chemo-preventive phytochemicals. Sample preparation by derivatization of metabolites making the metabolites compatible for analytical platform (Wang et al. 2013)

widely applied in gas chromatography GC-MS analysis as it improves the separation, detectability and sensitivity of metabolite detection. In recent years, chemical derivatization is widely applied in LC-MS analysis (Halket et al. 2005; Wang and Chen 2013). Derivatization reactions are designed based on the functional groups such as amino, carboxyl, carbonyl, and hydroxylmoieties in the metabolites. The two most desired outcomes of this approach are increased hydrophobicity and chargeability (Santa 2011).

8.2.3.2 Analytical Platform and Data Analysis of Metabolomics

Sophisticated platforms available viz. NMR, MS, infrared spectroscopy (Gamache et al. 2004) and electrochemical array (Schattka et al. 2011) enables us to detect chemo-preventive bioactive molecules in plants. Moreover, these methods unravel

the interactions between phytochemicals and biological systems. NMR and MS are the most widely used platforms for metabolomics analysis. Under electromagnetic field, MS determines mass to charge ratio of ions, while NMR measures the resonant frequency of nuclei. As compared to MS, NMR is less sensitive for detecting low abundance metabolites. Besides, NMR is non-destructive in nature and capable of providing more structural information. Chromatographic separation is not required for NMR analysis whereas majority of MS-based metabolomics approaches, GC, LC, capillary electrophoresis (CE) requires, prior to sample introduction and mass detection in MS system. Volatile metabolites or the metabolites which becomes volatile on derivatization are excellently suited for analysis in GC platform and polar compounds separation is performed by capillary electrophoresis (Wolfender et al. 2005). Metabolites in the biological sample are better compatible with LC platforms as compared to the other MS-based metabolomics approaches. Ultra-high pressure liquid chromatography (UPLC/UHPLC) is preferred over high pressure liquid chromatography as UPLC has features which use small particles, faster flow rate and high pressure as compared to HPLC thereby improving the chromatographic resolution and reduced running time in LC system (Wang et al. 2011). On elution from GC, CE or LC the analytes are subjected to ionization for detection by mass detectors in MS-based chemical analysis. Data analysis of NMR and MS-based metabolomics approaches is the crucial step for annotation of metabolites in metabolomic studies. NMR data comprises chemical shift and signal intensity, whereas GC-MS and LC-MS data comprise retention time (RT), mass to charge ratio, and signal intensity. In order to conduct metabolomic analysis, these data are deconvoluted to suitable data matrix so as to perform multivariate data analysis.

8.2.3.3 Metabolomics Based Identification of Bioactive Phytochemicals

Bioassay-based screening of crude plant extracts followed by fractionation and purification of chemo-preventive phytochemicals are the initial strategies to be adopted for identification of potent bioactive phytochemicals. The two types of high-throughput bioassay approaches for screening candidate chemo-preventive phytochemicals are; (i) cell-based approach for determining anti-proliferative and cytotoxicity against variety of cancer cell-lines such as 60 human cancer cell-lines of National Cancer Institute (NCI) representing nine human cancers (Shoemaker 2006); (ii) mechanism-based approach in which signalling molecules responsible for regulating proliferation and apoptosis of cancerous cells (*Ras, p53, bcl-2*) or enzymes (such as histone deacetylase, DT-diapharose) are used as biomarkers of screening assays (Kinghorn et al. 2003; Holbeck 2004). This is followed by validation on animal models and human trials. Fractionation and purification of bioactivity-guided compounds is a daunting task considering the chemical properties of the phytochemicals and the mechanism of bioactivity. Moreover, if chemo-preventive phytochemicals acts synergistically, unravelling the bioactivity of pure phytochemicals by bioassays becomes challenging (Rochfort 2005). Advanced analytical platforms such as NMR and MS-based metabolomics, which are widely used, is capable

qualitatively and quantitatively defining chemical composition of multiple plant extracts and identifying major differences among them through PCA modelling. A number of studies have illustrated the application of metabolomics approach for the identification of chemo-preventive phytochemicals from potent anticancer plants (Yuliana et al. 2011; Xie et al. 2015; Noorolahi et al. 2016).

8.3 Conclusions and Future Prospects

Sophisticated and powerful analytical platforms used in metabolomics have the capacity to measure numerous chemicals simultaneously and detect subtle differences among sample groups. Integration of traditional knowledge of cancer therapy with metabolomics provides a holistic approach for discovering novel candidates for anticancer drugs from plant extracts and elucidating the mechanism of action of potent chemo-preventive phytochemicals on regulation of cancer in biological systems. However, this field of study is still in its infancy as majority of metabolomics studies on the metabolic effects of chemo-preventive phytochemicals remain in the observational level. To elucidate the structure of the potent chemo-preventive phytochemicals, chemical analogues can be developed for finding novel anticancer drugs. Metabolomics is a promising domain for finding lead compounds considering the great need for novel chemo-preventive phytochemicals.

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Chapter 9
CADD Studies Applied to Secondary Metabolites in the Anticancer Drug Research

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Abstract Natural products have had an important and decisive role in the development of modern medicinal chemistry and drug design. The development of new bioassay techniques, biotech methods, bio-guided phytochemical studies, automated high-throughput screening, and high performance analytical methods has introduced both new concepts and possibilities for rational drug design and drug discovery. With the development of new spectroscopic techniques, organic chemists have been able to elucidate the complex molecular structures of natural constituents quickly. Secondary metabolites, namely, alkaloids, flavonoids, and terpenoids as anticancer molecules, involving various strategies of treatment, have been discussed with special reference to topoisomerases (Topo), cyclooxygenases (COX), lipoxygenase (LOX), and aromatase as enzymatic targets for various types of cancers. In silico methods or CADD (computer-aided drug design) studies are increasingly being used in both industries and universities. They involve an understanding of the molecular interactions from both qualitative and quantitative points of view. These methods generate and manipulate three-dimensional (3D) molecular structures, calculate descriptors and the dependent molecular properties, model constructions, and employ other tools that encompass computational drug research. Analysis of the molecular structure of a given system allows relevant information to be extracted and to predict the potential of bioactive compounds. Furthermore, in view of the recent advances made in the field of computer-aided drug design, the aim of present chapter is to discuss the use of computational approaches such as ADMET, molecular docking, molecular dynamics simulation, and QSAR to asses and predict the safety, efficacy, potency, and identification of natural potent anticancer molecules.

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9.1 Introduction

The kingdom plantae contributed significantly to the discovery of useful substances that may treat various human diseases. The development of organic chemistry in the nineteenth century occurred in parallel with the study of plants. The plants are the source of a large number of natural products having an undoubtedly unique development and occurrence of complex metabolites (Cragg and Newman 2014). The natural products often resulted from an optimized evolutionary process in which chemicals have been under the selective forces of coevolution, organisms producing substances in the presence of their predators. These products have been utilized by humans since ancient times to treat and cure their diseases. Traditional Chinese medicine is the best example of natural product efficiency, especially in the discovery of new active chemical entities. These compounds are present in fruits and vegetables and are important components of our daily diets. Of the existing plants in the world, most of which are unknown from a scientific point of view, only about 5% of the approximately 250–500 thousand species have been biologically studied and evaluated. Natural products are often phenolic compounds, flavonoids, alkaloids, or terpenes, secondary plant metabolites that may provide several benefits to our health. These benefits include cosmetic action, cardioprotective effects, anti-inflammatory activity, and usefulness in the treatment of cancer and the neglected diseases (Chan et al. 2013; Karioti et al. 2015). The use of natural products has been the single most successful strategy for discovering new medicines, and many medical breakthroughs are based on natural products. Half of the top 20 best-selling drugs are natural chemical compounds, and their total sales amount to US$ 16 billion yearly. These numbers suggest that natural chemicals may well be considered pre-optimized for bioactive potential and therefore possess “drug-like properties” (Kennedy et al. 2009; Wang et al. 2011; Clement 2014). New molecules are continually being reported in the literature, many with relevant pharmacological activity, such as Taxol, forskolin, artemisinin, etc. (Fig. 9.1). It is important to remember that plants have contributed over the years to obtaining various widely used drugs, such as morphine, emetine, vincristine, colchicine, rutin, etc. (Fig. 9.1). In the 1980s, consumers in the USA paid more than 8 billion dollars for prescriptions with active natural products, and about 80% of all people use natural compounds in the treatment of their diseases (Mills et al. 2005; Mishra et al. 2008; Moran et al. 2009).

Several therapeutically active metabolites were isolated from marine organisms, which could be used as effective modulators of biological targets such as phospholipases, adenosine receptors in tumor models (e.g., manoalide, lufarolide, and azidovudine (Fig. 9.2)) inspired by the chemical structure of a substance of

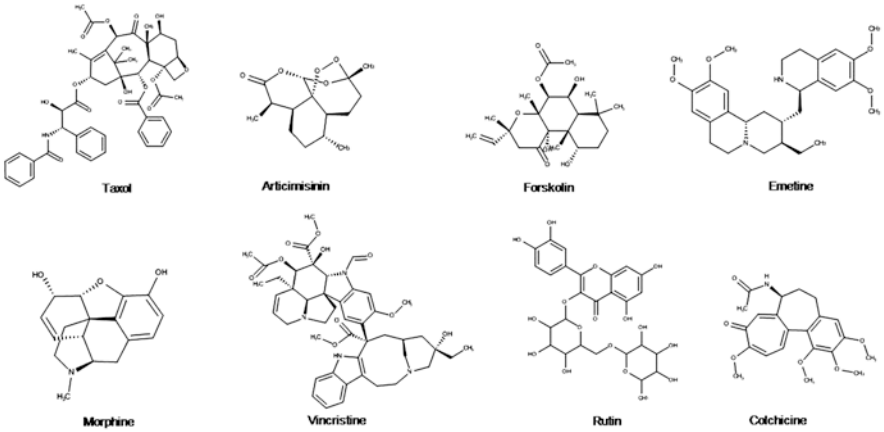

Fig. 9.1 Structure of some natural products

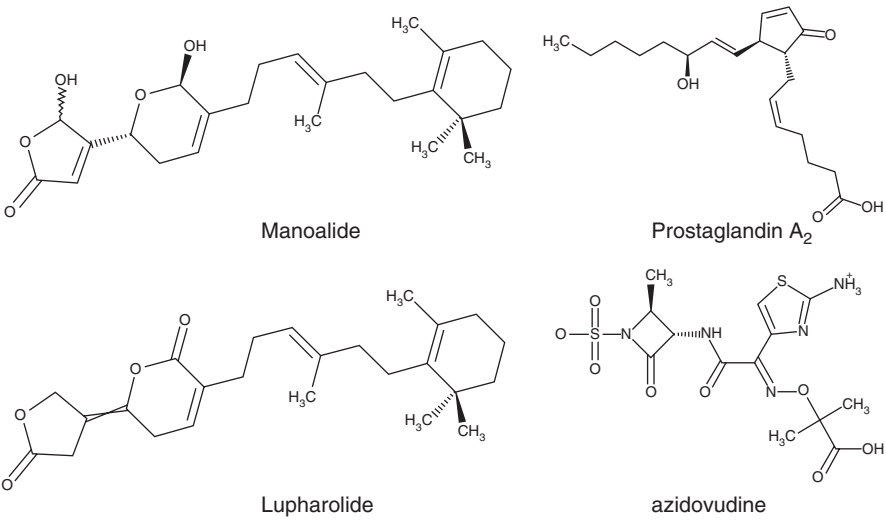

Fig. 9.2 Structure of some marine natural products

marine origin which proved to be active on HIV reverse transcriptase, agent responsible for acquired immunodeficiency syndrome (AIDS), and prostaglandin A_2 (Fig. 9.2) (Scotti et al. 2012a; Anjos et al. 2012; Souza et al. 2012; de Araújo et al. 2013; Hatae et al. 2015). New chemical bioactive entity studies done by industrial research laboratories have adopted techniques such as combinatorial chemistry to obtain more compounds. Through this technology, reactions are done in several steps, occurring in parallel or in mixtures, with few reagents. Products are reagent combinations, and therefore large numbers of new compounds can be generated. Thus, the aim of the present chapter is to discuss the use of computational approaches such as ADMET, molecular docking, molecular dynamics simulation, and QSAR to

asses and predict the safety, efficacy, potency, and identification of natural potent anticancer molecules.

9.2 Medicinal Chemistry and Its Significance

It is dedicated to understanding the molecular mechanism, chemical relationships, and pharmacological activity involved in drug action thru pharmacodynamic and pharmacokinetic factors. The introduction of new technologies has become a prime concept in medicinal chemistry expanding its interdisciplinary character. Most drugs are small bioactive molecules that interact with specific macromolecules or receptors, resulting in their therapeutic effects. Modern computational methods can determine the different qualitative and quantitative contributions of the structural subunits of different drugs. Pharmacokinetic and toxicity factors of new drug candidates can be evaluated virtually using modern computational tools. The computer has become an inseparable ally, allowing computational studies that model the chemistry and molecular dynamics of medicinal chemistry (Tang et al. 2014; Liu et al. 2015; Ding et al. 2015). Using several computational tools, researchers can create new virtual candidate ligands for receptor sites in three dimensions (3D). Pharmaceutical companies report that spending on research and development in 2004 was about 33 billion US dollars, representing real growth year to year in investment; this does not correspond, however, to a proportional increase in discoveries of new active molecular candidates for innovative drugs in the market (Fiorani et al. 1999). Natural products have had an important and decisive role in the development of modern medicinal chemistry and drug design. It has been observed (during the last 200 years) that the complexity, chemical diversity, and biological properties of natural products have all aided in the discovery of important new drugs (von Pawel et al. 2014).

In the past 30 years, the development of new bioassay techniques, biotech methods, bio-guided phytochemical studies, automated high-throughput screening, and high performance analytical methods has introduced both new concepts and possibilities for rational drug design and drug discovery. With the development of new spectroscopic techniques, organic chemists have been able to elucidate the complex molecular structures of natural constituents quickly. Gossypol obtained from the cottonseed oil (*Gossypium* sp.) is widely used in China as a male contraceptive, and hypericin isolated from Saint John's wort (*Hypericum perforatum*) extract is used as an antidepressant (Congur et al. 2015). However, another natural substance of oriental origin is artemisinin isolated from *Artemisia annua*, a plant known and used in Chinese medicine and whose structural complexity has inspired new, useful drugs for the treatment of malaria. Moreover, the active compounds β-artemether, arteether, and sodium artesunate were also obtained without limitations of bioavailability (Fig. 9.2) (Sonderstrup et al. 2015). In silico methods or CADD (computer-aided drug design) studies are increasingly being used in industries or universities which involved an understanding of molecular interactions from both qualitative and

quantitative points of views. These methods generate and manipulate three-dimensional (3D) molecular structures, calculate descriptors and the dependent molecular properties, model constructions, and employ other tools that encompass computational drug research. Analysis of the molecular structure of a given system allows relevant information to be extracted and to predict the potential of bioactive compounds (Grunnet et al. 2015). Theoretical studies using in silico methods have aided in the process of drug discovery. Technological advances in the areas of structural characterization, computational science, and molecular biology have contributed to faster planning of new feasible molecules (Yeo et al. 2013). Chemoinformatic studies showed that a large fraction of natural products have structural and physicochemical properties that render them as potential drugs. Some investigators have suggested "natural product-likeness score" as a means to filter large chemical databases and find new entities suitable for testing activity and these products subjected to increase the interest in phytochemistry, biochemistry, and related field of research.

9.3 Docking and Drug Discovery

Drug discovery is a lengthy and expensive process that can take up to 15 years and cost upward of billions of dollars. Most of the drug candidates were failed in clinical trial. Of every 10,000 compounds tested, only 1 or 2 are marketed. Traditionally, new drugs have been discovered through studies of natural or synthetic compounds with biological activity. Today, theoretical methodologies have the advantages of both effectively reducing costs and speeding up drug discovery, which results in earlier drug marketing. Examples are captopril, from Bristol-Myers Squibb used for the treatment of hypertension and heart failure; dorzolamide, an antiglaucoma agent developed by Merck; saquinavir, which is a protease inhibitor used for HIV therapy and produced by Hoffmann-La Roche; and zanamivir, a neuraminidase inhibitor used for influenza treatment (Camacho et al. 2015; Cui et al. 2015).

Computer-aided drug design (CADD) studies used several methodologies of computational chemistry to discover, enhance, and study drugs and their related biologically active molecules. Computational methods help in minimizing not only the number of drug candidates but also their ADME profile and toxicological properties. It is assumed that the biological activity of a drug is generally related to its interaction with a protein or a nucleic acid. The drugs are designed (in silico), based on their interaction (ligand with the macromolecule) observed in three dimensions, using molecular docking, in a structure-based drug design (SBDD) technique. This method is used to investigate and predict how the candidate drug (ligand) interacts at the molecular level, by binding to the target protein or nucleic acid (receptor), and to analyze the energies and interactions involved between them (Campos et al. 2011; Frampton 2013; Brown et al. 2014).

Docking involves molecular biology and computer-assisted drug design. The objective of this method is to predict the predominant binding mode of a ligand to proteins, using complex ligand-protein docking of the known three-dimensional

structures. Docking methods can effectively search the high-dimensional spaces which occur and apply a scoring function that correctly ranks candidate dockings. Through docking, it is possible to perform virtual screenings of large libraries of compounds, ranks, results, and their proposed structural hypotheses on of how the ligands might inhibit a target (Echenique and Alonso 2007; da Rocha et al. 2011; Decker 2011). Technological advances, better performing algorithms, and increasing computing power allow timeline molecular docking with thousands of ligands; this is of great importance in the pharmaceutical industry. In recent years, the growing number of publications based on molecular docking demonstrates its importance and effectiveness in drug discovery (Lavecchia and Di Giovanni 2013).

9.4 Treatment of Cancer by Plants and Their Derived Products

Cancer is the set of chronic diseases caused by mutations in protein-coding genes leading to disordered growth and multiplication of abnormal cells to form tumors that destroy tissue and other organs (Charifson and Walters 2014). Being caused by a genetic disorder, the disease development can be simple and fast. It was estimated that 18% of cancer cases reported in 2002 were associated with infections such as hepatitis B and C and papilloma virus (90% of patients with cervical cancer) (Duffy et al. 2012; Chrea et al. 2014). In addition, about 30% of cancers are associated with tobacco smoking and inhalation of pollutants and another 35% to eating habits (Shekhar 2008). Devi et al. (2015) reported that lung, stomach, colorectal, liver, and breast cancers are the major causes of death in the world. Breast cancer is the most common cancer in women, while prostate cancer and lung cancers are common in men (Kothandan and Ganapathy 2014). According to an estimate, 56% of 12.7 million cases and 64% of 7.6 million deaths in 2008 occurred in developed countries, and it is expected to increase up to 11.5 million till 2030 (Kothandan and Ganapathy 2014). At present, the chemotherapy is the most effective method of cancer treatment, but it does not destroy the tumor cells completely or diagnosed in late stage of cancer. Moreover, the disease's stage and resistance of tumor cells toward drugs and their side effects are main causes behind the failure of the treatment (Talele et al. 2010).

In this regard, the therapeutic potentials of plant and their derived products (alkaloids, flavonoids, and terpenoids) with special reference to topoisomerases (Topo), cyclooxygenases (COX), lipoxygenase (LOX), and aromatase provide an alternative way to target various types of cancers. The computational approaches, namely, ADMET, molecular docking, molecular dynamics simulation, and QSAR, are used in assessing and predicting the safety, efficacy, potency, and identification of these potent anticancer therapeutic molecules. According to the World Health Organization (WHO), 80% of the world's population utilizes the plants or their derived products to treat the various diseases in developing countries. The plant-derived natural prod-

ucts are of great promise for discovery and development of new pharmaceuticals against diverse human ailments including cancer. At present, out of 121 drugs prescribed for cancer treatment till date, 9 are derived from plants (Talele et al. 2010; Kothandan and Ganapathy 2014). Furthermore, among the FDA-approved anticancer drugs between 1984 and 1994, 60% were isolated from plants (Talele et al. 2010). Among 65 new drugs registered for cancer treatment during the period 1981–2002, 48 were obtained from plants (Chen et al. 2002).

9.4.1 Anticancer Molecules

Tan et al. (2014) investigated the neutral and cationic benzo[c]phenanthridine alkaloids against HCT-116 (colon tumor cells) and HL-60 (promyelocytic leukemia cells) and through computer-aided drug design. The cationic alkaloids (7,8-oxygenated benzo[c]phenanthridine alkaloids, chelerythrine, and NK109) showed better anticancer activity compared to neutral derivatives because these compounds formed shorter bonds between ring A and the substituents influencing the resonance, increasing the inhibition of topoisomerases I and II as evident in the electrostatic potential. Similarly, camptothecin, a monoterpenoid indole alkaloid showing anticancer activity, is isolated from the *Camptotheca acuminate* (Sagar et al. 2006) and acts as a topoisomerase I inhibitor (Jeong et al. 1999). Researchers in medicinal chemistry developed three semisynthetic syntheses: (1) water-soluble analogue of the plant alkaloid camptothecin, (2) topotecan, and (3) irinotecan, used against ovarian, cervical, colorectal, and lung cancers (Wheat and Currie 2008; Polo and Bravo 2006; Yang and Dou 2010; Thoppil and Bishayee 2011). Similarly, Cui et al. (2015) studied the genes from *Catharanthus roseus* with strictosidine synthase and geraniol 10-hydroxylase, introduced into *Ophiorrhiza pumila* hairy roots, generating the transgenic compound. They concluded that there was an increase in the production of camptothecin, which showed antitumor activity against leukemia K562 cell line.

The alkaloid camptothecin was developed as a potent anticancer drug directed against Topo I in the year 1958. Two derivatives of camptothecin, namely, topotecan and irinotecan, are being used as FDA-approved drugs against Topo I (Chalabi et al. 2007; Cheah et al. 2008). Similarly, the first Topo II inhibitor, namely, etoposide, an analogue of alkaloid podophyllotoxin, is also an FDA-approved anticancer drug coming from natural product (Anjos et al. 2012; Souza et al. 2012). Iriodenine has been reported to inhibit both Topo I and II by trapping the cleaved DNA-enzyme intermediate and preventing the release of enzymes (Li et al. 2002). Similarly, the alkaloid eleutherin has been reported to inhibit the action of Topo II by inducing relegation and dissociation of the enzyme from DNA (Vipin et al. 2015). Alkaloids dicentrine (both Topo I and II) and lunacridine (Topo II only) have been reported to inhibit respective topoisomerases by intercalating the DNA helix (Dave and Panchal 2012; Scotti et al. 2012b). However, alkaloids (matrine and tetrandrine) are reported

to exert their anticancer activity by induction of cell cycle arrest, apoptosis, as well as inhibition of metastasis and angiogenesis (Morris and Lim-Wilby 2008).

Anonaine is a potential anticancer agent, which inhibits cell proliferation by DNA damage and cell cycle arrest in human lung cancer cell lines H1299 (von Pawel et al. 2014). The anticancer activity of oliveroline has been reported through cytotoxicity in MCF-7 mp53 breast cancer cell lines expressing mutant p53 (Taylor et al. 2002). Furthermore, oliveroline has also been reported to act as an anticancer compound inhibiting proliferation of cells undergoing DNA damage at G_2 checkpoint (de Avila and de Azevedo 2014). Sanguinarine has been reported to exert its anticancer activity by induction of cell cycle arrest at different phases or apoptosis in a variety of cancer cell lines (Fiorani et al. 1999). Piperine has been reported to inhibit breast stem cell proliferation without causing toxicity to differentiated (normal) cells (Yuriev and Ramsland 2013). Evodiamine has been reported to exhibit anticancer activities under both *in vitro* and *in vivo* conditions by inducing the cell cycle arrest or apoptosis, thereby inhibiting the initiation of proliferation, angiogenesis, invasion, and metastasis in a variety of cancer cell lines (Fiorani et al. 1999; Sonderstrup et al. 2015). Berberine has been reported to inhibit multiple aspects of tumorigenesis and tumor progression under both *in vitro* and *in vivo* conditions including the induction of cell cycle arrest at the G_1 or G_2/M phases and apoptosis (Frampton 2013; Brown et al. 2014; Camacho et al. 2015; Cui et al. 2015).

Tan et al. (2011) reported the antitumor activity of dimers of triphenylethylene-coumarin hybrid containing one amino side chain. The authors attributed the anticancer activity to DNA metabolic enzymes, such as the topoisomerases. Later, Zhu et al. (2015) synthesized monomers and dimers of triphenylethylene-coumarin hybrid containing two amino side chains through condensation of three dicarboxylic acids with the amino monomeric hybrids. These compounds were tested against MCF-7 (human breast cancer), A549 (human lung cancer), K562 (chronic myeloid leukemia), and Hela (cervical carcinoma). The active compounds were evaluated as DNA inhibitors by UV-Vis, fluorescence, and CD spectroscopies and a DNA thermal denaturation experiment to observe interactions, properties, and also conformational changes in DNA morphology. The dimers showed better results than the monomers. The authors supposed that the length of the bond and the basic amino group had an influence on the antiproliferative activity of the coumarins.

In vitro and *in vivo* studies of a number of plant-derived flavonoids such as isoliquiritigenin, glabridin, protocatechuic acid, apigenin, fisetin, baicalin, daidzein, and gingerol have been reported to exhibit antiangiogenic activity (de Atenção 2013). Various naturally occurring flavonoids and their derivatives have been reported to be active against breast cancer with inhibitory effects on aromatase (Aggarwal et al. 2009; Grivennikov et al. 2011). Out of a total of 282 natural compounds proven with anticancer activity against aromatase, 125 were flavonoids (Ahmedin Jemal et al. 2011). Genistein, a phytosterol belonging to flavonoid family, has been reported to inhibit tyrosine kinase, angiogenesis, arrest of cell cycle in G_2/M phase, and induction of apoptosis in human promyelocytic HL-60 cancer cell lines (Tu et al. 2016). Biocalein has been reported to induce apoptosis in cell lines of human hepatocellular carcinoma (HCC) as well as inhibition of Topo II (Wang

et al. 2012). Similarly, quercetin has been also reported to induce apoptosis by stimulating release of cytochrome-c to the cytosol and activating caspase 9. A number of phenolic compounds, belonging to flavonoids, extracted from rhizome of ginger, have been reported to exhibit cytotoxic activity in cancer cells. However, resveratrol, a phytoalexin found in grapes, has also been reported to induce apoptosis, through CD95 signaling pathway in breast carcinoma HL60 and T47D cell lines (Qurishi et al. 2010). Similarly, curcumin, an important flavonoid, has been shown to be effective in a variety of cancers including colorectal, pancreatic, gastric, and prostate. It has been reported to be effective on different stages of carcinogenesis, namely, proliferation, angiogenesis, and metastasis. Moreover, curcumin has also been shown to act as a chemo-sensitizer, resulting in the increased activity of other anticancer factors in cases of multidrug-resistant and chemotherapy-resistant cancers (Kumar et al. 2012; Safarzadeh et al. 2014).

Geraniol, an acyclic dietary monoterpenoid, has been shown to inhibit the growth of HepG2 human hepatic carcinoma cell lines by inhibiting 3-hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the major rate-limiting enzyme in cholesterol biosynthesis in mammals (Mukherjee et al. 2001). Excisanin A, a diterpenoid, exhibited inhibition of growth of human Hep3B liver cancer cell lines via AKT signaling pathway (Brown et al. 2014). Oridonin (diterpenoid) exhibited antiproliferative activity toward prostate cancer cells. Actein (triterpenoid) was found to inhibit the growth of p53-positive HepG2 cancer cell lines. A triterpenoid, betulinic acid, was reported to inhibit growth of breast cancer cells (Houghton et al. 1991). Ursolic acid, an apentacyclic triterpene, has potent cancer-preventive activity and great therapeutic potential. Breast cancer MCF-7 cells exhibited typical apoptotic features, including chromatin clumps and aggregation and DNA fragmentation after ursolic acid treatment, which was in correlation with the downregulation of Bcl-2 and upregulation of caspase-3 (Kanzawa et al. 2001). Different *in vivo* studies have shown that xanthorrhizol (sesquiterpenoid) inhibits formation and development of tumors via reducing ornithine decarboxylase, COX-2, and NF-κB signaling activity (Hande 1998). Lycopene (tetraterpenoid) modulated breast cancer gene expression pertaining to various molecular pathways, such as apoptosis, cell communication, mitogen-activated protein kinase (MAPK) pathway, and cell cycle (Fortune and Osheroff 2000). Similarly, it has been reported to trigger G_2/M arrest and suppress Bcl-2 expression in breast cancer MCF 7 cell lines (Liu et al. 2009).

9.4.2 Topoisomerase Inhibition by Diterpenes

During the processes of replication and transcription along a stretch in the anterior and posterior region of DNA, strands are separated due to the formation of spirals. Topoisomerases act on the control of spirals, relaxing the DNA, and modifying its tertiary structure without changing the primary structure (Prescott et al. 2007; Brastianos et al. 2007; Konkimalla and Efferth 2010; Chen et al. 2011; Lu et al. 2012). These macromolecules are classified according to the cleavage of DNA

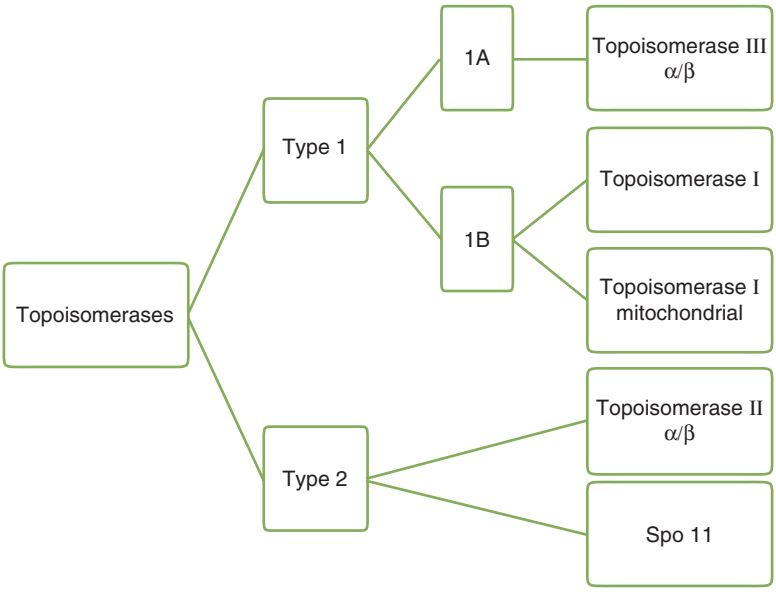

Fig. 9.3 Classification of topoisomerases

strands and the location of the covalent link between the enzyme and the DNA strands. In humans, there are two types of topoisomerases (Fig. 9.3). Enzymes type 1 break only one of the DNA strands, passing it through the intact tape and then reconnecting it. The type 1 topoisomerases (Topo I) can be 1A or 1B. The first, 1A, covalently links the 5′ portion of the DNA and does not need cofactors, while the second, 1B, covalently binds to the phosphate portion 3′ of the DNA using strand and Mg^{2+} or Zn^{2+} as cofactor. 1A is formed by topoisomerase type III α and β and type 1B by topoisomerase I and topoisomerase I mitochondrial. Type 2 enzymes (Topo II), performing the process, is similar to type 1; however, instead of a single-stranded DNA, both strands are broken. This group of topoisomerase II α and β and Spo 11 bind to 5′ and are dependent on ATP (Kakarala et al. 2010; Burgeiro et al. 2011; Yang et al. 2012; Millimouno et al. 2014). The findings of previous studies showed that the diterpenes have proven their anticancer activity (Tavares et al. 2006; da Silva et al. 2009; Pita et al. 2012; Scotti et al. 2014; Ishiki et al. 2014).

9.4.3 Molecular Modeling and Docking

The three-dimensional structures were drawn using HyperChem 8.0.3 software and energy minimized employing the MM+ (Dewar et al. 1985; Cohen 1996) force field without any restrictions. Subsequently, we performed a new geometry optimization based on the semiempirical AM1 method (Leach 2001). The optimized structures were subjected to conformational analysis using a random search method (Staker

Table 9.1 Energies (kcal/mol) obtained from the interaction of the ligands and enzymes

Compound	Topo I	Topo II
T1	−77.220	−73.918
T2	−66.860	−54.315
T3	−65.073	−62.102
A1	−48.066	−37.733
A2	−55.703	−48.965
A3	−65.204	−55.666

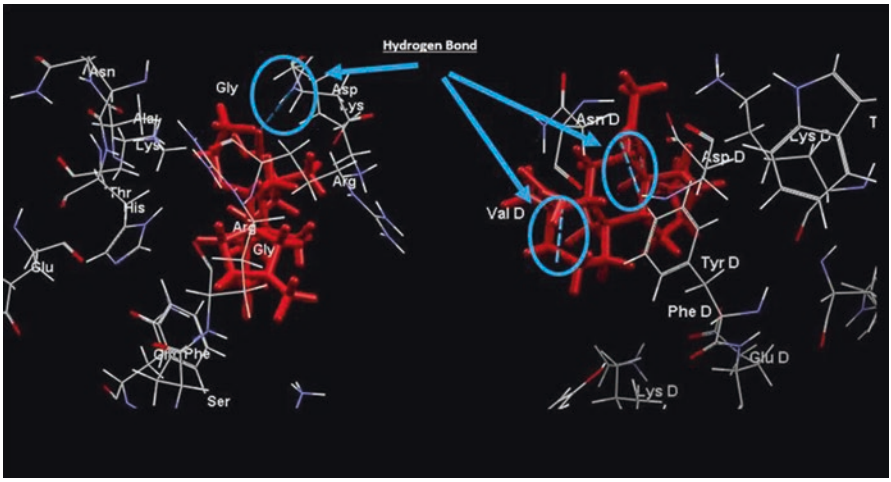

Fig. 9.4 Hydrogen bonds between the diterpene T1 and Topo I and II

et al. 2005). Selected dihedral angles were evaluated by rotation in accordance with the standard (default) conditions of the Spartan program (Graille et al. 2008). The diterpene ligands showed two enzymes: topoisomerases I and II (Motohashi et al. 2013). The enzymes were imported from the Protein Data Bank in the Molegro Virtual Docker 6.0 program with a template of complex ligands for the GRID. We selected the MolDock SE algorithm with ten runs for each ligand. The energies (kcal/mol) obtained from the interaction of the ligands and an enzyme are summarized in Table 9.1. We observed that all compounds had the best interaction with Topo I. Compound T1 best interacted in both receptors; on the other hand, A1 showed the highest energy with the two enzymes. We noted that compound T1 forms one hydrogen bond when submitted to docking with Topo I (with the ASP533 residue) and two with residues in Topo II (THR213 and TYR188) (Fig. 9.4). The atisane diterpene forms only steric interactions with ARG364 Topo I and Topo II of TYR188. We concluded that the stability difference observed in the energy of formation can be attributed to hydrogen-bond interactions. Other studies reported the same, as the observations of Laco et al. (2002) which H-bond interactions between the camptothecin and top1/DNA active site are reflected in the values of the energy scores.

9.5 Conclusions and Future Prospects

The successful treatment of cancer yet remains a challenge due to the lack of selectivity, toxicity, and development of multidrug-resistant cells to the currently available drugs. The plant-derived products offer a high selectivity, strong activity, low side effects, as well as cancer prevention role by enhancing body immunity. The development of cancer prevention and health-care products from natural products has a broader prospect and greater economic and social benefits when compared with prevalent synthetic anticancer drugs. In this regard, the bioinformatics tools have play a very crucial role in designing and developing new lead therapeutic molecules for the designing of antitumor drugs that use natural products as enzyme inhibitors of human topoisomerases (I and II). These may play an important role in DNA metabolism and inhibition of tumor cells. Some flavonoids and alkaloids like camptothecin and lamellarin D have been extensively examined for the basis for new compounds. In addition, the docking studies, performed with Brazilian diterpenes and both enzymes, showed that the trachylobane diterpene formed a more stable complex due to a hydrogen bond with Top I (with the ASP533 residue) and two hydrogen bonds with residues of Top II (THR213 and TYR188). This study may serve for the basis of compounds which may belong to class II or drug catalytic topoisomerase inhibitors, which interferes with the function of the topoisomerases.

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Chapter 10
Anticancerous Plant Compounds Affecting the Power House of Cancerous Cells: A Possible Herbal Mitocan

Shalini Mani, Nancy Taneja, Sweekriti Jain, and Manisha Singh

Abstract Mitochondria are semiautonomous organelles that play an essential role in cellular metabolism and programmed cell death pathways. Emerging evidences suggest that cancer cells exhibit various degrees of mitochondrial dysfunctions and metabolic alterations. Some of those alterations may provide a selective advantage to cells, allowing them to survive and grow under different stresses. Transformed cells have a very different metabolic and mitochondrial function from their normal counterparts. This is the reason that mitochondrial alterations in cancer cells can offer a great opportunity for efficient and selective anticancer therapy. The mechanism of action of mitochondria-targeted anticancer drugs relies on their ability to disrupt the energy-producing systems of cancer cell, mitochondria leading to increased reactive oxygen species, and activation of the mitochondrial-dependent cell death signaling pathways inside cancer cells. This emerging class of drugs is called as mitocans that affect the activities related to mitochondria, such as hexokinase inhibition, electron transport/respiratory chain blockage, activation of the mitochondrial membrane permeability transition pore targeting constituent protein subunits, and inhibition of Bcl-2 anti-apoptotic family proteins and Bax/Bid pro-apoptotic mimetic. A great deal of pharmaceutical research and refinement in the isolation and structure elucidation techniques has been able to identify various anticancer herbs. Many of these herbs are recognized for their valuable bioactive anticancer compounds. A great progress has been further made to identify the role of these active compounds on targeting mitochondria for cancer treatment. The present chapter described the various natural compounds such as curcumin, capsaicin, berberine, sanguinarine, and lamellarin D, which preferentially target the cancer cells by hindering the mitochondrial function via different mechanisms as "herbal mitocans" and also lowering the side effects of other synthetic anticancer drugs in the treatment of cancer.

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10.1 Introduction

Cancer is defined as an abnormal growth of cells which finally spreads in the system through uncontrolled cell division. Globally around 11 million people are diagnosed with cancer, and this number is predicted to increase to 16 million by 2020. There has been an enormous investment in the area of medical science to treat cancers during the past few decades worldwide. However, this condition is still considered as a leading health threat. The treatment of any malignancies involves surgery, radiotherapy, and/or drug therapy. This complex approach is not efficient enough to cure a large number of cancer patients, but this just prolonged survivals or is even without benefits.

Numerous novel approaches to study the role of mitochondria in cancer have led to the discovery of various structural and functional differences of this organelle between cancer and normal cells. A large number of attempts are being made to utilize these differences as new and site-specific targets for chemotherapy. In different preclinical studies, a good number of compounds targeting mitochondria have been tested. These groups of compounds, which target the mitochondria in cancerous cells, are collectively called as "mitocans." As a result of different studies, these mitocans have shown some efficacy in selectively killing the cancer cell. Mitocans, based on their target and varied mechanism of action, have been divided into a total of eight groups (Neuzil et al. 2013). Similar to various synthetic mitocans, some of natural compounds which are anticancerous in nature are also found to target the function of mitochondria, either directly or indirectly (Wang et al. 2010; Gibellini et al. 2015). As these compounds are natural and target the mitochondria of cancer cells, it may also be termed as "herbal mitocans." The present chapter describes various natural compounds such as curcumin, capsaicin, berberine, sanguinarine, and lamellarin D, which preferentially target the cancer cells by hindering the mitochondrial function via different mechanisms. The chapter further synopses the potential herbal mitocans, their sources and detailed mechanism of action.

10.2 Mitochondria

Mitochondrion is a rod-shape double membranous organelle, found in the eukaryotic and prokaryotic cells (Fig. 10.1). It is known as "powerhouse" of the cells, as they generate most of the energy required for the cellular processes in the form of adenosine triphosphate (ATP). The mitochondria are also involved in different cellular functioning like cell signaling, differentiation, cell cycle, and growth (Antico Arciuch et al. 2012). The membranes are composed of phospholipids and proteins.

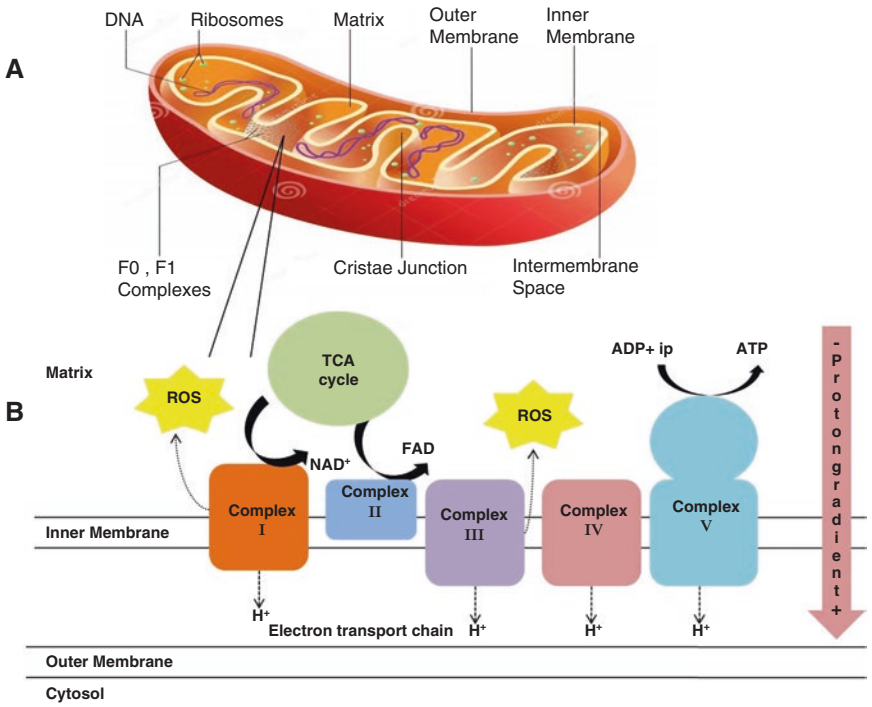

Fig. 10.1 Diagram showing the (**a**) structure and (**b**) function of mitochondria in ATP generation and ROS production

The inner mitochondrial membrane is folded into numerous folds known as the cristae. These cristae and the various proteins help in the ATP production. The inner membrane is strictly permeable, it is permeable only to oxygen, and it also helps in metabolite transportation across the membrane. The space between the outer and inner membrane of the mitochondria is known as intermembrane space. The mitochondrial matrix consists mixture of proteins, enzymes which plays an important role in the synthesis of ATP, tRNAs, and mitochondrial DNA (Voet et al. 2006).

10.2.1 Mitochondria in ATP Production

Mitochondria are the "powerhouse" of eukaryotic cells as they play a central role in the aerobic metabolism. The metabolic yield from the carbohydrate and lipid metabolism in the form of reduced "nicotinamide adenine dinucleotide (NADH)" and flavin adenine dinucleotide ($FADH_2$) act as substrates for oxidative phosphorylation for the ATP production (Bertram et al. 2006). For the synthesis of ATP, electrons are transferred from NADH and $FADH_2$ to O_2 via inner mitochondrial membrane electron transfer complexes (Fig. 10.1). The electron transport

complexes (I–V) are the pivotal for the processes like mitochondrial respiration and ATP production (Schagger and Pfeiffer 2000). The mitochondrial electron chain complexes accept electrons from NADH and succinate via complexes I and II, respectively, and transport them to the oxygen to form H_2O. In normal state, the inner mitochondrial membrane is comparatively impervious to the proton reflux thus creating an electrochemical gradient. This generates a proton gradient cross the inner mitochondrial membrane and further aids ATP production by ATP synthase (complex V) (Hatefi 1993).

10.2.2 Mitochondria in ROS Generation

Mitochondria are the major source of reactive oxygen species (ROS) in the tissues. Under normal biological functions, 1–2% of the oxygen is converted to ROS as a by-product of OXPHOS (Ott et al. 2007). Mitochondria are the major source of the ATP required for normal cellular functions. Coupled with oxidative phosphorylation, a few of the electrons possibly leaks from complexes I and III are seized by O_2 to form superoxide radical O_2^- and can also be changed to several types of ROS like peroxides, hydroxyl radicals, reactive nitrogen species (RNS), etc. (Fig. 10.1). There are growing indications which suggest that complex II can be a chief controller of mitochondrial ROS production in the normal and diseased state (Le et al. 2007; Drose 2013).

ROS play an important role in the cell signaling mediating the cellular proliferation, differentiation, and transcription processes (Weinberg and Chandel 2009). ROS activity, if not regulated, can damage intracellular proteins and also damages the cellular membrane integrity. Elevated ROS levels are damaging for nuclear DNA as well as mtDNA. As mitochondrial DNA is in the close proximity to the ROS production site and also lacks introns and histones, DNA repair mechanism makes it more susceptible to ROS damage. Thus, ROS levels can damage the mitochondrial respiratory complexes by causing the genotoxicity to the mtDNA leading to alterations in the mitochondrial functions. Severe oxidative stress can cause unalterable oxidative damage and leads to the cell death (Lee and Shacter 1999; Le et al. 2007). Mitochondria also play vital role in cellular metabolism and programmed cell death pathways. Functional, structural, and genomic mitochondrial alterations are also known to be associated with carcinogenesis in several cells.

10.2.3 Mitochondria in Intrinsic Apoptosis

In addition to being a source of the cellular energy in the form of ATP, mitochondria are also the core of the cell death signaling which includes apoptosis, necrosis, and autophagy. To maintain the cell homeostasis, the process of genetically regulated

cell death is crucial. Mitochondria also play a crucial function in the intrinsic apoptosis pathway, which is an ATP-dependent pathway and is controlled by various signaling factors (Elmore 2007). The intrinsic apoptosis pathway can be activated by different physiological or diseased cell stressors like toxins, infections, oxidative stress, and damaged DNA. Suppression of the intrinsic apoptotic pathway is also seen in a various malignancies, as it is involved in cancer initiation, progression, and metastasis (Wong 2011). This is due to the defective regulation of mitochondrial outer membrane of Bcl-2 family proteins which is also known to be involved in the both overexpression and under-expression of anti-apoptotic proteins (Raffo et al. 1995). This malfunction of cancerous cell helps in their accumulation and survival under acute environment, like hypoxic or acidic surroundings, which are frequent in cancer.

10.3 Differences in the Mitochondria of Cancer vs Normal Cells

In normal cells consisting healthy mitochondria, the majority of ATP for cells is generated by process of oxidative phosphorylation (OXPHOS). However, cancerous cells have certain mitochondrial dysfunction due to a variety of factors, like oncogenic signals and mutations in the mtDNA, and thus produce ATP via glycolytic pathway rather than mitochondria. This increase in aerobic glycolysis in cancer cells is known as "the Warburg effect," i.e., increased uptake of the glucose by the cells, elevated rate of glycolysis in the presence of adequate O_2, and increased lactic acid production (Warburg 1956). For the first time, Otto Warburg observed that aerobic glycolysis is in high rate in different tumor cells and suggested that this can be due to the impaired respiratory capacity of the cells (Warburg and Dickens 1930). Recent studies show that there are few cancer cells which have a standard ability for OXPHOS that can generate majority of the ATP from this process (Fan et al. 2013; LeBleu et al. 2014; Tan et al. 2015). Additionally, facts also suggest that this increased glucose uptake and aerobic glycolysis in rapidly dividing cancerous cells are due to their larger requirement of the glucose metabolites for the amino acid synthesis (Vander Heiden et al. 2011). Warburg's initially observed dissimilarity between the mitochondria of noncancerous and cancerous cells such as change in the size, number, shape, translation rates, lipid, and protein profiles of the inner mitochondrial membrane (Kroemer 2006; Modica-Napolitano et al. 2007; Fogg et al. 2011). Metabolic anomalies coupled with mitochondrial bioenergetics in cancer cells also include the difference in the choice of respiratory substrates, rates of electron transport, calcium uptake, and loss of enzyme actions of different processes including electron transport chain (ETC), OXPHOS, etc. (Modica-Napolitano et al. 2007). The higher mitochondrial membrane potential has also been observed in the different types of cancerous cells as compared to their normal cells (Marino et al. 1994; Houston et al. 2011).

Carcinoma cells also reveal elevated ROS levels than the healthy cells, which can be the cause of the development of the cancerous phenotype of the cells. The high ROS levels can trigger mutations in mtDNA and vice versa, i.e., mutations in the genes encoding respiratory chain complexes can also lead to elevated ROS generation. Activation of the oncogene is also a known cause for the enhanced mitochondrial ROS. In the hypoxic condition of cancerous cells, mitochondria also act as an oxygen sensors and augments ROS production, as an adaptive response (Guzy and Schumaker 2006; Miyata et al. 2011; Paul 2011).

10.4 Bioenergetics of Cancer

Survival of the cancer cells is due to their adaptation to consume different alternate energy sources as compared to the normal cells. Quickly dividing cancerous cells exhaust the nutrients with a faster rate, which leads to ailment and rapid weight loss and thus is used as a diagnostic characteristic for cancer. The cancerous cells deprived of glucose are fulfilling their energy demands by mitochondrial ATP synthesis or through catabolic pathways, i.e., the oxidation of fatty acids and amino acids. This switch toward the catabolism pathway is vital for the energy-deprived cancer cells and serves as a survival mechanism till the nutrients like glucose are restored. In such energy-deficient conditions, the mitochondria aid by providing energy to the cancer cells to sustain their survival (Wallace et al. 2010; Wallace 2012). Alterations in the bioenergetic metabolism in the cancer cells lead to the low or high capacity of mitochondrial functions even though the cells still rely on essential mitochondrial functions. Mitochondrial dysfunction is well known in various cancer types; this can be because of the mtDNA mutations, loss of tumor suppressor gene (p53), alterations in the respiratory chain complexes, etc. (Matoba et al. 2006; Galluzzi et al. 2008, 2010). In view of the fact that mitochondria are crucial for ATP, ROS production, redox signaling, apoptosis, and mitochondrial dysfunction would affect these significant cellular processes, which could possibly be a biochemical source for affecting the cancer cells.

10.4.1 Dysfunction of the Mitochondria and Variations in Energy Metabolism

In cancer cells, a major modification in energy metabolism is augmented aerobic glycolysis (Warburg effect), which leads to “respiration injury” and could be the initial cause of the cancer phenotype (Warburg 1956). Even a little damage in mitochondrial respiration would need a significant enhanced glycolysis process for the

energy compensation. It is also known that this glycolytic process also supplies crucial transitional metabolites for growth and proliferation of the cancer cell, and this could be the reason of extremely increased glucose uptake and consumption by the cancer cells. Dysfunction of the mitochondria is caused by the mutations in the mtDNA and carcinogenic signals, and high ROS levels can also be a driving force for the cancer cells to rely on the glycolysis for ATP generation. Glycolysis may also be highly operative even in cancer cells with normal mitochondrial function, and therefore multiple targets are required for the destruction of the cancerous cells (Moreno-Sánchez et al. 2010). Additionally, due to high rate of lactate production as a by-product of glycolysis, it gives rises to an acidic environment to the cells which may further initiate the invasion and metastasis of the tumor (Weinberg and Chandel 2009). It has been established that few nuclear genes which encode for mitochondrial proteins may act as tumor suppressors, and deformities in these suppressors result in tumor expansion (Gottlieb and Tomlinson 2005).

10.4.2 Dysfunction of the Mitochondria and Redox Imbalance in Cancer

Elevated ROS levels are observed in the cancer cells as compared to the noncancerous cells of the same tissue (Szatrowski and Nathan 1991). While the precise causes of the high ROS levels in carcinomas are still not apparent, dysfunction of the mitochondria could be the potential explanation (Brandon et al. 2006; Pani et al. 2009). Various research studies support the assumption that lack of functional p53 results in fluctuations in the mtDNA, which are also known to be associated with the augmented ROS levels in the cells (Achanta et al. 2005). These mutations in mtDNA encoding respiratory protein complexes of the ETC have also been interrelated with the increased ROS stress in various cancer types (Brandon et al. 2006).

Deformities of ETC could be a consequence of the mtDNA mutations and escalate the leakage of electrons forming the ROS (Adam-Vizi and Chinopoulos 2006; Brandon et al. 2006). Increased mitochondrial membrane potential in cancerous cells also leads to increased ROS production. The oxidative stress possibly will play a major function in gaining the indications of the cancerous cell proliferation, survival, and apoptosis (Trachootham et al. 2008 a, b). ROS act as a "double-edged sword" that may inflict diverse effects on the cells, depending on the level and time period of the ROS exposure. In case of increased ROS levels above the certain threshold level, it may cause a cytotoxic effect leading to death of cancerous cells and thus decreases the chances of cancer progression (Fruehauf and Meyskens 2007). As an adaptive mechanism, the increased oxidative stress could also promote the cell survival mechanisms and stimulation of the antioxidant pathways (Sullivan et al. 2008).

10.4.3 Dysfunction of the Mitochondria and Resistance to Apoptosis

Equilibrium between cell growth and death is necessary for the tissue homeostasis, and the misbalance in this process fails to abolish the cells by apoptotic pathway leading to accumulation of cancer cells, like the case of lymphocytic leukemia (Terwilliger and Abdul-Hay 2017). The role of mitochondria is crucial in the apoptosis regulation. In the mitochondrial intrinsic apoptotic pathway, the Bcl-2 family proteins are important for the cell survival. Impairment in the regulation of Bcl-2 is observed in different cancer types like brain, ovarian, stomach, and blood cancer (Mestre-Escorihuela et al. 2007). Fascinatingly, few specific tumor suppressor genes and oncogenes which regulate the cell apoptosis also affect Bcl-2 expression (Herbst et al. 2005).

Mitochondrial apoptotic events also include a significant action of opening of the "mitochondrial permeability transition pore (MPTP)" which leads to depolarization of mitochondrial membrane leading to the release of certain apoptotic factors. MPTP consists of core molecules like "adenine nucleotide translocase (ANT)," "voltage-dependent anion channel (VDAC)," and "cyclophilin D (CypD)" with a set of regulatory proteins such as "creatine kinase (CK)," "peripheral benzodiazepine receptor (PBR)," and "hexokinase (HK)" and is necessary for the mitochondrial functioning (Halestrap 2009). MPTP aids in the transport of small-sized molecules through the mitochondria, but under apoptotic signals, the opening of MPTP leads to the impairment of mitochondrial ETC and also allows the influx of p53 (Berridge 2009). These apoptotic signals also influence efflux of the factors like "cytochrome c, apoptosis-inducing factor (AIF), endonuclease G (endoG), and second mitochondria-derived activator of caspases (Smac)" and can activate the cell death pathways. MPTP aberrations are also associated with the disease phenotype like cancer, aging, and neurodegeneration (Cavalieri et al. 2009). Increased protein levels including VDAC, CypD, and ANT were described in cancerous cells and might apparently restrain cell apoptosis (Chen et al. 2009a).

10.5 Targeting Mitochondria for Cancer Therapy: Mitocans

Cancer cells have metabolic and mitochondrial needs, which is different from their normal counterparts. These mitochondrial alterations in cancer cells might act as a great window of opportunity for efficient and selective anticancer therapy. As a result, in the past few years, the developments of those drugs that can precisely target the metabolic activity of mitochondria in malignant cells have gained a lot of interest. Recently, the term "mitocan" has been proposed to identify and classify these agents targeting the mitochondria (Neuzil et al. 2007; Ralph and Neuzil 2008, 2010a, b). Mitocans act by destabilizing the mitochondria, affecting their apoptotic potential, which leads to the efficient death of cancer cells and suppresses the growth of tumor.

Based upon their specificity for tumor cells as well as a result of different studies, mitocans seem to hold great potential to be developed into an effective anticancer drug (Fulda and Kroemer 2011; Kepp et al. 2011). The recent findings suggest that mitochondria can be used as an important and perspective target in those tumors where a large number of genes are differentially expressed and/or the nature of mutation is uncommon even in the same type of tumor (Gerlinger et al. 2012). This suggests that it will be impractical to target cancer by targeting few genes or few pathways that may alter in cancer patients and that can be mutated. Instead, it is crucial to search for a new target that is not variable across different cancer types and whose exploitation can lead to design a common strategy for effective treatment in various cancer types. It appears clear that mitochondria are such targets that are up to certain extent functional in the vast majority of cancers, if not all (Ralph et al. 2010a, b). As being the "powerhouse" of the cell, mitochondria are reservoirs of a number of proteins which also promote intrinsic apoptotic pathway. This phenomenon helps in getting the cancerous cells into the commitment phase and finally leads to cell death (Kroemer et al. 2007). It is also important to highlight that defect in mitochondrial metabolism has already been reported in different cancer cells (Koppenol et al. 2011). Thus it is believed that targeting the mitochondria, for treatment of different types of tumor, may lead to an important future breakthrough in the management of malignancies in recent future. The different classes of mitocan includes those compounds that affect different activities which are exclusively associated with mitochondria such as inhibition of the Bcl-2 anti-apoptotic proteins and blocking of the electron transport chain, hexokinase inhibition, activation of the mitochondrial permeability transition pore (MPTP), etc. These drugs seem to improve the efficacy of the current standard cancer therapy as their mechanism of action is entirely different from the existing therapies that kill cancer cells. Based on their mode of action, there is a total of eight classes of mitocans known in the literature (Table 10.1).

10.5.1 Class 1: Inhibitors of Hexokinase

Different compounds which target hexokinase belong to this class of mitocans. Hexokinase (HK) is an enzyme and converts glucose to glucose-6-phosphate (G6P) by phosphorylation process. G6P is a substrate for metabolic pathways which gets ultimately coupled with ATP generation. A great correlation has been observed between the levels of HK activity and growth of carcinomas (Bustamante et al. 1981).This phenomenon of continuous phosphorylation of glucose by using ATP reduces the phosphate level available for oxidative phosphorylation. As a result, it interferes with reaching the high rate of respiration (Baggetto and Testa-Parussini 1990). Apart from converting glucose to G6P, HK is also associated with the cytosolic site of the porin-like voltage-dependent anion channel (VDAC). It is a transmembrane protein in the outer membrane of mitochondria (Pedersen 1978). Due to its presence in mitochondria localization, HK helps in stabilizing this tiny organelle,

Table 10.1 Different classes of mitocans with examples and their mechanisms of action

Class	Nature	Examples of mitocans	References
1	Inhibitors of exokinase	2-deoxyglucose (2DG), 3-bromopyruvate (3BP	Ko et al. (2004), Mathupala et al. (2006), Simons et al. (2007), and Chen et al. (2009a, b, c)
2	Bcl-2 family protein-targeting compounds	ABT-263, Gossypol, ABT-737, antimycin A, α-TOS	Pelicano et al. (2003) and van Delft et al. (2006)
3 and 4	Inhibitors of thiol redox and VDAC/ANT-targeting drugs	Arsenic trioxide, phenyl-ethylisothiocyanate, lonidamine, analogues of steroids like CD437	Xu and Thornalley (2001), Miller (2002), Pelicano et al. (2003), Don et al. (2003), and Trachootham et al. (2009)
5	Drugs targeting electron redox chain	Adaphostin, α-TOS, MitoVES, 3BP, tamoxifen	Le et al. (2007), Dong et al. (2008), Da Silva et al. (2009), and Dong et al. (2009, 2011a, b)
6	Lipophilic cations targeting the inner membrane	Rhodamine-123, (KLAKKLAK)2, F16	Johnson et al. (1980), Lampidis et al.(1983), and Ellerby et al.(1999)
7	Tricarboxylic acid cycle-targeting drugs	Dichloroacetate (DCA), BP	Bonnet et al. (2007), Da Silva et al. (2009), and Dell'Antone (2009)
8	Mitochondrial DNA-targeting drugs	Vitamin K3 (menadione), fialuridine, 1-methyl-4-phenylpyridinium, MitoVES	Lewis et al. (1996), Miyako et al. (1997), Umeda et al. (2000), and Sasaki et al. (2008)

suppresses apoptotic death of cancer cells, and promotes their survival (Mathupala et al. 2006). The compounds which inhibit the HK activities are glucose metabolites, 2-deoxyglucose, oxamate, and 3BP. These compounds collectively form a group of mitocans, which is known to selectively induce apoptosis in cancer cells. Out of all these inhibitors, 2-deoxyglucose (2DG) and 3-bromopyruvate (3BP) are the most studied HK inhibitors (Ko et al. 2004; Da Silva et al. 2009; Azevedo-Silva et al. 2015; Yasmin et al. 2015). Considerable focus has been on 2DG that inhibits HK activity which leads to compromised rate of glycolysis in cancer cells. Due to binding of 2DG to HK, the interaction between HK and VDAC is known to get compromised. As a result, the malignant cells become more susceptible toward other forms of treatments (Simons et al. 2007). 3BP is an alkylating agent, and apart from inhibiting the HK activity, it also binds with the mitochondrial complex II. This is the reason 3BP is also included in mitocan classes 2 and 5 as well. As per recent studies, 3BP covalently binds to HK; as a result, HK gets dissociated from VDAC (Chen et al. 2009b). Studies conducted in animal models have suggested that 3BP causes the rapid depletion of ATP in cancer cells and thus suppresses tumor growth in animal models (Mathupala et al. 2006; Dell'Antone 2009). This is the reason that 3BP is a potential candidate used in different cancer clinical trial-based studies. In different preclinical and clinical models of cancer, compounds of this

class showed specific toxicity against anaerobically metabolizing malignant cells (Pelicano et al. 2006; Azevedo-Silva et al. 2015; Yasmin et al. 2015).

10.5.2 Class 2: Bcl-2 Family Protein Targeting Compounds

This class includes mostly the mimetics of the Bcl-2 homology-3 (BH3) domains, which is an integral part of Bcl-2 family proteins. The peculiar nature for the action of this class of mitocans is based upon the fact that the pro-apoptotic Bcl-2 family proteins and anti-apoptotic proteins interact via their BH3 domains. The compounds of this class prevent activity of the BH3 domain containing proteins Bak and/or Bax and thus inhibit the formation of large channels or pores in the outer membrane of mitochondria (Youle and Strasser 2008). These BH3 mimetics are mostly the natural polyphenolic compound as gossypol. This agent has been observed to interact with BH3-binding domains, and thus it interferes with the interaction between Bcl-xLBcl-2 and the pro-apoptotic proteins such as Bax/Bak. It results in the oligomerization of Bax or Bak to form channels which ultimately help in activating the post-mitochondrial apoptotic signals (Oliver et al. 2005). The group of vitamin E analogues such as α-tocopheryl succinate (α-TOS) compound is known to interact with the BH3-binding domain of Bcl-2 and Bcl-xL, which affects their interaction with Bak protein, arresting proliferation of cancer cells. α-TOS promotes Bax translocation to the outer membrane of mitochondria, and ensuing cytochrome c release causes programmed cell death. Recent findings have revealed novel functions of the Bcl-2 family members, disclosing their role in mitochondrial metabolism, dynamics, and biogenesis also (Suen et al. 2008; Zhao et al. 2009; Shamasdin et al. 2013; Wei et al. 2013), which further highlights the significance of targeting these proteins.

10.5.3 Classes 3 and 4 of Mitocans: Inhibitors of Thiol Redox and VDAC/ANT Targeting Drugs

The permeability transition pore complex (PTPC) is a super channel and made up of the VDAC/ANT of proteins which are buried in the mitochondrial outer and inner membrane, respectively. PTPC interconnects the cytosol with mitochondrial matrix and acts as a transporter for a variety of small molecules and solutes including ATP and ADP (Zhivotovsky et al. 2009). It is noted that disturbance in the VDAC/ANT complex formation leads to apoptosis in cancer cells (Brenner and Lemoine 2014). Mainly the thiol redox inhibitors and the VDAC/ANT targeting drugs are integral compounds of classes 3 and 4 of mitocans. The activity of these compounds is based upon the redox environment of malignant cells, as cancer cells exhibit higher levels of ROS and relatively compromised level of antioxidants, in comparison to that of normal cells (Huang et al. 2000). Thus cancer cells are more susceptible toward

those compounds which further induce the rise in oxidative stress. Therefore, compounds oxidizing thiol group and/or further depleting the mitochondrial GSH pool may eventually lead to trigger apoptosis of cancer cells (Fulda et al. 2010; Trachootham et al. 2009). Agents like arsenic trioxide (Miller 2002) or isothiocyanates (Zhang et al. 2003), represented by phenylethylisothiocyanate (PEITC), are known to possess relative specificity in killing cancer cells by targeting the VDAC/ANT system (Trachootham et al. 2006).

10.5.4 Class 5 Mitocans: Drugs Targeting Electron Redox Chain

Mitochondrial ROS is generated due to the leakage of electrons in the ETC (complex I and complex III) (Ralph et al. 2011). Adequate ROS level within the range is not damaging for the cellular system; balanced ROS levels are obligatory for the cell signaling. Exposure of high ROS levels for a longer time may lead to carcinogenesis (Murphy 2009). On the contrary, a rapid increase in ROS levels above the threshold may also impart the cell to undergo cell death. Class 5 mitocans contain a broad collection of compounds that aims the mitochondrial respiratory complexes which further leads to increased cellular ROS levels (Fulda et al. 2010; Wang et al. 2010). One of the most common drugs of class 5 is tamoxifen; it is used to cure estrogen-positive breast cancer by inducing the cell death through interfering the FAD- binding site of complex I of mitochondria (Moreira et al. 2006; Higgins and Stearns 2011). In this class, α-TOS is another promising anticancer drug as it is known to target the cancerous cell apoptotic pathway as well as proposed to bind mitochondrial respiratory complex II (Birringer et al. 2003). α-TOS competes with ubiquinone for binding to the Q site located on complex II of the respiratory chain. This binding of α-TOS to complex II of mitochondrial electron transport (Dong et al. 2008, 2009) chain disrupts the electron flux to destabilize the mitochondrial membrane consequently and produces superoxide anion. Exposure of α-TOS to the cancerous cells increases the ROS levels further leading to cell death (Stapelberg et al. 2005). The increase in the ROS levels plays crucial role in pro-apoptotic activity, and this is one important reason to choose the analogues of vitamin E for targeting the cancer cells (Neuzil 2004; Dong et al. 2011a, b).

10.5.5 Class 6 Mitocans: Lipophilic Cations Targeting the Inner Membrane

Mitochondrial membrane potential (MMP) across the membranes is an important feature of mitochondria. It is generated due to the biochemical reactions of mitochondrial respiratory chain. The reduced state of NADH and FADH is maintained

by the TCA cycle and fatty acid oxidation; these reduced nucleotides are source of electrons to the ETC, finally accepted by the oxygen. This movement of electrons generates a proton gradient across the membrane and provides the force for the ATP synthase for ATP generation. Cancer cells are known to have greater MMP than the normal cells, but the reason is not clearly understood. As the mitochondrial membrane potential in cancer cells is highly negative, (−150 to −170 mV), they act as a targets for lipophilic cations like Rhodamine-123, a fluorescent dye (Rho123), and salt of tetraphenylphosphonium ion (TPP). These cations are known to penetrate the hydrophobic barriers of the plasma and mitochondrial membranes, and this is the reason it gets accumulated in mitochondria of cancer cells (Modica-Napolitano et al. 2007). The negative MMP of the cancer cells results in tenfold higher TPP accumulation in the cancerous cells. Rhodamine-123 mitochondrial accumulation was also reported in src-transformed cells indicating the specificity for the cancerous cells (Lampidis et al. 1983). A lipophilic cationic peptide $(KLAKKLAK)_2$ was also found to be specific for the tumor cells (Ellerby et al. 1999; Constance and Lim 2012).

10.5.6 Class 7 Mitocans: Tricarboxylic Acid Cycle Targeting Drugs

The tricarboxylic acid (TCA) cycle is the ultimate source of electrons which is further important for generation of electrochemical proton gradient, necessary for ATP production by electron transport chain. Compounds of class 7 mitocans target the different enzymes and substrate of TCA. The TCA cycle starts with the interaction of acetyl-CoA with oxaloacetate, and after different intermediate steps, it leads to formation of citrate. A number of compounds are known to target the TCA cycle as well as the very initial step where the pyruvate gets converted to acetyl-CoA. The conversion of pyruvate to acetyl-CoA is a mandatory step, and, only in the form of acetyl-CoA it can enter into the mitochondria and the TCA cycle. This reaction is catalyzed by the enzyme pyruvate dehydrogenase which is under the control of the pyruvate dehydrogenase kinase (PDK) enzyme. The inhibition of PDK leads to rise in the activity of pyruvate dehydrogenase, and eventually it leads to higher activity of the TCA cycle. Dichloroacetate (DCA) is known to suppress the activity of PDK and helps in selective killing of cancer cells (Bonnet et al. 2007). By promoting the pyruvate dehydrogenase activity, DCA causes a shift from anaerobic glycolysis to oxidative glycolytic which leads to a dramatic increase in ROS level followed by altered mitochondrial membrane potential. All these events are specific for cancer cells (Bonnet et al. 2007). DCA is already in use for the treatment of mitochondrial deficiencies. Hence, its development as an anticancer drug could be easier than dealing with a completely novel agent.

10.5.7 Class 8 Mitocans: Mitochondrial DNA Targeting Drugs

Mitochondria are different from other cell organelles because they carry their own genetic information encoded in the form of mitochondrial DNA (mtDNA). Group 8 of mitocans deals with that group of compounds which target mtDNA. Till date, a good number of compounds are known to interfere with the structure and function of mtDNA by different mechanisms. For example, vitamin K3 inhibits the activity of polymerase-γ which is important for replication of mtDNA (Sasaki et al. 2008). Similar observations have been made in the case of fialuridine also, which was found to induce the mitochondrial structural defects (Lewis et al. 1996). Further, Parkinsonian toxin 1-methyl-4-phenylpyridinium is also known to cause a reduction in the total content of mtDNA by destabilizing D-loop structure of the mitochondria (Umeda et al. 2000; Bajpai et al. 2012).

10.6 Herbal Mitocan

Several drugs are known to cure cancer, but they have a lot of side effects. This makes it important to move toward herbal medicines which have no to fewer side effects. Out of 5278 anticancerous plants listed in the database, a total of 346 have been successfully tested for their property in 60 different cell lines. These herbs are known to affect the cancerous cell in different ways. A lot of interest has been dedicated toward the understanding of natural compounds obtained from plants, commonly known as phytochemicals. These compounds have been a part of human diet in appropriate concentrations to have a medicinal effect. There are a lot of synthetic antitumor drugs available, but efforts are to find natural anticancerous molecules which would slow or inhibit the cancer growth with more precision and efficiency. It is also estimated that 50% of the anticancerous molecules are obtained from plant derivatives. Many of these natural compounds are seen to show their effects by targeting the mitochondria either indirectly or directly. These compounds when targeting mitochondria either affect their enzyme activity or signaling pathways. So similar to synthetic molecule targeting mitochondria in cancer, these natural compounds can also be termed as "herbal mitocans." Thus, herbal mitocans are a class of herbal medicine which targets the mitochondria for exhibiting its anticancerous activity. Mitochondria produce ROS which apart from being harmful to the cell also regulate various cellular processes like autophagy, apoptosis, etc. Various phytochemicals like curcumin, PEITC, and honokiol which are the naturally occurring molecules derived from plants have shown great antioxidant potential and can be used for regulating the cellular ROS in several ways. In the last 20 years, the effect of naturally occurring compounds on apoptosis has been a topic of vast investigation, with an effort to identify molecules that can selectively cause cancerous cell death. However, the result interpretation is a difficult task because of differences in *in vivo* and *in vitro* models, dose-dependent effect of many compounds, and the

capability of some compounds to have a survival effect on cancerous cell by promoting mitochondrial biogenesis and cell proliferation. Some potential herbal mitocans and their mode of action are discussed below (Table 10.2).

Table 10.2 List of potential natural compounds and their mode of action as herbal mitocans

Active compound	Herbs	Possible mechanism of action	References
β-Phenylethyl-isothiocyanate (PEITC)	Watercress	Increase in ROS levels	Huang et al. (2000), Rose et al. (2003), Achanta et al. (2005), Schumacker (2006), and Trachootham et al. (2009)
		Inhibition of GSH antioxidant pathways	
		Induction of conformational change in structure of Bax	
Honokiol	*Magnolia officinalis*	Induction of apoptosis	Yang et al. (2002), Ishitsuka et al. (2005), Li et al. (2007), and Chen et al. (2009a, b, c)
		Cell cycle arrest by interfering with the Rb function and inhibition of E2F1 transcriptional activity	
		Downregulating the Ras and Akt/mTOR pathways	
		Inhibiting angiogenesis by modulating NF-κB pathway	
α-Tocopherol succinate (α-TOS)	–	Disrupts electron transport chain by competing with ubiquinone for complex II	Turánek et al. (2009) and Zhao et al. (2009)
		Cytochrome c-mediated cell death	
Epigallocatechin-3-gallate (EGCG)	Green tea	Induces the release of mitochondrial apoptogenic proteins	Yamamoto et al. (2003), Nakazato et al. (2005), Nihal et al. (2005), Shanafelt et al. (2006), and Schroeder et al. (2009)
		ROS generation	
		Activation of caspases	
Curcumin	*Curcuma longa*	Upregulation of cytoprotective effect	Gogada et al. (2011a)
		Bcl-2 and Bcl-X1 downregulation and Bax upregulation-mediated apoptosis	
Pancratistatin	*Pancratium littorale*	Increase in levels of ROS	Pandey et al. (2005) and Siedlakowski et al. (2008)
		Decrease in mitochondrial transmembrane potential	
		Activation of caspases-3	

(continued)

Table 10.2 (continued)

Active compound	Herbs	Possible mechanism of action	References
OSW-1	*Ornithogalum saundersiae*	Apoptosis by Bcl-2 cleavage dependent on caspase-8	Zhu et al. (2005) and Zhou et al. (2005)
		Activation of calcium dependent apoptotic pathway	
Resveratrol	Grapes, blueberries, and raspberries	Interferes with PI3K/AKT, JAK/STAT, and MAPK pathways and induces apoptosis	Gogada et al. (2011b)
		Induces mitochondrial biogenesis by decreasing the acetylation of PGC-1alpha	
Vitamin K3	–	Hinders the calcium homeostasis and causes a decrease in the cellular levels of glutathione (GSH)	Gold (1986), Nutter et al. (1991), and Sasaki et al. (2008)
		Increases ROS and induces apoptosis	
Bezielle (BZL101)	*Scutellaria barbata*	Inhibition of oxidative phosphorylation	Vivian Chen et al. (2012)
		Increases ROS	
Dracocephalum kotschyi	–	Increase in ROS	Mojtaba Talari et al. (2014)
		Mitochondrial membrane permeabilization	
		Mitochondrial swelling	
		Cytochrome c release	
Sanguinarine	–	Increase in ROS levels	Sun et al. (2010)
		Mitochondrial depolarization and cytochrome c release	
Berberine	Plants of the Berberidaceae family	Inhibits complex I of mitochondria	Letasiová et al. (2006)
		Interacts with ANT	
Quercetin	Vegetables, fruits, seeds, tea	Inhibits mitochondrial ATP synthesis	Zheng et al. (2012)
Dichamanetin	*Piper sarmentosum*	Increases ROS	Yeonjoong Yong et al. (2013)

10.6.1 β-Phenylethylisothiocyanate (PEITC)

PEITC is a promising natural anticancerous molecule which is abundantly eaten in its glucosinolate form, found mainly in watercress. The leaves of watercress have been consumed by people of Asia and Europe from a long time. Lots of studies have been conducted which aim to find the possible mechanism of action of PEITC. A study conducted on HepG2 cells showed that a conformational change in the structure of Bax was induced by PEITC which led to the translocation of Bax to mitochondria. This accumulation of Bax in mitochondria resulted in loss of membrane potential and respiratory chain function in mitochondria. It was also seen that this accumulation is responsible for cell death by caspase-dependent mechanisms (Rose et al. 2003).

Most of the ROS generation occur in the mitochondrial respiratory chain, and mitochondrial dysfunction may lead to the increased ROS levels. This elevated ROS levels in cancerous cell may make them more susceptible to oxidative stress (Huang et al. 2000). This difference in levels of ROS in cancerous and normal cells may serve as potential basis of target for killing of cancerous cells (Schumacker 2006). However, to neutralize the oxidative stress generated by an increase in ROS, the antioxidant activity of the cancerous cells may be unregulated. As a result, the redox difference is balanced, and cancerous cells become resistant to drugs targeting the redox difference (Ogasawara and Zhang 2009). This drug resistance can be overcome by use of compounds that disable the upregulation of antioxidant activity (Trachootham et al. 2009). PEITC is one of the molecules which are known for its anticancerous property exhibited by ROS-modulating mechanisms. Recent studies have suggested that PEITC is an effective anticancerous agent which acts on cancerous cells by increasing their ROS levels and has little toxicity on normal cells (Satyan et al. 2006; Trachootham et al. 2009).

It was also demonstrated that PEITC efficiently kills the cancerous cells which have become resistant to standardized chemotherapeutic molecules like Gleevec (imatinib), cisplatin, and fludarabine. The anticancer activity of PEITC was analyzed using tumor xenograft models and cancer cell line from leukemia patients (Matoba et al. 2006). The effective anticancerous mode of action of PEITC is due to its ability to impair the glutathione antioxidant system (GSH), which is required by cancer cells to balance the redox difference. The increase in the amounts of GSH helps the survival of cancer cells and provides resistance against anticancer drugs targeting the redox difference (Trachootham et al. 2009). The elevated ROS levels make the cancerous cells rely on the GSH system for stabilizing the redox difference. PEITC disables the GSH antioxidant system by reducing the amounts of cellular GSH and inhibiting glutathione peroxidase, a redox-modulating enzyme (Trachootham et al. 2009).The inhibition of GSH system may result in increased levels of ROS in the cancer cells with mitochondrial dysfunction and induce apoptosis.

10.6.2 Honokiol

It is a natural compound obtained from various parts like bark, leaves, and seeds of the plant *Magnolia officinalis*. It shows an anticancer effect on various cancerous experimental models such as in breast, ovarian, and lung cancer, myeloma, and leukemia (Munroe et al. 2007; Fried and Arbiser 2009). It is also an active constituent of the Chinese herb medicine *houpo* and has been used for its medicinal properties since ages. It is hypothesized that it leads to cancer cell death by affecting the mitochondria and was initially thought to have antioxidant properties, which are still not very clear. This compound is reported to scavenge lipid peroxides and hydroxyl radicals and suppresses NADPH oxidase. Other studies have also suggested that it might be responsible for increasing the ROS levels in a variety of cell lines (Li et al. 2007; Chen et al. 2009c). Honokiol is a polyphenolic compound and might act as both antioxidant and prooxidant depending on the redox microenvironment (Liu et al. 2008). A lot of mechanism of actions of honokiol have been suggested like induction of apoptosis, cell cycle arrest by interfering with the retinoblastoma protein (Rb) function and inhibition of E2F1 transcriptional activity, downregulation of the Ras and Akt/mTOR pathways, and inhibition of angiogenesis by modulating NF-κB pathway.

Of all the possible mechanisms, the effect on mitochondria and the mitochondria-dependent apoptotic pathways are the major modes of actions of honokiol. It may also help to reduce chemotherapeutic drug resistance in myeloma by caspase-independent and caspase-dependent apoptosis (Ishitsuka et al. 2005). It was seen in a study that reduced expression of caspase-3 leads to honokiol resistance. The authors also observed that treatment with honokiol led to cleavage of anti-apoptotic protein Mcl-1 and the pro-apoptotic protein Bad was upregulated which resulted in the release of apoptosis-inducing factors from the mitochondria to initiate apoptosis. Another study on lung cancer cells revealed that honokiol is responsible for upregulation of Bad proteins and downregulation of Bcl-X1, which further releases cytochrome c from mitochondria to the cytosol and activates the caspase cascade. Very similar effects were also reported in ovarian and breast cancers (Yang et al. 2002).

10.6.3 α-Tocopherol Succinate (α-TOS)

α-Tocopherol succinate (α-TOS) is an admirable candidate in cancer therapy. By nature it is a semisynthetic compound and derivative of vitamin E. This vitamin is present in most of the seed oils, so α-TOS may also be placed in the category of herbal mitocans. Its possible mechanism of action has already been discussed in Sect. 10.5. This compound has shown promising anticancerous activity in various cancer types both *in vitro* and *in vivo*. Various vitamin E analogues are currently under research, aiming to improve the therapeutic activity (Turánek et al. 2009).

10.6.4 Epigallocatechin-3-Gallate (EGCG)

Green tea has been long identified for its cancer risk-reducing potential. Many people who drank green tea had lesser cancer incidences and lower reappearance rates in a variety of cancer (Nakachi et al. 1998; Jian et al. 2004). Epigallocatechin-3-gallate (EGCG) is found in abundance in green tea and is supposed to be the active component of green tea that exhibits its anticancer property. It has been reported that EGCG was able to cause rapid cell death by targeting the mitochondria-related apoptotic events like release of mitochondrial apoptogenic proteins, ROS generation, and activation of caspases in various types of malignant B-cell line (Nakazato et al. 2005). This induced apoptosis could be reduced by antioxidants, catalase, and SOD2 showing that ROS may play an important role in mitochondria-mediated apoptosis. Tumor cells which did not have caspase-3 expression did not undergo cell death when treated with EGCG. Also, it also affected cancerous cells and was not toxic to normal cells (Nihal et al. 2005). It was seen that EGCG caused an increase in ROS levels in tumor cells, whereas the ROS of normal cells decreased. This might be explained by another study, and according to which, the catalase activity was higher in normal cells as compared to tumor cells (Yamamoto et al. 2003). EGCG was also seen to have synergetic effect when it was given along with other chemotherapy drugs like erlotinib and arsenic acid (Nakazato et al. 2005; Zhang et al. 2008).

The exact mechanism of action of EGCG against cancer cells is not yet known. However, a study demonstrated that most of the EGCG gets accumulated in the mitochondria of a cell. This accumulation might have protective effect for normal cells (Schroeder et al. 2009) and damaging effect for cancer cells which had a different mitochondrial function. It is possible that this mitochondrial dysfunction makes the tumor cells more susceptible to EGCG. However, the exact binding target of EGCG is not yet known. EGCG has shown encouraging effects in various clinical trials exhibiting partial response against tumor cells in 75% of the patients having B-cell malignancies (Shanafelt et al. 2006).

10.6.5 Curcumin

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a diarylheptanoid obtained from the rhizome of *Curcuma longa*, which shows anticancer effect in both *in vivo* and *in vitro* conditions by suppressing the cancer cell growth and proliferation. Curcumin is known to have direct antioxidant properties and scavenges free radicals such as hydroxyl, nitric oxide, and peroxynitrite. It also have indirect anticancer effects by upregulating the cytoprotective response by altering the expression of various genes like superoxide dismutase (SOD), catalase, and other proteins with antioxidant properties. And these direct and indirect effects

of curcumin have been beneficial against cancer in both *in vitro* and *in vivo* models (Singh and Khar 2006; Tuorkey 2014).

Curcumin is the major component of turmeric powder and has shown lung cancer cell death by Bcl-2 and Bcl-X1 downregulation and Bax upregulation and is currently in the phase II and III clinical trials in various types of cancer. Curcumin is seen to induce apoptosis in cancer cells (Gogada et al. 2011a) and had no toxic effects in normal cells which might be attributed to its possible mode of action which might be the recognition of increased levels of ROS in cancer cells due to mitochondrial dysfunction (Tuorkey 2014).

10.6.6 Pancratistatin

Pancratistatin (PST) is a naturally occurring compound obtained from spider lily *Pancratium littorale* (Ludueña et al. 1992). It is known to specifically target mitochondria of cancer cells and induce apoptosis while having no to less toxic effects in normal cells (Pandey et al. 2005). A study on human breast cancer cells showed that incubation of cells with PST resulted in increased levels of ROS and decreased cellular ATP and MMP. A research study also showed that the activation of caspases-3 and exposure of phosphatidylserine on the outer leaflet of the cell membrane were observed 1 h after the tumor cells were incubated with PST (Pandey et al. 2005). It also showed synergistic effects when given with tamoxifen in breast cancer.

10.6.7 OSW-1

OSW-1 (3β, 16β, 17α-trihydroxycholest-5-en-22-one16-*O*-{*O*-(2-*O*-(4-methoxybenzoyl)-β-Dxylopyranosyl)-(1 → 3)-2-*O*-acetyl-*α*-arabino pyranoside) is a glycoside which is found in the bulbs of *Ornithogalum saundersiae* and has also been synthesized chemically (Deng et al. 1999). It is very effective in a broad range of cancerous cell lines, even in small concentration, and is generally not toxic to normal cells (Ma et al. 2001). A study conducted on Chinese hamster ovary (CHO) demonstrated that OSW-1 induces apoptosis by Bcl-2 cleavage dependent on caspase-8 (Zhu et al. 2005). The study also showed that OSW-1 damaged the mitochondrial membrane and cristae because of excess of calcium that damages the membrane integrity and activated the calcium-dependent apoptosis pathway in human pancreatic and leukemia cell lines (Zhou et al. 2005). Additionally, the clones of the cells that lacked mtDNA and had damaged ETC, showed resistance to OSW-1 suggesting the significant role of mitochondria in mediating cytotoxic action of OSW-1 (Zhou et al. 2005).

10.6.8 Resveratrol

Resveratrol (3,5,4-trihydroxystilbene) is a stilbenoid which is naturally produced by various plants in case of injury or environmental stress and is found in a variety of fresh fruits like grapes, blueberries, and raspberries. Resveratrol improves the function of mitochondria by inducing mitochondrial biogenesis by decreasing the acetylation of PGC-1alpha which is one of the major regulators of mitochondrial biogenesis and enhancing the expression of genes responsible for oxidative phosphorylation. It has also been seen that resveratrol prevented skin cancer development in mice that were treated with carcinogen and induced the apoptosis of cancer cells.

The exact mechanism of action is not yet known. However, a study has shown that it induces apoptosis in colon cancer. It was also seen that resveratrol induces cell death by interfering with various signaling pathways involving PI3K/AKT, JAK/STAT, and MAPK (Roy et al. 2009). RSV can act directly by binding to various sites in the electron transport chain by competing with ubiquinone sites. It also interacts with complex V and F0-F1 ATP synthase. It is normally considered as an antioxidant, but it can also act in favor of oxidative stress and induce apoptosis through mitochondrial pathway. Studies have also shown that it acts as a ROS scavenger, and this antioxidant activity results in cytoprotective effects on several cell types (Gogada et al. 2011b).

10.6.9 Vitamin K3

Vitamin K3 (2-methyl-1,4-naphthoquinone; menadione) is a synthetic molecule, which can act as a precursor to various forms of vitamin K in the body. The tumor-inhibiting activity of vitamin K3 has been seen in various *in vivo* and *in vitro* models (Gold 1986; Nutter et al. 1991). Vitamin K3 is able to hinder the calcium homeostasis and causes a decrease in the cellular levels of glutathione (GSH) by oxidizing GSH to form glutathione disulfide (GSSG) (Di Monte et al. 1984). Studies show that vitamin K3 specifically inhibits DNA polymerase γ which is responsible for mtDNA replication leading to elevated ROS levels and apoptosis (Sasaki et al. 2008; Aoganghua et al. 2011).

10.6.10 Bezielle (BZL101)

It is an aqueous extract obtained from the herb *Scutellaria barbata* and is a ROS regulator which has cytotoxic effects against various types of cancer cells both *in vitro* and *in vivo*. Studies showed that cancer cells when incubated with Bezielle

showed higher levels of ROS and reduced cellular ATP and NAD. It is also seen to inhibit oxidative phosphorylation. And it was seen that the cells lacking a functional mitochondria were resistant to cellular death by Bezielle showing that mitochondria are a major target of Bezielle-mediated selective cancer cell death (Chen et al. 2012).

10.6.11 Dracocephalum kotschyi

It is an Iranian medicinal plant that has been used for a variety of human cancers along with the plant *Peganum harmala*. It has been seen in studies on hepatocellular carcinoma (HCC) that *Dracocephalum kotschyi* extracts caused increase in ROS, mitochondrial membrane permeabilization, mitochondrial swelling, and cytochrome c release in tumor cells and had no such toxic effect in normal cells. This property makes it a promising herbal mitocan (Talari et al. 2014).

10.6.12 Sanguinarine

It is a natural benzylisoquinoline alkaloid and is known to interfere with the calcium loading capacity of mitochondria and increases the p53 expression. It is reported that sanguinarine targets complex II of the mitochondria and affects respiration. Other studies have also shown that it leads to apoptosis caused by increase in ROS levels, mitochondrial depolarization, and cytochrome c release (Sun et al. 2010).

10.6.13 Berberine

It is an alkaloid that is obtained from plants of the Berberidaceae family. It has been seen that berberine acts as an anticancer agent and directly targets various mitochondrial functions like inhibition of complex I and also interacts with ANT. It is among the several natural compounds that are known to directly or indirectly target the mitochondrial dysfunction in cancer cells with little to no harmful effect on normal cells (Letašiová et al. 2006).

10.6.14 Quercetin

Quercetin is a major dietary flavonoid found in vegetables, fruits, seeds, red wine, and tea. Quercetin is known to accumulate in the mitochondria which make it important to study the effect of it on mitochondrial biochemical pathways. Quercetin

inhibits mitochondrial ATP synthesis similar to a well-known inhibitor of electron transport chain. It also greatly affects the succinate oxidase and the NADH oxidase activity. It also acts as a ROS scavenger (Zheng et al. 2012).

10.6.15 Dichamanetin

It is a flavanone which is produced as a secondary metabolite in *Piper sarmentosum*, a plant used as spice in southern regions of Asia. In a study, incubation of dichamanetin with cancer cells and estimation of various mitochondrial pathways showed that this compound caused a significant reduction in viability in various tested cell lines because of the increase in levels of ROS (Yong et al. 2013).

10.7 Conclusions and Future Prospects

The most important aspect in targeting the mitochondria for the treatment of cancer should be its therapeutic selectivity (Fig. 10.2). A large number of mitochondrial functions are crucial for cell survival and normal functioning. Thus any compound that inhibits one of these essential functions (directly/indirectly) may be harmful for even normal cells by exhibiting the toxic side effect. Thus, a potential anticancerous drug should preferentially target those components/activities which are clearly

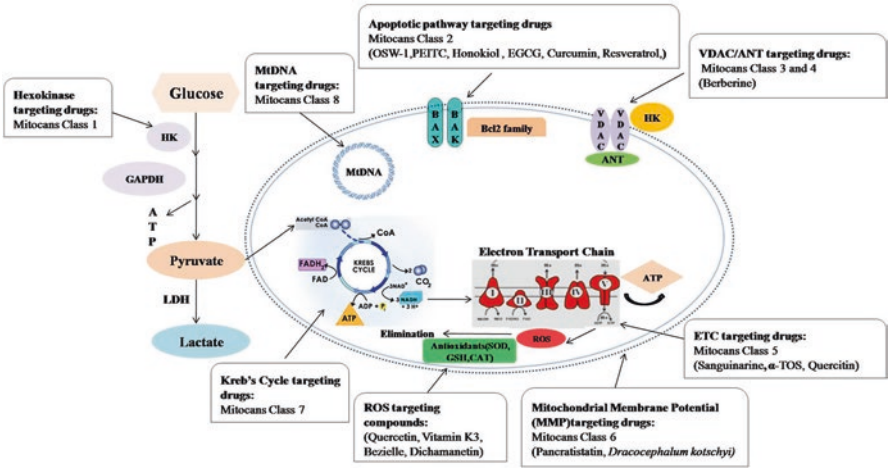

Fig. 10.2 Schematic diagram showing the potential mitocans belonging to different classes and their specific target with respect to structure and metabolism of mitochondria

different in normal and malignant cells. As per various research in recent past, mitochondrial activities have been found to differentiate cancerous and normal cells, which makes them a hot spot as therapeutic targets. Taking this difference in the account, the potential mitocan should target the mitochondria-related abnormalities of malignant cells, instead of showing a nonspecific inhibitory effect on mitochondria without distinguishing the normal and cancer cells. Among the various types of herbal mitocans identified, vitamin E analogues, PEITC, and honokiol have extensively been evaluated in recent studies. As per various *in vitro* and *in vivo* studies, these natural compounds are known to show a promising anticancer activity. One feature which is common in all these compounds is that they preferentially kill the cancer cells and exhibit very low cytotoxicity to normal cells. These compounds provide new avenues to improve cancer therapy. However, the investigation of the underlying mechanisms of many other herbal components may provide new and potential therapeutic targets in cancer biology in terms of altering the mitochondrial activity and their interactions with therapeutic compounds. Thus, the identification of such potent new compounds and further testing for their ability to specifically target the mitochondria in cancer cells may be important tasks in future research.

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Chapter 11
Phytoestrogens as a Natural Source for the Possible Colon Cancer Treatment

Suresh Challa, Rajanna Ajumeera, and Naresh Venna

Abstract Phytoestrogens (PEs) are naturally available bioactive compounds widely available in a spectrum of sources such as plant foods and are said to exhibit estrogen-like, antioxidant, and anticancer properties. There are wide range of PE-containing sources which are usually consumed by humans such as isoflavones (IF), coumestans, and lignans. Many of the fruits, vegetables, and whole grains are known to contain PE. For example, soybeans mainly contain IF, and flaxseeds mostly contain lignans, while clover, alfalfa, and soybean sprouts are rich in coumestans. There are many factors which affect the way these compounds act in a cell type such as estrogen receptor (ER)-α and ER-β levels and the amount of co-activators and corepressors present. The proposed mechanism by which these PEs work is by exerting their antioxidant effects through the inhibition of tyrosine kinase as well as DNA topoisomerase activities and also by suppressing the process of angiogenesis. Findings from molecular, cellular, and animal studies suggest that PE may potentially confer health benefits related to colon cancer (CC) pathology. High incidence of CC might be resulted with the intake of high-calorie diet including consumption of saturated fat and practicing sedentary lifestyle, whereas PEs from fiber-rich food could serve as prophylactics. The aim of this chapter is to elucidate the mechanistic approaches of different plant-based estrogens in combating colon cancer and their possible beneficial and clinical effects and therapeutic implications.

Keywords Benefits · Colon cancer · Consumption · Isoflavones · Phytoestrogens

11.1 Introduction

Developing countries are witnessing colon cancer as the most predominant type of cancer with roughly 2.2 million people suffering from the disease worldwide (Arnold et al. 2017). A fundamental reason could be that the changes in the food

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consumption pattern lead to colon cancer development, especially in the Western world. Phytoestrogens are among the most widely studied dietary bioactive components which have potential curative properties and can reduce the growing prospects of heart diseases and bone-related disorders as well as alleviate the menopausal symptoms and some hormonal cancers. Till today, several studies have shown that high consumption of PEs results in low incidence of developing tumors in the prostate, ovary, and colon (Hwang and Choi 2015; Gupta et al. 2016; Rietjens et al. 2017; Hussain and Green 2017). Parkin et al. (2002) have shown that the higher incidence of cancer in Western population compared to Asian population might be because of low dietary intake of PE which plays a protective role against cancer possibly by lowering down the unconjugated sex hormones in the circulation. Among sex hormones, estrogens regulate the development of colon cancers through its receptors, particularly ER-α and ER-β. Several studies have indicated the fact that PE structure is similar to mammalian hormone estrogen and, metabolically, they act as estrogen agonist and antagonist. Binding of PE to ERs and subsequent interaction with process of sex steroid biosynthesis result in reducing the cancer risk (Hwang and Choi 2015; George et al. 2017; Amawi et al. 2017). In addition, PEs not only show hormonal activity, but they also show some important non-hormonal activities that are involved in cancer prevention. The other mechanisms involved in differentiation of colon cancer are by inhibiting the activity of kinases and obliterating the process of tumor angiogenesis as well as DNA topoisomerase 1, which are involved in induction of cancer cell apoptosis (Shafiee et al. 2016; Rietjens et al. 2017). Colon cancer incidence rate is higher in Western population when compared to Asian population because of food habits. Individual's age and sex are also important factors in the increased incidence of colon cancer. However, population with the age group of 50 and above has shown greater risk of colon cancer incidence, and mortality rate appears to be greater in male population when compared with women. Dietary habits also strongly influence the risk of colon cancer. Few reports suggest that people consuming high-fat diet and red and processed meat and not taking proper amount of fruits and vegetables are having a higher risk of colon cancer. Moreover, several lifestyle factors such as alcohol consumption, lack of physical activity, and metabolic diseases also influence the incidence rate of colon cancer. In addition, environmental factors are also responsible for the cause of colon cancer such as migration of people from one type of climate region to another region (Janout and Kollarova 2001; de Jong et al. 2005; Larsson and Wolk 2006; Wiseman 2008; Haggar and Boushey 2009). Tumor occurs over an extended period in a multistage process in humans and is dependent on several factors. Modifications in both genetic and an epigenetic factor appear to be responsible for conversion of normal to cancerous cells. Malignant cells vary from normal cells by many properties, such as continuous cell proliferation, resistance to growth inhibition, and resistance to apoptosis, and supporting angiogenesis and metastasis. These mechanisms also involve physiological changes and alteration of signal transduction pathways (Gupta et al. 2010).

Cancer of both colon and rectum is often considered as a colorectal cancer, which affects the lower part of the digestive system. Several studies have shown that the

risk of colon cancer will be enhanced by adenomas and polyps. In most cases, colon cancer started as small noncancerous clusters of cells called adenomatous polyps; in some of the cases, the polyps are converted to colon cancer. Adenomas are categorized into two types, conventional adenomas and sessile serrated polyps. In most of the cases, these two types of adenomas are responsible for colorectal cancer (Strum 2016). Predominantly adenomas are seen in distal colon in the age group of below 60 years, and above this age they are mostly found in the proximal colon (Zauber et al. 2012). The microsatellite instability pathway and chromosomal instability pathways are responsible for the conversion of adenomas into carcinoma. In both pathways, genes affected by somatic mutation are responsible for the most diverse cancers (Lin et al. 2003; Leary et al. 2008; Nishihara et al. 2013; Strum 2016). Prevalence of colon cancer is sporadic in majority of the cases, and 2–6% cases are hereditary disease due to mutation of autosomal dominant genes. Most commonly mutated tumor suppressor genes (TSGs) are APC and TP53, and most commonly mutated oncogenes are BRAF, KRAS, and P13KCA genes. There are two types of inherited conditions found in colorectal cancer such as familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. Mutation of mismatched repair genes and tumor suppressor genes is responsible for familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC or lynch syndrome), respectively, and mutation of TSG-APC might lead to familial adenomatous polyposis. The MLH1 and MLH2 genes function as DNA mismatch repair pathway; mutation of these two genes is responsible for HNPCC (Papadopoulos et al. 1994; Smith et al. 2002; Wang et al. 2004; Pampaloni et al. 2013; Crockett et al. 2015; Strum 2016). The aim of this chapter is to elucidate the mechanistic approaches of different plant-based estrogens in combating colon cancer, their possible beneficial and clinical effects and therapeutic implications.

11.2 Mechanism of Carcinogenesis

Three different mechanisms are responsible for colorectal cancer: (1) chromosomal instability (CIN), (2) microsatellite instability (MSI), and (3) CpG island methylation (CIM). One or combination of these three pathways is involved in the development of colorectal cancer (Fig. 11.1). CIN is involved in the mutations of adenomatous polyposis coli (APC), followed by mutation of TSGs and tumor oncogenes. Sixty to seventy percent of sporadic colorectal cancer cases arise by chromosomal instability. This pathway is associated with aneuploidy, which is improper segregation of chromosomes in mitotic divisions. Defect in checkpoints in mitotic divisions is responsible for chromosome mis-segregation, and this condition leads to aneuploidy. Mutation of the following genes such as mitotic arrest deficient (Mad1 as well as Mad2); budding uninhibited by benzimidazoles 1 (BUB1); hZw10, hZwilch/FLJ10036, and hROD/KNTC genes; and kinesin family member 11 (KIF11) can result in chromosomal instability. Several other mechanisms are also driven to chromosomal instability: abnormal centromere number and function,

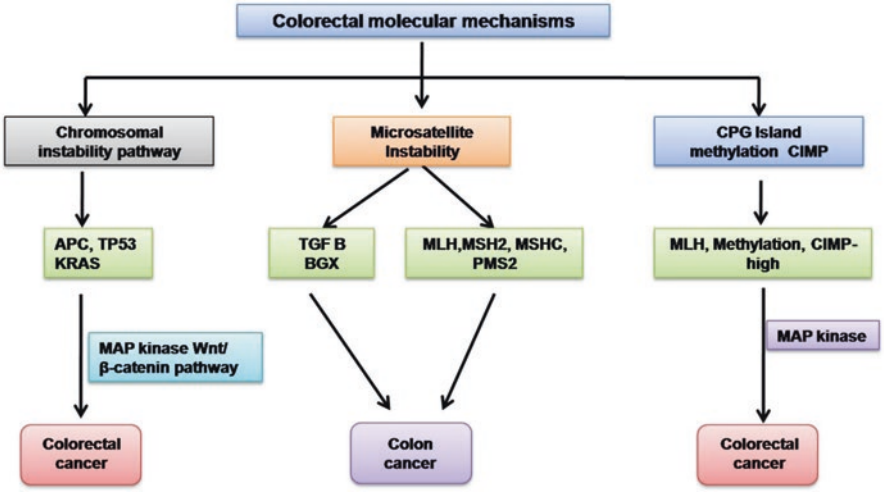

Fig. 11.1 Molecular mechanism of colorectal carcinogenesis

telomere dysfunction, loss of heterozygosity, defect in DNA damage response, and mutation of proto-oncogenes and TSGs (KRAC, APC, and TP53). In colorectal carcinogenesis, the earliest genetic event is Wnt pathway activation. The tumor suppressor gene APC regulates the beta-catenin/Tcf pathway. Mutation of APC gene allows accumulation of beta-catenin in cytoplasm. This beta-catenin binds to DNA-binding protein transcription factor and moves to the nucleus, followed by transcription of genes containing TCF-DNA-binding sites which regulates proliferation, migration, and adhesion of colorectal cells (Smith et al. 2002; Wang et al. 2004; Roper and Hung 2013; Colussi et al. 2013; Tariq and Ghias 2016).

Microsatellite instability pathway is responsible for 12–17% of all colorectal carcinogenesis with only 3% associated with hereditary nonpolyposis colorectal carcinogenesis and remaining are sporadic cases driven by the hypermethylation of the MLH1 gene. Microsatellite is driven by inactivating the mutation of DNA mismatch repair genes. The main function is correcting DNA replication error; DNA mismatch repair system has important components including hPMS1, hPMS1, hMLH1, hMSH2, hMSH3, and hMSH6. Another target gene in the microsatellite colorectal carcinogenesis pathway is the TGF-β receptor 2 (TGF-βR2). TGF-β is a negative regulator of proliferation in colon epithelial cells. Mutation of Smad2 and Smad4 genes regulates the TGF-β pathway that leads to MSI colorectal carcinogenesis. Another mutational target gene of microsatellite instability colorectal carcinogenesis is active in type 2 receptors (ACVR2) and tumor suppressor gene BAX. ACVR2 executes an important function in differentiation and growth suppression by phosphorylating Smad2 and Smad3 proteins. Several studies have shown ACVR2 mutation to frequently occur with TGF-β2 mutations. Tumor suppressor gene BAX regulated the intrinsic apoptosis mechanism. Fifty percent of colorectal carcinogenesis cases are caused by homologous frameshift mutation of

BAX gene and cells escaping from the intrinsic apoptosis mechanism. CpG island methylation phenotype (CIMP), methylation of DNA at the cytosine base of CpG islands are done by DNA methyltransferase enzyme. An initial CIMP tumor occurrence could be a quick shift in the BARF proto-oncogenes, and usual colonic epithelial cell apoptosis is prevented. CpG island methylation takes place in the mismatch repair gene MLH1, and subsequently its transcriptional inactivation leads to the formation of malignant tumor (Takayama et al. 2006; Zhang et al. 2010; Roper and Hung 2013; Colussi et al. 2013; Tariq and Ghias 2016).

11.3 Metastasis

Maximum carcinoma-related fatalities are due to complication in correlation with metastasis. Cancer that spread from tissues to organs from the site of origin to a distant target within the body ends up in generating metastasis. Tumor formation requires multifarious cancer cells, and from these everyday at least 10% of the cells infiltrate into the circulation. This particular event ends up in replacing normal cells with cancerous tumor cells in different kinds of body tissues. Predominantly, metastasis occurs in the late phase in the development of carcinoma; henceforth surgery is a good option to treat the small tumor that is being formed in the initial stages (Guillerey and Smyth 2015). This metastatic process is termed as a battery of independent, rate-limiting, and continuous process. The brief events that take place during metastasis process are as follows.

11.3.1 Angiogenesis

It is the process of fabrication of fresh blood capillaries, and it involves important events like migration, growth, and proliferation of the cells that are endothelial in nature. The cells are lined up inside the wall of the blood vessels (Folkman 2002).

11.3.2 Intravasation

It is the process of cancer cell invasion through basal membrane in the circulation either in blood or lymphatic system. Critically during this process, the cancerous cells break out from their primary sites.

11.3.3 Survival in Circulation

Largest number of circulation cancer cells happens to die within 24 h by different processes such as cytotoxicity or by lysis by NK cells. After intravasation, tumor cells normally will be protected by platelets and platelet-derived microparticles (Sakurai and Kudo 2011).

11.3.4 Extravasation

It is known that tumor cells exudate through venules and then migrate to arterioles where the oxygenation for the tissue is more.

11.3.5 Secondary Tumor Formation

Tumor cells often spill into the circulation possibly through the process of apoptosis and get exterminated by immune cells (Hedley and Chambers 2009). Ultimately to become a secondary tumor, cancerous cells should divide and have to undergo angiogenesis. Some of the studies indicates that early angiogenic stages of metastatic growth in bone-marrow-derived EPC, while few suggest that co-option of normal vessels is a mechanism for metastasis vascularization (Folkman 2002; Guillerey and Smyth 2015; Bielenberg and Zetter 2015).

11.4 Treatment of Colon Cancer

Treatment for cancer varies from one individual to another, and treatment options and recommendations depend on several factors. The major factors include stage and type of cancer, age of the individual, and health condition of the patient. The most common treatment options are surgery, radiation therapy, chemotherapy, and targeted therapy.

11.4.1 Surgery

Surgery is the most common treatment for early stages of colorectal cancer. It involves removal of the tumor and small amount of surrounding healthy tissue and is often called surgical resection. Surgical options for colorectal cancer are

polypectomy and colectomy. When the tumor is removed as part of the polyps, then it is called polypectomy. Removal of complete colon or part of the colon and surrounding lymph nodes during surgery is called colectomy. A complete removal of the colon is called total colectomy, and if only a part of the colon is removed, then it is called partial colectomy. In general, the side effect of surgery is pain and tenderness in the area of operation.

11.4.2 Radiation Therapy

Radiation therapy uses high-energy rays (X-rays or gamma rays) or charged particles to kill the cancer cells. Sometimes the tumor cannot be completely removed by surgery; hence radiation therapy can be used to treat the cancer cells. Radiation therapy in combination with chemotherapy acts effectively on few colorectal cancers. These two treatments are together referred to as chemoradiation therapy. Treatments of colorectal cancers involve the use of different types of radiation therapy which are listed below.

11.4.2.1 External Beam Radiation Therapy

External beam radiation therapy which is used to treat colorectal cancers uses X-rays coming from outside of the body through a machine and is delivered to where the tumor is located.

11.4.2.2 Internal Radiation Therapy

Brachytherapy is most commonly used for the treatment of colorectal cancer. The convenience of this treatment is that the radiation doesn't cross the skin and other tissues to cure the tumor; henceforth the side effects are very less.

11.4.2.3 Stereotactic Radiation Therapy

Stereotactic radiation therapy uses radiation at a very high dose. This is also a type of external beam radiation therapy that is mainly used if a tumor is in metastasis stage. The advantage of this technique can help avoid removing parts of the tissues that might be removed during surgery. In general, the possible side effects from radiation therapy are skin irritation, nausea, fatigue, stomach upset, painful bowel movement, sexual problems, and infertility in both male and female.

Table 11.1 FDA-approved drugs for chemotherapy

Drug name	Mechanism of action
Fluorouracil (5-FU)	Disrupts DNA and RNA synthesis
	Inhibits the enzyme thymidylate synthase – key enzyme in the creation of the DNA nucleotide dTMP – and impairs DNA synthesis in the S phase of the cellular replication cycle
	Creates incorrect nucleotides which are incorporated into DNA and interferes with normal protein production leading to cell death
Capecitabine (Xeloda)	Capecitabine inhibits de novo synthesis of DNA by inhibiting thymidylate synthase. It is a key enzyme for the synthesis of thymidine monophosphate
	Capecitabine is metabolized to 5-fluorouracil by thymidine phosphorylase; this enzyme's expression is more in cancer cells compared to normal cells
Irinotecan (Camptor)	Disrupts DNA replication and transcription
	Inhibits DNA topoisomerase I, relaxes super-coiled double-stranded DNA, and prevents DNA religation
Oxaliplatin (Eloxatin)	Induces apoptotic cell death
	Causes inter- and intra-strand DNA cross-links and halts replication and transcription
Trifluridine	Trifluridine is a standard antiproliferative agent with two types of mechanisms of action; it inhibits thymidylate synthase (TS) and is also integrated into DNA

11.4.3 Chemotherapy

Chemotherapy uses one or more anticancer drugs to kill the cancer cells. This treatment uses single or combination of different drugs to treat the individual at the same time. Several research studies have shown that combination of chemotherapy and target therapies is increasing the survival rate of cancer patients. In systematic chemotherapy, drugs are directly injected into the veins or given in the form of pills or capsules. The given drug is distributed all over body through the bloodstream. In adjuvant chemotherapy, treatment is given to the patient after all the visible and known tumors have been removed by surgery and will be used to prevent the reoccurrence of cancer. Neoadjuvant chemotherapy is the administration of drugs prior to the surgery or radiation therapy. The main approach of this chemotherapy is reduction in size of the tumor and inhibition of spreading of tumor. Frequently used FDA-approved chemotherapy drugs for treating colorectal cancer (Table 11.1) include fluorouracil (5-FU), capecitabine (Xeloda), irinotecan (Camptor), oxaliplatin (Eloxatin), and trifluridine (André et al. 2004; Ciombor et al. 2015; Hammond et al. 2016). Chemotherapy effects depend on type and dose of drugs advised and period of consumption. Loss of hair, anorexia, bleeding, fatigue, mouth ulcers, and nausea are regular adverse effects of chemo drugs, diarrhea being the most common. Among FDA-approved chemo drugs, oxaliplatin causes nerve damage with tingling sensation and numbness in hands and feet, skin rashes, and trouble in

Table 11.2 FDA-approved target therapy drugs

Drug name	Mechanism of action
Regorafenib	Inhibits the vascularization and growth
Bevacizumab	Reduces the formation of new vasculature
	Induces local hypoxia and blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A)
Aflibercept	Regression of tumor vasculature and new vascular growth
	Binds to circulating VEGFs and inhibits the activity of the vascular endothelial growth factor subtypes VEGF-A and VEGF-B, as well as placental growth factor (PGF)
Cetuximab	Inhibits cell growth and survival
	Binds to the external domain of the EGFR – receptor is internalized and degraded without activation or phosphorylation
	Induces antibody-mediated cytotoxicity
	Downregulates the VEGF expression
Panitumumab	Panitumumab selectively binds to epidermal growth factor receptor (EGFR) and induces internalization of EGFR
	Induces apoptosis
	Inhibits cell proliferation, decreases the expression of pro-inflammatory cytokines and VEGF

breathing being the major symptoms, and capecitabine or 5-FU (when given as an infusion) when used for hand and foot treatment causes redness in hands and feet initially and ends up in pain and sensitivity.

11.4.4 Target Therapy

Target therapy is different from the conventional chemotherapy, as in this treatment drugs are targeted to specific genes, proteins, or tissue environments that support tumor growth and survival. Target therapy mainly involves two kinds of drugs to treat cancer; monoclonal antibodies inhibit specific target outside the cancer cells or the target of surrounding environment of cancer cells and are administered intravenously (IV). Monoclonal antibodies can also be delivered through toxic substance and directly sent to cancer cells. Next to monoclonal antibodies, small molecular drugs are targeted to inhibit the process that supports cancer cell metastasis and growth. Generally these drugs will be taken as pills or capsules orally. There are two main types of target therapies for colorectal cancer (Table 11.2).

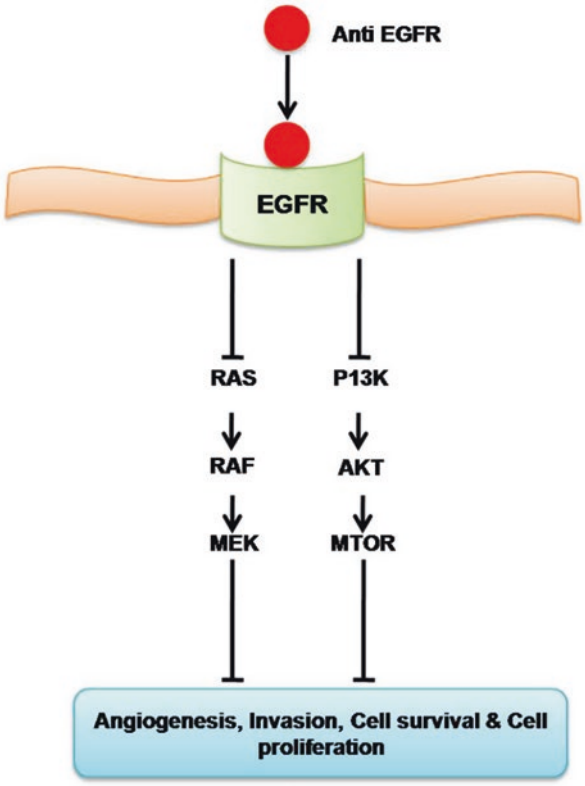

Fig. 11.2 Possible mechanism of anti-EGFR drugs and its targets

11.4.4.1 Anti-angiogenesis Therapy (Inhibition of VEGF)

Anti-angiogenesis therapy uses drugs to target and inhibit vascular endothelial growth factor (VEGF) which is mainly involved in tumor angiogenesis. Commonly used anti-angiogenesis therapy drugs are bevacizumab (Avastin), regorafenib (Stivarga), ziv-aflibercept (Zaltrap), and ramucirumab (Cyramza).

11.4.4.2 Epidermal Growth Factor Receptor (EGFR) Inhibitors

EGFR is overexpressed on the surface of cancerous cells and is mainly involved in the progression of tumor. The possible mechanisms of EGFR are that epidermal growth factor receptor ligands (EGFRL) bind the extracellular domain of EGFR, initiate receptor activation, and stimulate downstream signaling pathways such as PI3K/AKT and RAS/RAF/MAPK pathways. These signaling pathways are involved in cell growth, proliferation, angiogenesis, and metastasis (Fig. 11.2). Cetuximab or panitumumab drugs (anti-EGFR drugs) block ligand binding to EGFR, thus inhibiting EGFR downstream signaling pathways. Cetuximab (Erbitux) and panitumumab

(Vectibix) are the most commonly used drugs that act as inhibitors of epidermal growth factor receptors and hamper the tumor progression (André et al. 2004; Ciombor et al. 2015; Hammond et al. 2016).

11.4.4.3 Possible Side Effects of Drugs of Ziv-Aflibercept and Bevacizumab

Lethargy, high blood pressure, bleeding, low white blood cell counts, mouth sores, food aversions, and diarrhea are the most common side effects of these drugs, while blood clots, severe bleeding, holes forming in the colon (called *perforations*), kidney problems, allergic reactions, and slow wound healing are rare but possible serious adverse effects.

11.4.4.4 Possible Side Effects of Drugs Cetuximab (Erbitux), Panitumumab (Vectibix), and Regorafenib (Stivarga)

Regular side effects of these drugs include skin problems which can sometimes lead to infections. Panitumumab causes serious skin problems that lead to skin peeling. Other side effects include fever and diarrhea and allergic reaction during the infusion, which could cause low blood pressure and breathing problems. Regorafenib causes fatigue, loss of appetite, hand-foot syndrome, diarrhea, high blood pressure, weight loss, and abdominal pain. Severe bleeding and perforations in the stomach or intestines could be categorized as more serious side effects but are found to be less common.

11.5 Lifestyle, Nutrition, and Cancer

Lifestyle of an individual is responsible for nearly 90–95% incidence of all cancers, and the remaining 5–10% is associated with improper function of genes. Many epidemiological studies suggest that proper nutritious diet could reduce cancer deaths by up to 35%, and certain cancers could be totally avoided by up to 80–90% by consuming appropriate diet. Many plant-derived dietary compounds are known for their multi-targeting activities and, hence, referred as nutraceuticals (nutrition and pharmaceutical). Nutraceuticals are defined as compound considered being a food or part of the food that protect against pathological conditions and provide health benefits. These are biologically active compounds and can be used to target tumor cell development processes at various steps (Gupta et al. 2010; Pampaloni et al. 2013). Several studies at epidemiological level have shown tumor incidence at many sites which is negatively correlated to consumption of fruits and vegetables. Certain foods including fruits and vegetables reduce the risk of colon cancer. Several case control studies lowered the incidence rate of cancer by increased uptake of

fiber-containing foods. In addition, studies have shown 40–50% significant decrease in colon cancer and 50% decrease in colorectal cancer with higher uptake of fiber-containing fruits and vegetables. Dietary fibers, antioxidants (e.g., β-carotene, vitamin C), and anticarcinogenic constituents (e.g., protease inhibitors, PE) in these vegetables, fruits, and grains might have a potential protective effect, reducing the tumor risk (Block et al. 1992; Howe et al. 1992; Pampaloni et al. 2013).

11.6 Phytoestrogens

Phytoestrogens belong to a diverse class of compounds structurally and functionally similar to mammalian hormone estrogen (17-estradiol). These compounds are available in greens, grains, fruits, and vegetables. Phytoestrogens have a diverse biological activity because they can act both as agonists showing similar activity of endogenous estrogens and antagonists by inhibiting the estrogenic activity of estrogen hormone. Similar to estrogen agonists, these phytoestrogens cause estrogen-like effects; as estrogen antagonists, these phytoestrogens are categorized into mainly three important groups; they are isoflavones, lignans, and coumestans. Other classes of phytoestrogens are anthraquinones, flavones, prenylflavonoids, chalcones, and saponins.

11.6.1 Isoflavones

These are the most important PEs found in legume, soya, peanuts, and clover. Naturally occurring isoflavones, namely, daidzein (4,7-dihydroxyisoflavone), genistein (4,5,7-trihydroxy isoflavones), formononetin, and biochanin A (Ososki and Kennelly 2003; Dixon 2004) (Fig. 11.3), show similar activity to mammalian estrogen hormone. Once mammals consume isoflavones, they are metabolized to daidzein and genistein in the gastrointestinal tract, and further, biochanin A and formononetin are metabolized, respectively, to genistein and daidzein (Kurzer and Xu 1997). Genistein is the most effective compound of all the isoflavones with anticancer and antioxidant properties (Hussain and Green 2017). Several studies have tested genistein and showed that cancer cells are inhibited under high concentration and proliferated in low concentration. However, there are some studies on PEs showing to have a very little effect as antioxidants; soymilk and supplementary soy isoflavones are protective in lipoprotein against oxidation and oxidative DNA damage in postmenopausal women. In humans glutathione peroxidase is the most important enzyme in antioxidation, and genistein helps in increasing of antioxidant enzymes (Anderson et al. 1999; Brownson et al. 2002; Li et al. 2012; Shafiee et al. 2016).

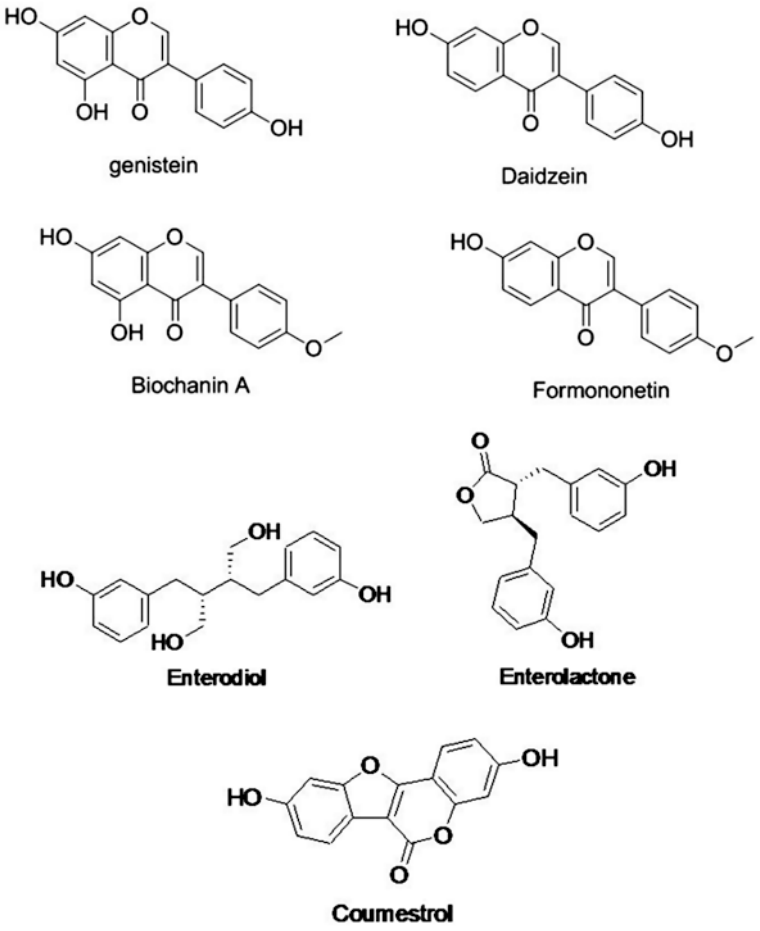

Fig. 11.3 Structures of isoflavones (genistein, daidzein, biochanin A, and formononetin), lignans (enterodiol and enterolactone), and coumestans (coumestrol)

11.6.2 Lignans

Lignans are another group of PE first recognized in plants. Lignans are later found in the biological fluids of mammals. It is a di-phenolic plant compound containing 2,3-dibenzyl butane structure formed by two cinnamic acid residues. Plant lignans' chemical structure differs from that of mammalian lignans as they lack the phenyl hydroxyl groups in aromatic ring at meta-position of the ring (Fig. 11.3). Lignans are highly available in rye bread, legumes, whole grains, vegetables, fruits, and oilseeds. High concentrations of lignans are found in the flaxseed. Seed coat, bran layer of seeds, and wooden portion of plants are sources of lignans with matairesinol

and secoisolariciresinol being the most well-known. These two plant lignans are converted into enterodiol and enterolactone, respectively, by bacterial action in the gut. In plants, lignans are conjugated with sugar moiety and are converted into unconjugated lignans through a series of metabolic reactions by gastric hydrochloric acid and anaerobic microbe-derived β-glycosidases. The administration of antibiotic- or microbe-free environment inhibits production and excretion of lignans. In mammals, once lignans are absorbed in the epithelial border of the intestine, they are re-conjugated in the liver by enzymes of UDP-glucuronosyltransferase and sulfotransferase. Intake of plant lignans has many health benefits; particularly, they reduce the risk of incidence of cancers. In addition, plant lignans also possess anticarcinogenic, antioxidant, antiproliferative, and apoptotic activities. As lignans are structurally similar to mammalian hormone β-estradiol, they influence the hormonal cancers via estrogen-mediated signal transduction pathways. Lignans are also involved in estrogen-independent pathways via insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor.

11.6.3 Coumestans

Coumestans are an important group of plant PE isolated from plants. The structure of coumestans is similar to mammalian hormone estrogen and shows estrogenic activity (Fig. 11.3). This group of PE is first reported by Bickoff and coworkers in 1957. Coumestans were first isolated from alfalfa or lucerne (*Medicago sativa*), ladino clover (*Trifolium repens*), and strawberry clover (*T. fragiferum*). Some of the plant coumestans were shown to have uterotropic activities. Coumestrol and 4-methxycoumestrol are important coumestans and shown to have estrogenic activity. These two coumestans are found in alfalfa and ladino clover. Important dietary sources of coumestans are sprouts of alfalfa and mung bean, and coumestans are especially higher in clover. It is showed that feeding of female rats with coumestrol resulted in suppression of estrous cycle and also negatively affected the sexual behavior of male offsprings. Coumestrol also regulates the metabolic effects and increases the lipid synthesis and glycogen catabolism (Kurzer and Xu 1997; Nogowski 1999; Ososki and Kennelly 2003; Dixon 2004).

11.7 Phytoestrogens and Colon Cancer Treatment

Colon cancer is considered to be an important disease, leading to majority of cancer deaths worldwide. In the last decade, there were numerous reports indicating that the increased prevalence of CRC could be because of alterations in lifestyle, improper nutrition, and environmental factors. The increased drug resistance and side effects resulting from the use of conventional radiotherapy, chemotherapy, and target therapy are a major problem in the treatment of colon cancer. Thus, it is

necessary to investigate effective anticancer drugs with low side effects for the treatment of colon cancer. Phytoestrogens, a diverse group of plant-derived polyphenolic bioactive compounds, are identical to estrogen based on their function and chemical structure. It is most commonly found in soy food. In humans, soy is a primary source of plant-derived proteins. A wide variety of biologically active chemical compounds are found in soy and soy food products which independently or combinedly contribute to health. Soy is rich in isoflavones, which has several health benefits. Isoflavones are structurally identical to estrogen molecule. PEs are considered to possess therapeutic properties and can prevent cancer. Reports indicate that exposure to PEs can inhibit the transition of G_2/M in tumor cells and can upregulate the cell cycle inhibitory molecule. PEs have several other mechanisms of action like EGF receptor inhibition, vascular endothelial growth factors, and TNFα. They can act as antioxidants, 3β- and 17β-hydroxysteroid dehydrogenase inhibitors, suppressors of angiogenesis, and inhibitors of aromatase mRNA expression and activity (Krazeisen et al. 2001; Li et al. 2005; Rice et al. 2006; Haggar and Boushey 2009; Virk-Baker et al. 2010; Barnes 2010; Hwang and Choi 2015).

Several epidemiological surveys indicate that a curtailed incidence of cancers is associated with hormones in Asia, where one can witness regular consumption of soy-based food. Thus, soybean consumption results in the reduction of cancer rates, showing the key role of PEs in cancer prevention, which are known to be highly potent antioxidants. However, soybeans are rich in trypsin inhibitors; they also contain other proteins like sphingolipids phosphatidylinositol, and saponins, which impart various advantages relating to health care. In summary, all three compounds present in soybean possess tumor-preventive properties in animal models (Birt et al. 2001; Pampaloni et al. 2013; Amawi et al. 2017). Several studies epidemiologically reported the association between reduced colorectal risk of cancer and soy food intake. Studies conducted in Asia and Hawaii also reported the same, but these findings need to be reassessed (Oba et al. 2007; Akhter et al. 2008; Yang et al. 2009; Budhathoki et al. 2011; Shin et al. 2015). Factors like gender differences contribute majorly to the incidence of CRC. The influence of female sex steroid hormones in women has a major role in lowering the death rate associated with colorectal cancer. Hormone therapy (combination of estrogen and progestin) on its pros side has protective role in development of CRC and is also known to reduce the risk of colon cancer to 32% in postmenopausal women, but on its cons side, it is associated with other risks especially showing higher incidence of heart diseases (Cotterchio et al. 2006; Barone et al. 2008; Nüssler et al. 2008; Pampaloni et al. 2013).

Some studies suggested that the PEs may show their protective role through the activation of estrogen receptor (ER)-β in the gastrointestinal tract, where ER-β is the predominant subtype of ER. Steroid hormone receptor members, namely, ERs and nuclear receptors, activate upon binding of the ligand forming a stable dimer resulting in the initiation of ER-specific response target gene transcription. The above dimer formation hampers in the absence of the ligand and initiates the binding of ER to shock protein. There are two main types of ERs, alpha (ER-α) and beta (ER-β). Estrogens exert their effects on target tissues by these ligand-activated transcription factors. They also show a varied distribution in tissues; for example, the

mammary glands and uterus mainly contain ER-α, while the endothelial cells, central nervous system, and colonic mucosa mainly contain ER-β (Pampaloni et al. 2013). Clinical studies have reported that CRC highly expresses ERs. Very low expression levels of ER-α and high expression levels of ER-β associated with the cellular differentiation and CRC stage were detected in normal or pathological colonic mucosa (adenoma and carcinoma). However, ER-β expression levels were less along the pathological mucosa, respectively. These findings showing the expression of ER-β protein getting lower in malignant tumors with respect to normal tissue have helped to develop a hypothesis that ER-β may act as a cancer suppressor, further preventing malignant transformation and uncontrolled proliferation (Glazier and Bowman 2001; Pampaloni et al. 2013; Williams et al. 2016).

11.7.1 Possible Mechanisms of Colon Cancer Prevention by PE Treatment

In addition to hormonal activities, some of the non-hormonal mechanisms of PE exist in treatment of tumor which include reduction in proliferation, changes in cell signaling, induction of detoxification enzymes, and induced cell cycle arrest, apoptosis, and anti-inflammatory and antioxidative properties. Wang et al. (1998) have demonstrated that dietary PE can have a new mode of action in CRC chemoprevention. In a study, six prominent PEs were examined in Colo205 cells for their potency to induce NADPH: quinone reductase (QR). However, there was no significant change in QR mRNA expression and activity upon daidzein or formononetin, enterolactone, and genistein treatment in a dose-dependent manner. However, effects of biochanin A and coumestrol QR expression were in moderation. Further, in this study, cell proliferation was most effectively inhibited by enterolactone (20%), followed by genistein (7%) and biochanin A (4%) (Wang et al. 1998; Lechner et al. 2005). Another interesting target for cancer chemoprevention is cyclooxygenase 2 (COX-2). Many studies have shown that colon tumor development involves COX-2 overexpression followed by prostaglandin overproduction. Among the PEs, however, genistein reportedly inhibits COX-2 expression in different cell types, such as gingival fibroblasts (Noguchi et al. 1996) and endothelial cells (Blanco et al. 1995). Suppressed COX-2 promoter activity is observed with increasing dose when Mutoh et al. (2000) examined the effects of genistein in DLD-1 human colon cancer cells that were transfected with the promoter sequence of the COX-2 gene in fusion with the β-galactosidase reporter gene. In addition, Mutoh et al. (2000) explained that the attributed effect was not only with mechanism involved in inhibition of tyrosine kinase but also suggested that the resorcin moiety in the genistein structure is critical since it is shared by other substances (Noguchi et al. 1996; Mutoh et al. 2000; Koehne and Dubois 2004; Lechner et al. 2005).

As per the literature, genistein is known for G2/M cell cycle arrest among various tumor cell lines. Among them, Park et al. (2001) for the first time studied colon

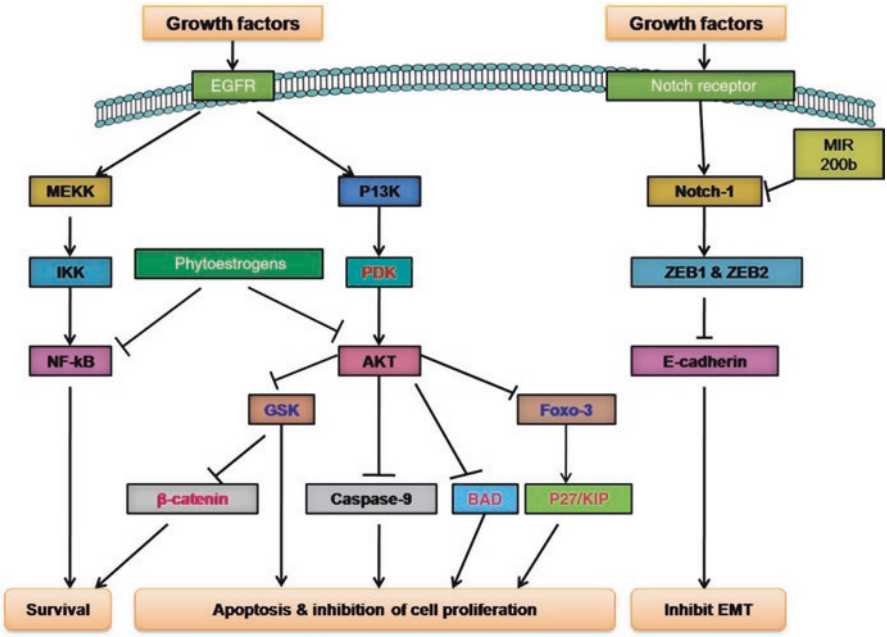

Fig. 11.4 Role of PE in signaling pathways, apoptosis, EMT, and cell proliferation

cancer cells and the effect of genistein in cell cycle progression. Genistein treatment showed increased expression and activation of p21waf1/cip1 protein and promoter reporter construct in Colo320 cells. In addition, authors have considered that genistein is a potential chemotherapeutic agent especially in combination with dexamethasone because of its correlation with G2/M arrest due to the activation of the cyclin-dependent kinase inhibitor p21waf1/cip1. A major component of soy products, genistein has been shown to have anticancer properties. In colon cancer cells, genistein has shown antiproliferative function via PI3K/Akt pathway by promoting FOXO3 activity and by inhibiting EGF-induced FOXO3 phosphorylation. Further, genistein increased FOXO3 activity by inhibiting EGF-induced FOXO3 disassociation from p53 (mut), increasing the expression of the p27kip1 cell cycle inhibitor, further inhibiting proliferation in colon cancer cells. These reports suggested that inactivation of FOXO3 is a key step in EGF-mediated proliferation (Fig. 11.4) (Sarkar and Li 2003; Qi et al. 2011a, b; Ganai and Farooqi 2015).

Further, there are reports of nearly 90% cancer-related deaths caused by tumor metastasis. The cancer cells possessing EMT properties lead to cancer metastasis by increasing the expression levels of motility-related proteins in the cell and increased migration and invasion to different parts of the body. Studies also indicate genistein targets various signaling pathways by specifically regulating the EMT process that results in the inhibition of cancer metastasis. Notch-1 signaling pathway contributes significantly to EMT phenomena by upregulating EMT markers such as ZEB1, ZEB2, Slug, and vimentin. Genistein treatment has been shown to reactivate miR-

200b, repressed by Notch-1 signaling, and hence suppress EMT process in AsPC-1 cells. Whenever miR-200b is reexpressed, reduced ZEB1 and vimentin expression, as well as enhanced E-cadherin expression, was observed thereby inhibiting the EMT process (Fig. 11.4) (Bao et al. 2011; Lee et al. 2016).

Lignan's role against colon tumor has been shown mostly by *in vitro* or *in vivo* studies. Enterodiol (EDL) and enterolactone (ENL) inhibited activation of c-fos in different breast and colon tumor cell lines. A new anticancer drug has been identified from *Phyllanthus urinaria*, a medicinal plant for cancer treatment. Drug is a substituted methylenedioxy lignin (7′-hydroxy-3′,4′,5,9,9′-pentamethoxy-3,4-methylenedioxy lignin), competent of activating caspases 3 and 8 and also c-myc in cancer cell lines through its ability to inhibit telomerase and bcl2. This compound may be serving as a chemotherapeutic drug after further evaluations (Giridharan et al. 2002; Webb and McCullough 2005). Lignans influence cancer via estrogen-mediated pathway, because of their structural similarity to 17-β estradiol. In addition, growth hormones, namely, insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF), in estrogen-dependent or estrogen-independent manner mediate the action of lignans. IGFs constitute a number of mitogenic factors which are anabolic in nature and help regulate growth and development along with various cellular processes. Also, few studies report IGF-1 showing effective mitogen characteristics involved in several cancers, like prostate, breast, and colon cancer. An investigation conducted in nude mice model with an ER-negative breast tumor showed a significant association with downregulation of IGF-1 and decrease in tumor growth and metastasis upon 10%-flaxseed diet. However in one study, estrogenic mechanisms had a little effect as observed in ER-negative experimental model. Thus, this study suggested that lignans have a similar mode of action as that of soy-based phytoestrogen, genistein. It has also been reported to play a maximum role in disrupting signal transduction pathways and growth factor transcription by inhibiting tyrosine protein kinase (Yu and Rohan 2000; Chen et al. 2002; Sandhu et al. 2002).

VEGF plays a major role in normal vascular development and tumor progression. Dabrosin et al. (2002) showed that feeding 10% diet of flaxseed to nude mice causes decreased tumor growth by possibly decreasing extracellular VEGF levels. Dabrosin also showed reduced metastatic events with traditional human breast cancer tumors. In this study, researchers contemplated that lignans are the most notable active components in flaxseeds which decrease VEGF through two possible pathways: an estrogen-dependent pathway and estrogen-independent pathway. VEGF consists of estrogen response element (ERE), which contributes to the reduction of circulating estrogens by lignans. Alternatively, lignans in flaxseed may cause decrease in cancer size or restriction of cancer growth through unknown mechanism in which there is an inhibition of hypoxic state. Epidemiologically, *in vitro* and animal studies reported that lignans via various mechanisms like antiproliferative and anti-angiogenic acquire tumor inhibitory properties (Hyder et al. 2000; Dabrosin et al. 2002; Hausott et al. 2003). Several *in vitro* and *in vivo* studies explored the effective role of lignans against colon cancer supporting the antiproliferative activities with decreased tumor number, size, and volume and also suppressed tumor

growth rate. Estrogen-independent growth inhibitory effect was demonstrated in four colon cancer cell lines, namely, LS174T, Caco-2, HCT-15, and T-84, ENL and EDL, each at 100 μM concentrations. A significant association was observed in normalized β-catenin levels with supplementation of 10% rye to Min mice in comparison to wheat-, beef-, or inulin-supplemented diets (Sung et al. 1997; Mutanen et al. 2000; Webb and McCullough 2005).

11.8 Conclusions and Future Prospects

Phytoestrogens are naturally available bioactive compounds widely available in a spectrum of sources such as plant foods. The main dietary sources rich in phytoestrogens are soybean, flaxseed, oil seeds, nuts, kala chana, whole grains, mung bean, red lentils, tofu, green beans, red clover, fruits, and vegetables. They have diverse biological activities because of their capability to behave both as estrogen antagonists and agonists. Phytoestrogens showed both hormonal and non-hormonal activities involved in cancer prevention. The other mechanisms involved in inhibiting the progression of tumor growth are through the embargo of tyrosine protein kinases and inhibition of angiogenesis and DNA topoisomerase 1, which are also involved in the induction of cancer cell apoptosis. In today's world, Westernized diet and lifestyle factors have a major impact in many cancers, particularly colorectal cancer, as it is becoming a worldwide serious health issue. Despite improvements in surgical and chemotherapeutic treatments, colorectal cancer has a poor survival rate. Thus, in this scenario there is a need for natural therapeutic agents with minimal side effects which are required to control the progression of colorectal cancer. Data on the role of PEs in *in vitro* and animal and human studies show that they decrease the risk of different types of tumors. Among them, naturally available bioactive PEs are known to possess antiproliferative and anticancer properties decreasing colorectal cancer and pathologies associated with colon cancer. In spite of a lot of epidemiological and animal data being available that suggest the fact that PEs might show a protective role against colon cancer, the effects observed in colon tumor cell lines should be understood cautiously due to the complications in comparing the exposure of PE to the cells at tissue level in human clinical trials. Data on the suppressing effects of PE on the progress of unchanged normal cells needs to be elucidated if higher concentrations of PE intake are to be extensively recommended. In addition, the exact correlations between dietary PE exposure in humans and the development of cancer need to be explored. Also, there is an urgent need to understand the regulatory mechanisms associated with the absorption of PE and also the interactions with other dietary constituents especially zinc and iron.

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Chapter 12
Bioinformatics Approaches for Genomics and Post Genomics Applications of Anticancer Plants

Avni Mehta and Yasha Hasija

Abstract After the culmination of Human Genome Project in 2003, it was prophesied that the upcoming era in modern biotechnology would pose as a real acid test. While the pre-genomic era was marked by the efforts to sequence the human genome, advancements into the post-genomic era are characterized by the challenge of reaping benefits from these genomic texts. Formidably a large data is generated from high throughput techniques, and it cannot be used efficiently in probing the plant genome and evolution without the aid of bioinformatics. The goal of this field is thus, to provide computational approaches and in silico methodologies for coping with, and interpreting this genomic data to develop new cost-effective, accessible, safe and reliable treatments for diseases such as cancer. A major aspect of cancer research focuses on studying clinically useful plant-derived anticancer agents and promising new plants with anticancer potential. Also, certain agents that have failed in earlier clinical studies are considered for evaluation to obtain novel anticancer drugs using bioinformatics approaches and this field has triggered more interest among researchers in recent years. The aim of this chapter is to merge the sphere of computer-based methods in 'omics' technologies with the anticancer analysis of plant sources, and also to cover the sophisticated bioinformatics softwares and tools adopted in the process.

Keywords Bioinformatics · Genomics · Metabolomics · Proteomics · Transcriptomics

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12.1 Introduction

With an increase in the number of cancer patients across the world, it has become more imperative to reap maximum benefits from anticancer agents. Plants are a crucial source for such compounds and are being researched vigorously for generating lead molecules in the drug development process (Katiyar et al. 2012; Atanasov et al. 2015). However, this process is impaired without the aid of advancing omics technologies that have made non-targeted identification of signaling molecules possible and much more efficient. The understanding of biological processes has improved substantially with the examination of genes and gene products associated with cell growth, apoptosis, necrosis and cellular metabolism. Providing major breakthroughs in the field of oncology, omics has led to an earlier detection and more accurate treatment of cancer (Sallam 2015; Armitage and Southam 2016; Dijkstra et al. 2016; Uzilov et al. 2016).

Genetics and molecular biology experienced a face lift with the emergence of genomics, an interdisciplinary field concerned with the study of genomes, the functions of genes as a unit of inheritance, their interactions with the environment and most importantly, techniques such as DNA sequencing and applications of recombinant DNA. Further, the flow of genetic information through transcriptional and post-transcriptional modifications of DNA gives an entire set of messenger RNA called transcriptome. While microarray analysis is highly prevalent, it is being replaced by RNA-Seq that incorporates next-generation sequencing. In the next step, mature mRNA is decoded by ribosomes, where translation occurs. The resulting proteins are researched through techniques like mass spectrometry and two-dimensional gel electrophoresis under proteomics, a rapidly growing field. Another aspect of post genomics is metabolomics, one of the newest 'omics' sciences. Concerned with the study of metabolites through methods like nuclear magnetic resonance spectroscopy, it explores cellular processes and the physiology of cells (Horgan and Kenny 2011). To analyze and interpret biological data from omics, and overcome the obstacle of integration of these technologies, bioinformatics is employed. With a wide range of applications in cancer and plant research, this multifaceted field has become indispensable. Thus, the aim of the chapter is to merge the sphere of computer-based methods in 'omics' technologies with the anticancer analysis of plant sources, and also to cover the sophisticated bioinformatics softwares and tools adopted in the process.

12.2 Omics in Anticancer Plants

Coupled with developments in technology, improved sample handling, validated study designs and statistical solutions for data interpretation, omics provide a holistic approach to the interplay between surroundings and human health (Vlaanderen et al. 2010). Since these involve examination of huge numbers of genes, their

associated expressions or proteins, the techniques involved are called high throughput. High-throughput screening methods provide effectual assessment of agents or conditions in biological assays (Szymanski et al. 2012) and are pivotal for the discovery of new chemotypes (Macarron et al. 2011). Coalescence of multiple omics data in higher plants is essential to remodel complex networks that typify the phenotypes in the cell. Genomics, transcriptomics, proteomics and metabolomics techniques pave the way for robust and practical plant metabolic engineering (Yonekura-Sakakibara et al. 2013). Exploitation of these technologies has led to the authorization and subscription of many new anticancer plant-derived materials as medicines, due to reduced side effects and efficacious chemoprevention activity (Fridlender et al. 2015).

12.2.1 Genomics in Anticancer Plants

Genome sequences incorporate critical information regarding plant origin, development and epigenomic regulation that serves as the cornerstone of decoding genome diversity and chemodiversity at the microscopic level (Hao and Xiao 2015). High-throughput sequencing of medicinal plants highlights the biosynthetic mechanisms of anticancer compounds, especially secondary metabolites (Boutanaev et al. 2015) and their regulatory pathways.

RAD-Seq (restriction-site associated DNA sequencing) (Rubin et al. 2012), a fractional genome sequencing strategy can construct a RAD library and carry out low-coverage genome sequencing of candidate species. This is an efficient approach for assessing the heterozygosity of the representative genome. Genetic map and physical map are other essential tools for the aggregation of complex plant genomes and research in functional genomics. The results obtained are used in map-based cloning. Other applications include metabolic gene mapping and marker-assisted selection of anticancer traits. This genomic data from the RAD-Seq and genotyping by sequencing (GBS) method can also be utilized to determine the origin and the spatial distribution pattern of existing anticancer plants (Hao and Xiao 2015).

A recent study states that sequencing of the entire genome of *Ocimum tenuiflorum* has helped to delineate that amino acid mutations present at the loci of genes involved in biosynthesis, confer exceptional pharmaceutical attributes to this herb. As a defense mechanism, this plant generates specialized metabolites like ursolic acid, luteolin, apigenin, taxol, oleanolic acid, sitosterol and eugenol that have anticancer potential. With the genome laid out, the specific genes responsible for curing cancer can be known and can be used to develop targeted drugs (Upadhyay et al. 2015).

Capsicum annuum (Qin et al. 2014), *Coffea canephora* (Denoeud et al. 2014), *Brassica napus* (Chalhoub et al. 2014) and *Phalaenopsis equestris* (Cai et al. 2015) are some representative plant species that have undergone whole-genome sequencing. A comparison between the genome sequences of cultivated pepper Zunla-1 (*C. annuum*) and its wild progenitor Chiltepin (*C. annuum var. glabriusculum*) gave an understanding about *Capsicum* domestication and differentiation. The *Capsicum*

reference genome, along with tomato and potato genomes, provides an insight into the evolution of other Solanaceae species, including the well-known *Atropa* medicinal plants. This strategy can be applied to probe a number of plant-derived substances exhibiting anticancer activity such as Camptothecin derivatives, Podophyllotoxin derivatives, Taxanes, Vinca alkaloids, etc. (Fridlender et al. 2015).

Microsatellite markers such as Simple Sequence Repeats (SSRs) are present in high frequency in transcribed regions of plants, especially in untranslated regions (UTR) and also possess the ability to associate with many phenotypes. Thus, they have several applications in plant genomics. They aid in plant reproduction, genome regulation, evaluation and organisation. Microsatellite markers have been identified for many anticancer plant families and genera, and these results are useful for gene evolution and protection of genetic resources of these plants. A recent Korean study focussed on *Viscum coloratum*, a hemiparasitic plant with anticancer properties and 19 novel polymorphic microsatellite loci were developed to aid this plant's ecological conservation and population genetics studies (Kim et al. 2017). Present in the genome sequences, these markers are crucial for recombination and quantitative genetic variation. Through the current study on evolutionary genomics, there is a great scope of improvement in resolution, making it possible to identify the particular genes responsible for specific innovations. More information on plant evolution may improve the understanding on botanical diversity, including medicinally important traits such as anticancer, antiallergic, anti-inflammatory, etc. properties (Hao and Xiao 2015). Genome sequencing of plant genomes has qualified as a reliable process to delve into a diverse set of concepts of medicinal importance.

12.2.2 Transcriptomics in Anticancer Plants

Whole-genome sequencing is an expensive process. It is also challenging when the genome comprises of a high proportion of repeat sequences, high heterozygosity and non-diploids (Chen et al. 2010). Large-scale comparative transcriptome studies of anticancer plants are more feasible than comparative genomics. Transcriptomics is thus, an effective strategy to retrieve genomic data from several non-model medicinal plants that do not have a reference genome. This information delineates certain relevant traits pertaining to secondary metabolite formation and for studying pharmaceutically important molecular mechanisms (Hao et al. 2015a).

Curcuma longa, commonly known as turmeric, is recognized as an herbal remedy and alternative medicine for cancer. Research has shown that this herbaceous member of the ginger family reduces incidences of gastrointestinal cancers due to the presence of secondary metabolite, Curcumin in its rhizomes. Rhizome transcriptomes of various varieties of *Curcuma longa* were sequenced via Illumina reversible dye terminator sequencing. This resulted in the availability of transcripts related to terpenoid biosynthesis and biosynthetic pathways of other anticancer phytochemicals like vinblastine, taxol and curcumin. This helped to reinstate the biosynthetic pathways to synthesize various terpenoids in *C. longa* and contributed to its tran-

scriptomic database(Annadurai et al. 2013).Also, researchers have used the phylotranscriptomic approach to test phylogenetic hypotheses to give information about the evolution of the fundamental anticancer plant traits attributed for their myriad chemodiversity (Hao et al. 2015b). Another medicinal plant species, *Chlorophytum borivilianum* that derives its medicinal value for its higher saponin content (approximately 17% by dry weight), exhibits antitumor and anticancer properties (Kumar et al. 2010) due to the presence of cytotoxic steroidal glycosides, saponinchloromaloside-A and spirostanol-pentaglycosides embracing beta-D-apiofuranose. High throughput transcriptome sequencing of its leaf RNA was performed through Illumina's HiSeq 2000 sequencing platform. Bioinformatics tools such as SOAP denovo, Contig Assembly Program (CAP3) assembler and Kegg Orthology-Based Annotation System (KOBAS) were used for the assembly and annotation of the transcriptome. Further, molecular insight into the flavonoid and steroid biosynthesis pathways of this endangered species and bioinformatics analysis showed that its combination with other herbs could be instrumental in oncological treatment (Kalra et al. 2013).

Transcriptomics using DNA microarray has become another effective tool for the study of anticancer plants because of high throughput, sensitivity, precision, specificity, and duplicability. It is implemented widely in Chinese herbal medicine and provides a practical approach that enables researchers to examine the expression of numerous genes concurrently (Lo et al. 2012).Whole transcriptome shotgun sequencing (WTSS), also known as RNA sequencing (RNA-seq), provides whole-transcriptome expression profiles of certain plant extracts as well, hence making integrated analysis of transcriptomics and metabolomics possible in any plant species (Yamazaki et al. 2013). It allows the probing of genes involved in metabolite synthesis of plant specialized products, and the integration of transcriptome data with metabolic profiling data sets to reveal the relationship between genes and metabolites in anticancer plants. Its prime feature is that it makes it possible to obtain gene sequences from plants without a reference genome. A 2013 study used Illumina-derived short read sequences for deep transcriptome analysis of cell suspension cultures and the hairy roots of *Ophiorrhiza pumila*, a Rubiaceae species that accumulates the anticancer monoterpenoidindole alkaloid, camptothecin. This yielded a 2GB sequence for each sample, and expedited prediction of new and novel genes committed in secondary metabolic pathways. Bioinformatics tools such as the Oases assembler, CAP3 program, Bowtie package and Cufflinks aided this research (Yamazaki et al. 2013).

One of the most medicinally important plants is the *Withania somnifera* that synthesizes bioactive secondary metabolites known as withanolides. Chemoprofiling of leaf and root tissues of this plant imply dissimilarities in the composition and properties of withanolides in various chemovars. To identify the genes involved in chemotype and tissue-specific withanolide biosynthesis, transcriptomes of leaf and root tissues of discrete chemotypes were established (Gupta et al. 2015). Several medicinal phytometabolites have also been discovered in the buttercup family, Ranunculaceae. Some of them like alkaloids, terpenoids, saponins, and polysaccharides, have expressed antitumorigenic behaviour both *in vitro* and *in vivo*.

Gene expression profiling and relevant transcriptomics platforms provided an insight into the distinctive effects of plant metabolites on cancer cells with varying physical characteristics (Hao et al. 2017).

12.2.3 Proteomics in Anticancer Plants

Proteomics is a powerful platform to analyse drug-regulated proteins on a large scale and investigate signalling pathway perturbations in cells. It mainly characterizes protein functions, protein-protein interactions *in vitro* and *in vivo*, and protein modifications, and has various applications in the research on anticancer plants (Lao et al. 2014). It can also substantiate post-translational protein modifications such as phosphorylation, glycosylation, acetylation, and proteolysis (Zhang et al. 2011). These modifications can occur as cancer progresses or after drug treatment, and can be analysed by proteomic approaches. The mechanism of action of a drug is studied through macro-analysis of protein alterations through proteomic technologies and through the identification of modified proteins as prospective drug targets (Lao et al. 2014). Traditional Chinese medicine (TCM) is an abundant source of anticancer drugs. Bioactive secondary metabolites isolated from TCMs project substantial antitumor effects, although their pathways are still ambiguous. Terpenoids, flavonoids, glycosides and other bioactive TCM products have been studied extensively via proteomics to describe their antitumor activities in various cancers. A significant number of natural agents extracted usually perform tumour-suppression by exclusively targeting mitochondria in malignant cells (Wang et al. 2015).

Analysis of natural flavones such as Baicalein, Tangeretin and Luteolin exhibit anticancer properties but their mechanisms of action are still unclear. However, a proteomic study delineated that Baicalein led to the up-regulation of peroxiredoxin-6, which reduced the generation of reactive oxygen species (ROS) and inhibits colorectal cancer cell proliferation (Huang et al. 2012). Another flavonoid, luteolin demonstrates similar anticancer activity against several forms of cancers, including human hepatic cancers. Proteomics is a multifunctional tool that thus, provides a methodical approach towards understanding the molecular mechanisms of TCM in tumor cells and investigating protein-drug interactions. Stable Isotope Labeling with Amino acids in Cell culture (SILAC) and Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) are chief quantitative approaches for the process (Wang et al. 2015).

Tripterygium wilfordii, a representative TCM has been widely and successfully used to treat numerous diseases such as rheumatoid arthritis and psoriasis. Its anticancer activity and intrinsic action mechanisms have also been investigated intensively and a proteomics study showed that diterpenoid epoxide triptolide, an important bioactive metabolite, has applications in curing colon cancer (Liu et al. 2011). Triptolide treatment induces cell division and the perinuclear translocation of 14–3-3ξ, a key protein pertaining to cell cycle arrest and cell death (Liu et al. 2012). The plant extracts of *A. Paniculata* have also been found to contain diterpene

compounds that encompass medicinally relevant properties against cancer, pathogenic bacteria, virus and hepatitis (Valdiani et al. 2012). There is a lack of comprehensive molecular genetic studies on this medicinal herb from the family Acanthaceae and thus, a huge volume of useful information is obtained from its protein profiling. Proteomic analysis is thus, an efficient methodology to obtain advanced knowledge on the inheritable traits and physiology of anticancer plants (Talei et al. 2014).

Periplocin, sourced from the bark and stems of *Periploca graeca*, can prevent both lung and colon cancer *in vitro* and *in vivo*. It exhibits anticancer effects on the cells via beta-catenin/TCF signalling pathway by inducing apoptosis. According to a study in 2014, quantitative proteomics technologies like tandem mass spectrometry and two-dimensional gel electrophoresis were used to explore the effect of periplocin treatment on human lung cancer cell lines A549. The western blot was used to authenticate the modified proteins and the protein-protein interactions between them were also investigated (Lu et al. 2014). The antioxidant, antineoplastic, antiangiogenic and particularly, anticancer properties of Curcumin and its derivatives have also been thoroughly researched. Several studies have used proteomics to investigate the potential of curcumin against different cancer cell lines. From a study in 2011, proteomic analysis distinguished 12 differentially expressed proteins that boost multiple functional activities in the MCF-7 breast cancer cell line. These functions include DNA transcription, mRNA splicing and translation, amino acid synthesis, protein synthesis, folding and degradation, lipid metabolism, glycolysis, and cell motility (Fang et al. 2011).

Berberine, a natural product obtained from the subterranean part of *Coptis chinensis*, has also gained interest due to its anti- proliferative properties. Differentially expressed proteins in HepG2 liver cancer cells investigated through proteomic analysis. The results revealed that berberine led to G_0 cell cycle arrest and apoptosis (Wang et al. 2016). Gambogic acid, a natural chemical extracted from the brownish or orange resin of *Garcinia hanburyi* was intensely investigated and found to hinder the growth of various cancer cells via multiple signalling pathways. This xanthonoid has shown promising antitumor activity in clinical trials (Chantarasriwong et al. 2010; Anantachoke et al. 2012; Chen et al. 2012). Research through applied proteomics has shown that stathmin could be a potential target of gambogic acid in hepatocellular carcinoma. Later, more than 80 compounds with anticancer potential were identified from the *Garcinia* species. Bioassay guided fractionation and systematic proteomic analysis aimed at studying the possible action mechanisms of these active compounds were employed. They showed that 1,3,6,7-tetrahydroxyxanthone, a bioactive metabolite isolated from *G. oblongifolia*, curtailed cell proliferation by the up-regulation of p16 and 14–3-3σ in hepatocellular carcinoma cells (Fu et al. 2012a). The proteomics data also revealed that 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone, which is also derived from *G. oblongifolia*, can induce cancer cell death by suppressing Heat shock protein 27 (Hsp27) (Fu et al. 2012b). Through 2-DE analysis, it has become evident that Hsp27 plays a crucial role. Tanshione IIA, a phenanthrene quinine extracted from the root of *Salvia miltiorrhiza* has also been shown to down-regulate Hsp27

expression in cervical cancer cells (Lao et al. 2014). In some cultures, dietary components such as fenugreek seeds are also used for the treatment of cancer. In 2014, the proteomic profiles of these seeds showed that an incidence of primary CNS T cell lymphoma reacted to fenugreek treatment and led to tumor regression. The *in vitro* effect of fenugreek as a substance that causes cancer cell destruction through cytotoxins, points to the significance of this seed as a treatment for cancer (Alsemari et al. 2014). Proteomics is thus, an essential tool to predict the protein targets of bioactive compounds present in anticancer plants.

12.2.4 Metabolomics in Anticancer Plants

Plants synthesize a vast number of primary and secondary metabolites. Hence, they are being probed extensively to find new chemical entities (NCEs) for drug discovery and development. As chemotherapeutics has many side effects such as fatigue, hair loss, resistance, mouth sores, nausea, blood disorders and nervous system effects, recent attention has shifted to plants that provide a good opportunity for complementary cancer cure (Tecza et al. 2015). More than 50% of anticancer drugs used in therapeutics today are sourced from natural products, whose relevance, however, was undermined due to the arduous method of conventional lead generation (Cragg and Newman 2013; Newman and Cragg 2016). Hence, a dire need of an efficient strategy for the detection of bioactive compounds was felt. Medicinal plant-based metabolomics, a rapidly emerging field, has become a study of prime importance since it has the potential to prevent natural product research from reaching an impasse, aid discovery of anticancer drugs and improve the effectiveness of lead-finding (Kim et al. 2010; Mukherjee et al. 2016; Okada et al. 2010). This approach is being exploited in a wide range of applications including medical science, synthetic biology, Ayurvedic medicine and predictive modelling of plant systems. The working principle of metabolomics basically involves sample preparation, separation of compounds, identification, data processing and finally analysis.

Several therapeutically important secondary metabolites like paclitaxel (taxol), camptothecin (irinotecan, topotecan) and podophyllotoxins (etoposide, teniposide), etc. have been investigatedand have reported to possess anticancer activity. The metabolic profiling and anti-tumorigenic activities of certain widely cultivated plants of the family Compositae were also evaluated. Anticancer potential was studied for human hepatocellular carcinoma (HepG-2) and breast adenocarcinoma (MCF-7) cell lines. Plant species portrayed variable metabolomic profiling. While *Artemisia* revealed the highest concentration of secondary metabolites, *Pulcaria crispa* was found to have the most effective *in vitro* anticancer activity. The latter depicted the maximum inhibition concentration of 50% (IC_{50}) in comparison with the extracts investigated against these cell lines (El-Naggar et al. 2015). With several applications in cancer research such as identification of biomarkers for disease detection, supervision of drug response and analysis of potential cytotoxic effects, metabolomic analysis can carry out extensive studies in a non-invasive way. It dis-

seminates knowledge about cancer metabolites involved in the *in situ* methodology. It is based on the analysis of the metabolic fingerprint of cancer cells before and after treatments with plant extracts. Nuclear Magnetic Resonance (NMR) spectroscopy based metabolomics is effective for the juxtaposition of metadata of two different measurements to detect any changes. The spectra obtained can be subtracted to reveal the new signals using Heteronuclear Single Quantum Coherence (HSQC) spectroscopy (Kim et al. 2010). According to a study in 2016, NMR spectroscopy and multivariate analysis methods were also used to study the metabolic profile and inhibitory effects of *Aloe Vera* on Raji cells with an IC_{50} of 40 μg/ml. The metabolome elaborated on the influence of the surrounding conditions on the genome of an organism. It was reported to exert influence on human hepatocellular carcinoma cells by increasing expression of p53 and Bcl-2 gene. A wonder plant with many beneficial properties, it has significant anticancer and anti-tumorigenic properties (Noorolahi et al. 2016).

Certain secondary metabolites are not detected in plants due to low therapeutic activity and low concentrations. However, because of several intrinsic constituents in herbs and their formulations, synergistic biological activities are produced. In such cases of drug analysis, metabolomics acts as an efficient strategy to comprehend the phytochemical basis of a spectrum of these therapeutically active plant constituents. Owing to the technological boom and high-tech breakthroughs in the isolation and detection of metabolites, the technique of metabolomics is thus, rapidly emerging as a powerful platform for exploitation of anticancer plants, for biomarker-driven drug discovery and development. It offers a promising approach to plant metabolite fingerprinting and such research is urgently needed for better utilization of anticancer plants (Mukherjee et al. 2016).

12.3 Need for Bioinformatics

With emerging ground-breaking technologies, current cancer research has plunged into the era of systems biology, which is characterized by massive data generation through omics advances. In the last decade, with developments in modern biological technologies such as pyrosequencing, next generation sequencing and third generation sequencing (Fernandes et al. 2011), scientific discoveries have gained great momentum and sequence generation has become more cost-efficient and speedy. But these high throughput sequencing studies also face drawbacks such as data management, interpretation and quality control (QC) issues. Rapid medical achievements in the laboratory regarding the framework of diseases like cancer are certainly laudable. But, they have not necessarily translated into effective ‘treatment’ breakthroughs. Data sets these days extend from tens to hundreds of gigabytes per run due to which, data storage is another major concern. In other words, biological data are exploding, both in size and complexity. So, next generation analytical tools demand improved robustness, flexibility and cost efficiency. Comprehensive omics investigations, to understand cancer progression and treatment response, integrated

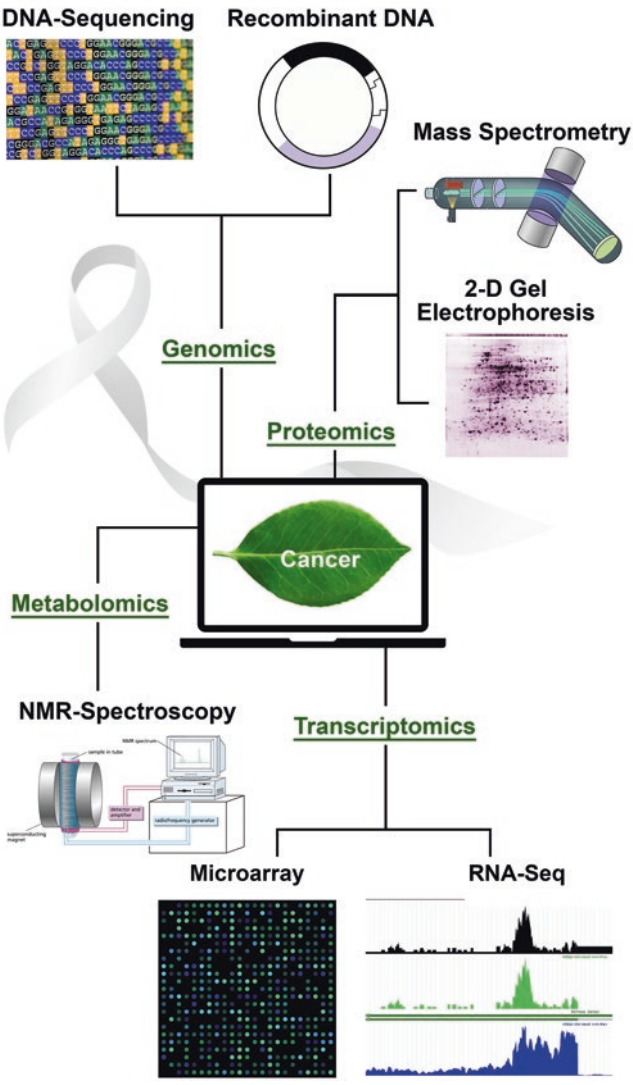

Fig. 12.1 Integration of omics technologies with bioinformatics in research of anticancer plants

with the power of bioinformatics allow exploitation of these achievements for future diagnostic, prognostic and therapeutic purposes. The challenges posed are being tackled effectively by incorporation of bioinformatics, computational biology and wet lab sciences (Al-Haggar et al. 2013) to understand the molecular and genetic bases of diseases like cancer (Fig. 12.1). They also help to enable system-level understanding correlation and dependencies between the molecular components involved.

Network analysis has applications in mining of biological knowledge from these data with high-fidelity and performing data-driven biology. Coupled with the newly emerging genome editing advances and exhaustive gene expression using microarrays, it has become a standard tool for studying gene function as well as metabolic pathways that promote cancer cell survival and growth (Yonekura-Sakakibara et al. 2013). Bioinformatics also allows researchers to search biologic databases, compare gene sequences and protein data on a vast scale in order to determine sequences or proteins that vary among cancerous and physiologic cells and tissues or different phenotypes of the same disease. In conjugation with omics, it is used for identification of therapeutic targets and drug design. Relevant bioinformatics technologies have really transformed extant thinking on nuclear genome, transcriptome, proteome and metabolome evolution.

12.4 Bioinformatics Approaches for Applications of Anticancer Plants

There are several bioinformatics tools, programs, web servers, softwares and pipelines that have contributed towards genomics and postgenomicsstudies on anticancer plants or have the potential to do so.

12.4.1 Genomics Applications

This section states the bioinformatics approaches for genomics applications of anticancer plants (Tables 12.1 and 12.2).

12.4.1.1 Genome Assembly

12.4.1.1.1 Phrap

Phrap, a program that assembles shotgun DNA sequence data, can handle large datasets. It permits utilization of the read as a whole and not just the trimmed high quality part. In the presence of repeats, it improves the process of assembly by using a combination of user-supplied and internally computed data quality information. Instead of a consensus, it forms the contig sequence as an assortment of the highest quality parts of the reads. Phrap also provides information regarding assembly to also carry out trouble-shooting of assembly programs successfully (Machado et al. 2011).

Table 12.1 Bioinformatics approaches for classified genomics applications of anticancer plants

Genomics applications	Bioinformatics approaches	References
Genome assembly	Phrap, CAP and CAP3, BaCCardI, Velvet, SOAPdenovo and SOAPdenovo2	Li et al. (2010), Zerbino (2010) and Machado et al. (2011)
Whole genome sequencing analysis	SeqMap, RMAP, ZOOM, NGSEP, MAQ, SAMtools	Duitama et al. (2014) and Perea et al. (2016)
Nucleotide sequence homology search	BLASTN, TBLASTX, BLASTP, TBLASTN	Madden (2013)
Comparative genome visualisation	SynBrowse, CMap and CMap3D	Duran et al. (2010), Segal et al. (2012) and Lee et al. (2016)
RAD-Seq	pyRAD, RADIS, stacks	Eaton (2014)
Protein-coding gene prediction	Augustus, Exonerate	Cruaud et al. (2016)
Tandem repeat prediction	PolySSR, SA-SSR, SAT, poly	Pickett et al. (2016)
Synteny block detection	SyMAP, SynChro	Soderlund et al. (2011) and Drillon et al. (2014)

Table 12.2 Bioinformatics approaches for other genomics applications of anticancer plants

Other genomics applications	Bioinformatics approaches	References
Sequence alignment	Consed	Gordon and Green (2013a, b)
Genetic mapping	MultiPool	Edwards and Gifford (2012)
Genotyping by sequencing (GBS) analysis	Tassel-GBS	Glaubitz et al. (2014)
Genome annotation pipeline	MAKER	Campbell et al. (2014)
Fragment recruitment	Bowtie	Langmead (2010)

12.4.1.1.2 Contig Assembly Program (CAP) and CAP3

The Contig Assembly Program finds the shortest common superstring of a set of fragments. It supports DNA shotgun sequencing with the help of a filter that removes DNA fragment pairs that cannot overlap. For the remaining fragments, computation of maximal-scoring overlapping alignment is done. Then, the fragments are assembled in order of alignment scores. The CAP sequence assembly program has been improved to give the CAP3 program. It uses forward-reverse constraints to rectify assembly errors and connect contigs, and base quality values to compute overlaps between sequence reads, create multiple sequence alignments of reads and give consensus sequences. CAP3 also has the ability to automatically clip 5′ and 3′ poor regions of reads. Compared to Phrap, it is better at constructing scaffolds on low-pass data.

12.4.1.1.3 BAC Card I

Though there are several tools that carry out post-processing of sequence assemblies, there aren't as many for construction of virtual clone maps from assembly data. These maps are useful for whole genome shotgun assembly validation and integration of different types of genomic data. The BAC Card I tool is used for this purpose. It computes contig scaffolding, looks for misassemblies and carries out intergenome comparison between related strains as well.

12.4.1.1.4 SOAP Denovo and SOAP Denovo 2

SOAP denovo is a method to assemble short-reads, especially from Illumina GA. It can carry out analyses of large plant genomes that have not been explored. However, it needed several improvements to be more efficient. This is solved by its successor, SOAPdenovo2, which has enhanced accuracy, continuity and coverage, especially for repeat regions in contig assembly and scaffold construction (Li et al. 2010).

12.4.1.1.5 Velvet

Velvet is a novel set of de novo assembly methods. It is based on the manipulation of de Brujin graphs for short read sequencing data. It can remove errors, build contigs and simplify repeated regions both in the presence and absence of read pair information. It is particularly useful when an organism without a reference genome needs to be studied (Zerbino 2010).

12.4.1.2 Whole Genome Sequencing Analysis

12.4.1.2.1 SeqMap

SeqMap can be used for mapping millions of short sequences to a genome of billions of nucleotides, using an index-filtering algorithm. It detects the positions in the reference genome where the sequences are located along with several substitutions, insertions and deletions of nucleotide bases. This is extremely important for high-throughput sequencing analysis.

12.4.1.2.2 RMAP

RMAP is aimed at accurately mapping paired-end reads from next-generation sequencing technology. It uses more advanced quality scores that are indicators of error probability. The length of the read must be between 20 and 64bp. The user can fix the maximum number of mismatches between a read and the location to which it maps.

12.4.1.2.3 Zillions of Oligos Mapped (ZOOM)

With next generation sequencing technologies, huge amounts of short reads are generated. However, for the identification of SNPs or unpopular transcripts, there is a need for software that can map these to a reference genome. ZOOM is used for mapping of oligonucleotides back to the genomes through spaced seeds. It is unparalleled in speed and very sensitive. It has applications in personalized medicine for cancer and re-sequencing.

12.4.1.2.4 Next Generation Sequencing Eclipse Program (NGSEP)

A need was felt for this bioinformatics software tool to carry out accurate and efficient analysis of data generated by high throughput sequencing simultaneously with every GBS protocol. NGSEP is useful for genetic mapping of complex traits and other downstream analyses as well. It can play a major role in genetic improvement of anticancer plants and genomic selection through phenotype prediction (Duitama et al. 2014; Perea et al. 2016).

12.4.1.2.5 Mapping and Assembly with Quality (MAQ)

Particularly designed for Illumina-Solexa 1G Genetic Analyzer, Maq is a program for measuring alignment quality of reads generated by next-generation sequencing technologies. It does ungapped alignment at the mapping stage and calls the consensus at assembling stage. There are up to two or three mismatches in the hits in case of single-end reads and around one in case of paired-end reads.

12.4.1.2.6 SAMtools

This is a set of programs for interacting with high-throughput sequencing data. The DNA sequence read alignments can be in SAM (Sequence Alignment/Map), BAM (Binary Alignment/Map) and CRAM formats. They can be viewed, sorted and indexed with the help of the provided tools, and are also inter-convertible. Two key features are that there is a provision to operate compressed BAM files without having to un-compress them and there is also a provision to compress large SAM files for saving space.

12.4.1.3 Nucleotide Sequence Homology Search

12.4.1.3.1 Nucleotide-Nucleotide Basic Local Alignment Search Tool (BLASTN)

The user specifies a nucleotide sequence. BLASTN searches the nucleotide database to carry out comparison with the nucleotide query (Madden 2013).

12.4.1.3.2 Nucleotide 6-Frame Translation-Nucleotide 6-Frame Translation (TBLASTX)

This program is the slowest one in the BLAST family since it incorporates a complex algorithm. The user specifies a nucleotide sequence. TBLASTX translates this query in all six possible reading frames and compares it to all six reading frames of the nucleotide sequence database. This is helpful in detecting distant relationships between nucleotide sequences (Madden 2013).

12.4.1.3.3 Protein-Protein Basic Local Alignment Search Tool (BLASTP)

The user specifies a protein sequence. BLASTP searches the protein sequence database to carry out comparison with the protein query (Madden 2013).

12.4.1.3.4 Protein-Nucleotide 6-Frame Translation (TBLASTN)

The user specifies a protein sequence. TBLASTN compares this query with all six reading frames of the nucleotide sequence database. This is helpful in identification of proteins in uncharacterized genomes (Madden 2013).

12.4.1.4 Comparative Genome Visualisation

12.4.1.4.1 Connectivity Map (cMap) and cMap3D

cMap is a graphical utility for comparing genetic and physical maps within and between related species. It has three components – database cMapDB that can be used for a variety of mapping applications, the user interface and a data retriever. This tool specifies loci, positions and linkage groups. However, it has a limitation of carrying out comparison of only adjacent aligned maps. CMap3D solves this

problem by allowing comparsion of several genetic maps in three-dimensional space (Duran et al. 2010; Segal et al. 2012).

12.4.1.4.2 SynBrowse

SynBrowse is a tool for generic sequence comparison that helps in the visualization and analysis of genome alignments within and between species. It is useful for the user to study synteny, homologous genes and other conserved elements between sequences. By carrying out comparison with a reference genome, it also helps in identifying unspecified genes, putative regulatory elements and distinguishable features of a species (Lee et al. 2016).

12.4.1.5 Restriction Site Associated DNA Sequencing Analysis (RAD-Seq)

12.4.1.5.1 pyRAD

pyRAD is a software for the assembly of de novo RAD-seq loci. It has the ability to assemble datasets at fast pace due to its parallel processing and optional hierarchical clustering methods. This pipeline has an advantage over Stacks since there is no disruption of homologous loci clusters by indels in PyRAD (Eaton 2014).

12.4.1.5.2 Radis

Illumina sequencing generates large amounts of data which can be processed for phylogenetic inference using the perl pipeline, RADIS. It makes exploration of RAD-Seq data easier and faster. However, it depends on Stacks for eliminating PCR duplicates, establishing loci and separating data from the multiplex system (Cruaud et al. 2016).

12.4.1.5.3 Stacks

Stacks are a widely used pipeline for converting short-read sequences produced by the Illumina platform to specific loci. Its main function is de novo assembly of RAD sequences for genetic maps. It can be used to examine anticancer plants that have or do not have a reference genome and is hence, flexible software. It can also be used for conducting population genomics to understand adaptation in wild plants with anticancer properties.

12.4.1.6 Protein-Coding Gene Prediction

12.4.1.6.1 Augustus

AUGUSTUS is a web server that predicts genes in eukaryotic genomic sequences by the means of a Generalized Hidden Markov Model (GHMM), a generative probabilistic and statistical model. It helps the server by considering both intrinsic and extrinsic information to give hints about potential protein-coding regions. Finally, the most likely gene structure that is in accord with the constraints specified by the user is the result though there are cases in which no such structure exists. The constraints are significant especially when only a segment of the gene structure is known.

12.4.1.6.2 Exonerate

Exonerate is a genome annotation tool for protein-coding gene prediction. It allows pairwise sequence juxtaposition and gives the user the ability to align sequences using several alignment models that involve either dynamic programming or are a hands-on interaction.

12.4.1.7 Tandem Repeat Prediction

12.4.1.7.1 PolySSR

PolySSR is a novel approach to identify short sequence repeats (SSRs) as well as putatively polymorphic SSRs. The information regarding sequences is derived from public EST databases that include heterozygous individuals and different genotypes. The success rate for potential polymorphic SSR markers is elevated since it considers the presence of SNPs in the flanking regions for PCR-primer design.

12.4.1.7.2 SA-SSR

SA-SSR is accurate and comprehensive software for efficient detection of SSRs in a database with multiple sets of sequences. It is based on suffix and longest common prefix arrays and addresses most of the problems faced by extant tandem repeat prediction algorithms. One of its key features is that it gives a lot of control to the user (Pickett et al. 2016).

12.4.1.7.3 SSR Analysis Tool (SAT)

Microsatellites are one of the most powerful genetic markers. One method for their development is by constructing genomic DNA libraries, followed by DNA sequencing to get SSR markers from the bulk sequence data. But this is a highly intensive and slow process. The SSR Analysis Tool (SAT), a Web application, addresses this challenge effectively. It facilitates the integration, analysis and display of sequence data from these libraries. It can also design PCR primers specifically.

12.4.1.7.4 Poly

There are many programs that detect SSRs tracts but are unable to analyse the results quantitatively. Poly is a tool that carries this out effectively by easily quantifying the frequencies at which SSRs are located relative to other DNA sequence elements, and the tract length as well. It is not a fast program but is technically sounder than its substitutes.

12.4.1.8 Synteny Block Detection

12.4.1.8.1 Synteny Mapping and Analysis Program (SyMAP)

SyMAPv4.0 is a software package that detects, computes, displays, analyses and queries genome syntenic relationships. It has applications only in medium-to-high divergent eukaryotic genomes, and is particularly for studying monocotyledons and eudicotyledons. It has multiple display modes such as circular, side-by-side, dotplot, closeup, etc. (Soderlund et al. 2011).

12.4.1.8.2 SynChro

This tool is created to reconstruct conserved synteny blocks between comparisons of genomes in pairs. It is formulated on a method that simply calculates the Reciprocal Best-Hits (RBH) to build the synteny blocks. It then, finishes off these blocks with non-RBH syntenic homologs automatically. SynChro functions at a fast pace by adjusting a few parameters only and with the help of multiple essential visualization tools (Drillon et al. 2014).

12.4.1.9 Others

12.4.1.9.1 Consed

After sequence assemblies are created with phrap, they need to be viewed. The user should be directed to the variant sites one by one. The incorrect assemblies need to be detected, edited and aligned. All this is done with the help of Consed tool. It picks primers for amplification and templates efficiently. Furthermore, it includes BamScape, which is able to view bam files with unlimited number of reads as well as promote editing of the reference sequence in targeted regions (Gordon and Green Gordon and Green 2013a, b).

12.4.1.9.2 MultiPool

MultiPool is used for mapping of genetic elements from pooled genotyping. When sequencing depth and marker spacing are varied, noise levels become more non-uniform. In such a case, this computational method is used. It allows information sharing across the variant locations as well and uses a dynamic Bayesian network (DBN). This method can be extended to any number of replicates (Gordon and Green Gordon and Green 2013a, b).

12.4.1.9.3 Tassel-GBS

The bioinformatics pipeline, Tassel-GBS was designed to identify and call SNP genotypes by processing next-generation raw genotyping by sequencing data. It can aid GBS in the process of breeding anticancer plants and studying genomic diversity. This method has extremely high utility primarily for two reasons. Firstly, it can be used by those who do not have access to sophisticated computing resources. Secondly, it has the ability for large analyses but it can also be run at smaller scales (Glaubitz et al. 2014).

12.4.1.9.4 Maker

MAKER is a pipeline that allows plants with less complex genomes to annotate their genomes and create databases. It is portable, easily trainable and configurable. In the initial runs, the outputs automatically develop the gene prediction algorithm leading to a better quality gene model in the following runs, and are directly entered into the Generic Model Organism Database (GMOD). The quality indices of the annotations play an instrumental role in the functioning of MAKER (Campbell et al. 2014).

12.4.1.9.5 Bowtie

The Bowtie package is an extremely fast and memory-efficient bioinformatics tool. It aligns short sequencing reads output by next generation sequencing technologies. These alignments can be used to build genome indices and call consensus sequences (Langmead 2010).

12.4.1.9.6 Blast2GO

Blast2GO is an all-inclusive bioinformatics platform for the functional annotation of genomic datasets through high-throughput technologies. Subsequently, data mining is done on the output based on GO vocabulary. With an interactive and user-friendly interface and easy operability, it is a highly suitable software tool for plant genomics research. One of the prime reasons for this is that it can be extended to a vast number of species and be customised accordingly.

12.4.2 Transcriptomics Applications

This section states the bioinformatics approaches for transcriptomics applications of anticancer plants (Table 12.3).

Table 12.3 Bioinformatics approaches for transcriptomics applications of anticancer plants

Transcriptomics applications	Bioinformatics approaches	References
Gene expression analysis	DAVID, EGAssembler, KOBAS	Xie et al. (2011)
miRNA prediction	IPA, psRNATarget	Henderson-Maclennan et al. (2010) and Dai and Zhao (2011)
Spliced read alignment	SpliceMap, TopHat and TopHat2	Au et al. (2010) and Kim et al. (2013)
De novo transcriptome assembly	Oases, and SOAPdenovo-trans	Schulz et al. (2012) and Xie et al. (2014)
Alternative splicing	Cufflinks	Trapnell et al. (2010)
miRNA prediction pipeline	miRCat and miRCat2	Paicu et al. (2017)

12.4.2.1 Gene Expression Analysis

12.4.2.1.1 Database for Annotation, Visualisation and Integrated Discovery (DAVID)

DAVID is a web-based program that provides functional annotation tools for large lists of genes or proteins. It facilitates the transition from data collection to biological meaning. Its tools summarize the datasets according to Gene Ontology terms, protein functional domains and motifs, and biochemical pathways. They also associate genes with diseases, detect interacting proteins and explore gene names in batch.

12.4.2.1.2 EGAssembler

EGAssembler is a web server which analyses expressed sequence tags and has the ability to process large volumes of data rapidly. It can merge and align genomic fragments generated from shotgun sequencing to give the initial sequence. It has in-built tools as well as tools that can be modified by the user. They are capable of clustering and EST assembly as well.

12.4.2.1.3 KeggOrthology-Based Annotation System (KOBAS)

KOBAS is a web server that has a reservoir of knowledge regarding diseases, gene ontology and enriched pathways, derived from significant databases like KEGG, BioCyc, OMIM, etc. Its improved versions - KOBAS 2.0 and KOBAS 3.0 can be accessed online. It is a popular tool for gene expression analysis that performs statistical tests to give output (Xie et al. 2011).

12.4.2.2 miRNA Prediction

12.4.2.2.1 Ingenuity Pathway Analysis (IPA)

IPA is a powerful web-based software application that has a wide range of features that enable searching, viewing, modelling, understanding, analysing and visualising complex omics' data. This data can be derived from miRNA and SNP microarrays, RNA-Seq and other omics experiments. It is a popular tool that provides an insight into biological and chemical interactions pivotal to research, and discovers the processes for diseases like cancer, neurological disease, cardiovascular disease, etc. (Henderson-Maclennan et al. 2010).

12.4.2.2.2 psRNATarget

psRNATarget is a novel miRNA target prediction server that features the ability to differentiate between translational and post-translational inhibition. This tool is designed for the analysis of data generated from high-throughput sequencing and transcriptomics technologies. It allows reverse complementary matching between non-coding RNA and the RNA that has already been transcribed, along with the evaluation of the target site (Dai and Zhao 2011).

12.4.2.3 Spliced Read Alignment

12.4.2.3.1 TopHat and TopHat 2

TopHat is a bioinformatic tool for high throughput alignment of shotgun transcriptomiccDNA sequencing reads. It does fast splice junction mapping for RNA-Seq reads by alignment carried out as a two-fold process. Firstly, unspliced reads are aligned with the ultra high-throghput short read aligner Bowtie. Then, the mapping reads are analyzed to discover RNA splice junctions de novo. An improved version of TopHat is TopHat2, which can align reads of various lengths across small indels accurately and produce sensitive alignments even for unfavourable genomes (Kim et al. 2013).

12.4.2.3.2 SpliceMap

SpliceMap is a highly sensitive de novo splice junction discovery tool. It offers support for arbitrarily long RNA-seq read lengths. Though more inclined toward mammalian genomes, it can also be used on anticancer plant genomes (Au et al. 2010).

12.4.2.4 De Novo Transcriptome Assembly

12.4.2.4.1 SOAPdenovo-Trans

RNA-Seq had become a favorable method to tackle the increase in throughputs cost-effectively. But short reads from next generation sequencing make their assembly to recover full transcript sequences a herculean task. SOAPdenovo-Trans, a *de novo*transcriptome assembler, has been designed specifically to solve this problem. It adjusts with alternative splicing and variable expression levels amid transcripts. This assembler is faster and provides higher contiguity as well as lower obsolescence (Xie et al. 2014).

12.4.2.4.2 Oases

Oases is a de novo transcriptome assembler that performs its functions even when there is no reference genome. It assembles RNA-Seq reads from technologies such as Illumina, SOLiD or 454 to produce transcripts. It uses an array of hash lengths, a dynamic noise filter, a resolution of alternative splicing events and merging of multiple assemblies (Schulz et al. 2012).

12.4.2.5 Others

12.4.2.5.1 Cufflinks

Cufflinks is a great tool for measurement of de novo transcript isoform expression. It carries out assembly of transcripts by assembling the alignments into transcript sets. It is also responsible for estimating their abundances and determining differential expression. Cufflinks regulates RNA-Seq samples by accepting the aligned reads. Then, depending on how many read support each transcript, their relative abundances are estimated (Trapnell et al. 2010).

12.4.2.5.2 miRCat and miRCat2

miRCat can identify mature miRNAs and their precursors from high-throughput plant sRNA datasets. Thus, it does not require a putative precursor sequence since it is predicted by the program itself. A tool depicting high sensitivity and specificity, miRCat searches for sRNA-covered genomic regions after the sequences are mapped to the input genome. After a list of loci has been created, it is probed further for likely miRNA candidates. All reads must have a certain abundance, whose level can be varied using the minimum abundance parameter. Finally, the sRNA read with the most abundance within a locus is identified as the likely miRNA. miRCAt2, an improved version of miRCat, has much lower high false positive and false negative rates, and predicts miRNA loci using a novel entropy-based approach (Paicu et al. 2017).

12.4.3 Proteomics Applications

This section states the bioinformatics approaches for proteomics applications of anticancer plants (Table 12.4).

Table 12.4 Bioinformatics approaches for proteomics applications of anticancer plants

Proteomics applications of Anticancer Plants	Bioinformatics approaches	References
Protein sequence analysis	Cd-hit, CDD	Fu et al. (2012a, b, c) and Marchler-Bauer et al. (2013, 2015)
Protein-protein interaction prediction	STRING, pathway studio	Henderson-Maclennan et al. (2010) and Szklarczyk et al. (2015)
Protein database search	Psi-blast, BLASTX	Madden (2013)
Mass spectrometry based proteomics	MassLynx, mascot, MFPaq, IsobariQ	Arntzen et al. (2011)
Protein-protein docking	HexServer	Macindoe et al. (2010)
Protein-structure analysis	MODELLER	Webb and Sali (2014, 2016)

12.4.3.1 Protein Sequence Analysis

12.4.3.1.1 Cluster Database at High Identity with Tolerance (CD-HIT)

CD-HIT is a popular program that clusters and compares proteins that meet a similarity threshold to reduce redundancy and correct the bias within a dataset. This enhances the analyses of other sequences as well and helps the user in understanding data structures. It can handle large databases and works at a fast pace, hence reducing manual curation (Fu et al. 2012c).

12.4.3.1.2 Conserved Domain Database (CDD)

Conserved Domain Database is a public resource that incorporates domain models derived from databases like Pfam, SMART, etc. It is primarily responsible for proteins' annotation. One of its key features is that it can identify conserved domains in protein sequences. The NCBI-curated domains use three-dimensional structural data to elucidate on structure relationships (Marchler-Bauer et al. 2013, 2015).

12.4.3.2 Protein-Protein Interaction Prediction

12.4.3.2.1 Search Tool for the Retrieval of Interacting Genes/Proteins (STRING)

STRING is a biological database and web resource that evaluates and integrates physical and functional protein-protein interactions from co-expression data. These associations can be predicted or can be previously known, and are statistically analyzed by the confidence scores generated. STRING incorporates classification systems like GO, Pfam and KEGG as well as sources like experimental data, computational prediction methods and public text collections. It is accessible to all and is updated within regular intervals of time (Szklarczyk et al. 2015).

12.4.3.2.2 Pathway Studio

Pathway Studio is pathway software developed for the visualisation of proteomics data and analysis of protein interaction maps. It has applications in interpretation of gene expression, metabolomics and other high throughput data as well. It is most useful for budding researchers who wish to gain further insight into their independent discoveries and prevents them from carrying out investigations about subjects that have already been deeply explored (Henderson-Maclennan et al. 2010).

12.4.3.3 Protein Database Search

12.4.3.3.1 Position-Specific Iterative BLAST (PSI-BLAST)

PSI-BLAST is tool similar to BLAST with the difference that the former uses position-specific scoring matrices (PSSM) generated during the search. It also searches the protein databases one by one for sequences matching the protein query. It can search the target databases several times using multiple alignments of sequences above a certain score threshold. With every round of searching, a new PSSM is generated. This PSSM is further used to search the database for new matches (Madden 2013).

12.4.3.3.2 Nucleotide 6-Frame Translation-Protein (BLASTX)

The user specifies a nucleotide sequence. BLASTX searches the protein databases to carry out comparison with the translated nucleotide query. It occurs in one step itself (Madden 2013).

12.4.3.4 Mass Spectrometry Based Proteomics

12.4.3.4.1 MassLynx

MassLynx software manages, edits, analyzes and shares mass spectrometry information. It improves the MS system with features like instinctive interface and good instrument control. This software package is extremely versatile and flexible.

12.4.3.4.2 Mascot, and Mascot File Parsing and Quantification (MFPaq)

Mascot software is a benchmark for the identification of proteins from primary sequence databases. This powerful search engine is used for characterization and quantitation of proteins using mass spectrometry data. Unlike its contemporaries, it is capable of integrating all proven search methods like peptide mass fingerprinting,

sequence query and identification of fragment ions from uninterpreted MS/MS data (MS/MS Ion Search). The Mascot result files can be easily verified by the web application MFPaQ. It also carries out data quantification using isotopic labeling methods like stable isotope labeling with amino acids in cell culture (SILAC) or isotope-coded affinity tags (ICAT). It can also do so via label-free approaches such as spectral counting or MS signal comparison. MFPaQ also creates and juxtaposes non-redundant protein lists.

12.4.3.4.3 IsobariQ

Isobariq involves isobaric labeling of proteins that plays an integral role in quantitative mass spectrometry. It has applications in data derived from isobaric peptide termini labeling (IPTL), isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass tags (TMT). The user can study the proteomes extensively and through an interactive graphical user interface. This tool uses variance stabilizing normalization (VSN) algorithms (Arntzen et al. 2011).

12.4.3.5 Others

12.4.3.5.1 HexServer

HexServer is a protein docking server that blazed a trail. Based on the mathematical concept of the Fourier-transform, it was the first one of its kind to be powered by graphics processers. Structures can be inputted from the protein data bank (PDB) to generate a series of docking predictions without the need of any sophisticated computing infrastructure (Macindoe et al. 2010).

12.4.3.5.2 MODELLER

MODELLER is a computer program used for analysis of three-dimensional protein structures and in some cases, quaternary structures as well. The user inputs a sequence alignment that is then, modelled homologically. This software tool is based on satisfaction of spatial restraints, a method that has been derived from the transition of NMR spectroscopy data to three-dimensional structures (Webb and Sali 2014, 2016).

12.4.4 Metabolomics Applications

This section states the bioinformatics approaches for metabolomics applications of anticancer plants (Table 12.5).

Table 12.5 Bioinformatics approaches for metabolomics applications of anticancer plants

Metabolomics applications	Bioinformatics approaches	References
Metabolite library	ReSpect, METLIN	Sawada et al. (2012)
Metabolomic network data and analysis	MetaCrop, MetaCore	Schreiber et al. (2012)
Mass spectrometry based metabolomics	MetPa, MetaboAnalyst	Xia and Wishart (2010) and Xia et al. (2012, 2015)
NMR-based metabolomics	ProMetab, MetaboHunter	Tulpan et al. (2011)

12.4.4.1 Metabolite Library

12.4.4.1.1 RIKEN Tandem Mass Spectral Database (ReSpect)

RIKEN tandem mass spectral database is an online database of tandem mass spectroscopy data that has applications in plant metabolomics research. One of its major uses is that it helps in narrowing down complex phytochemical structures to candidate structures. It is constructed on the basis of a fragmentation association rule that helps in assessing confidence levels of annotations obtained by the user, as well as the structural characterization of metabolites (Sawada et al. 2012).

12.4.4.1.2 Metabolite and Tandem MS Database (METLIN)

METLIN is a repository that stores and manages data pertaining to metabolites as well as tandem mass spectrometry. With multiple searching capabilities, it provides MS/MS data at varying collision energies through a positive as well as a negative ionization approach. It helps the user with basic information such as the name, theoretical mass, chemical formula, structure and elemental composition of the metabolite along with its fragment structure. METLIN is also linked with databases like KEGG and HMDB to help the user in compound identification.

12.4.4.2 Metabolomic Network Data and Analysis

12.4.4.2.1 MetaCrop

MetaCrop is a manually-updated database that not just provides high-quality information about metabolic pathways, but also allows its automatic export for building metabolic models. It aids plant metabolomic research by improving yields of crops with potential anticancer properties. It also helps the user with details such as the location of the crop, its transport and related reaction kinetics. Its successor, MetaCrop 2.0 was released in 2011 (Schreiber et al. 2012).

12.4.4.2.2 MetaCore

MetaCore is integrated software suite based on a database of chemical metabolism, molecular interactions and pathways. This database is regularly updated and manually managed. It also provides information regarding toxicity, gene-disease associations, functional analysis of Next Generation Sequencing and many more molecular classes.

12.4.4.3 Mass Spectrometry Based Metabolomics

12.4.4.3.1 MetPa

An integration of statistical enrichment methods with pathway topological characteristics, MetPA is a user-friendly and fully-featured tool. It is responsible for studying relevant metabolic pathways and aids data visualisation through a network system with several features just like Google Maps. It makes data analysis possible by generating a report automatically with the help of statistical procedures (Xia and Wishart 2010).

12.4.4.3.2 MetaboAnalyst

MetaboAnalyst is an online pipeline for analysis and interpretation of data generated from high-throughput metabolomics technologies. Its successors, MetaboAnalyst 2.0 and MetaboAnalyst 3.0 were released in January 2012 and April 2015 respectively. It offers numerous approaches for processing, normalization and annotation of the metabolomic data. It involves advanced statistical analysis methods for carrying out metabolomic studies (Xia et al. 2012, 2015).

12.4.4.4 Nuclear Magnetic Resonance (NMR) Based Metabolomics

12.4.4.4.1 ProMetab

ProMetab is a nuclear magnetic resonance based metabolomics tool that is responsible for data processing. It facilitates the transition from raw NMR spectra to a format for multivariate chemometric and biometric analysis.

12.4.4.4.2 MetaboHunter

MetaboHunter is a user-friendly and freely accessible web server application that enables automatic identification in H-NMR spectra of metabolites. It provides fast-paced metabolic fingerprinting and several effective search methods. Based on

compound peak lists and intuitive plotting, it aids in visualisation and is capable of identifying more than 80% of detectable metabolites on an average from spectra of complex mixtures (Tulpan et al. 2011).

12.5 Conclusions and Future Prospects

Recent developments in technologies and instrumentation allow large-scale as well as nano-scale examination of biological samples. Ranging from turmeric (*Curcuma longa*) to *Aloe vera* to holy basil (*Ocimum tenifluorum*) to flowering plants of the coffee and even, asparagus family, plants have multifarious applications in cancer treatment and prevention. The anticancer agents derived from these plants have been classified into vinca alkaloids, epipodophyllotoxins, taxanes and camptothecin derivatives, and have proven to be extremely successful in the discovery of novel medicines. They have untapped potential for novel molecular target discovery and can contribute significantly to the drug development process. Medicinal plants can be probed for compounds with anticancer properties through omics strategies. Since the completion of the first draft of the human genome, an exceptional abundance of biological data has been churned out. Hence, in order to expedite cancer research, it is crucial to integrate systems biology, omics-based technology, bioinformatics and computational biology all together. Interactions and networks between genes and proteins are pivotal for the examination of cancer molecular mechanisms. Weight has shifted from genes to gene products. Hence, it is not only important to strategize and research at the level of the genome, but also at the level of proteome, transcriptome and metabolome. High-throughput sequencing has given an entire facelift to genomics and transcriptomics. Recombinant DNA technology and microarray technology have also become significantly important genomics and transcriptomics techniques respectively. In order to study proteomes, an increasing number of plant biologists are adopting mass spectrometry that aids protein identification, quantitation and structure determination. De novo peptide sequencing is also a popular mass spectrometry technique to determine a peptide sequence without any prior knowledge of the constituent amino acids. It is usually carried out in conjunction with two-dimensional gel electrophoresis that has applications in protein analysis. To study plant metabolism, the omics research technique of nuclear magnetic resonance spectroscopy is also adopted.

In modern biology and medicine, the analysis of huge amount of digital data derived from omics technologies has been overseen by the progressing science of bioinformatics. It allows effortless retrieval, assimilation, prediction and storage of DNA and protein sequence data with the help of efficient software programs and biological databases. With applications in not just genome sequence analysis, but also analysis of gene variation and expression, gene regulation dynamics, protein structure and function, this burgeoning field is being stressed upon globally. It assists molecular biologists in harvesting the fruits put forth by computational biology. This is all the more relevant since disorders are no more investigated by just

probing isolated genes. It has been expanded to gene networks, their interactions and roles in diseases like cancer. This has set the ball rolling for a whole new era of personalised medicine. As research in the field of anticancer plants progresses, more and more bioinformatics tools are being developed to make data extraction, processing, management, storage, analysis, interpretation and integration exceedingly efficient. They play a vital role in today's plant science. The challenge is thus, to employ these technologies effectively and optimally so as to solve oncological problems.

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Chapter 13
Fruits of Rosaceae Family as a Source of Anticancer Compounds and Molecular Innovations

Muhammad Sameeullah, Muttalip Gündoğdu, İhsan Canan, Turan Karadeniz, Muhammad Aasim, and Khalid Mahmood Khawar

Abstract Fruits of the family, Rosaceae (Apple, cherry, Peach, strawberry, rose, raspberry) are rich source of phenolic and antioxidant compounds having anticancer properties. The present chapter discusses the detail information about anticancer compounds of strawberry, raspberry, peach, apple, cherry and rose and also the genes responsible for the biosynthesis, accumulation and transport of anticancer compounds during growth and maturation of fruits. The transcriptome expression was performed to find putative genes responsible for anticancer compounds during the biosynthesis and transporter genes. It is revealed form the promoter analysis that *cis*-acting element is responsible for the regulation of anticancer compounds. Thus, CRISPR/Cas9 enhanced the biosynthesis of anticancer compounds during fruit development and maturation stages. CRISPR/Cas9 will be used for the silencing of genes which putatively inhibit the formation of anti-cancer compounds and also up-regulate biosynthesis and transporter genes mediated by CRISPR/Cas9to enhance their accumulation in these fruits.

Keywords Antioxidants · *cis*-acting elements · CRISPR/Cas9 · Gene families · Phenolics

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13.1 Introduction

Almond, peach, apple, strawberry, rose, cherry, pear, raspberry, black berry and plum are important members of Rosaceae family, and their fruits are important source of phenolics, antioxidants and contains biological properties to suppress the cancer cells. In last decade tremendous reports showing the health beneficial activities of antioxidants against different kind of cancer cells have been reported. These studies propose that the addition of phytochemical rich nutrient sources greatly prevent human body from diseases; such that the fruits of cherry (Velioglu et al. 1998) strawberry, apple and pear (Wang et al. 1996) peach and plum (Lea et al. 2008) in Rosaceae family got attention as sources of poly phenols and anthocyanin, plentiful different anti-oxidant and effective anticancer compounds (Ames et al. 1993; Willett 1994; Lea et al. 2008; Sharma et al. 2010; Johnson et al. 2011). Cancer is recognized as one of the major public health related issues and causing 585,720 deaths in 2014 alone in USA (Siegel et al. 2014). The present chapter highlights the potentiality of Rosaceae family fruits and their compounds in the cancer treatments. Furthermore, the possible enhancing role of CRISPR/Cas9 technology in the biosynthesis and production of anticancer compounds in the plant species is also discussed.

13.2 Anticancer Compounds of Family Rosaceae

13.2.1 Anticancer Compounds of Strawberry

The strawberry fruits are rich in phenolic compounds such as anthocyanins, flavanols, hydrolysable tannins and condensed tannins (Table 13.1). Among polyphenols anthocyanins are the most important. Vitamin C also is also reported to have curing effects on the cancer cells (Steinmetz and Potter 1996). Polyphenol-rich strawberry extract (PRSE) characterization revealed the higher amount of vitamin C and also polyphenols that were determined by Total Phenolic Contents (TPH), Total Flavonoids Content (TFC), and Total Anthocyanin Contents (ACY). The PRSE could reduce the survival of A17 a highly tumorigenic breast cancer cell while normal cells showed less sensitivity compared to cancer cells (Amatori et al. 2016); such that biological potential role of PRSE was established by *in vitro* and *in vivo* studies using strawberry extract with high effectiveness against breast cancer cells (Giampieri et al. 2012; Amatori et al. 2016). Hydrolysable tannins (ellagic acid) have been considered to be protective against breast and prostate cancer (Giampieri et al. 2012), and also has anti-inflammatory effect on colon cancer cells (Landete 2011). Similarly, Vitamin C (ascorbate) is an important radical scavenging antioxidant which prevents in cancer by reducing nitrite and inhibits the growth of tumor cell (Steinmetz and Potter 1996).

13.2.2 Anticancer Compounds of Apple

Apple is rich in phloretin 2′-glucoside (Ph) compound (Table 13.1), which is a natural polyphenol and has been demonstrated to exert anticancer activity via restriction of protein kinase C (PKC) action and introduction of apoptosis (Kern et al. 2007) in inhibiting liver cancer cells. Human liver cancer cells (Hep G2 and Hep 3B) were treated with Ph showed severe growth inhibition by the (paclitaxel) PTX-induced apoptosis mechanism (Yang et al. 2009). More recently, phloretin has been reported as an anticancer agent for human lung cancer cells A549, Calu-1, H838 and H520. Phloretin could suppress the growth of non-small cell lung cancer (NSCLC) by the down-regulation of Bcl-2, MMP-2 and -9, and up-regulation of cleaved-caspase-3 and -9. It also inhibits the activation of PCD and suppressed the infiltration and movement of the cells through coordinating PCD pathways and MMPs (Ma et al. 2016a). Ph and its derivatives exerted conclusive antitumor action against human cancer cell lines such as Bel 7402 liver cancer cell, HT-29 human colon cancer cell, A549 human lung cancer cell, HepG2 human ileocecal cancer cell (Qin et al. 2015). Polyphenolic compound found in apples and pears, has been shown to exert antitumor activity through inhibition of protein kinase C (PKC) activity and its induction of apoptosis.

Table 13.1 Anticancer compounds in selected fruits

Plants	Anticancer compounds	References
Cherry	Ellagic acid (ND)	Jakobek et al. (2007)
	Anthocyanin (192.5 mg/kg)	Jakobek et al. (2007)
	Vitamin C (7 mg/100 g FW)	USDA (2015)
Apples	Ellagic acid (0.07 mg/g DW)	Williner et al. (2003)
	Anthocyanin (50 nmol/cm^2)	Merzlyak et al. (2003)
	Vitamin C (4.6 mg/100 g)	USDA (2015)
Peach	Anthocyanin (27.91 mg /100 g FW)	Ceccarelli et al. (2016)
	Vitamin C (6.6 mg/100 g FW)	USDA (2015)
Raspberry	Ellagic acid (1642 mg/100 g)	Määttä-Riihinen et al. (2004)
	Anthocyanin (952.4 mg/100 g DM)	Wang and Lin (2000)
	Vitamin C (8.2–15.9 μmol gDW$^{-1)}$	Miret and Munné-Bosch (2016)
Rose	Ellagic acid (1461.2 μg/g DW)	Nowak (2006)
	Anthocyanin (16 mg %)	Oprica et al. (2016)
	Vitamin C (426 mg/100 g FW)	USDA (2015)
Strawberry	Ellagic acid (341 μg/g WT)	Atkinson et al. (2006)
	Anthocyanin (448.5 mg/100 g DM)	Wang and Lin (2000)
	Vitamin C (0.57 mg/g)	Amatori et al. (2016)

13.2.3 Anticancer Compounds of Peach

Peaches are rich in polyphenolic compounds (Table 13.1). The fruit extracts were tested against MDA-MB-435 breast cancer cell line that effectively constrained the generation of cancer cells. The concentration of extract to inhibit 50% (IC_{50}) growth was much lower (~42 mg/l) for cancer cells compared to normal cells (130 mg/l). Thus, it was established that these phenolic acids extract chlorogenic and neo-chlorogenic acids can act as high potential chemo preventive dietary source; with high growth inhibition against MDA-MB-435 cells recording compared to normal MCF-10A cells (Noratto et al. 2009).

13.2.4 Anticancer Compounds of Raspberry

Raspberries that are rich in anthocyanin, polyphenols and ellagitannins compounds are a rich source of the antioxidant compounds (Table 13.1). These phytochemicals possibly reach the colon and proficiently inhibit several important stages in colon carcinogenesis (Coates et al. 2007). The berries are reported to diminish procreation and enhanced apoptosis in colon tumors without interfering normal cells (Kristo et al. 2016). The short term effect of berry treatment was observed against colon tumors that suggested the role of this fruit as a potential source of anticancer (Wang and Stoner 2008).

13.2.5 Anticancer Compounds of Cherry

Cherry contains enormous amount of anthocyanin and ascorbic acid (Table 13.1); both of which are considered to effective anticancer agents (Aluko 2012; Amatori et al. 2016). The cherry extract containing anthocyanins inhibited the proliferation of human colon cancer cells HCT 116 and HT 29 (Kang et al. 2003). Bobe et al. (2006) demonstrated that the cherry extract in combination with sulindac reduced the incident of tumorigenesis in small intestine and also inhibited the proliferative activities in human (MCF-7) and mouse (4 T1) breast cancer cells (Ogur et al. 2014).

13.2.6 Anticancer Compounds of Rosehip

Rosehips (*Rosa rugose*) are rich in vitamin C, phenolics, flavonoids and carotenoids (Table 13.1). Recently, Zhong (2017) has reported the synergistic effect of vitamin C with rosehip triterpene on the effective inhibition and proliferation of MCF-7 breast cancer cells without inducing negative effects on normal breast cells

MCF-10A. Furthermore, xanthophyll esters derived from rosehips also inhibited cancer stem cell sub-populations and prevented cell migration.

13.2.7 Other Anticancer Compounds of Rosaceae Family Fruits

Ellagic acid is predominant phenolic in strawberry, raspberry and rosehip (Table 13.1). Ellagic acid exhibited active anti-proliferative exertion against the breast, prostatic and colon cancer cells. Ellagic acid exposure diminished cancer cell activity as demonstrated by decline in ATP levels of the cancer cells. Ellagic acid manifested a discriminatory cytotoxicity and anti-proliferative activity, and induced apoptosis in cancer cells without posing lethal developments on the activity of normal human lung fibroblast cells (Losso et al. 2004). Anthocyanin has been described to show anti-carcinogenic exertion in opposition to multiple cancer cell types *in vivo* and *in vitro* (Wang and Stoner 2008). *In vitro* studies showed that the anthocyanin has antioxidant effects by scavenging reactive oxygen species (ROS) radicles in liver, breast and leukemic cells. Anthocyanin arrest cell proliferation by intercepting the triggering of mitogen-activated protein kinase (MAPK) pathway. Anthocyanin mediate program cell death (PCD) independent to caspase regulated PCD in cancer cells (Feng et al. 2007). Further anthocyanin has anti-inflammatory, anti-angiogenesis, anti-invasiveness. *In vivo* studies demonstrated in model animals showed the powerful effect of anthocyanin to prevent esophageal, colon, skin and lung cancers such as (Wang and Stoner 2008). Vitamin C has been believed to be having anticancer properties since 1930 (van der Reest and Gottlieb 2016). The millimolar concentration of vitamin C exerted cytotoxic effect on cancer cells. This amount of vitamin C was effective to impair tumor growth in mouse intestinal cancer cells (Yun et al. 2015). High doses of ascorbat reported to be effective in proliferation of cancer cells (Yun et al. 2015; Mastrangelo et al. 2016).

13.3 Biosynthesis and Transporter Genes for the Anticancer Compounds

13.3.1 Biosynthesis of Ellagic Acid Genes

The ellagic acid biosynthesis genes identified and characterized in strawberry. It was observed that green immature fruits accumulate high amount of ellagic acid in consonance with the gene *GT2* expression levels. Reverse genetic approach also confirmed the function of *FaGT2* in biosynthesis of ellagic acid (Schulenburg et al. 2016). The ellagic acid biosynthesis genes belong to UDP glycosyltransferases gene families. In cultivated strawberry (*Fragaria* × *ananassa*) *FaGT2* and wild type

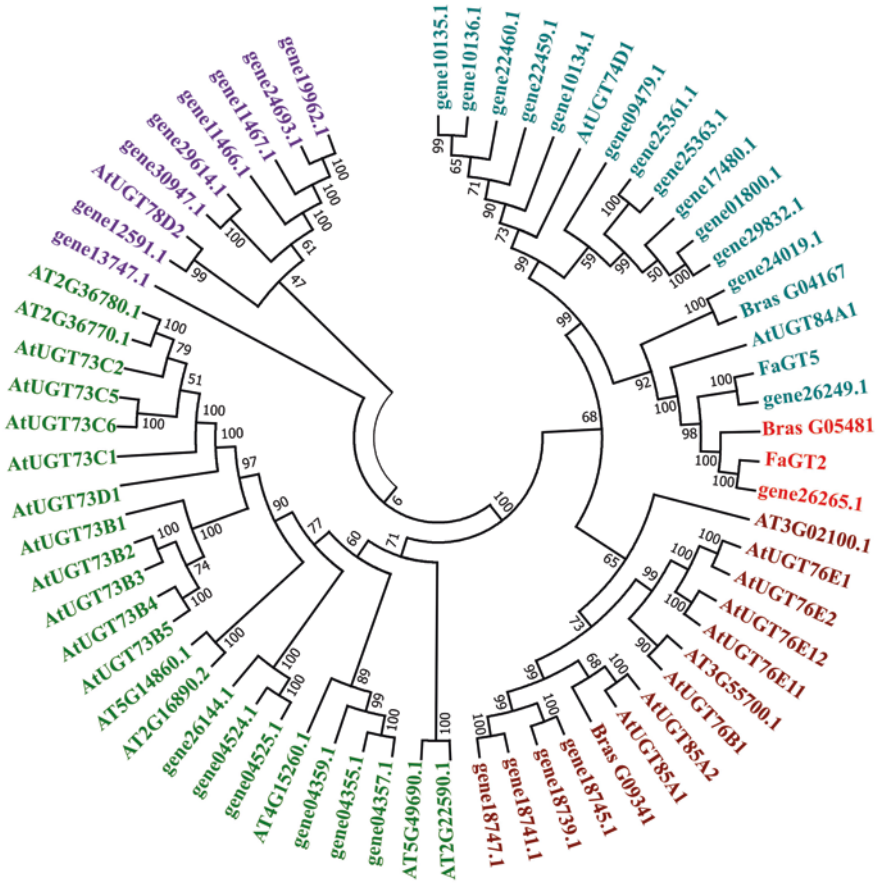

Fig. 13.1 Biosynthesis of ellagic acid genes

strawberry (*Fragaria vesca*) *gene26265.1* are responsible for the biosynthesis of ellagic acid. Another rosaceae family member raspberry is highly rich in ellagic acid (Table 13.1). The gene *Bras G05481*is believed to be putatively responsible for ellagic acid biosynthesis in black raspberry as it falls under the same clade of *FaGT2* and *gene26265.1* as shown in Fig. 13.1.

13.3.2 Biosynthesis of Anthocyanin Genes

Lin-Wang et al. (2010) has reported the biosynthesis of anthocyanin genes in plant species related to rosaceae family. This gene family belongs to R2R3 MYB transcription factor for the anthocyanin development in fruit flesh and flowers. Down-regulation of *FaMYB10* inhibited the production of anthocyanin in strawberry

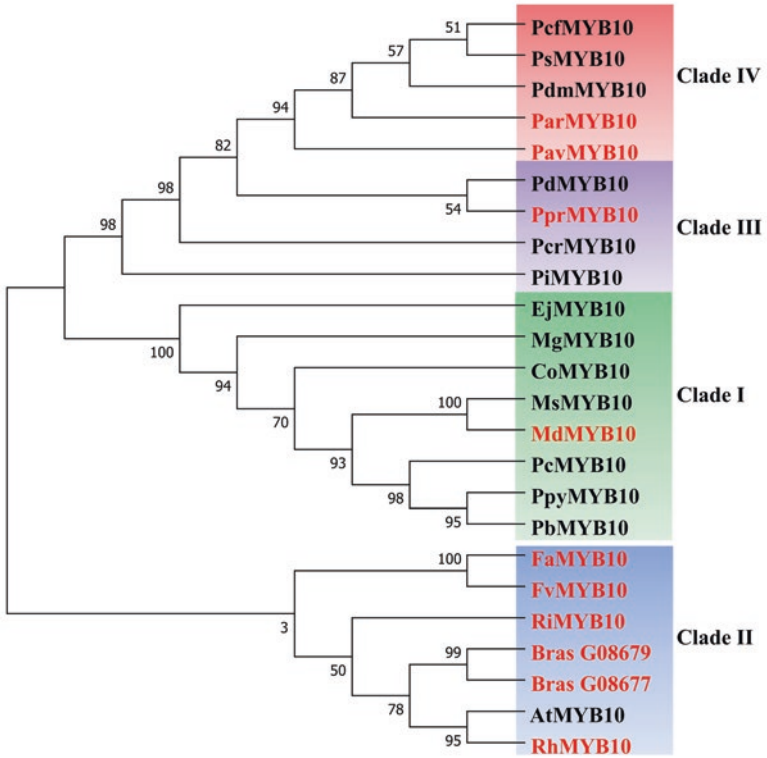

Fig. 13.2 Biosynthesis of anthocyanin genes

receptacles (Medina-Puche et al. 2014). Over-expression of *FvMYB10* induced the higher level of anthocyanin production in receptacles but also in leaves and flower in wild strawberry. On the other hand, RNAi silencing of *FvMYB10* diminished the anthocyanin level in the tissues (Lin-Wang et al. 2014). In apple, anthocyanin level was highly correlated with the expression of *MdMYB10* and fruit flesh color suggesting that *MdMYB10* is responsible for anthocyanin production (Espley et al. 2007). In peach fruit anthocyanin pigment development also regulate by *PprMYB10* (Ravaglia et al. 2013). Further the anthocyanin biosynthesis genes in rose, cherry and raspberry are also reported to be governed by MYB10 genes (Fig. 13.2).

13.3.3 Biosynthesis of Vitamin C Transporter Genes

The first vitamin C transporter gene in *Arabidopsis* plant was reported recently. Vitamin C produce in mitochondria but high level of vitamin C accumulation in chloroplast revealed by functional characterization of AtPHT4;4 which belongs to phosphate family 4 (Miyaji et al. 2015). This chloroplast envelop localized

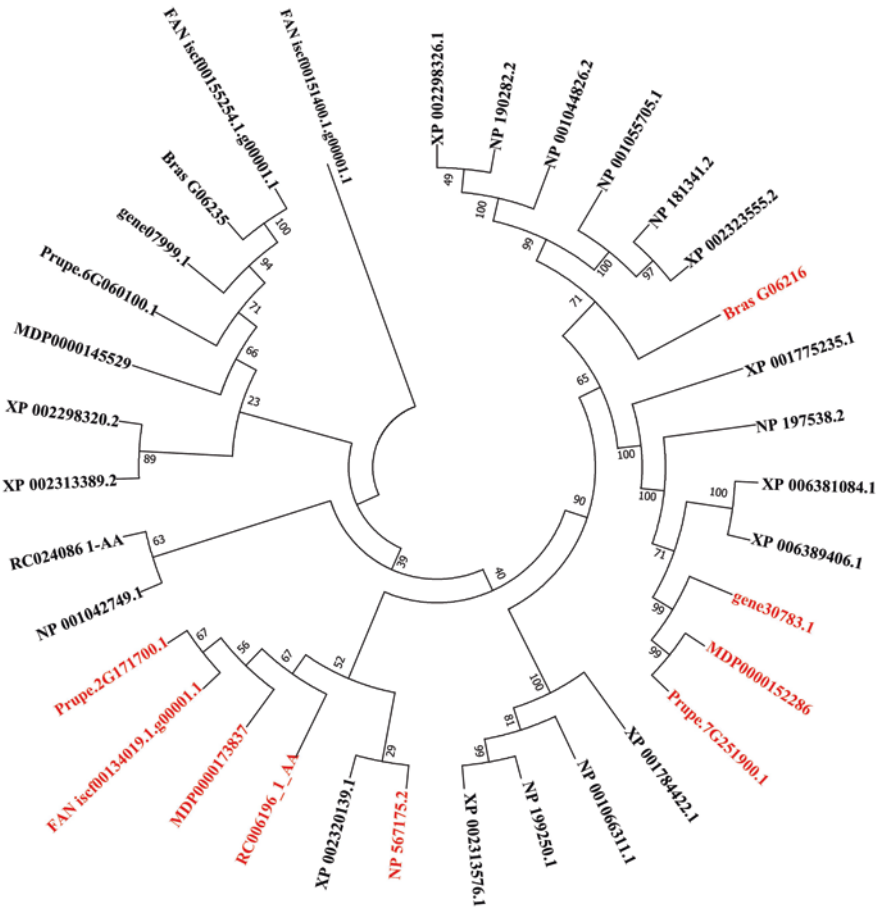

Fig. 13.3 Biosynthesis of Vitamin C transporter genes

transporter may have role in protection from strong sun light stress (Brunetti et al. 2013; Miyaji et al. 2015). Homology based searching in the rosaceae family gene database revealed close homologue of AtPHT4;4 in rose (RC006196_1_AA), apple (*MDP0000173837*, *MDP0000152286*), garden strawberry (*FAN iscf00134019.1.g00001.1*), peach (Prupe.2G171700.1, Prupe.7G251900.1), wild strawberry (*gene30783.1*) and black raspberry (*Bras G06216*) (Fig. 13.3) the functional characterization of these putative ascorbate genes in rosaceae would lead to reveal their evolutionary relationship for the transport of vitamin C in different organs of fruit plants.

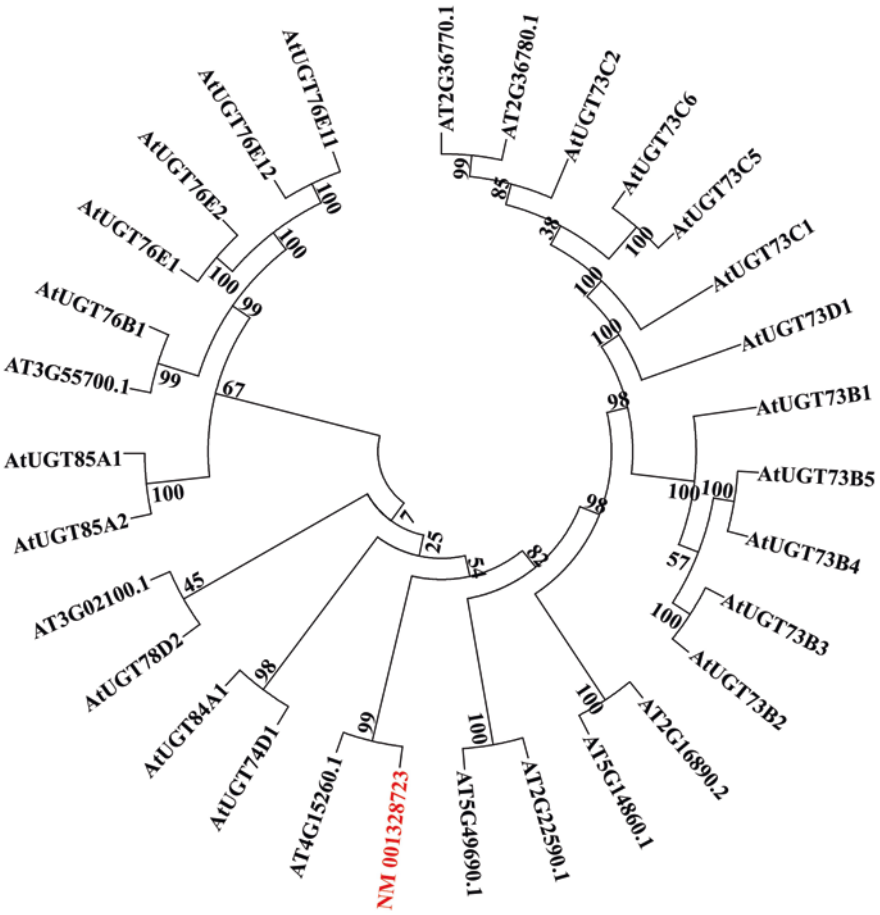

Fig. 13.4 Biosynthesis of Phloretin genes

13.3.4 Biosynthesis of Phloretin Genes

Phloretin 2′-glucoside (phlorizin) is the dominating phenolic compound is in all tissues of apple. These phenolic metabolites contribute to fruit color and flavor. The gene belongs to UDP-glycosyltransferase 88 family. MdPGT1 (NM 001328723) converts phloretin to phlorizin in the presence of uridine diphosphate glucose (Jugdé et al. 2008) (Fig. 13.4).

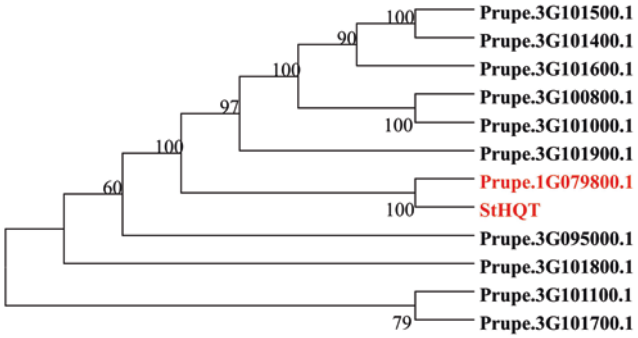

Fig. 13.5 Biosynthesis of Chlorogenic acid genes

13.3.5 Biosynthesis of Chlorogenic Acid Genes

Chlorogenic acid (CGA) is predominant phenolic compound in peach fruit. Hydroxycinnamoyl CoA:quinate hydroxycinnamoyl transferase (HQT) gene has been characterized in tobacco, tomato (Niggeweg et al. 2004) and potato (Payyavula et al. 2015). The functional characterization of HQT in tomato by overexpression and down regulation in potato revealed that biosynthesis of CGA is dependent on this gene. A close homologue to potato HQT in peach was found by database mining. This functional characterization of the peach HQT (Prupe.1G079800.1) would reveal its tissue specificity and accumulation in peach fruit during maturation and developmental stages (Fig. 13.5).

13.4 Regulation of *cis*-Acting Elements by Phytohormones in Biosynthesis Genes and Transporters of Anticancer Compounds

The upstream region approximately 1000 bp of ellagic acid biosynthesis genes was retrieved from the Rosaceae family genome database (https://www.rosaceae.org). These promoter sequences were analyzed with PlantCare database (Lescot et al. 2002) to reveal *cis*-acting elements responsive to phytohormones. Since, more often genes for substrate production or transport are dependent on phytohormones for their up-regulation (Wang and Irving 2011; Sameeullah et al. 2013). Therefore, prediction of phytohormones responsive elements would be beneficial to up-regulate or controlled expression of anti-cancer compound biosynthesis genes or transporters by the application of phytohormones. In *Fragaria vesca* the promoter sequence of ellagic biosynthesis gene (*gene26265*) contains two auxins and one salicylic acid responsive element. Black raspberry also contains one auxin response and a gibberellic acid response element (Table 13.2). However, anthocyanin biosynthesis

Table 13.2 Presence of *cis*-acting elements responsive to phytohormones in the promoters of anticancer compounds related biosynthesis genes and transporters

Gene name	*Cis*-element	Sequence	Functions	Copies/ promoter	Upstream location of *cis* elements
Ellagic acid *gene26265 Fragaria vesca*	AuxRR-core	GGTCCAT	Auxin responsive element	2	−400, −408
	TCA-element	CCATCTTTTT	Salicylic acid responsive element	1	−615
Bras_G05481 Rubus occidentalis	AuxRR-core	GGTCCAT	Auxin responsive element	1	−967
	TATC-box	TATCCCA	Gibberellin responsive element	1	−659
Anthocyanin *MDP0000259614 Malus domestica*	ABRE	TACGTG	Abscisic acid responsive element	1	−174
	CGTCA-motif	CGTCA	MeJA responsive element	1	−827
FvMYB10 Fragaria vesca	ABRE	TACGGTC	Abscisic acid responsive element	1	−846
	CGTCA-motif	CGTCA	MeJA responsive element	2	−386, −422
PprMYB10 Prunus persica	TGACG-motif	TGACG	MeJA responsive element	2	−954, −989
Bras_G08679 Rubus occidentalis	ABRE	TACGTG	Abscisic acid responsive element	1	−658
	CGTCA-motif	CGTCA	MeJA responsive element	1	−171
	TCA-element	CCATCTTTTT	Salicylic acid responsive element	2	−579

(continued)

Table 13.2 (continued)

Gene name	*Cis*-element	Sequence	Functions	Copies/ promoter	Upstream location of *cis* elements
Bras G08677 Rubus occidentalis	ABRE	CGTACGTGCA TACGTG	Abscisic acid responsive element	2	−834, −836
	GARE-motif	AAACAGA	Gibberellin-responsive element	1	−171
	TCA-element	CCATCTTTTT	Salicylic acid responsive element	1	−611
Vitamin C *Prupe.2G171700.1 Prunus persicap*	ABRE	ACGTGGC CACGTG	Abscisic acid responsive element	2	−902, −904
	CGTCA-motif	CGTCA	MeJA responsive element	1	−530, −587
	TGA-element	AACGAC	Auxin-responsive element	1	−267
MDP0000173837 Malus domestica	ABRE	CGTACGTGCA TACGTG	Abscisic acid responsive element	2	−834, −836
	GARE-motif	AAACAGA	Gibberellin-responsive element	1	−171
	TCA-element	CCATCTTTTT	Salicylic acid responsive element	1	−611
Bras_G06216 Rubus occidentalis	ABRE	GACACGTGGC CACGTG	Abscisic acid responsive element	2	−646, −648
	CGTCA-motif	CGTCA	MeJA responsive element	1	370
	GARE-motif	AAACAGA	Gibberellin response element	1	−42
	TGA-element	AACGAC	Auxin response element	3	−157, −897, −806

gene30783.1 Fragaria vesca	TCA-element	GAGAAGAATA	Salicylic acid response element	1	−893
MDP0000152286 Malus domestica	CGTCA-motif	CGTCA	MeJA responsive element	2	−607, −960
Prupe.7G251900.1 Prunus persica	CGTCA-motif	CGTCA	MeJA responsive element	1	−606
Phloretin *MDP0000219282 Malus domestica*	ABRE	GACACGTGGC ACGTGGC TACGTG	Abscisic acid responsive element	3	−724, −727, −726
	GARE-motif	TCTGTTG	Gibberellin response element	4	−15, −391, −198, −643
	TGA-element	AACGAC	Auxin response element	1	−825
Chlorogenic acid *Prupe.1G079800 Prunus persica*	GARE-motif	AAACAGA	Gibberellin response element	1	−472
	P-box	CCTTTTG	Gibberellin response element	1	−209

genes from apple (*MDP0000259614*) contain one MeJA (methyl jasmonic acid) and one (ABRE) abscisic acid response element. *Fragaria vesca* gene *FvMYB10* has two MeJA and one ABRE. *Prunus persica* (peach) *PprMYB10* has two MeJA elements. Black raspberry gene *Bras_G08679* has one ABRE and MeJA while two salicylic acid (SA) elements. Another black raspberry gene *Bras G08677* has two ABRE and one each for gibberellin and salicylic acid (Table 13.2).

The putative transporter gene of vitamin C from peach has two ABRE and one for MeJA and auxin response element. Apple *MDP0000173837* gene has two response element for ABRE and one each for gibberellin and salicylic acid. Black raspberry gene *Bras_G06216* has one *cis*-acting element each for gibberellin and MeJA, two for ABRE and three auxin response elements. *Fragaria vesca gene 30783*.1 has 1 SA, apple gene *MDP0000152286* that contain 2 MeJA and peach has 1 MeJA *cis*-acting element (Table 13.2). Putative phloretin biosynthesis gene *MDP0000219282* (*MdPGT1*) in apple has three ABRE, 4 gibberellin and one for auxin cis-acting element (Table 13.2), while, putative gene for chlorogenic acid biosynthesis in peach has one cis-acting element for auxin and two different gibberellic acid response elements (Table 13.2).

13.5 CRISPR/Cas9 Strategies to Improve Biosynthesis and Transport of Anticancer Compounds in Rosaceae Family Plants

CRISPR/Cas9 technology got attention in all disciplines of biological sciences for gene editing and modification without introducing the risk of gene pollution and environmental risk. This eco-friendly technique in last few years has been tested in various plant species (Sameeullah et al. 2017). This state-of-the art technology has facilitated either modification in a single gene of multiple gene (Lowder et al. 2015; Ma et al. 2016b). Therefore, for the enhancement of anticancer compounds in rosaceae family plants multiple target genes can be over expressed by manipulating the promoters of the anticancer biosynthesis genes or transporters. The modification of promoter by utilizing fused complex dCas9-VP64 in native promoter regions of ellagic acid, anthocyanin, phloretin and chlorogenic acid biosynthesis genes and vitamin C transporter genes would enhance the accumulation corresponding metabolites in fruits. Thus this environmental friendly approach would be feasible to produce anticancer compounds in rosaceae family to meet the human needs and create a healthy society.

13.6 Conclusions and Future Prospects

The fruits of rosaceae family such as strawberry, apple, peach, rose, cherry and black raspberry are naturally rich in anticancer compounds such as ellagic acid, anthocyanin, vitamin C, phlorizin and chlorogenic acid. These health beneficial compounds are not fully tapped from rosaceae family due to effluence of genetic and environmental effects on the plants. The biosynthesis and transport of anticancer compounds would be enhanced by the application of phytohormones and by up-regulating the genes due to presence of phytohormones specific *cis*-acting elements in the promoters of the corresponding genes. The application of phytohormones in common practice in progressive farmer's community. Another approach is the utilization of CRISPR/Cas9 technology to boost the anticancer compounds accumulation in fruits by modification in the promoters of the target genes. Thus it would be possible to enrich the rosaceae family fruits with ellagic acids, anthocyanin, vitamin C, phlorizin and chlorogenic acid by phytohormones application or adapting CRISPR/Cas9 to build healthy and cancer free society.

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Chapter 14
Mechanism of Action of Anticancer Herbal Medicines

Mohd Gulfishan, Muhammad Afzal, Imran Kazmi, Anwarulabedin Mohsin Quazi, Tariq Ahmad Bhat, and Ajmat Jahan

Abstract Cancer is a main health challenge for the world due to unavailability of standard treatments and severe side effects of chemotherapy. It is the second major cause of casualty worldwide after cardiac disorder. Medicinal plants are used for the treatment of various diseases since ancient time. Due to toxicity of allopathic medicines, peoples are coming back toward the use of natural medicines for the treatment of diseases. Approximately 38% of Americans are using alternative medicine or herbal medicines and spend around $34 billion dollars yearly on it. Constituents isolated from plants showed a crucial role in the development of useful anticancer agent. These include etoposide, vincristine and vinblastine from *Catharantus roseus*, camptothecin from *Camptotheca acuminata*, podophyllotoxin from *Podophyllum peltatum*, etc. The aim of the chapter is to describe various anticancer plants and their possible mechanisms of action in the prevention of cancer.

Keywords Action mechanism · Alternative therapy · Health care · Herbal medicine · Phytoconstituents

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14.1 Introduction

Cancer, one of the deadliest diseases of the world and became a public health burden all over, results in premature loss of life of many people. Cancer cells did not respond to any signals which control the irregular behavior of cells, invade normal tissues, and spread throughout the body. The continuous and uncontrolled proliferation of the cancer cell is the main abnormality in the development of cancer. The loss of growth control showed by the cancer cell is the main difference between the cancer cell and the normal ones. Around the world, cancer is the second leading cause of death and consumed 8 million lives in 2015. Cancer cases are projected to increase by 50% from 14 million to 21 million and cancer death by 60% from 8 million to 13 million. Among the different types of cancer death, the most common are lung (1.69 million deaths), liver (788000 deaths), colorectal (774000 deaths), stomach (754000 deaths), and breast (571000 deaths). In the coming decades, the number of cancer cases is expected to rise by about 70%, and the maximum death can occur in low- and middle-income countries. Tobacco and alcohol use, lack of physical activity, high body mass index, and low fruit and vegetable intake are the major behavioral and dietary risk which results more than one third of death from cancer. Based on the existing database, by reducing the risk factors and current prevention strategies, 30–50% of cancers can be cured. Early diagnosis and adequate treatments can also prevent many cancers.

The development of cancer is a multistep and complex process, and many agents such as radiations, viruses, chemicals, and environmental pollution have been found to cause cancer in both experimental animals and humans. Virus induced approximately 15% of all cancers. Human T-cell lymphotropic virus type I (HTLV-I), hepatitis B virus (HBV), several human papillomavirus (HPV) types, Epstein-Barr virus (EBV), hepatitis C virus (HCV), and human immunodeficiency virus type I (HIV-I) are oncogenic in nature (Boccardo and Villa 2011). The infection caused by these viruses may transform normal cells into a cancerous cell after many years of infection latency (Weinberg 1994; Butel 1999). The infections caused by these viruses are sexually transmitted because of unsafe and nonuse of contraception, and some of them are associated with unsafe health (WHO 2009; Boccardo and Villa 2011). The International Agency for Research on Cancer (IARC) declared 150 chemicals and other agents, ionizing radiation, occupational (workplace) and environmental airborne particles, some drugs, as well as foods and other consumer products as potential carcinogens (WHO 2009). Out of the total, 8% of lung cancer is due to the exposure to microscopic airborne particle at workplaces and compared to 12% deaths because of chronic obstructive pulmonary disease. Such type of cancer may be prevented by minimizing the exposure and by enclosing the workplace area safer to the human being (WHO 2009). Radiation and chemicals induce mutation by damaging the DNA which eventually leads to the development of cancer. Solar ultraviolet radiation (major cause of skin cancer), tobacco, and aflatoxin (a liver carcinogen) are some of the carcinogen which results in cancer development. About 80–90% of the lung cancers and oral cavity-related cancers are caused by smoking

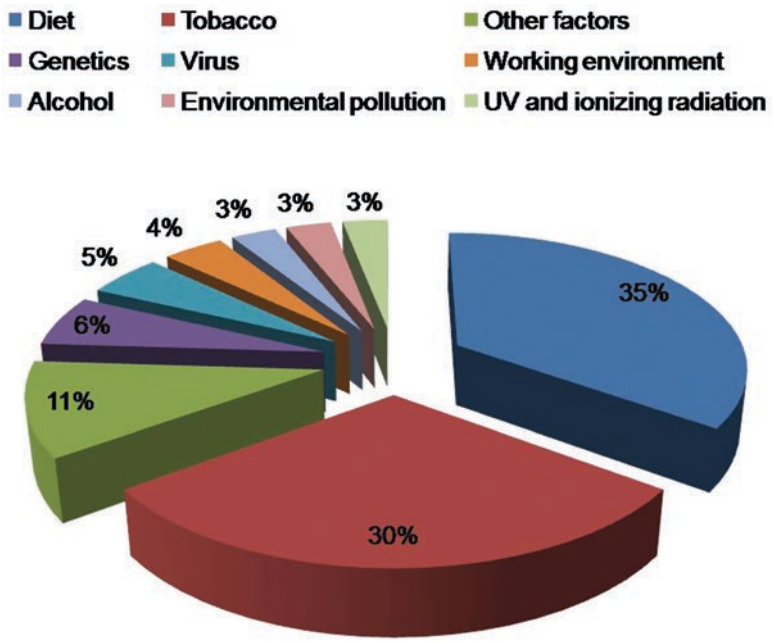

Fig. 14.1 Percentage of different factors affecting induction of cancers in humans

or by tobacco chewing. In addition, the balance between oxidants and antioxidants is necessary for the basic events of the cell regulation. When this balance is disturbed, the organism is said to be in oxidative stress (Bankson et al. 1993; McCord 1993). Oxidative stress produces reactive oxygen species (ROS) which can damage DNA and subsequently disrupt normal transcription and replication and induce mutations (Ames et al. 1995; Beckman and Ames 1998; Cavalieri et al. 2000; Evans et al. 2004). Such oxidative damage to macromolecules can deregulate signaling pathways which results in the development of cancer (Hietanen et al. 2004; Kawanishi and Hiraku 2006; Sawa and Ohshima 2006). Percentage of different factors affecting induction of cancers in humans is depicted in Fig. 14.1. The aim of this chapter is to describe the various anticancer plants and their possible mechanisms of action in the prevention of cancer.

14.2 Role of Phytoconstituents in the Treatment of Cancer

Plants have been used in treating different types of diseases, and different afflictions are still treated with habitual traditional medicines (Fabricant and Farnsworth 2001; Alviano and Alviano 2009). For 80% people living in rural areas, plants and their derived biologically active compound are important constituents of health care system (Sakarkar and Deshmukh 2011). About 500,000 plant species reported on earth

are tested for potency of their biologically active compound to treat different kinds of ailments, till date (Verpoorte 2000). Cyanogenic glycosides and glucosinolates, saponins, sulfur compounds, phenolic glycosides, phenols, unsaturated lactones, etc. are some of the plant-derived natural products with the potential to treat different afflictions (Mukherjee et al. 2001; Quiroga et al. 2001). Several plants and their active biological component have been a source of medicinal agents, and many of them possess anticancer and cytotoxic properties (Rao et al. 2016). Alkaloids, terpenes, and polyphenols have been reported to show the biological potential of medicinal plants (Balunas and Kinghorn 2005; Hu 2011). The cytotoxic nature of triterpenoids such as ursolic acid, oleanolic acid, boswellic acids, pomolic acid, avicins, and fomitellic acids is well documented (Dzubak et al. 2006). The anticancer activities of flavonoids such as kaempferol, myricetin, quercetin, and rutin have also been reported (Ren et al. 2003). Moreover, the anticancer activities of alkaloids such as matrine and sanguinarine have also been reported (Lu et al. 2012). Some of the medicinal plants with their isolated phytochemicals that possessed anticancer activity are listed in tabular forms (Table 14.1 and 14.2). The different classes of phytochemicals that are used in the cure of cancer are depicted in Fig. 14.2

14.3 Anticancer Plants and Their Mechanism of Action

*14.3.1 Aloe Plant (*Aloe vera*)*

Aloe juice is well known for their purgative potential and hair conditioning (De Caro et al. 2015; Ganesan and Choi 2016). It also possessed anticancer activity due to presence of a chemical emodin (Tabolacci et al. 2015). Lin et al. (2006) reported that emodin inhibited the cell viability and also stimulated the arrest of G2/M cell cycle.

*14.3.2 Asafoetida (*Ferula asafoetida*)*

Asafoetida is a perennial herb generally obtained from mountains of Afghanistan and India. The chief active constituent of this plant is ferulic acid, which is known for their antioxidant activity, prevention of oxidative stress, induction of nuclear translocation of Nrf2, and scavenging of reactive oxygen species (Das et al. 2017). It contains luteolin and α-pinene, which play a significant role in the inhibition intracellular production of ROS (Porres-Martinez et al. 2016)

Table 14.1 The anticancer activity of various medicinal plants and their derived phytochemicals

Plant	Phytochemicals	Anticancer activities
Alangium salviifolium	Isoquinoline alkaloids and derivatives	Ehrlich ascites carcinoma
Aloe vera	Aloin	Inhibition of human neuroectodermal tumors
Azadirachta indica	Limonoids	Murine Ehrlich carcinoma (EC) and B16 melanoma
Apium graveolens	Polyacetylenes	Leukemia cell lines
Alisma orientale	Triterpenes	HepG2, MDA-MB-231, and MCF-7 cell lines
Alstonia yunnanensis	Isoquinoline alkaloids and derivatives	Colon cancer
Aristolochia cucurbitifolia	Isoquinoline alkaloids and derivatives	Human liver cancer cell line
Aristolochia manshuriensis	Isoquinoline alkaloids and derivatives	Bone cancer
Atractylodes macrocephala	Sesquiterpenes	Lung carcinoma cells
Berberis vulgaris	Berberine	Breast, liver, and colon cancer cell lines
Brucea javanica	Triterpenes	Bladder cancer
Clausena harmandiana	IAD	Cholangiocarcinoma
Daphniphyllum glaucescens	Terpenoids, alkaloids	General treatment of cancer
Dictamnus dasycarpus	Triterpenes	Human breast cancer cells
Emblica officinalis	Alkaloids	Antitumor activity
Euphorbia fischeriana	Diterpenes	General treatment of cancer
Ginkgo biloba	Terpenoids	Human breast cancer cell line
Goniothalamus amuyon	IAD	General treatment of cancer
Gynura pseudochina	Terpenoids, alkaloids	Breast cancer
Hedyotis biflora	Benzopyrones	General treatment of cancer
Oroxylum indicum	Flavonoid	HeLa cells
Petroselinum crispum	Polyacetylenes	Leukemia cell lines
Piper longum	Amide alkaloids	HL60 and MCT-7 cell lines
Rhinacanthus nasutus	Rhinacanthins	HeLaS3 cells
Rubia cordifolia	Quinones	P-388 cancerous cell line
Schisandra henryi	Triterpenes	Leukemia and HeLa cells
Vitex rotundifolia	Diterpenes	Leukemia/myeloma, colon cancer
Winchia calophylla	Indole alkaloids	P-388 and A-549 tumor cell lines

Table 14.2 The anticancer activities of isolated compounds from different medicinal plants

Medicinal plant	Isolated compounds	Anticancer activities
Taxus baccata	Cabazitaxel	Metastatic castration-resistant prostate cancer
Colchicum autumnale	Colchicine	Multiple solid tumors (acts on matrix metalloproteases)
Combretum caffrum	Combretastatin	Human breast cancer
Taxus species	Docetaxol	Breast cancer, ovarian cancer, non-small-cell lung cancer (NSCLC)
Glycyrrhiza uralensis	Isoliquiritigenin	Human NSCLC, A549 lung cancer cell line
Taxus baccata	Larotaxel	Metastatic breast cancer, Bladder cancer, HSCLC, pancreatic cancer
Podophyllum peltatum *Podophyllum emodi*	Podophyllotoxin	Lymphomas, bronchial and testicular cancers
Taxus brevifolia	Paclitaxel	Breast cancer, ovarian cancer, NSCLC
Polygonum roots, peanut seeds, berries, and grapes	Resveratrol	Hepatoblastoma HepG2 and colorectal tumor SW480 cells
Broussonetia papyrifera	2S-abyssinone II, verubulin	Glioblastoma, brain tumors
Catharanthus roseus	Vinblastine, vincristine, vindesine	Lymphocytic leukemia
	Vinflunine, vinorelbine	Leukemias, lymphomas, testicular cancer, breast and lung cancers, Kaposi's sarcoma, advanced breast cancer, advanced NSCLC

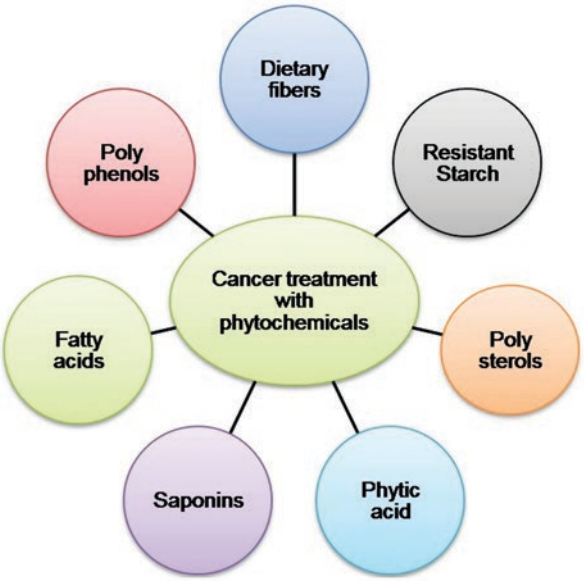

Fig. 14.2 Role of different phytochemicals in the cure of cancer

14.3.3 Artemisia (*Artemisia annua*)

It is also known as sweet wormwood, sweet annie, sweet sagewort, and sweet fern (Wang et al. 2017a, b). It contains artemisinin, which had protective effect against malaria and also possesses cancer activity (Mizushina et al. 2010; Blazquez et al. 2013). Artemisinin induces apoptosis in the cells of prostate cancer and showed efficacy against cancer of the colon, leukemia, cancer of the breast, and other cancerous cells (Mizushina et al. 2010). It also increased the downregulation of testes-specific protease 50 (abnormal protein) formed in cancer by overexpression of YSP50 gene in cancer cell, and inhibition of its expression can decrease proliferation of the cell and induce cell apoptosis (Wang et al. 2016a, b).

14.3.4 *Barberry* (Berberis vulgaris)

It is used in traditional medicine of Ayurveda since 2500 years ago. It is utilized in the treatment of diarrhea, fever, stomachic upset, fatigue, and nausea but recently identified as an anticancer plant (Abd El-Wahab et al. 2013). It showed antibiotic, antioxidant, and anti-inflammatory activities (Cybulska et al. 2011). It contains an active chemical compound berberine, which stimulates the genetic expression of caspase-3, caspase-8, and caspase-9 in mitochondria (Ho et al. 2009).

14.3.5 *Bitter Melon* (Momordica charantia)

It is utilized as a folk medicine in the treatment of different diseases. It reduced the cell proliferation and increased the apoptosis in the breast cancer cell (Ray et al. 2010). Apart from this it also showed effect in cervical and prostate cancer (Pongnikorn et al. 2003; Ru et al. 2011). It contains the active chemical compound charantin and BG-4 peptide having anticancer effect by apoptosis and reduction in the expression of Bax and Bcl-2 in cell, lead to increase in expression of caspase-3 and affecting cell cycle proteins CDK2 and p21 expression (Dia and Krishnan 2016).

14.3.6 *Blackberry* (Rubus fruticosus)

It is used as nutritional food due to presence of large amount of vitamin K, dietary fibers, vitamin C, carbohydrate, protein, fats, and minerals (Bushman et al. 2004; Jakobsdottir et al. 2013). The anticancer property in blackberry is due to presence of ellagic acid (EA). It possessed strong antioxidant activity and induces cancerous

cell growth inhibition by apoptosis through decreased ATP. It also had cytotoxicity and antiproliferative activities (Losso et al. 2004). EA showed anticancer activity by tumor cell proliferation inhibition, breaking carcinogen binding to DNA, induction of apoptosis, inhibiting angiogenesis, and disrupting the process of drug resistance and inflammation needed for metastasis and tumor growth (Zhang et al. 2014).

*14.3.7 Black Walnut Hulls (*Juglans nigra*)*

Black walnut is a very important herbal medicine known as walnut and used in the treatment of different diseases as a conventional and complementary medicine. Different parts of *J. nigra* are used for the management of several varieties of diseases like diarrhea, helminthiasis, stomachache, sinusitis, asthma, arthritis, scrofula, eczema, diabetes mellitus, skin disorders, thyroid dysfunction, anorexia, infectious diseases, and cancer (Panth et al. 2016). Walnuts contain bioactive compounds such as phytosterols and α-linolenic acid which may exert anticancer effects. It shows anticancer effect by induction of apoptosis by modulation in expression of apoptic genes and prevents proliferation. It increases expressions of tp53 genes, Bax, and caspase-3 and their corresponding biomolecule in cancerous cells (Vanden Heuvel et al. 2012).

*14.3.8 Bloodroot (*Sanguinaria canadensis*)*

It is a conventional medicinal plant utilized by American natives for the treatment of a wide range of diseases (Croaker et al. 2016). It contains bioactive alkaloids like chelerythrine, sanguinarine, sanguilutine, sanguirubine, chelilutine, and chelirubine and two protopin alkaloids allocryptopine and protopine (Bambagiotti-Alberti et al. 1991). Among these chemical constituents, sanguinarine suppresses Akt phosphorylation and phosphoinositide 3-kinase in KB cells and induces apoptosis (Lee et al. 2016).

*14.3.9 Burdock Root (*Arctium lappa*)*

Burdock is a native of northern Asia and Europe, and its root is traditionally used for the purification of blood. In China it is utilized in the treatment of venereal diseases, skin conditions, kidney problems, and respiratory infections. In European countries, it was utilized to treat several diseases like gout, rheumatism, scurvy, and respiratory infections. Burdock root eliminates cancer-promoting toxic matters that are stored in the intestine from improper food digestion (Wang et al. 2016a, b). Among the various active chemical compounds, arctigenin and Lappaol F are of prime

importance. Arctigenin inhibits the multiplication of cancer cells and induces apoptosis by caspase-3 activation in the cells of ovarian cancer. Suppression of STAT3/NO/iNOS signaling is basically associated with anticancer potential of arctigenin compound (Huang et al. 2014; Feng et al. 2016). However, Lappaol F showed anticancer activity by arresting cell division in G2 stage and induction of apoptosis (Sun et al. 2014).

*14.3.10 Cacao (*Theobroma cacao*)*

Cacao beans are a very good source of polyphenols. Polyphenols constitute around 10% of the weight of the bean. Cacao one of the derivatives of chocolate, especially dark chocolate, is a well-known antioxidant in the American diet after vegetables and fruits (Rusconi and Conti 2010). It is a rich source of caffeine and procyanidin. Procyanidin has anticanceractivity against breast cancer cells (Ramljak et al. 2005) by generating reactive oxygen species and stimulating apoptosis finally. Procyanidin is associated with the downregulation of various cell cycle regulatory proteins and site-specific dephosphorylation (Tabolacci et al. 2016).

*14.3.11 Camptotheca Plant (*Camptotheca acuminata*)*

Camptotheca plant is known as Chinese happy tree. It contains a natural alkaloid named camptothecin obtained from the bark. It is recognized as a potent anticancer agent and acts as a DNA topoisomerase I poison with an interesting antitumor activity, and its use is limited by low stability and unpredictable drug interactions (Martino et al. 2017).

*14.3.12 Cascara Sagrada (*Rhamnus purshiana*)*

Cascara bark is a native of North America and is known as bearberry, chitticum bark, and Chinook Jargon (Lans et al. 2007). The bark of this plant is used as a laxative in different parts of the world. The main active chemical constituents are aloe emodin and cascarosides A, B, C, and D. Aloe emodin inhibited cancer cell growth and suppresses the matrix metalloproteinase-9 and metalloproteinase-2 expression in cancer cells mediated by p38 mitogen-activated protein kinase signaling pathway and suppression of extracellular signal mediated by Akt/phosphatidylinositol 3-kinase and kinase signaling pathways (Lin et al. 2016).

14.3.13 Catharantus Plant *(*Catharantus roseus*)*

It is generally known as Madagascar periwinkle and found in different parts of the world. It contains two biologically active alkaloids vinblastine and vincristine used to treat cancer (Chan et al. 2016). These alkaloids may disrupt the function of microtubule responsible for the formation of mitotic spindle apparatus, which may be arrested in the metaphase arrest (Himes 1991).

14.3.14 Chamomile (Matricaria retutita*)*

It has been widely used as a conventional Tunisian herbal drug because of having the powerful health benefits (Jabri et al. 2016). It contains several bioactive compounds. Among these compounds, apigenin is chemically a flavone with anticancer and antioxidant activities. It is associated with a reduced cancer risk, especially cancers of the digestive tract, breast, blood, prostate, and skin (Shukla and Gupta 2010). Apigenin inhibited the apoptosis in cancer cells through the generation of reactive oxygen species as well as stress of endoplasmic reticulum in extrinsic and intrinsic mitochondrial pathway (Chen et al. 2016a, b; Wang and Zhao 2016).

14.3.15 Creosote Bush *(*Larrea tridentata*)*

This plant is a well-known North American shrub of deserts. It is widely used as a conventional herbal drug for the treatment of more than 50 different kinds of diseases (Arteaga et al. 2005). Among the various isolated bioactive compounds, nordihydroguaiaretic showed anticancer potential. This compound affects the neu/HER2/c-erbB2 and IGF-1 receptors responsible for the inhibition of growth of cancer cells (Youngren et al. 2005).

14.3.16 *Clove Bud (*Eugenia aromaticum*)*

Volatile oil is extracted from clove bud. It is usedas topical remedy for pain, flavoring and fragrance agent. The main pharmacologically active compounds of the oil are phenylpropanoids like eugenol, carvacrol, cinnamaldehyde, and anthocyanins and thymol. Anthocyanins inhibit the growth of cancerous cells (Chaieb et al. 2007). It arrests the growth of tumor and promotes arrest of G0/G1 cycle of cell as well as apoptosis (Liu et al. 2014).

14.3.17 Dandelion *(*Taraxacum officinale*)*

Leaves of this plant are utilized by medical practitioners of Chinese medicine and Ayurveda for the treatment of abscesses, cysts, and tumors and for retention of water (Hu et al. 2017). It is also utilized as a medicament for various diseases of women like uterus and breast cancer and gallbladder and liver disorders (Koo et al. 2004). It shows anticancer activity by induction of apoptosis in human cancer cells (Yoon et al. 2016).

14.3.18 Dioscorea *sp. (*Dioscorea bulbifera, D. membranacea, D. collettii*)*

The rhizome of this plant is extensively used in Thailand as a complementary and traditional medicine for the treatment of hepatic carcinoma and cholangiocarcinoma (Thongdeeying et al. 2016). It contains 5,6-dihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene and steroidal saponin diosgenin possessing anticancer activities. Diosgenin induces autophagy and apoptosis by inhibition of the mTOR/PI3K/Akt pathway signaling (Nie et al. 2016), and 5,6-dihydroxy-2,4-dimethoxy-9,10-dihydrophenanthrene arrests cell division in G2/M cell cycle phase and induces apoptosis (Duangprompo et al. 2016).

14.3.19 Foxglove *(*Digitalis purpurea*)*

Digitalis is an important and useful plant for atrial fibrillation, congestive heart failure, and cardiac arrhythmia (Ziff and Kotecha 2016). The main cardioprotective chemical constituents of the plant are digitoxin and gitoxin glycosides. These cardiac glycosides also showed cancer-inhibiting properties (Patel 2016). Cardiac glycosides arrest cell division in G0/G1 phase. Intracellular pathways indicated that it may modulate protein molecules involved in autophagy, apoptosis, proliferation, and cell cycle (Kaushik et al. 2016).

14.3.20 Frankincense *(*Boswellia serrata*)*

Frankincense oleo-gum resin is used in conventional medicine for the treatment of several disorders of inflammation like arthritis, asthma, pain, chronic bowel disease, and various other diseases (Khan et al. 2016). Among the various active constituents, boswellic acid shows anticancer activity. It inhibited protein synthesis (Casapullo et al. 2016) and metastasis by downregulation of cancer biomarkers (Yadav et al. 2012).

*14.3.21 Gentian Root (*Gentiana triflora*)*

Gentian is an herb found in the high pastures of the Alps and the Himalayas. It is used as bitters tonic, teas, general tonics, and tinctures (Pan et al. 2016). Its extract shows anticancer activity by cytotoxicity and chromosomal DNA degradation (Matsukawa et al. 2006).

*14.3.22 Ginger Root (*Zingiber officinale*)*

Ginger is a valuable and an economically significant plant used in most of the parts of the world. Ginger is utilized as spice, food, medicine, ornament, and condiment (Ismail et al. 2016). Gingerol is a bioactive compound isolated from ginger and is effective in the treatment of various types of cancers, most especially in the treatment of ovarian cancer. Gingerol induces cancer cell death in humans (Rhode et al. 2007). In ovarian cancers, it boosts immunological function and reduces inflammation, and it also helps protect the colon against colon cancer (Jeong et al. 2009).

14.3.23 Seeds of Grapes *(*Vitis vinifera*)*

Extract of grape seeds showed inhibition of colorectal cancer cell growth. The extract of grape seed is also useful against colorectal cancer (Derry et al. 2013). The phytochemicals isolated from seeds of grapes have both nonnutritive and nutritive values that showed significant antitumor property. Among these compounds, proanthocyanidins are the main biologically active compounds, which may inhibit the spread of pancreatic cancer cells to different parts of the body (Prasad and Katiyar 2013). Moreover, it inhibited the angiogenesis process triggered by colon cancer and also suppresses the growth of tumor in the colon (Huang et al. 2012). Due to poor absorption rate of proanthocyanidins, it is accumulated in high concentration in the colon and gastric tract. It inactivates the PKB/PI3-kinase pathway and enhanced apoptosis in cell line of colon cancer (Engelbrecht et al. 2007).

*14.3.24 Guava (*Psidium guajava*)*

Guava contains quercetin 3-glucuronide, d-glucuronic acid, xanthyletin, and loganin. These chemicals reduce lung cancer cell metastasis and the activity and expression of matrix metalloproteinase 2 and matrix metalloproteinase 9 by down-regulation of ERK1/2 activity in cells (Im et al. 2012). Leafy extract of guava is effective in inhibition of brain-derived metastatic carcinoma due the presence of

flavonoids and high polyphenolic compounds and acts as a chemopreventive agent (Chen et al. 2007). The compound of this plant also prevents cancer cells through various signaling cascades and induces the growth of tumors (Ryu et al. 2012).

14.3.25 Jasmine (Jasminum grandiflorum)

Jasminum is an important plant used in different parts of the world. It is widely utilized by tribal communities of India for the treatment of various diseases like toothache, body pains, ulcers, stomachache, and sexual dysfunction and impotency (Arun et al. 2016). Ethanolic extract of Jasmine flower showed chemopreventive effects under *in vivo* condition in mammary carcinoma. The bioactive compound responsible for their therapeutic activity is not fully explored, till date. Moreover, it also exhibited anti-lipid peroxidative potential and enhanced antioxidant defense in the living system (Kolanjiappan and Manoharan 2005).

14.3.26 Liquorice Root (Glycyrrhiza glabra)

Roots of liquorice are a well-known Chinese integrative and alternative medicine and most widely used as an antiulcer, antiviral, and anti-inflammatory agent. It gives protection against the damage of DNA that may be due to the effect of carcinogens. Liquorice contains polyphenols which induce apoptosis in cancerous cells (Wang and Nixon 2001). The root extract of liquorice suppresses the multiplication of human breast cancer cells. The root gives protection against human breast cancer by modulating expression of the Bax/Bcl-2 family of apoptotic regulatory factors (Jo et al. 2004).

14.3.27 Medicinal Mushrooms

Medicinal mushrooms contain a wide range of biologically active compounds and showed anticancer potential against the treatment of various types of cancers. These chemical constituents showed a variety of therapeutic activities like free radical scavenging, immunomodulating, antibacterial, anti-inflammatory, antiviral, antifungal, antidiabetic, hepato-protective, and cancer preventing. The bioactive constituents of medicinal mushrooms disrupt the intracellular signaling pathways related to differentiation of cell, inflammation and apoptosis, survival, progression of tumor, metastasis of cancer cells, and angiogenesis (Petrova 2012). It also inhibited the multiplication and spread of breast cancer cells by the arrest of cell cycle at G2/M phase and the regulation of gene expression. The potential of breast cancer cells to invade, migrate, and adhere is also prevented by medicinal mushrooms (Jiang and Sliva 2010).

*14.3.28 Milk Thistle (*Silybum eburneum, S. marianum*)*

The milk thistle plant is conventionally used as a natural medicine for the treatment of different types of diseases like CNS disorders such cerebral ischemia and Parkinson's and Alzheimer's disease for more than 2000 years (Devi et al. 2016). The pericarp extract of the plants contains flavonolignan mixture (isosilibinin, silibinin, silidianin, and silicristin). Among various chemical constituents, silibinin is the main active compound, which may possess *in vitro* anticancer activity against prostate adenocarcinoma cells, estrogen-independent and estrogen-dependent breast cancer cells, ecto-cervical cancer cells, colon cancerous cells, and both non-small- and small-cell human lung cancer (Bhatia et al. 1999; Hogan et al. 2007; Mokhtari et al. 2008). It reduces the risk of prostate cancer by inhibiting signaling mediated by HIF-1α, lipogenesis, and angiogenesis in cancerous cells (Deep et al. 2016) and also migrates and proliferates human hepatic cells (Ezhilarasan et al. 2016).

14.3.29 Nightshades (Solanum nigrum*)*

Solanum nigrum is called as black nightshade, while other species like *S. lyratum* and *S. dulcamara* are commonly known as bittersweet nightshade. The alkaloid isolated from various *Solanum* species showed anticancer activity by different mechanisms like interfering with the function and structure of tumor cell membrane, disturbing RNA and DNA synthesis, and changing the distribution of cell cycle. A glycoprotein isolated from *S. nigrum* showed anticancer potential by blockage of the NF-kappaB antiapoptotic pathway, caspase cascade reaction activation, and increase of the nitric oxide production (An et al. 2006). *Solanum* glycoalkaloid solamargine isolated from *S. incanum* shows anticancer activity by apoptosis induction in human liver cell (Kuo et al. 2000).

*14.3.30 Onion (*Allium cepa*)*

Onion showed a wide range of therapeutic action like antibiotic, anti-inflammatory, and anticancer activities (Griffiths et al. 2002; Park et al. 2007). It contains a flavonoid quercetin and is used for the treatment of multiple types of cancers (Galeone et al. 2006). However, quercetin showed anticancer effect by apoptosis and PI3K/Akt activation by regulating reactive oxygen species (Lu et al. 2017).

*14.3.31 Podophyllum Plant (*Podophyllum peltatum*)*

It is also known as American mandrake, may apple, wild mandrake, or ground lemon. It contains the mixture of toxic compounds known as podophyllotoxin. Etoposide is a semisynthetic derivative of phyllotoxin and causes inhibition of mitosis by microtubule assembly blocking. Etoposide inhibits progression of cycle cell at a pre-mitotic phase and DNA synthesis (Sinkule 1984).

*14.3.32 Rosemary (*Rosmarinus officinalis*)*

Extracts of rosemary plant exhibit anti-inflammatory, antioxidant, anticancer, and antidiabetic activities. The extract contains various chemical constituents like carnosic acid mixed with polyphenols, rosmarinic acid, carnosol, sageone, etc. (Moore et al. 2016). Carnosol, a phenolic diterpene, showed significant anti-inflammatory, antioxidant, apoptotic, and antiproliferative effects (Chun et al. 2014). Sageone, another diterpene isolated from *R. officinalis*, exhibited anticancer activity in gastric cancerous cells of humans (Shrestha et al. 2016). Moreover, the carnosic acid from this plant inhibits the signaling of STAT3 and increases apoptosis by reactive oxygen species generation in human colon cancer cell lines (Kim et al. 2016).

*14.3.33 Rue (*Ruta graveolens*)*

It is commonly known as a rue plant. The crude extracts and oil of this plant are used in many countries for the treatment of various diseases. Traditionally *Ruta graveolens*is used for the treatment of eye problems, pain, dermatitis, and rheumatism (Richardson et al. 2016). Furanoacridones extracted from the plant are effective against breast cancer cell line of humans (Schelz et al. 2016). Graveoline, extracted from *Ruta* plant, induces autophagy and apoptosis in melanoma cells (Ghosh et al. 2014).

*14.3.34 Saffron (*Crocus sativus*)*

It is the stigma of the flower of *Crocus sativus* which is widely used as herbal remedy for the enhancement of health and as an immune modulator in the Southeast Asian and Middle East countries (Mashmoul et al. 2016). Crocin, the main pharmacologically active compound isolated from stigma of saffron flower, showed a wide variety of bioactivities. It exhibited pharmacological activities like immune enhancer, antioxidant, and anticancer activities and significantly suppressed the proliferation of human lung adenocarcinoma cells (Chen et al. 2015).

*14.3.35 Tea (*Camellia sinensis*)*

It is commonly known as a tea and is a rich source of catechins. Tea leaf contains more than ten catechins, among which epigallocatechin gallate is the most prevalent (Xiang et al. 2016). It promotes the apoptosis in cells of B lymphoma by pathway dependent on caspase and by modulation of Bcl-2 protein family (Wang et al. 2015). However, theobromine isolated from this plant also showed antiproliferative and apoptotic effects (Wu et al. 2016).

*14.3.36 Gokhru (*Tribulus terrestris*)*

Tribulus terrestris belongs to the family Zygophyllaceae. It is generally known as puncture vine, Gokharu, or Gokshur and is widely used in Chinese and Indian traditional medicine for the treatment of a variety of disorders. It contains a variety of chemical compounds which are therapeutically important, like flavonol glycosides, flavonoids, alkaloids, and steroidal saponins (Chhatre et al. 2014). Terrestrosin D, a steroidal saponin isolated from *T. terrestris*, inhibits angiogenesis and growth of prostate cancer in humans (Wei et al. 2014).

*14.3.37 Turmeric (*Curcuma longa*)*

It is widely used as a condiment and spice and cultivated widely in Indian and Asian countries. Turmeric is used as a traditional medicine against many diseases like hepatic disorders, diabetes, and cough (Rezaee et al. 2016). Curcumin isolated from its turmeric is a polyphenol which showed anticancer potency against various types of cancer (Dasiram et al. 2016). It induces apoptosis in the cell via cleavage of poly(ADP-ribose) polymerase-1 (Mishra et al. 2016). It also modulated the P-glycoprotein in cancerous cell (Lopes-Rodrigues). Curzerene is another constituent from rhizome that induces the GSTA1 protein downregulation and expressions of mRNA in human lung adenocarcinoma cells (Wang and Zhao 2016).

*14.3.38 Thuja Plant (*Thuja occidentalis*)*

Thuja, also known as white cedar and arborvitae, in Europe is popular as an ornamental tree. It is utilized in different classes of traditional medicines like homeopathy and folk medicine and for treatment of bronchial catarrh, psoriasis, amenorrhea, uterine cancer, rheumatism, enuresis, and cystitis (Chang et al. 2000). It is traditionally used as an anticancer remedy due to the presence of its bioactive constituent

thujone. It decreases the viability of the cell and showed pro-apoptotic, antiproliferative, and antiangiogenic activities and promotes neoplasia regression and also inhibited the markers of angiogenesis like CD31, Ang-4, and VEGF in tumor cells (Torres et al. 2016).

14.4 Conclusions and Future Prospects

Numerous medicinal plants are used in various parts of the world for the treatment and prevention of cancers in various conventional ways for generations to generations. Use of phytoconstituents in the treatment of cancer is increasing rapidly and in an area of promising research. Bioactive compounds exhibited potential anticancer activity with specific mechanism of action on human body in clinical trials. Some specific compound of plant responsible for their anticancer effect along with their mechanism of action is the subject of interest for the researcher. The current chapter summarized the detail of anticancer plants with their specific mechanism of action in humans. Further clinical and toxicity studies are required on many mentioned plants in this chapter for the development of clinically approvable novel anticancer drugs.

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